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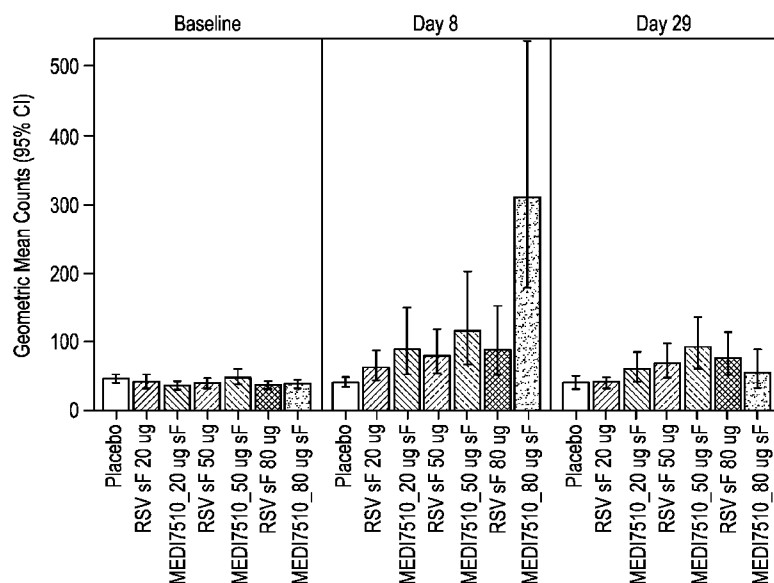
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(54) Title: VACCINE DOSE AND USE THEREOF

FIG. 1



(57) Abstract: Disclosed are methods of enhancing respiratory syncytial virus (RSV) immunity in a human subject. The methods encompass administering to a subject a single intramuscular dose of a composition comprising about 20 μ g, about 50 μ g, 80 μ g or about 120 μ g RSV soluble F protein. The composition further contains an adjuvant of glucopyranosyl lipid A (GLA) in a squalene-based stable emulsion. The RSV soluble F (sF) protein is amino acids 1-524 of RSV soluble F protein from human strain A2 lacking a transmembrane domain (SEQ ID NO: 1). In some embodiments the subject is human and is at least about 60 years old, or between at least about 60 years old and about 87 years old. In additional embodiments, the method of enhancing RSV immunity in the subject results in enhancing a Th1 biased cellular immune response in the subject, inducing neutralizing antibodies against RSV in the subject, reducing RSV viral titers in the subject, inducing an immune response to RSV in the subject, and/or preventing RSV infection in the subject.



— with sequence listing part of description (Rule 5.2(a))

VACCINE DOSE AND USE THEREOF

Reference to Sequence Listing Submitted Electronically

The content of the electronically submitted sequence listing in ASCII text file (Name: RSVFseqlist.txt; Size: 46,196 bytes; and Date of Creation: February 17, 2016) filed with the application is incorporated herein by reference in its entirety.

BACKGROUND

[0001] Respiratory syncytial virus (RSV) is a significant cause of respiratory disease, including pneumonia, in older adults and children. Symptomatic RSV re-infection occurs commonly throughout life. RSV outbreaks occur in a seasonal pattern that is similar, but not identical, to that of influenza, generally spanning the months of November to April in the temperate zone of the Northern Hemisphere; however, unlike influenza, RSV re-infection is not dependent on seasonal antigenic variation and is thought to occur because the duration of immunity after infection is relatively short (Hall et al, Immunity to and frequency of reinfection with respiratory syncytial virus. *J Infect Dis.* 1991;163(4):693-8; Falsey Serum antibody decay in adults following natural respiratory syncytial virus infection. *J Med Virol.* 2006 Nov;78(11):1493-7). Most adult RSV infections are undiagnosed because adults are rarely tested for the virus. Recent epidemiologic data highlight the importance of RSV as a cause of respiratory illness in adults, including illness resulting in hospitalization and death, particularly in older adults or in those with underlying cardiac or pulmonary disease (Falsey et al, Respiratory syncytial virus infection in elderly and high-risk adults. *N Engl J Med.* 2005;352:1749-59.; Walsh et al, Risk factors for severe respiratory syncytial virus

infection in elderly persons. *J Infect Dis.* 2004;189(2):233-8; Widmer et al, Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults. *J Infect Dis.* 2012;206(1):56-62. doi: 10.1093/infdis/jis309; Mullooly et al, Vaccine Safety Datalink Adult Working Group. Influenza- and RSV-associated hospitalizations among adults. *Vaccine.* 2007;25:846-55; United States Centers for Disease Control and Prevention (CDC), (2014). Older adults are more likely than younger adults to exhibit severe manifestations of RSV infection (Mullooly et al, 2007; Walsh et al, Viral shedding and immune responses to respiratory syncytial virus infection in older adults. *J Infect Dis.* 2013;207(9):1424-32. doi: 10.1093/infdis/jit038; Zhou et al, Hospitalizations associated with influenza and respiratory syncytial virus in the United States, 1993-2008. *Clin Infect Dis.* 2012;54(10):1427-36. doi: 10.1093/cid/cis211).

[0002] In healthy adults, RSV infection manifests predominantly as an upper respiratory tract disease causing mild, cold-like symptoms (nasal congestion, cough, wheezing and low-grade fever) leading to recovery within a week or two. However, older adults frequently succumb to symptomatic RSV infections (Evans, A. S., eds., 1989, “Viral Infections of Humans. Epidemiology and Control,” 3rd ed., Plenum Medical Book, New York at pages 525-544). For instance, several RSV infection epidemics have been reported among nursing home patients and institutionalized young adults (Falsey, A. R., *Infect. Control Hosp. Epidemiol.*, 12:602-608, 1991; and Garvie et al., *Br. Med. J.*, 281:1253-1254, 1980). RSV infection can cause serious health consequences in immunosuppressed persons, particularly bone marrow transplant patients (Hertz et al., *Medicine*, 68:269-281, 1989).

[0003] Recent epidemiological studies show that older adults are more prone to serious health consequences of RSV infection. Complications of RSV infection in older adults can lead to pneumonia infection and eventually, death. RSV infection is estimated to cause approximately 10,000 to 14,000 US deaths annually in persons at least 65 years old (Mullooly et al, 2007; Falsey et al, 2005; Kurzweil et al, Translational sciences approach to RSV vaccine development. *Expert Rev Vaccines.* 2013;12(9):1047-60. doi: 10.1586/14760584.2013.824706; US CDC, 2014). Further, although most frequently studied in the US, recent data show that RSV is clearly a cause of disease in older adults globally (Falsey et al, Respiratory syncytial virus and other respiratory viral infections in older adults with moderate to severe influenza-like illness. *J Infect Dis.* 2014 Jun 15;209(12):1873-81. doi: 10.1093/infdis/jit839). For instance, in a study modeling mortality data

from the Netherlands, RSV was found to be responsible for more deaths in persons 65 years old, or older, than deaths attributable to influenza A infection (van Asten et al, Mortality attributable to 9 common infections: significant effect of influenza A, respiratory syncytial virus, influenza B, norovirus, and parainfluenza in elderly persons. *J Infect Dis.* 2012 Sep 1;206(5):628-39. doi: 10.1093/infdis/jis415). In another study performed in England and Wales, RSV was reported to account for 1% and 2% of deaths among persons aged 45 to 74 and persons at least 75 years old, respectively, or approximately 1,200 and 4,000 deaths, respectively, each winter season (Hardelid et al, Mortality caused by influenza and respiratory syncytial virus by age group in England and Wales 1999-2010. *Influenza Other Respir Viruses.* 2013 Jan;7(1):35-45. doi: 10.1111/j.1750-2659.2012.00345.x). In the Netherlands, the total absolute number of RSV-associated excess hospitalizations was highest and approximately similar among adults 65 years old or older, and the youngest children, 0 to 1 year of age. RSV-active periods were associated with excess mortality among 50- to 64-year-olds and the elderly (Jansen et al, Influenza- and respiratory syncytial virus-associated mortality and hospitalisations. *Eur Respir J.* 2007;30:1158-66). The rate of RSV hospitalization has been estimated to be 25.4 per 10,000 residents 65 years old or older in the US (Mullooly et al, 2007). In a prospective 3-year study of hospitalized US patients at least 50 years old, annual rates of hospitalization due to RSV and influenza were estimated to be 15.01 and 11.81 per 10,000 residents, respectively (Widmer et al, 2012). Even more recently, the hospitalization rate associated with RSV in the US, assessed prospectively, was 11.24 (95% confidence interval [CI]: 0.83, 3.82) per 10,000 persons at least 50 years old, and emergency department visit rates were about 2 times higher: 19.48 (95% CI: 9, 40.8; Widmer et al, Respiratory syncytial virus- and human metapneumovirus-associated emergency department and hospital burden in adults. *Influenza Other Respir Viruses.* 2014 May;8(3):347-52. doi: 10.1111/irv.12234). The estimate from the US CDC (2014) is that each year, on average, there are 177,000 hospitalizations and 14,000 deaths among adults older than 65 years in the US. The annual US cost of RSV hospitalization in patients 65 years old or older has been estimated at between \$150 and \$680 million (Han et al, Respiratory syncytial virus pneumonia among the elderly: an assessment of disease burden. *J Infect Dis.* 1999; 179:25-30). In a recently completed US-based 4-year assessment of patients with polymerase chain reaction (PCR)-confirmed RSV-associated respiratory disease that resulted in medical provider contact, the

seasonal incidence of RSV disease was 1.79% (95% CI: 1.47% to 2.18%) in patients at least 60 years old (McClure et al, Seasonal incidence of medically attended respiratory syncytial virus infection in a community cohort of adults ≥ 50 years old. PLoS One. 2014;9(7):e102586. doi:10.1371/journal.pone.0102586). In a slightly older population of healthy older adults at least 65 years old followed for respiratory illness for 4 consecutive seasons, the incidence of RSV infection (not necessarily medically attended) diagnosed by culture, PCR, or serology was 3% to 7% annually (Falsey et al, 2005).

[0004] Treatment options for RSV infection are limited. Illness can be prolonged, lasting an average of 16 days, and one third of patients were reported to have sought medical care. In the healthy elderly with RSV illness, 15% called their physician and 17% made an office visit (Falsey et al, 2005). Patients with high risk conditions (chronic heart or lung disease) and RSV illness were even more likely to have medical care contact: about half consulted a physician, 9% visited an emergency room, and 16% were hospitalized (Falsey et al, 2005).

[0005] Severe RSV infection of the lower respiratory tract often requires considerable supportive care, including administration of humidified oxygen and respiratory assistance (Fields et al., eds, 1990, Fields Virology, 2nd ed., Vol. 1, Raven Press, New York at pages 1045-1072). The antiviral agent ribavirin was approved for treatment of infection (American Academy of Pediatrics Committee on Infectious Diseases, *Pediatrics*, 92:501-504, 1993). Ribavirin, a guanosine analog, was shown to be effective in the treatment of RSV pneumonia and bronchiolitis, modifying the course of severe RSV disease in immunocompetent children (Smith et al., *New Engl. J. Med.*, 325:24-29, 1991). Use of ribavirin is limited because it requires prolonged aerosol administration and due to potential health risk to pregnant women exposed to the drug in hospital settings.

[0006] Presently there is no vaccine available that protects against RSV infection. Safety issues present a major obstacle to development of an RSV vaccine. For instance, a formalin-inactivated vaccine was developed and though the vaccine provided some immunogenicity, the vaccine unexpectedly caused a higher and more severe incidence of RSV-linked lower respiratory tract disease in immunized infants than in infants immunized with a similarly prepared trivalent parainfluenza vaccine (Kim et al., *Am. J. Epidemiol.*, 89:422-434, 1969; and Kapikian et al., *Am. J. Epidemiol.*, 89:405-421, 1969). Despite over 50 years of subsequent research, attempts to develop

suitable vaccines against RSV have met with no success. Thus, given the current absence of any preventive or therapeutic strategy for RSV in older adults, there remains a compelling unmet medical need in this population for a safe and efficacious vaccine against RSV.

SUMMARY

[0007] Disclosed are methods of enhancing respiratory syncytial virus (RSV) immunity in a human subject. The methods comprise administering to a subject a single intramuscular dose of a composition comprising about 20 µg, about 50 µg, about 80 µg, or about 120 µg RSV soluble F protein. The composition further comprises an adjuvant of glucopyranosyl lipid A (GLA) in a squalene-based stable emulsion. The RSV soluble F (sF) protein is amino acids 1-524 of RSV soluble F protein from human strain A2 lacking a transmembrane domain, as show below (SEQ ID NO: 1):

METGLLELEILELELYSALAASNALAIETHRTHRILELETHRALAVALTHRPHECYSPHE
 ALASERGLYGLNASNILETHRGLGLPHETYRGLNSERTHRCYSSERALAVALSERLYSGL
 YTYRLESERALALEARGTHRGlyTRPTYRTHRSERVALILETHRILEGLLESERASNILEL
 YSLYSASNLYSCYSASNGLYTHRASPALALYSVALLYSLEILELYSGLNGLLEASPLYST
 YRLYSASNALAVALTHRGLLEGLNLELEMETGLNSERTHRPRALATHRASNASNARGA
 LAARGARGGELLEPRARGPHEMETASNTYRTHRLEASNASNALALYSLYSTHRASNVAL
 THRLESERLYSLYSARGLYSARGARGPHELEGLYPHELELEGLYVALGLYSERALAILEA
 LASERGLYVALALAVALSERLYSVALLEHISLEGLGLYGLVALASNLYSILELYSSERAL
 ALELESERTHRASNLYSALAVALVALSERLESERASNGLYVALSERVALLETHRSELYS
 VALLEASPLELYSASNTYRILEASPLYSGLNLELEPRILEVALASNLYSGLNSERCYSSERI
 LESERASNILEGLTHRVALILEGLPHEGLNGLNLYSASNASNARGLELEGLILETHRARG
 GLPHESERVALASNALAGLYVALTHRTHRPRVALSERTHRTYRMETLETHRASNSEGL
 LELESERLEILEASNASPMETPRILETHRASNASPGLNLYSLYSLEMETSERASNASNVAL
 GLNILEVALARGGLNGLNSERTYRSEIRILEMETSERILEILELYSGLGLVALLEALATYRV
 ALVALGLNLEPRLETYRGLYVALILEASPTHPRCYSTRPLYSLHISTHRSEPRLECYS
 THRTHRASNTHRLYSGLGLYSERASNILECYSLETHRARGTHRASPARGGLYTRPTYRC
 YSASPASNALAGLYSERVALSERPHEPHEPRGLNALAGLTHRCYSLYSVALGLNSERAS
 NARGVALPHECYSPHMETASNSERLETHRLEPRSERGLVALASNLECYASASNVAL
 ASPILEPHEASNPRLYSTYRASPCYSLYSILEMETTHRSELYSTHRASPVALSERSESER
 VALILETHRSEERLEGLYALAILEVALSERCYSTYRGLYLYSTHRLYSCYSTHRALASERA
 SNLYSASNARGGLYILEILELYSTHRPHESEASNGLYCYSPTYRVALSERASNLYSG
 LYVALASPTHRVALSERVALGLYASNTHRLETYRTRYRVALASNLYSGLNGLGLYLYSSE
 RLETYRVALLYSGLYGLPRILEILEASNPHETYRASPPRLEVALPHEPRSERASPGLPHEA
 SPALASERILESERGLNVALASNGLLYSILEASNGLNSEERLEALAPHEILEARGLYSSERA
 SPGLLELEHISASNVALASNALAGLYLYSSERTHRTHRASN

[0008] In some aspects the subject is human and is at least about 60 years old, or between at least about 60 years old and about 87 years old.

[0009] In some aspects, the enhanced RSV immunity provided to the subject includes an increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells. The baseline level of RSV F-specific T cells can be the level in the subject prior to administration of the composition, the mean level found in a pool of subjects who have not received the composition, or the mean level found in a pool of subjects administered a non-adjuvanted composition comprising about 20 µg, about 50 µg, about 80 µg, or about 120 µg RSV soluble F protein.

[0010] In the disclosed methods, where the enhanced immunity includes an increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells, the increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells can be at least one- to about ten-fold, at least one- to about seven-fold, or one- to about three-fold.

[0011] In some aspects, when the composition comprises about 20 µg or about 50 µg RSV soluble F protein, the increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells can be at least a six- to about a ten-fold increase. In another aspect, the composition comprises about 80 µg RSV soluble F protein, and the subject is about 60 years old to about 69 years old. For instance, in one aspect, the subject is more than 69 years old, and the increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells can be at least about eight- to ten-fold, at least about nine- to ten-fold, at least about four- to six-fold, or at least about five- to six-fold.

[0012] In aspects where the enhanced immunity includes an increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells, the T cell activity can be determined at about 8 days after vaccination by IFN γ Enzyme Linked Immunosorbent Spot Assay (ELISPOT) assay.

[0013] In other aspects, the enhanced RSV immunity provided to the subject includes an increase in the RSV microneutralizing antibody titer of the subject over a baseline RSV microneutralizing antibody titer. The baseline level of RSV microneutralizing antibody titer can be

the level in the subject prior to administration of the composition, the mean level found in a pool of subjects who have not received the composition, or the mean level found in a pool of subjects administered a non-adjuvanted composition comprising about 20 µg, about 50 µg, about 80 µg, or about 120 µg RSV soluble F protein. In other aspects, these levels can be assessed via geometric mean titres (GMTs).

[0014] In such aspects, the subject can be between at least about 60 years old and about 87 years old. Further, where the enhanced RSV immunity provided to the subject includes an increase in the RSV microneutralizing antibody titer of the subject over a baseline RSV microneutralizing antibody titer, the baseline RSV microneutralizing antibody titer can be increased by at least one-fold, at least about one- to four-fold, at least about one- to three-fold, three-fold, at least about 2.5- to 4.0-fold, at least about 3.0- to 4.0-fold, or at least about 3.5- to 4.0-fold.

[0015] In some aspects, the enhanced immunity is indicated by an increase in the anti-RSV F protein-specific antibody titer in the subject over a baseline anti-RSV F protein-specific antibody titer, such as, for instance, a 5-fold increase in the anti-RSV F protein-specific antibody titer in the subject over a baseline anti-RSV F protein-specific antibody titer. The baseline level of anti-RSV F protein-specific antibody titer can be the level in the subject prior to administration of the composition, the mean level found in a pool of subjects who have not received the composition, or the mean level found in a pool of subjects administered a non-adjuvanted composition comprising about 20 µg, about 50 µg, about 80 µg, or about 120 µg RSV soluble F protein.

[0016] In such aspects, the subject can be between at least about 60 years old and about 87 years old. Further, where the enhanced RSV immunity provided to the subject includes an increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer, the baseline anti-RSV F protein-specific antibody titer can be increased by at least about one-fold, at least about 5- to 25-fold, at least about 5- to 15-fold, or at least about 10- to 15-fold increase.

[0017] In some aspects, the composition comprises about 80 µg RSV soluble F protein, and the enhanced immunity provided to the subject includes an increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer of at least about 15- to 25-fold, at least about 20- to 25-fold, or at least about 20-fold.

[0018] In other aspects, the subject is more than about 69 years old, and the composition comprises about 80 µg RSV soluble F protein. In such aspects, the enhanced immunity provided to the subject includes an increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer of at least about 5- to 15-fold, at least about 10- to 15-fold, or at least about 10-fold.

[0019] In one aspect, the anti-RSV F protein-specific antibodies are IgG antibodies.

[0020] In another aspect, the enhanced immunity is indicated by an increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer as determined by a competitive ELISA (cELISA) using the antibody palivizumab as the competitor antibody, such that the ELISA measures the titer of antibodies with binding characteristics that are sufficient to block binding of palivizumab. Further, in such aspects, where the enhanced RSV immunity provided to the subject includes an increase in the RSV-specific antibody titer of the subject over a baseline RSV-specific antibody titer as measured by cELISA, the baseline RSV-specific antibody titer can be increased by at least about 5- to 35-fold, or at least about 10- to 30-fold.

[0021] In some aspects, the composition comprises about 20 µg or about 50 µg RSV soluble F protein, and the baseline RSV-specific antibody titer can be increased by at least about 5- to 25-fold, or at least about 10- to 25-fold.

[0022] In some aspects, the composition comprises about 50 µg RSV soluble F protein, and the baseline RSV-specific antibody titer can be increased by at least about 10- to 25-fold, at least about 15- to 25-fold, at least about 15- to 20-fold.

[0023] In some aspects, where the composition comprises about 80 µg RSV soluble F protein, the baseline RSV-specific antibody titer can be increased by at least about 20- to 35-fold, or at least about 25- to 30-fold. In one such embodiment, the subject is more than about 69 years old, the composition comprises about 80 µg RSV soluble F protein, and the baseline RSV-specific antibody titer can be increased by at least about 25- to 35-fold, or at least about 30-fold.

[0024] In other embodiments, the subject is more than about 69 years old, the composition comprises about 80 µg RSV soluble F protein, and the baseline RSV-specific antibody titer can be increased by at least about 10- to 25-fold, at least about 15- to 20-fold, or at least about 20-fold.

[0025] In one aspect, a composition comprising about 120 µg RSV soluble F protein is administered to the subject which includes an adjuvant comprising about 1.0 µg, about 2.5 µg, or about 5.0 µg glucopyranosyl lipid A (GLA) in a squalene-based stable emulsion. In this embodiment, the RSV soluble F protein is amino acids 1-524 of RSV soluble F protein from human strain A2 lacking a transmembrane domain (SEQ ID NO: 1). In one aspect, the subject is human of at least about 60 years of age. For instance, in one aspect, the subject is about 60 years old, at least about 65 years old, or about 60 and 65 years old.

[0026] In one aspect, the composition comprises about 120 µg RSV soluble F protein and is administered intramuscularly.

[0027] In some aspects, the RSV soluble F protein can be recombinant RSV soluble F protein and can be in some instances produced *in vitro* by Chinese Hamster Ovary (CHO) cells.

[0028] In one aspect, the RSV soluble F protein is resuspended from lyophilized form in the adjuvant.

[0029] In some aspects, the composition is administered in a volume of about 0.5 mL.

[0030] In one aspect, the composition need only be administered annually.

In still other aspects, the composition is administered concomitantly with a composition intended to generate an immune response against influenza virus. The composition intended to generate an immune response against influenza virus can be administered contralaterally to the composition comprising RSV soluble F protein.

[0031] In one aspect, the subject is human and is RSV seropositive, and/or has been previously exposed to RSV.

[0032] In additional aspects, the method of enhancing RSV immunity in the subject results in enhancing a Th1 biased cellular immune response in the subject, inducing neutralizing antibodies against RSV in the subject, inducing an immune response to RSV in the subject, and/or preventing RSV infection or disease caused by RSV in the subject.

[0033] In some aspects, the adjuvant is about 2.5 µg glucopyranosyl lipid A (GLA) in a squalene-based stable emulsion of about 2% (v/v).

[0034] In aspects where an antibody titer is determined, the antibody titer in the subject is determined at about 20 days, about 50 days, about 100 days, about 150 days, about 200 days, about 250 days, about 300, or about 365 days after vaccination

[0035] In certain aspects where an antibody titer is determined, the antibody titer in the subject is determined at about 29 days after vaccination.

[0036] In other aspects where an antibody titer is determined, the antibody titer in the subject is determined at about 271 days after vaccination.

[0037] In additional aspects where an antibody titer is determined, the antibody titer in the subject is determined at about 361 days after vaccination.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0038] FIGURE 1. Response to RSV sF (non-adjuvanted) compared to MEDI7510 (containing RSV sF + 2.5 µg GLA in 2% (v/v) SE) administered at 20 µg, 50 µg, or 80 µg, w in RSV seropositive population, as determined by IFN γ ELISPOT assay (spot-forming counts 10^6 peripheral blood mononuclear cells [PBMCs]) at baseline, Day 8 and Day 29 post dose (CI = Confidence Interval). MEDI7510 (adjuvanted RSV sF) boosts cellular immunity in a seropositive population (baseline is similar to result obtained from placebo).

[0039] FIGURES 2A-C. Response to RSV sF (non-adjuvanted) compared to MEDI7510 (containing RSV sF + 2.5 µg GLA in 2% (v/v) SE) administered at 20 µg, 50 µg, or 80 µg in RSV seropositive population, as determined by microneutralization antibody titer (Figure 2A), anti-F IgG titer (Figure 2B), and competitive ELISA titer using palivizumab as competitor (Figure 2C) at Day 29 post dose (CI = Confidence Interval). Geometric mean titers (GMTs) are presented. MEDI7510 (adjuvanted RSV sF) boosts humoral immunity in a seropositive population.

[0040] FIGURE 3. Reverse cumulative distribution of microneutralization titers (log2) at Day 29 post dose as compared to baseline titers for RSV sF (non-adjuvanted) compared to MEDI7510 (containing RSV sF + 2.5 µg GLA in 2% (v/v) SE) administered at 20 µg, 50 µg, or 80 µg.

[0041] FIGURE 4. Correlation between assays for the detection of immune responses to RSV. Post baseline titer/count data from all active groups at primary timepoint (Day 8 post dose for ELISPOT assays, and Day 29 post dose for all the other biomarkers) combined. All comparisons

between humoral immunogenicity assays have P value <0.001, Pearson Correlation. Comparisons between humoral immunogenicity assays and ELISPOT assay have p value <0.02, Pearson Correlation, showing that assay results are highly correlated.

[0042] FIGURE 5. Baseline (BL) Titer Effects on Post-Dose Fold Rise from Baseline: BL < Median vs $\geq$ Median, by Assay. Fold change in antibody titers at day 29 post dose for microneutralization, RSV sF IgG and competitive ELISA assays, as well as fold change in IFN γ ELISPOT assay at day 8 post dose for RSV sF (non-adjuvanted) compared to MEDI7510 (containing RSV sF + 2.5 μ g GLA in 2% (v/v) SE) administered at 20 μ g, 50 μ g, or 80 μ g). Seropositive subjects with lower baseline titers have more pronounced antibody responses to vaccination.

[0043] FIGURE 6. MEDI7510 (containing RSV sF + 2.5 μ g GLA in 2% (v/v) SE) significantly shifts population antibody responses in an antigen dose-dependent manner. Microneutralization titers are given for RSV sF (non-adjuvanted) compared to MEDI7510 (containing RSV sF + 2.5 μ g GLA in 2% (v/v) SE) administered at 20 μ g, 50 μ g, or 80 μ g, with 2.5 μ g GLA in 2% (v/v) SE). Dotted lines represent 69th percentile of antibody titers at baseline. After dosing, 95% of subjects have microneutralizing antibody titers that exceed the 69th percentile of baseline titers.

[0044] FIGURE 7. Reverse cumulative distribution of interferon gamma ELISPOT responses (Spot forming cells/ 10^6 PMBC), at Days 8 and 29 post dose as compared with baseline for RSV sF (non-adjuvanted) compared to MEDI7510 (containing RSV sF + 2.5 μ g GLA in 2% (v/v) SE) administered at 20 μ g, 50 μ g, or 80 μ g .

[0045] FIGURE 8. Baseline (BL) antibody titer effects on post-dose titer: BL < Median vs $\geq$ Median by assay. Change in response (GMT) at day 29 post dose for microneutralization, RSV sF IgG and competitive ELISA assays, as well as (GMC) change in IFN γ ELISPOT assay at day 8 post dose for RSV sF (non-adjuvanted) compared to MEDI7510 (containing RSV sF + 2.5 μ g GLA in 2% (v/v) SE) administered at 20 μ g, 50 μ g, or 80 μ g,).

[0046] FIGURE 9. Age Effects on Post-Dose Titer: 60–69 Years vs >69 Years by Assay. Change in response (GMT) at day 29 post dose for microneutralization, RSV sF IgG and competitive ELISA assays, as well as (GMC) change in IFN γ ELISPOT assay at day 8 post dose for RSV sF

(non-adjuvanted) compared to MEDI7510 (containing RSV sF + 2.5 µg GLA in 2% (v/v) SE) administered at 20 µg, 50 µg, or 80 µg).

[0047] FIGURE 10. Age Effects on Post-Dose Fold Rise from Baseline: 60–69 Years vs >69 Years by Assay. Change in response (GMT) at day 29 post dose for microneutralization, RSV sF IgG and competitive ELISA assays, as well as (GMC) change in IFN γ ELISPOT assay at day 8 post dose for RSV sF (non-adjuvanted) compared to MEDI7510 (containing RSV sF + 2.5 µg GLA in 2% (v/v) SE) administered at 20 µg, 50 µg, or 80 µg.

[0048] FIGURE 11. Post-Dose (Day 8) RSV F-specific IFN γ ELISPOT Data. Day 8 Post Dose Count of RSV sF Spot-Forming Cells per Million PBMCs vs Baseline Count of RSV sF Spot-Forming Cells per Million PBMCs

[0049] FIGURE 12. Outline for the Phase 2 Efficacy Study for MEDI7510.

[0050] FIGURE 13. Outline for the Phase 3 efficacy study for MEDI7510.

DETAILED DESCRIPTION

Definitions

[0051] Unless otherwise defined herein, scientific and technical terms shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0052] The term "a" or "an" entity refers to one or more of that entity; for example, "an antibody," is understood to represent one or more antibodies. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0053] The term "about" as used herein refers to the range of error expected for the respective value readily known to the skilled person in this technical field.

[0054] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to

encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0055] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0056] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects or aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0057] As used herein, the term “non-naturally occurring” substance, composition, entity, and/or any combination of substances, compositions, or entities, or any grammatical variants thereof, is a conditional term that explicitly excludes, but only excludes, those forms of the substance, composition, entity, and/or any combination of substances, compositions, or entities that are well-understood by persons of ordinary skill in the art as being “naturally-occurring,” or that are, or can be at any time, determined or interpreted by a judge or an administrative or judicial body to be, “naturally-occurring.”

[0058] As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term “polypeptide” refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids are included within the definition of “polypeptide,” and the term “polypeptide” can be used instead of, or interchangeably

with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, and derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

[0059] A polypeptide as disclosed herein can be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides can have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides that do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded. As used herein, the term glycoprotein refers to a *protein* coupled to at least one carbohydrate moiety that is attached to the protein via an oxygen-containing or a nitrogen-containing side chain of an amino acid, e.g., a serine or an asparagine.

[0060] By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated as disclosed herein, as are native or recombinant polypeptides that have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0061] As used herein, the term "non-naturally occurring" polypeptide, or any grammatical variants thereof, is a conditional term that explicitly excludes, but only excludes, those forms of the polypeptide that are well-understood by persons of ordinary skill in the art as being "naturally-occurring," or that are, or can be at any time, determined or interpreted by a judge or an administrative or judicial body to be, "naturally-occurring."

[0062] Other polypeptides disclosed herein are fragments, derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. The terms "fragment," "variant,"

"derivative" and "analog" as disclosed herein include any polypeptides that retain at least some of the properties of the corresponding native antibody or polypeptide, for example, specifically binding to an antigen. Fragments of polypeptides include, for example, proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein. Variants of, e.g., a polypeptide include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. In certain aspects, variants can be non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions. Derivatives are polypeptides that have been altered so as to exhibit additional features not found on the original polypeptide. Examples include fusion proteins. Variant polypeptides can also be referred to herein as "polypeptide analogs." As used herein a "derivative" of a polypeptide can also refer to a subject polypeptide having one or more amino acids chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those peptides that contain one or more derivatives of the twenty standard amino acids. For example, 4-hydroxyproline can be substituted for proline; 5-hydroxylysine can be substituted for lysine; 3-methylhistidine can be substituted for histidine; homoserine can be substituted for serine; and ornithine can be substituted for lysine.

[0063] A "conservative amino acid substitution" is one in which one amino acid is replaced with another amino acid having a similar side chain. Families of amino acids having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. In certain embodiments, conservative substitutions in the sequences of the polypeptides and antibodies do not abrogate the binding of the polypeptide or antibody containing the amino acid sequence, to the antigen to which the antibody binds. Methods of identifying nucleotide and amino acid conservative substitutions that do not eliminate antigen-binding are well-known in the art. (*See,*

e.g., Brummell et al., *Biochem.*, 32:1180-1187, 1993; Kobayashi et al., *Protein Eng.*, 12(10):879-884, 1999; and Burks et al., *Proc. Natl. Acad. Sci. USA*, 94:412-417, 1997).

[0064] The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, e.g., messenger RNA (mRNA), cDNA, or plasmid DNA (pDNA). A polynucleotide can comprise a conventional phosphodiester bond or a non-conventional bond (e.g., an amide bond, such as found in peptide nucleic acids (PNA)). The terms "nucleic acid" or "nucleic acid sequence" refer to any one or more nucleic acid segments, e.g., DNA or RNA fragments, present in a polynucleotide.

[0065] By an "isolated" nucleic acid or polynucleotide is intended any form of the nucleic acid or polynucleotide that is separated from its native environment. For example, gel-purified polynucleotide, or a recombinant polynucleotide encoding a polypeptide contained in a vector would be considered to be "isolated." Also, a polynucleotide segment, e.g., a PCR product, that has been engineered to have restriction sites for cloning is considered to be "isolated." Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in a non-native solution such as a buffer or saline. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides, where the transcript is not one that would be found in nature. Isolated polynucleotides or nucleic acids further include such molecules produced synthetically. In addition, polynucleotide or a nucleic acid can be or can include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

[0066] As used herein, a "coding region" is a portion of nucleic acid that consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, e.g., on a single vector, or in separate polynucleotide constructs, e.g., on separate (different) vectors. Furthermore, any vector can contain a single coding region, or can comprise two or more coding regions, e.g., a single vector can separately encode an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. In addition, a vector,

polynucleotide, or nucleic acid can include heterologous coding regions, either fused or unfused to another coding region. Heterologous coding regions include without limitation, those encoding specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0067] In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid that encodes a polypeptide normally can include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. An operable association is when a coding region for a gene product, e.g., a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter can be a cell-specific promoter that directs substantial transcription of the DNA in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription.

[0068] In other embodiments, a polynucleotide can be RNA, for example, in the form of messenger RNA (mRNA), transfer RNA, or ribosomal RNA.

[0069] Polynucleotide and nucleic acid coding regions can be associated with additional coding regions that encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide as disclosed herein. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence that is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells

can have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain aspects, the native signal peptide, e.g., an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, can be used. For example, the wild-type leader sequence can be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse β -glucuronidase.

[0070] A portion of a polypeptide that is "amino-terminal" or "N-terminal" to another portion of a polypeptide is that portion that comes earlier in the sequential polypeptide chain. Similarly a portion of a polypeptide that is "carboxy-terminal" or "C-terminal" to another portion of a polypeptide is that portion that comes later in the sequential polypeptide chain. For example in a typical antibody, the variable domain is "N-terminal" to the constant region, and the constant region is "C-terminal" to the variable domain.

[0071] The term "expression" as used herein refers to a process by which a gene produces a biochemical, for example, a polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into messenger RNA (mRNA), and the translation of such mRNA into polypeptide(s). If the final desired product is a biochemical, expression includes the creation of that biochemical and any precursors. Expression of a gene produces a "gene product." As used herein, a gene product can be either a nucleic acid, e.g., a messenger RNA produced by transcription of a gene, or a polypeptide that is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, e.g., polyadenylation, or polypeptides with post translational modifications, e.g., methylation, glycosylation, the addition of lipids, association with other protein subunits, proteolytic cleavage, and the like.

[0072] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt or slow the progression of an existing diagnosed pathologic condition or disorder. Terms such as "prevent,"

“prevention,” “avoid,” “deterrence” and the like refer to prophylactic or preventative measures that prevent the development of an undiagnosed targeted pathologic condition or disorder. Thus, “those in need of treatment” can include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented.

[0073] By "subject" or "individual" or "animal" or "patient" or “mammal,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, swine, cows, bears, and so on.

[0074] As used herein, phrases such as “a subject that would benefit from therapy” includes subjects, such as mammalian subjects, that would benefit from administration of the disclosed antibody compositions.

[0075] The term "antibody" means an immunoglobulin molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing through at least one antigen recognition site within the variable region of the immunoglobulin molecule. As used herein, the term "antibody" encompasses intact polyclonal antibodies, intact monoclonal antibodies, antibody fragments (such as Fab, Fab', F(abc')₂, and Fu fragments), single chain Fu (scFv) mutants, multispecific antibodies such as bispecific antibodies generated from at least two intact antibodies, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen determination portion of an antibody, and any other modified immunoglobulin molecule comprising an antigen recognition site so long as the antibodies exhibit the desired biological activity. The term "antibody" can also refer to a Y-shaped glycoprotein with a molecular weight of approximately 150 kDa that is made up of four polypeptide chains: two light (L) chains and two heavy (H) chains. There are five types of mammalian Ig heavy chain isotypes denoted by the Greek letters alpha (α), delta (δ), epsilon (ϵ), gamma (γ), and mu (μ). The type of heavy chain defines the class of antibody, i.e., IgA, IgD, IgE, IgG, and IgM, respectively. The γ and α classes are further divided into subclasses on the basis of differences in the constant domain sequence and function, e.g., IgG1, IgG2A, IgG2B, IgG3, IgG4, IgA1 and IgA2. In mammals there are two types of immunoglobulin light chains, λ and κ . The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the

heavy or light chain of the antibody. The variable domains of the heavy chain and light chain are referred to as "VH" and "VL", respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

[0076] By "specifically binds," it is generally meant that an antibody or fragment, variant, or derivative thereof binds to an epitope *via* its antigen binding domain, and that the binding entails some complementarity between the antigen binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope, *via* its antigen binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" can be deemed to have a higher specificity for a given epitope than antibody "B," or antibody "A" can be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0077] As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with one or more antigen binding domains, e.g., of an immunoglobulin molecule. (See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 2nd ed., 1988, at pages 27-28). As used herein, the term "avidity" refers to the overall stability of the complex between a population of antigen binding domains and an antigen. (See, e.g., Harlow, at pages 29-34). Avidity is related to both the affinity of individual antigen binding domains in the population with specific epitopes, and also the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity. An interaction between a bivalent monoclonal antibody with a receptor present at a high density on a cell surface would also be of high avidity.

[0078] As use herein, the term "antigenic formulation" or "antigenic composition" refers to a preparation which, when administered to a vertebrate, especially a bird or a mammal, will induce an immune response.

[0079] As used herein the term "adjuvant" refers to a compound that, when used in combination with a specific immunogen in a formulation, will augment or otherwise alter or modify the resultant immune response. Modification of the immune response can include intensification or broadening

the specificity of either or both antibody and cellular immune responses. Modification of the immune response can also mean decreasing or suppressing certain antigen-specific immune responses.

[0080] As used herein, the stages of life include: youth, reproductive maturity, and elderly. The term “youth” refers to a mammal from newborn to the point at which the mammal has attained reproductive maturity. The term “reproductive maturity” refers to a mammal that is at an age where mammals of that species are generally capable of mating and reproducing. As used herein, the term “elderly” refers to a mammal from reproductive maturity to death. The term “elderly” can be defined in terms of chronology (i.e., age in years); change in social role (i.e. change in work patterns, adult status of children and menopause); and/or change in capabilities (i.e. invalid status, senility and change in physical characteristics). In terms of chronology, when referring to human mammals, the term “elderly” generally refers to a person that has attained the chronological age of at least about 50, 55, 60 or 65 years old.

[0081] As used herein, “viral fusion protein” or “fusion protein” or “F protein” refers to any viral fusion protein, including but not limited to, a native viral fusion protein or a soluble viral fusion protein, including recombinant viral fusion proteins, synthetically produced viral fusion proteins, and viral fusion proteins extracted from cells. As used herein, “native viral fusion protein” refers to a viral fusion protein encoded by a viral gene or viral RNA that is present in nature. The term “soluble fusion protein” or “soluble F protein” refers to a fusion protein that lacks a functional membrane association region, typically located in the C-terminal region of the native protein. As used herein, the term “recombinant viral fusion protein” refers to a viral fusion protein derived from an engineered nucleotide sequence and produced in an in vitro and/or in vivo expression system. Viral fusion proteins include related proteins from different viruses and viral strains including, but not limited to viral strains of human and non-human categorization. Viral fusion proteins include type I and type II viral fusion proteins. Numerous RSV-Fusion proteins have been described and are known to those of skill in the art.

[0082] As used herein, the terms “immunogens” or “antigens” refer to substances such as proteins, peptides, peptides, nucleic acids that are capable of eliciting an immune response. Both terms also encompass epitopes, and are used interchangeably.

[0083] As use herein, the term "immunogenic formulation" refers to a preparation which, when administered to a vertebrate, e.g. a mammal, will induce an immune response.

[0084] As used herein, "pharmaceutical composition" refers to a composition that includes a therapeutically effective amount of RSV-F protein together with a pharmaceutically acceptable carrier and, if desired, one or more diluents or excipients. As used herein, the term "pharmaceutically acceptable" means that it is approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopia, European Pharmacopia or other generally recognized pharmacopia for use in mammals, and more particularly in humans.

[0085] As used herein, the term "pharmaceutically acceptable vaccine" refers to a formulation that contains an RSV-F immunogen in a form that is capable of being administered to a vertebrate and that induces a protective immune response sufficient to induce immunity to prevent and/or ameliorate an infection or disease, and/or to reduce at least one symptom of an infection or disease. In one aspect, the vaccine prevents or reduces at least one symptom of RSV infection in a subject. Symptoms of RSV are well known in the art. They include rhinorrhea, sore throat, headache, hoarseness, cough, sputum, fever, rales, wheezing, and dyspnea. Thus, in one aspect, the method can include prevention or reduction of at least one symptom associated with RSV infection. A reduction in a symptom can be determined subjectively or objectively, e.g., self-assessment by a subject, by a clinician's assessment or by conducting an appropriate assay or measurement (e.g. body temperature), including, e.g., a quality of life assessment, a slowed progression of a RSV infection or additional symptoms, a reduced, severity of a RSV symptoms or a suitable assays (e.g. antibody titer and/or T-cell activation assay).

[0086] As used herein, the term "effective amount" refers to an amount of antigen necessary or sufficient to realize a desired biologic effect. The term "effective dose" generally refers to the amount of an antigen that can induce a protective immune response sufficient to induce immunity to prevent and/or ameliorate an infection or disease, and/or to reduce at least one symptom of an infection or disease. The term a "therapeutically effective amount" refers to an amount which provides a therapeutic effect for a given condition and administration regimen.

[0087] As used herein, the term "naïve" refers to a person or an immune system which has not been previously exposed to a particular antigen, for example, RSV. A naïve person or immune

system does not have detectable antibodies or cellular responses against the antigen. The term “seropositive” refers to a mammal or immune system that has previously been exposed to a particular antigen and thus has a detectable serum antibody titer against the antigen of interest. The term “RSV seropositive” refers to a mammal or immune system that has previously been exposed to RSV antigen. A seropositive person or immune system can be identified by the presence of antibodies or other immune markers in serum, which indicate prior exposure to a particular antigen.

[0088] As used herein, the phrase "protective immune response" or "protective response" refers to an immune response mediated by antibodies against an infectious agent or disease, which is exhibited by a vertebrate (e.g., a human), that prevents or ameliorates an infection or reduces at least one disease symptom thereof. The RSV-F protein vaccines described herein can stimulate the production of antibodies that, for example, neutralize infectious agents, blocks infectious agents from entering cells, blocks replication of the infectious agents, and/or protect host cells from infection and destruction. The term can also refer to an immune response that is mediated by T-lymphocytes and/or other white blood cells against an infectious agent or disease, exhibited by a vertebrate (e.g., a human), that prevents or ameliorates infection or disease, or reduces at least one symptom thereof.

[0089] As use herein, the term "vertebrate" or "subject" or "patient" refers to any member of the subphylum cordata, including, without limitation, humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species. Farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats (including cotton rats) and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like are also non-limiting examples. The terms "mammals" and "animals" are included in this definition. Both adult and newborn individuals are intended to be covered. In particular, infants and young children are appropriate subjects or patients for a RSV vaccine.

[0090] As used herein, the term "vaccine" refers to a preparation of dead or weakened pathogens, or antigenic determinants derived from a pathogen, wherein the preparation is used to induce formation of antibodies or immunity against the pathogen. In addition, the term "vaccine" can also refer to a suspension or solution of an immunogen (e.g. RSV-F protein) that is administered to a

vertebrate, for example, to produce protective immunity, i.e., immunity that prevents or reduces the severity of disease associated with infection.

Human Respiratory Syncytial Virus (RSV) Proteins

[0091] Human respiratory syncytial virus (RSV) is a member of the family *Paramyxoviridae*, subfamily *Pneumovirinae* and genus *Pneumovirus*. The *Paramyxoviridae* family also includes human parainfluenza virus and viruses that cause measles and mumps. RSV is divided into two subgroups, A and B, which are differentiated primarily on the variability of the G gene and encoded protein. RSV is an enveloped virus characterized by a single stranded negative sense RNA genome encoding three transmembrane structural proteins (F, G and SH), two matrix proteins (M and M2), three nucleocapsid proteins (N, P and L) and two nonstructural proteins (NS1 and NS2).

[0092] The two major protective antigens of RSV are the envelope fusion (F) and attachment (G) glycoproteins that are expressed on the surface of Respiratory Syncytial Virus (RSV), and have been shown to be targets of neutralizing antibodies. These two proteins are also primarily responsible for viral recognition and entry into target cells. G protein binds to a specific cellular receptor and the F protein promotes fusion of the virus with the cell. The F protein is also expressed on the surface of infected cells and is responsible for subsequent fusion with other cells, leading to syncytia formation. Thus, antibodies to the F protein can neutralize virus, block entry of the virus into the cell, and/or prevent syncytia formation. Although antigenic and structural differences between RSV A and RSV B subtypes have been described for both the G and F proteins, more significant antigenic differences reside on the G protein. Conversely, antibodies raised to the F protein show a high degree of cross-reactivity among subtype A and B viruses. Consequently, RSV F protein is an attractive pharmaceutical target for neutralizing RSV because it is present on the viral surface and accessible to immunosurveillance. Additionally, as noted, the sequence and structure of RSV F protein is less variable compared to other RSV proteins, such as the RSV G protein.

[0093] The RSV F protein is a type I transmembrane surface protein possessing an N-terminal cleaved signal peptide and a membrane anchor near the C-terminus. In nature, the RSV-F protein is expressed as a single inactive 574 amino acid precursor designated F₀. *In vivo*, F₀ oligomerizes in the endoplasmic reticulum of the infected cell and is proteolytically processed by an endoprotease to

yield a linked heterodimer containing two disulfide-linked subunits, F₁ and F₂. The smaller of these fragments is termed F₂ and originates from the N-terminal portion of the F₀ precursor. The N-terminus of the F₁ subunit that is created by cleavage contains a hydrophobic domain (the fusion peptide), which associates with the host cell membrane and promotes fusion of the membrane of the virus, or an infected cell, with the target cell membrane. In one aspect, the F-protein is a trimer or multimer of F₁/F₂ heterodimers.

[0094] The F glycoprotein contains multiple mouse and human CD8 and CD4 T cell epitopes (Olson MR and Varga SM, "Pulmonary immunity and immunopathology: lessons from respiratory syncytial virus," *Expert Rev. Vaccines*, 7:1239-1255, 2008). For instance, RSV-specific CD8⁺ T cell responses are detected in seropositive human adults (Cusi MG, et al., "Age related changes in T cell mediated immune response and effector memory to Respiratory Syncytial Virus (RSV) in healthy subjects," *Immun. Ageing*, 7:14, 2010) and contribute to clearing virus-infected cells and resolving RSV infection in animal models (Bangham CR, et al., "Cytotoxic T-cell response to respiratory syncytial virus in mice," *J. Virol.*, 56:55-59, 1985; Srikiatkachorn A and Braciale TJ, "Virus-specific CD8⁺ T lymphocytes downregulate T helper cell type 2 cytokine secretion and pulmonary eosinophilia during experimental murine respiratory syncytial virus infection," *J. Exp. Med.*, 186:421-432, 1997; Hussell T, et al., "CD8⁺ T cells control Th2-driven pathology during pulmonary respiratory syncytial virus infection," *Eur. J. Immunol.*, 27:3341-3349, 1997; and Munoz JL, et al., "Respiratory syncytial virus infection in C57BL/6 mice: clearance of virus from the lungs with virus-specific cytotoxic T cells," *J. Virol.*, 65:4494-4497, 1991). RSV-specific CD4 T cell responses promote both B cell antibody production and CD8 responses, with Th1-type CD4 responses promoting CD8 responses more effectively than Th2-type responses (Hurwitz JL, "Respiratory Syncytial Virus Vaccine Development," *Expert Rev. Vaccines*, 10:1415-1433, 2011).

[0095] Suitable RSV-F proteins for use in the compositions described herein can be from any RSV strain or isolate known in the art, including, for example, Human strains such as A2, Long, ATCC VR-26, 19, 6265, E49, E65, B65, RSB89-6256, RSB89-5857, RSB89-6190, and RSB89-6614; or Bovine strains such as ATue51908, 375, and A2Gelfi; or Ovine strains.

[0096] In one aspect, an RSV-F protein for use herein can include an amino acid sequence that is at least about 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an RSV-F

amino acid sequence provided herein, or can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid modifications with respect to an RSV-F amino acid sequence provided herein. For example, in one aspect, the RSV-F protein for use in the compositions described herein is the amino acid sequence of the wild-type RSV-F Human strain A2, is set forth in SEQ ID NO: 1.

[0097] Native, full-length viral fusion proteins typically include a membrane association region. Recombinant soluble viral fusion proteins can be generated that lack a functional membrane association region. The functional membrane association region is often located in the C-terminal region of the native protein. Recombinant soluble viral fusion proteins can be generated by deletion, mutation, or any mode of disruption known in the art, of the functional membrane associated region of a viral fusion protein. For example, any part or all of the membrane association region can be removed or modified provided: (i) that the membrane association region is not detectably functional (e.g. region no longer reside in the membrane), and (ii) a certain percent of the membrane association region remains (e.g., about 50% or less remains), is removed (e.g., about 50% or more removed), or is modified (e.g., about 50% or more modified). The extent to which the disrupted membrane associated region no longer confers association of the protein to the plasma membrane can be determined by any technique known in the art that can assess membrane association of proteins. For example, co-immunostaining of the viral fusion protein and a known membrane associated protein can be performed to visualize protein retained in the membrane. Examples of soluble viral fusion proteins are provided herein and include soluble RSV-F protein. Soluble RSV-F protein is also referred to herein as RSV-sF or simply sF. Soluble RSV-F can be generated, for example, by deletion of at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the 50 amino acid C-terminal transmembrane domain of the RSV-F protein, corresponding to amino acid 525-574 of SEQ ID NO: 2, which is the following sequence:

SEQ ID NO: 2

LETHRSERLYSVALLEASPLELYSASNTYRILEASPLYSGLNLELEPRIL

[0098] Three non-overlapping antigenic sites (A, B, and C) and one bridge site (AB) have been identified for the fusion glycoprotein of the A2 strain of respiratory syncytial virus (RSV-F A2). (Beeler and Wyke Coelingh, (1989) "Neutralization Epitopes of the F Glycoprotein of Respiratory Syncytial Virus: Effect of Mutation upon Fusion Function," J. Virol. 63(7):2941-2950). In one aspect, the RSV-F protein includes one or more intact A, B or C neutralizing epitopes. In one aspect, the RSV-F protein includes at least the A epitope. In another aspect, the RSV-F protein includes at least the B epitope. In another aspect, the RSV-F protein includes at least the C epitope. In other aspects, the RSV-F protein includes at least the A and B epitopes, at least the B and C epitopes, or at least the A and C epitopes. In another aspect, the RSV-F protein includes all three neutralizing epitopes (i.e., A, B and C).

Recombinant Expression of RSV-F

[0099] In one aspect, a composition includes RSV-F protein. As used herein, the term "RSV-F protein" refers to full-length wild-type RSV-F protein, as well as variants and fragments thereof, including, for example, RSV soluble F protein (also referred to as RSV-sF). In a one aspect, the composition includes recombinantly produced RSV-F protein. In a more particular aspect, the composition includes recombinantly produced soluble RSV-F protein.

[0100] To recombinantly produce an RSV-F protein, an open reading frame (ORF) encoding the viral fusion protein can be inserted or cloned into a vector for replication of the vector, transcription of a portion of the vector (e.g., transcription of the ORF) and/or expression of the protein in a cell. The term "open reading frame" (ORF) refers to a nucleic acid sequence that encodes a viral fusion protein, for example a soluble viral fusion protein, that is located between a start codon (AUG in ribonucleic acids and ATG in deoxyribonucleic acids) and a stop codon (e.g., UAA (ochre), UAG (amber), or UGA (opal) in ribonucleic acids and TAA, TAG, or TGA in deoxyribonucleic acids).

[0101] A vector can also include elements that facilitate cloning of the ORF or other nucleic acid elements such as those useful in replication, transcription, translation and/or selection. Thus, a vector can include one, or more, or all of the following elements: one or more promoter elements, one or more 5' untranslated regions (5'UTRs), one or more regions into which a target nucleotide sequence can be inserted (an "insertion element"), one or more ORFs, one or more 3' untranslated

regions (3'UTRs), and a selection element. Any convenient cloning strategy known in the art can be used to incorporate an element, such as an ORF, into a vector nucleic acid.

[0102] General texts that describe molecular biological techniques, which are applicable to the disclosed compositions and methods, such as cloning, mutation, cell culture and the like, include the following: Berger and Kimmel, *Guide to Molecular Cloning Techniques*, *Methods in Enzymology* volume 152 Academic Press, Inc., San Diego, Calif. (Berger); Sambrook et al., *Molecular Cloning--A Laboratory Manual* (3rd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 2000 ("Sambrook"), and *Current Protocols in Molecular Biology*, F. M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., ("Ausubel"). These texts describe mutagenesis, the use of vectors, promoters, and many other relevant topics related to, e.g., cloning and mutating the RSV-F protein. Additionally, cloning strategies for soluble viral fusion proteins are described more fully in at least WO 2012/103496, entitled *EXPRESSION OF SOLUBLE VIRAL FUSION GLYCOPROTEINS IN MAMMALIAN CELLS*. The disclosures of these references are hereby incorporated by reference herein in their entirety for all purposes.

[0103] The compositions described herein also encompass variants of RSV-F. The variants can contain alterations in the amino acid sequences of the RSV-F protein. The term "variant" with respect to a protein refers to an amino acid sequence that is altered by one or more amino acids with respect to a reference sequence. The variant can include "conservative" changes and/or "nonconservative" changes. Other variations can also include amino acid deletions, insertions, substitutions, or combinations thereof. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without eliminating biological or immunological activity can be found using computer programs well known in the art, for example, DNASTAR software.

[0104] In one aspect, the nucleic acids encoding a viral fusion protein provided herein can be modified by changing one or more nucleotide bases within one or more codons throughout the nucleotide sequence. As used herein, "nucleotide base" refers to any of the four deoxyribonucleic acid bases, adenine (A), guanine (G), cytosine (C), and thymine (T) or any of the four ribonucleic acid bases, adenine (A), guanine (G), cytosine (C), and uracil (U). As used herein, "codon" refers to a series of three nucleotide bases that code for a particular amino acid. Generally, each amino acid

can be encoded by one or more codons. **Table 1** presents substantially all codon possibilities for each amino acid.

Table 1

TABLE 1: DNA Codon Table			
Amino Acid	DNA Codons	Amino Acid	DNA Codons
Ala/A	GCT, GCC, GCA, GCG	Leu/L	TTA, TTG, CTT, CTC, GTA, GTG
Arg/R	CGT, CGC, CGA, CGG, AGA, AGG	Lys/K	AAA, AAG
Asn/N	AAT, AAC	Met/M	ATG
Asp/D	GAT, GAC	Phe/F	TTT, TTC
Cys/C	TGT, TGC	Pro/P	CCT, CCG, CCA, CCG
Gln/Q	CAA, CAG	Ser/S	TCT, TCC, TCA, TCG, AGT, AGC
Glu/E	GAA, GAG	Thr/T	AGT, ACC, ACA, ACG
Gly/G	GGT, GGC, GGA, GGG	Trp/W	TGG
His/H	CAT, CAC	Tyr/Y	TAT, TAG
Ile/I	ATT, ATC, ATA	Val/V	GTT, GTC, GTA, GTG
START	ATG	STOP	TAA, TGA, TAG

[0105] In one aspect, the nucleic acid encoding RSV-F can include one or more substitutions. The substitutions can be made to change an amino acid in the resulting protein in a non-conservative manner or in a conservative manner. A conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. In one aspect, the nucleic acid encoding RSV-F includes one or more conservative amino acid substitutions which do not significantly alter the activity or binding characteristics of the resulting protein.

[0106] As used herein, the term “conservative substitution” refers to a substitution in which one or more amino acid residues are substituted by residues of different structure but similar chemical characteristics, such as where a hydrophobic residues is substituted by a hydrophobic residue or where an acidic residue is substituted by another acidic residue or a polar residue for a polar residue or a basic residue for a basic residue. Nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. Amino acids containing aromatic ring structures are phenylalanine, tryptophan, and tyrosine. Polar neutral amino acids

include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. Positively charged (basic) amino acids include arginine, lysine and histidine. Negatively charged (acidic) amino acids include aspartic acid and glutamic acid. More specific examples of conservative substitutions include, but are not limited to, Lys for Arg and *vice versa*, such that a positive charge can be maintained; Glu for Asp and *vice versa*, such that a negative charge can be maintained; Ser for Thr, such that a free -OH can be maintained; and Gln for Asn, such that a free NH₂ can be maintained. In one aspect, the RSV-F immunogen includes one or more conserved or non-conserved amino acid substitutions. In one aspect, the RSV-F immunogen includes one or more conserved amino acid substitutions.

[0107] The term "identical" as used herein refers to two or more nucleotide sequences having substantially the same nucleotide sequence when compared to each other. One test for determining whether two nucleotide sequences or amino acids sequences are substantially identical is to determine the percent of identical nucleotide sequences or amino acid sequences shared.

[0108] Calculations of sequence identity can be performed as follows. Sequences are aligned for optimal comparison purposes, e.g., gaps can be introduced in one or both of a first and a second amino acid, or a nucleic acid sequence for optimal alignment, and non-homologous sequences can be disregarded for comparison purposes. The length of a reference sequence aligned for comparison purposes is sometimes 30% or more, 40% or more, 50% or more, 60% or more, and 70% or more, 80% or more, 90% or more, or 100% of the length of the reference sequence. The nucleotides or amino acids at corresponding nucleotide or polypeptide positions, respectively, are then compared between the two aligned sequences. When a position in the first sequence is occupied by the same nucleotide or amino acid as the corresponding position in the second sequence, the nucleotides or amino acids are deemed to be identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, introduced for optimal alignment (highest percent identity) of the two sequences.

[0109] Comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For instance, percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of Meyers & Miller

(CABIOS, 4:11-17, 1989), which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. Alternatively, percent identity between two amino acid sequences can be determined using the Needleman & Wunsch (*J. Mol. Biol.*, 48: 444-453, 1970) algorithm which has been incorporated into the GAP program in the GCG software package (available at the http address www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, for example. A set of parameters often used with a Blossum 62 scoring matrix includes a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. Percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at http address www.gcg.com), using NWSgapdna.CMP matrix and a gap weight of 60 and a length weight of 4.

[0110] Another manner for determining whether two nucleic acids are substantially identical is to assess whether a polynucleotide homologous to one nucleic acid will hybridize to the other nucleic acid under stringent conditions. As used herein, the term "stringent conditions" refers to conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 6.3.1 -6.3.6 (1989). Aqueous and non-aqueous methods are described in that reference and either can be used. An example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1 % SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1 % SDS at 55°C. A further example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1 % SDS at 60°C. Often, stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1 % SDS at 65°C. More often, stringency conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1 % SDS at 65°C.

[0111] In the past, studies of the fusion activity of RSV have been hindered by low recombinant expression levels. In particular, recombinant F protein expression levels from standard expression vectors tend to be low in comparison to the levels of F protein expression observed during RSV

replication (Huang et al., “Recombinant respiratory syncytial virus F protein expression is hindered by inefficient nuclear export and mRNA processing,” *Virus Genes*, 40:212-221, 2010). The difference could be due to the differences between viral and recombinant F protein expression. In general, there are two major differences between viral and recombinant F protein expression. First, transcription of the F gene during viral replication occurs in the cytoplasm of the host cell, whereas transcription occurs in the nucleus during recombinant F protein expression from standard mammalian expression vectors. Export from the nucleus to the cytoplasm of viral transcripts can be problematic, even for viruses that normally replicate in the nucleus. For viral transcripts, the inhibition is thought to be a product of AU abundance, which is relatively high in comparison to mammalian transcripts. Therefore, in one aspect, GC abundance in the F protein gene sequence can be modified to enhance transcription. (*Id.*).

[0112] In one aspect, the amino acid sequence of the RSV-Fusion protein is at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the protein encoded by a unmodified wild-type RSV-F sequence, such as the RSV-F sequence shown in SEQ ID NO: 3, shown below. In some aspects, the amino acid sequence encoded by the modified nucleotide sequence is 100% identical to the amino acid sequence encoded by the unmodified wild type nucleotide sequence for RSV-F shown in SEQ ID NO: 5 (shown below).

SEQ ID NO: 3

METGLLELEILELELYSALAASNALAIETHRTHRILELETHRMETGLLELEILELELYSAL
AASNALAIETHRTHRILELETHRRTYRGLNSERTHRCYSSERALAVALSERLYSGLYTYR
LESERALALEARGTHRGLYTRPTYRTHRSERVALILETHRILEGLLESERASNILELYSLY
SASNLYSCYSASNGLYTHRASPALALYSVALLYSLEILELYSGLNGLLEASPLYSTYRLY
SASNALAVALTHRGLLEGLNLEMETGLNSERTHRPRALATHRASNASNARGALAAR
GARGGLLEPRARGPHEMETASNTYRTHRLEASNASNALALYSLYSTHRASNVALTHRL
ESERLYSLYSARGLYSARGARGPHELEGLYPHELELEGLYVALGLYSERALAILEALASE
RGLYVALALAVALSERLYSVALLEHISLEGLGLYGLVALASNLYSILELYSSERALALEL
ESERTHRASNLYSALAVALVALSERLESERASNGLYVALSERVALLETHRSERLYSVAL
LEASPLELYSASNTYRILEASPLYSGLNLELEPRILEVALASNLYSGLNSERCYSSERILES
ERASNILEGLTHRVALILEGLPHEGLNGLNLYSASNASNARGLELEGLILETHRARGGLP
HESERVALASNALAGLYVALTHRTHRPRVALSERTHRTRYRMETLETHRASNSERGLLEL
ESERLEILEASNASPMETPRIETHRASNASPGLNLYSLYSLEMETSERASNASNVALGLN
ILEVALARGGLNGLNSERTYRSEIRILEMETSERILEILELYSGLGLVALLEALATYRVALV
ALGLNLEPRLETYRGLYVALILEASPTHPRCYSTRPLYSLEHISTHRSEIRPLECYSTR

THRASNTHRLYSGLGLYSERASNILECYSLETHRARGTHRASPARGGLYTRPTYRCYSA
 SPASNALAGLYSERVALSERPHEPHEPRGLNALAGLTHRCYSLYSVALGLNSERASNAR
 GVALPHECYASPTHMETASNSERLETHRLEPRSERGLVALASNLECYASNVALASPI
 LEPHEASNPRLYSTYRASPCYSLYSILEMETTHRSERLYSTHRASPVALSERSERSERVAL
 ILETHRSERLLEGLYALAILEVALSERCYSTYRGLYLYSTHRLYSCYSTHRALASERASN
 YSASNARGGLYILEILELYSTHRPHESERASNGLYCYASPTYRVALSERASNLYSGLYV
 ALASPTHRVALSERVALGLYASNTHRLETYRRTYRVALASNLYSGLNGLGLYLYSSERLE
 TYRVALLYSGLYGLPRILEILEASNPHETYRASPPRLEVALPHEPRSERASPGLPHEASPA
 LASERILESERGLNVALASNGLLYSILEASNGLNSERLEALAPHEILEARGLYSSERASPG
 LLELEHISASNVALASNALAGLYLYSSERTHRTHRASNILEMETILETHRTHRILEILE
 VALILEILEVALILELELESERLEILEALAVAGLYLELELETYRCYSLYSALAARGSERTH
 RPRVALTHRLESERLYSASPGLNLESERGLYILEASNASNILEALAPHESEASN

[0113] As indicated in Table 1, a subset of amino acids and the STOP codon can be encoded by at least two codon possibilities. For example, glutamate can be encoded by GAA or GAG. If a codon for glutamate exists within a nucleic acid sequence as GAA, a nucleotide base change at the third position from an A to a G will lead to a modified codon that still encodes for glutamate. Thus, a particular change in one or more nucleotide bases within a codon can still lead to encoding the same amino acid. This process, in some cases, is referred to herein as codon optimization.

[0114] Provided herein are examples of nucleotide sequences for RSV-F (set forth as SEQ ID NOs: 5, shown below). Also provided herein, for example, are nucleotide sequences for soluble RSV-F (set forth in SEQ ID NO: 4, shown below).

SEQ ID NO: 4

```
atggagttgc taatcctcaa agcaaattgca attaccacaa tcttactgct agtcacattt
tggttttgcct ctggtcaaaa catcactgaa gaattttatc aatcaacatg cagtgcagtt
agcaaaggct atcttagtgc tctgagaact ggttggtata ccagtgttat aactatagaa
ttaagtaata tcaagaaaaa taagtgtaat ggaacagatg ctaaggtaaa attgataaaa
caagaattag ataaatataa aaatgctgta acagaattgc agttgctcat gcaaagcaca
caagcaacaa acaatcgagc cagaagagaa ctaccaaggt ttatgaatta tacactcaac
aatgccaaaa aaaccaatgt aacattaagc aagaaaagga aaagaagatt tcttggtttt
ttgttaggtg ttggatctgc aatcgccagt ggcgttgctg tatctaaggt cctgcaccta
gaaggggaag tgaacaagat caaaagtgt ctactatcca caaacaaggc ttagtgcagc
ttatcaaattg gagtcagtg cttaaccagc aaagtgttag acctcaaaaa ctatatagat
aaacaattgt tacctattgt gaacaagcaa agctgcagca tatcaaatat agaaactgtg
```

atagagttcc aacaaaagaa caacagacta ctagagatta ccagggaatt tagtgттаат
 gcaggtgtaa ctacacctgt aagcacttac atgttaacta atagtgaatt attgtcatta
 atcaatgata tgcctataac aaatgatcag aaaaagttaa tgtccaacaa tgttcaaata
 gttagacagc aaagttactc tatcatgtcc ataataaaag aggaagtctt agcatatgta
 gtacaattac cactatatgg tgttatagat acaccctgtt ggaaactaca cacatcccct
 ctatgtacaa ccaacacaaa agaagggtcc aacatctgtt taacaagaac tgacagagga
 tgggtactgtg acaatgcagg atcagtatct ttcttcccac aagctgaaac atgtaaagtt
 caatcaaatc gagtattttg tgacacaatg aacagtttaa cattaccaag tgaagtaaат
 ctctgcaatg ttgacatatt caaccccaaa tatgattgta aaattatgac ttcaaaaaca
 gatgtaagca gctccgttat cacatctcta ggagccattg tgtcatgcta tggcaaaaact
 aaatgtacag catccaataa aaatcgtgga atcataaaga cattttctaa cgggtgcat
 tatgtatcaa ataaaggggt ggacactgtg tctgtaggta acacattata ttatgtaaат
 aagcaagaag gtaaaagtct ctatgtaaaa ggtgaaccaa taataaattt ctatgacca
 ttagtattcc cctctgatga atttgatgca tcaatatctc aagtcaacga gaagattaac
 cagagcctag catttattcg taaatccgat gaattattac ataatgtaaa tgccggtaaa
 tccaccacaa atatcatgat aactactata attatagtga ttatagtaат attgttatca
 ttaattgctg ttggactgct cttatactgt aaggccagaa gcacaccagt cacactaagc
 aaagatcaac tgagtggat aaataatatt gcatttagta acta

SEQ ID NO: 5

atggagttgc tcatcctcaa ggccaacgcc atcaccacga tcctcacggc agtcacattc
 tgtttcgctt ctggtcagaa catcactgag gaattctacc aatcgacgtg cagtgcagtt
 agcaagggct atctcagtgc tctgagaacg ggttggtata ccagtgtcat cactatcgag
 ttgagtaaca tcaagaagaa caagtgtaac ggaaccgatg cgaaggtaaa gttgatcaag
 caggagttgg acaagtacaa gaacgctgta acagagttgc agttgctcat gcagagcaca
 ccagcgacga acaaccgagc caggagagag ctaccaaggt tcatgaacta cacgctcaac
 aacgccaaga agaccaacgt gacattgagc aagaagagga agaggagatt cctcggtttc
 ttgttgggtg tcggatctgc aatcgccagt ggcgttgctg tctcgaaggт cctgcaccta
 gaaggggaag tgaacaagat caagagtgtc ctgctatcca cgaacaaggc tgtcgtcagc
 ttgtcaaacg gagtcagtgt cttgaccagc aagggtgttg acctcaagaa ctacatcgac
 aagcagttgt tacctatcgt gaacaagcaa agctgcagca tctcaaacat cgagactgtg
 atcgagttcc agcagaagaa caacagacta ctagagatca ccagggagtt cagtgtcaac
 gcaggtgtaa cgacacctgt cagcacttac atgttgacta acagtgagtt gttgtcattg
 atcaacgaca tgcctatcac caacgatcag aagaagttga tgtccaacaa cgtgcagatc

gtcagacagc agagctactc gatcatgtcc atcatcaagg aggaagtctt ggcatacgtat
gtacagttgc cactgtatgg tgtcatcgac acaccctgct ggaagctgca cacgtcccct
ctatgtacga ccaacacgaa ggaagggtcc aacatctgct tgaccaggac tgacagagga
tggtactgcg acaacgcagg atccgtgtcg ttcttcccac aggctgagac ctgcaaggtc
cagtccaacc gagtcttctg cgacacgatg aacagcttga cgttgccgag tgaggtaaac
ctctgcaacg tcgacatctt caaccccaag tacgactgca agatcatgac gtccaagacc
gatgtcagca gctccgtgat cacatcgctc ggagccatcg tgtcatgcta cggcaagacc
aagtgcacag cgtccaacaa gaaccgtgga atcatcaaga cgttctcgaa cgggtgcgac
tacgtctcaa acaagggggt ggacactgtg tctgtaggca acacattgta ctacgtaaac
aagcaggaag gtaagagcct ctacgtcaag ggtgaaccaa tcatcaactt ctacgacccg
ttggtcttcc cctctgacga gttcgacgca tcgatctctc aggtcaacga gaagatcaac
cagagcctag cattcatccg gaagtccgac gagttgttgc acaacgtgaa tgccggtaag
tccaccacaa actaaatgga gttgctcatc ctcaaggcca acgccatcac cacgatcctc
acggcagtca cattctgttt cgcttctggt cagaacatca ctgaggaatt ctaccaatcg
acgtgcagtg cagttagcaa gggctatctc agtgctctga gaacggggtg gtataccagt
gtcatcacta tcgagttgag taacatcaag aagaacaagt gtaacggaac cgatgcgaag
gtaaagttga tcaagcagga gttggacaag tacaagaacg ctgtaacaga gttgcagttg
ctcatgcaga gcacaccagc gacgaacaac cgagccagga gagagctacc aaggttcatg
aactacacgc tcaacaacgc caagaagacc aacgtgacat tgagcaagaa gaggaagagg
agattcctcg gtttcttggt ggggtgtcga tctgcaatcg ccagtggcgt tgctgtctcg
aaggctctgc acctagaagg ggaagtgaac aagatcaaga gtgctctgct atccacgaac
aaggctgtcg tcagcttgtc aaacggagtc agtgtcttga ccagcaagggt gttggacctc
aagaactaca tcgacaagca gttgttacct atcgtgaaca agcaaagctg cagcatctca
aacatcgaga ctgtgatcga gttccagcag aagaacaaca gactactaga gatcaccagg
gagttcagtg tcaacgcagg tgtaacgaca cctgtcagca cttacatggt gactaacagt
gagttgttgt cattgatcaa cgacatgcct atcaccaacg atcagaagaa gttgatgtcc
aacaacgtgc agatcgctag acagcagagc tactcgatca tgtccatcat caaggaggaa
gtcttggcat acgtagtaca gttgccactg tatgggtgtca tcgacacacc ctgctggaag
ctgcacacgt cccctctatg tacgaccaac acgaagggaag ggtccaacat ctgcttgacc
aggactgaca gaggatggta ctgcgacaac gcaggatccg tgtcgttctt cccacaggct
gagacctgca aggtccagtc caaccgagtc ttctgcgaca cgatgaacag cttgacgttg
ccgagtgagg taaacctctg caacgtcgac atcttcaacc ccaagtacga ctgcaagatc
atgacgtcca agaccgatgt cagcagctcc gtgatcacat cgctcggagc catcgtgtca
tgctacggca agaccaagtg cacagcgtcc aacaagaacc gtggaatcat caagacgttc

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tcgaacgggt gcgactacgt ctcaaacaag ggggtggaca ctgtgtctgt aggcaacaca
ttgtactacg taaacaagca ggaaggtaag agcctctacg tcaaggggtga accaatcatc
aacttctacg acccggttggc cttccctctc gacgagttcg acgcatcgat ctctcaggtc
aacgagaaga tcaaccagag cctagcattc atccggaagt cgcacgagtt gttgcacaac
gtgaatgccg gtaagtccac cacaactaa
```

[0115] In one aspect, the nucleotide sequences encoding RSV-F protein, including, for example, soluble RSV-F, can be modified by changing one or more nucleotide bases within one or more codons such that: a) the amino acid sequence of the encoded viral fusion protein is similar or identical to the amino acid sequence of the protein encoded by the unmodified nucleotide sequence, and b) the combined percent of guanines and cytosines (% GC) is increased in the modified nucleotide sequence compared to the unmodified nucleotide sequence. For example, the %GC in the modified nucleic acid sequence can be at least about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%. As indicated in Table 1, nucleotide base changes at the first, second and/or third codon positions can be made such that an A or a T is changed to a G or a C while preserving the amino acid and/or STOP codon assignment.

[0116] Provided herein is an example of nucleotide sequences for RSV-F that have been modified by changing one or more nucleotide bases within one or more codons, and wherein the combined percent of guanines and cytosines (% GC) is increased in the modified nucleotide sequence (for example, 58% GC) compared to the unmodified nucleotide sequence (for example, 35% GC).

[0117] The nucleotide sequences provided herein can be modified by changing one or more nucleotide bases within one or more codons such that: a) the amino acid sequence of the encoded viral fusion protein is similar or identical to the amino acid sequence of the protein encoded by the unmodified nucleotide sequence; b) the combined percent of guanines and cytosines (% GC) is increased in the modified nucleotide sequence compared to the unmodified nucleotide sequence; and c) the overall combined percent of guanines and cytosines at the third nucleotide codon position (% GC3) is increased in the modified nucleotide sequence compared to the unmodified nucleotide sequence. In one aspect, the % GC3 is at least about 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%,

63%, 64%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. As indicated in Table 1, most nucleotide base change possibilities reside at the third nucleotide codon position. In some aspects, every codon, including the STOP codon, either has a G or a C in the third nucleotide codon position already or can be modified to have a G or a C at the third nucleotide codon position without changing the amino acid assignment. Thus, for any given nucleotide sequence, it is possible to have up to 100% G or C at each third nucleotide codon position (GC3) throughout the nucleotide sequence.

[0118] In one aspect, the RSV-F protein, including in some aspects, soluble RSV-F protein, has an isolated nucleic acid sequence with a GC content of at least about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% and that encodes a RSV-F protein, including for example, soluble RSV-F protein, that has an amino acid sequence that is at least about 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 98%, 98%, 99% or 100 % identical to SEQ ID NO: 1. In another aspect, the nucleotide sequence is 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 98%, 98% , 99% or 100% identical to SEQ ID NO: 4. In one aspect, the soluble viral fusion protein lacks a functional membrane association region. In other aspects, the soluble viral fusion protein lacks the C-terminal transmembrane region amino acids corresponding to amino acids 525 to 574 of SEQ ID NO: 3.

[0119] In one aspect, the nucleic acid sequence encoding the RSV-F protein is at least about 60% 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 98%, 98% or 99% identical to SEQ ID NO: 1.

[0120] Recombinant viral fusion proteins can be further modified, such as by chemical modification, or post-translational modification. Such modifications include, but are not limited to, pegylation, albumination, glycosylation, farnesylation, carboxylation, hydroxylation, hasylation, carbamylation, sulfation, phosphorylation, and other polypeptide modifications known in the art. The viral fusion proteins provided herein can be further modified by modification of the primary amino acid sequence, by deletion, addition, or substitution of one or more amino acids.

[0121] In one aspect, the viral fusion protein is modified by post-translational glycosylation. A recombinant viral fusion protein can be fully glycosylated, partially glycosylated, deglycosylated, or non-glycosylated. In some aspects, a recombinant viral fusion protein, e.g., RSV-F fusion protein, can have a glycosylation profile similar to, substantially identical to, or identical to the glycosylation profile of the native counterpart protein (see, Rixon et al., *J. Gen. Virol.*, 83: 61 -66, 2002). Recombinant viral fusion glycoproteins can include any of the multiple glycosidic linkages known in the art.

[0122] RSV-F protein suitable for use in the compositions described herein can be expressed and purified using constructs and techniques known in the art. Systems and methods for producing and purifying viral fusion proteins such as RSV-F are known, and are described more fully in, for example, WO 2012/103496, entitled EXPRESSION OF SOLUBLE VIRAL FUSION GLYCOPROTEINS IN MAMMALIAN CELLS, the disclosure of which is hereby incorporated by reference herein in its entirety for all purposes.

Compositions

[0123] As discussed previously, development of a safe and easily manufacturable RSV vaccine has faced large technical hurdles. Although vaccines have been successfully developed for other viruses, such as influenza, to this day no vaccines have been successfully developed for RSV. In terms of vaccine development, respiratory viruses can be divided into two principle groups: (i) those that upon exposure generate long-term immunity, and whose continued survival requires constant mutation, and (ii) those that upon infection only incomplete immunity is generated and repeated infections are common, even with little or no subsequent viral mutation. Influenza virus and respiratory syncytial virus (RSV) typify the former and latter groups, respectively. (See, U.E. Power, "Respiratory syncytial virus (RSV) vaccines – Two steps back for one leap forward," *J. Clin. Virol.*, 41: 38-44, 2008). Consequently, although successful vaccines have been developed against influenza virus, this is not the case for RSV, despite many decades of research and exploration of several vaccine approaches.

[0124] While not wishing to be bound by any specific theory, it is possible that due to prior exposure to RSV in an elderly population, live attenuated RSV virus vaccine would be insufficiently immunogenic. Pre-existing RSV immunity could inhibit replication of the virus vaccine and

consequently diminish the ability of live RSV vaccine to boost RSV immunity. Therefore, a vaccine that could prevent RSV-related illness in the elderly could address an unmet and urgent medical need in this target population.

[0125] In one aspect, a composition is provided to achieve these medically relevant goals. In particular, the composition includes RSV-F protein as described herein. In one aspect, the composition includes recombinantly expressed RSV-F protein as described herein. In one aspect, the composition includes RSV soluble F protein as described herein. In one aspect, the RSV soluble F protein lacks a C-terminal transmembrane domain. In another aspect, the RSV soluble F protein lacks a cytoplasmic tail domain.

[0126] In a further aspect, the composition includes RSV soluble F protein in combination with an adjuvant. Frequently, purified protein antigens lack inherent immunogenicity. Therefore, immunogenic vaccine formulations often include a non-specific stimulator of the immune response, known as an adjuvant. Some adjuvants affect the manner in which antigens are presented. For example, in some instances an immune response is increased when protein antigens are precipitated by alum. In other instances, emulsification of antigens can prolong the duration of antigen presentation. Immunization protocols have used adjuvants to stimulate responses for many years, and as such, adjuvants are well known to one of ordinary skill in the art. Adjuvants are described in more detail in Vogel et al., "A Compendium of Vaccine Adjuvants and Excipients (2nd Edition)," herein incorporated by reference in its entirety for all purposes.

[0127] Examples of known adjuvants include, but are not to be limited to, complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*), incomplete Freund's adjuvants, and aluminum hydroxide adjuvant. Other known adjuvants include: granulocyte macrophage colony-stimulating factor (GM-CSF), Bacillus Calmette-Guérin (BCG), aluminum hydroxide, Muramyl dipeptide (MDP) compounds, such as thur-MDP and nor-MDP, muramyl tripeptide phosphatidylethanolamine (MTP-PE), RIBI's adjuvants (Ribi ImmunoChem Research, Inc., Hamilton MT), which contains three components extracted from bacteria, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion. MF-59, Novasomes®, major histocompatibility complex (MHC) antigens are other known adjuvants.

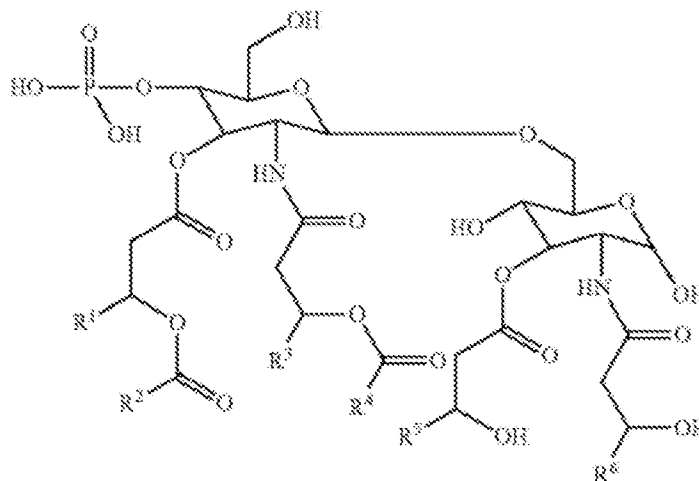
[0128] While alum is often used as an adjuvant for vaccines, it is known for boosting humoral immunity but not for induction of effective cellular immunity (Langley JM et al., “A dose-ranging study of a subunit Respiratory Syncytial Virus subtype A vaccine with and without aluminum phosphate adjuvantation in adults > or =65 years of age,” *Vaccine*, 27:5913-5919, 2009; Falsey AR, et al., “Comparison of the safety and immunogenicity of 2 respiratory syncytial virus (RSV) vaccines--nonadjuvanted vaccine or vaccine adjuvanted with alum--given concomitantly with influenza vaccine to high-risk elderly individuals,” *J. Infect. Dis.*, 198:1317-1326, 2008; and Kool M, et al., “Alum adjuvant: some of the tricks of the oldest adjuvant,” *J. Med. Microbiol.*, 61:927-934, 2012).

[0129] Novel adjuvant compounds incorporating Toll-like receptor (TLR) 9 agonists have been shown to improve Th1-biased cellular responses to RSV vaccines in mouse models (Hancock GE, et al., “CpG containing oligodeoxynucleotides are potent adjuvants for parenteral vaccination with the fusion (F) protein of respiratory syncytial virus (RSV),” *Vaccine*, 19:4874-4882, 2001; and Garlapati S, et al., “Enhanced immune responses and protection by vaccination with respiratory syncytial virus fusion protein formulated with CpG oligodeoxynucleotide and innate defense regulator peptide in polyphosphazene microparticles,” *Vaccine*, 30(35):5206-5214, 2012). TLR4-based adjuvants such as a Monophosphoryl Lipid A (MPL)/QS-21 combination or Protollin, a formulation of LPS complexed with meningococcal outer membrane proteins, have also been able to induce cellular IFN γ production to RSV vaccines in mice (Neuzil KM, et al., “Adjuvants influence the quantitative and qualitative immune response in BALB/c mice immunized with respiratory syncytial virus FG subunit vaccine,” *Vaccine*, 15:525-532, 1997; Cyr SL, et al., “Intranasal proteosome-based respiratory syncytial virus (RSV) vaccines protect BALB/c mice against challenge without eosinophilia or enhanced pathology,” *Vaccine*, 25:5378-5389, 2007).

[0130] Enterobacterial lipopolysaccharide (LPS) is a potent stimulator of the immune system. However, its use in adjuvants is avoided due to its toxicity. A non-toxic derivative of LPS, monophosphoryl lipid A (MPL), produced by the removal of the core carbohydrate group and phosphate from the reducing-end glucosamine has been produced, along with a further detoxified version of MPL, produced by the removal of the acyl chain from the 3-position of the disaccharide backbone, called 3-O-deacylated monophosphoryl lipid A (3D-MPL).

[0131] Another synthetic TLR4 agonist optimized for binding to the human MD2 molecule of the TLR4 complex is a synthetic hexylated Lipid A derivative called glucopyranosyl lipid adjuvant (GLA). GLA has been demonstrated to be a potent Th1-biasing adjuvant in both rodent and primate model systems (Coler RN, et al., “A synthetic adjuvant to enhance and expand immune responses to influenza vaccines,” *PLoS One*, 5:e13677, 2010; and Lumsden JM, et al., “Evaluation of the safety and immunogenicity in rhesus monkeys of a recombinant malaria vaccine for *Plasmodium vivax* with a synthetic Toll-like receptor 4 agonist formulated in an emulsion,” *Infect. Immun.*, 79:3492-3500, 2011).

[0132] GLA is described in detail in, for instance, U.S. Patent Application Publication No. 2011/0070290, entitled “Composition Containing Synthetic Adjuvant,” the disclosure of which is hereby incorporated by reference in its entirety for all purposes. As described in U.S. Patent Application Publication No. 2011/0070290, GLA comprises: (i) a diglucosamine backbone having a reducing terminus glucosamine linked to a non-reducing terminus glucosamine through an ether linkage between a hexosamine moiety at position 1 of the non-reducing terminus glucosamine and a hexosamine moiety at position 6 of the reducing terminus glucosamine; (ii) an O-phosphoryl group attached to the hexosamine moiety at position 4 of the non-reducing terminus glucosamine; and (iii) up to six fatty acyl chains, wherein one of the fatty acyl chains is attached to 3-hydroxy of the reducing terminus glucosamine through an ester linkage, wherein one of the fatty acyl chains is attached to a 2-amino of the non-reducing terminus glucosamine through an amide linkage and comprises a tetradecanoyl chain linked to an alkanoyl chain of greater than 12 carbon atoms through an ester linkage, and wherein one of the fatty acyl chains is attached to 3-hydroxy of the non-reducing terminus glucosamine through an ester linkage and comprises a tetradecanoyl chain linked to an alkanoyl chain of greater than 12 carbon atoms through an ester linkage. For example, GLA can be defined by the following structural formula:



wherein R^1 , R^3 , R^5 and R^6 , are C_{11} - C_{20} alkyl; and R^2 and R^4 are C_{12} - C_{20} alkyl. In some aspects, GLA is formulated as a stable oil-in-water emulsion (SE), which is referred to herein as GLA-SE.

[0133] In one aspect, the composition includes an adjuvant that is a TLR agonist. In one aspect, the disclosed composition includes an adjuvant that is a TLR4 agonist. Cytokines induced by TLR4 signaling, such as IL-6 and IFN γ , act as B cell growth factors and support class-switching to antibodies optimized for interactions with Fc receptors and complement (Finkelman FD, et al., "IFN-gamma regulates the isotypes of Ig secreted during in vivo humoral immune responses," *J. Immunol.*, 140:1022-1027, 1988; and Nimmerjahn F and Ravetch JV, "Fc-receptors as regulators of immunity," *Adv. Immunol.*, 96:179-204, 2007). These cytokines additionally recruit professional antigen presenting cells, inducing MHC I molecules and antigen processing protein upregulation to allow for better activation of T cells (Ramanathan S, et al., "Antigen-nonspecific activation of CD8 $^+$ T lymphocytes by cytokines: relevance to immunity, autoimmunity, and cancer," *Arch. Immunol. Ther. Exp.*, 56:311-323, 2008). Type I IFN induced by TLR4 signaling can enhance cross-presentation of protein antigens (Durand V, et al., "Role of lipopolysaccharide in the induction of type I interferon-dependent cross-priming and IL-10 production in mice by meningococcal outer membrane vesicles," *Vaccine*, 27:1912-1922, 2009), allowing induction of strong CD8 $^+$ T cell responses to associated ovalbumin protein (Lasarte JJ, et al., "The extra domain A from fibronectin

targets antigens to TLR4-expressing cells and induces cytotoxic T cell responses in vivo” *J. Immunol.*, 178: 748-756, 2007; MacLeod MK, et al., 2011). For instance, in one embodiment, compositions disclosed herein include an adjuvant that comprises GLA. In one embodiment, the composition is formulated as a particulate emulsion. In another embodiment, composition includes an adjuvant that includes GLA in a stabilized squalene based emulsion.

[0134] The dosage for the RSV composition can vary, for example, depending upon age, physical condition, body weight, sex, diet, time of administration, and other clinical factors and can be determined by one of skill in the art. In one aspect, the composition is formulated as a stable aqueous suspension having a volume of at least about 50 μl , 75 μl , or 100 μl and up to about 200 μl , 250 μl , 500 μl , 750 μl , or 1000 μl .

[0135] In one aspect, at least about 1 μg , 5 μg , 10 μg , 20 μg , 30 μg or 50 μg and up to about 75 μg , 80 μg , 85 μg , 90 μg , 95 μg , 100 μg , 105 μg , 110 μg , 115 μg , 120 μg , 125 μg , 130 μg , 135 μg , 140 μg , 145 μg , 150 μg , or as much as 200 μg of RSV soluble F protein, as described herein can be included in the disclosed compositions. In one aspect, the composition includes recombinant RSV-F immunogen at a concentration of at least about 0.01 $\mu\text{g}/\mu\text{l}$, 0.05 $\mu\text{g}/\mu\text{l}$, 0.1 $\mu\text{g}/\mu\text{l}$ and up to about 0.1 $\mu\text{g}/\mu\text{l}$, 0.2 $\mu\text{g}/\mu\text{l}$, 0.3 $\mu\text{g}/\mu\text{l}$, 0.4 $\mu\text{g}/\mu\text{l}$, 0.5 $\mu\text{g}/\mu\text{l}$, or 1.0 $\mu\text{g}/\mu\text{l}$.

[0136] In one aspect, the composition includes at least about 0.1 μg , 0.5 μg , 1 μg , 1.5 μg , 2 μg , or 2.5 μg , 3 μg , 4 μg , 5 μg , 10 μg , or 20 μg adjuvant. In one aspect, the adjuvant is GLA-SE and the composition includes at least about 0.1 μg , 0.5 μg , 1 μg , 1.5 μg , 2 μg , or 2.5 μg , 3 μg , 4 μg , 5 μg , 10 μg or 20 μg GLA in a squalene-based SE. In one aspect, the composition includes adjuvant at a concentration of at least about 1 $\text{ng}/\mu\text{l}$, 2 $\text{ng}/\mu\text{l}$, 3 $\text{ng}/\mu\text{l}$, 4 $\text{ng}/\mu\text{l}$ or 5 $\text{ng}/\mu\text{l}$, 0.1 $\mu\text{g}/\mu\text{l}$, 0.2 $\mu\text{g}/\mu\text{l}$, 0.3 $\mu\text{g}/\mu\text{l}$, 0.4 $\mu\text{g}/\mu\text{l}$, or 0.5 $\mu\text{g}/\mu\text{l}$. In one aspect, the adjuvant is GLA-SE and the composition includes at least about 1 $\text{ng}/\mu\text{l}$, 2 $\text{ng}/\mu\text{l}$, 3 $\text{ng}/\mu\text{l}$, 4 $\text{ng}/\mu\text{l}$ or 5 $\text{ng}/\mu\text{l}$, 0.1 $\mu\text{g}/\mu\text{l}$, 0.2 $\mu\text{g}/\mu\text{l}$, 0.3 $\mu\text{g}/\mu\text{l}$, 0.4 $\mu\text{g}/\mu\text{l}$ or 0.5 $\mu\text{g}/\mu\text{l}$, 1 μg , 1.5 μg , 2 μg , or 2.5 μg , 3 μg , 4 μg , 5 μg , 10 μg , or 20 μg GLA in a squalene-based SE.

[0137] In one aspect, the adjuvant comprises GLA in a stabilized oil-in-water emulsion having a GLA concentration of at least about 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, or 5%. In one aspect, the adjuvant comprises GLA in a stabilized oil-in-water emulsion (SE), wherein GLA has a

mean particle size of at least about 25 nm, 50 nm, 75nm, 100 nm, 125 nm, 150nm, 175 nm, or about 200 nm.

[0138] In another aspect, the composition includes between about 1 µg and 100 µg RSV-sF glycoprotein in combination with between about 1 µg and 10 µg GLA in between 2% to 5% SE in a final volume between of about 100 µl to about 500 µl. In another aspect, the composition is a liquid formulation that includes between about 10 µg and about 100 µg RSV-sF glycoprotein in combination with between about 1 µg and about 5 µg GLA, in between about 2% to 5% SE, in a final volume of between about 250 µl to about 1000 µl. In a further aspect, the composition is formulated for intramuscular injection and includes about 10 µg, 20 µg, 30 µg, 40 µg, 50 µg, 60 µg, 70 µg, 80 µg, 90 µg, 100 µg, 110 µg, 120 µg, 130 µg, 140 µg, or about 150 µg RSV-sF glycoprotein in combination with 1 µg, 1.5 µg, 2.0 µg, 2.5 µg, 3.0 µg, 3.5 µg, 4.0 µg, 4.5 µg, or 5 µg GLA in 2%, 3%, 4%, or 5% (v/v) SE in a final volume of about 500 µl.

[0139] The amount and frequency of administration can be dependent upon the response of the host. In one aspect, the composition is administered as a single dose, for instance annually. In another aspect the composition is administered according to a two dose regimen. In another aspect, the composition is administered on a dosage schedule, for example, an initial administration of the composition with subsequent booster administrations. In one aspect, the composition is administered according to a two-dose regimen in which the second dose is administered at least about 1, about 2, about 3, or about 4 weeks after the initial administration, or at least about 1, about 2, about 3, about 4, about 5, or about 6 months after the initial administration, or at least about 1 year or longer after the initial administration. In another aspect, the composition is administered on a dosage schedule in which a second dose is administered at least about 1, about 2, about 3, or about 4, weeks after the initial administration, or at least about 1, about 2, about 3, about 4, about 5, or about 6 months, after the initial administration, or at least about 1 year or longer after the initial administration, and a third dose is administered after the second dose, for example, at least about 1, about 2, about 3, about 4, about 5, about 6 months, or about one year after the second dose.

[0140] In another aspect, the composition includes a pharmaceutically acceptable carrier or diluent in which the immunogen is suspended or dissolved. Pharmaceutically acceptable carriers are known, and include but are not limited to, water for injection, saline solution, buffered saline,

dextrose, water, glycerol, sterile isotonic aqueous buffer, and combinations thereof. For parenteral administration, such as subcutaneous injection, the carrier can include water, saline, alcohol, a fat, a wax, a buffer or combinations thereof. A thorough discussion of pharmaceutically acceptable carriers, diluents, and other excipients is presented in Remington's Pharmaceutical Sciences (Mack Pub. Co. N.J. current edition), the disclosure of which is hereby incorporated by reference in its entirety for all purposes. The formulation should suit the mode of administration. In another aspect, the formulation is suitable for administration to humans, is sterile, non-particulate and/or non-pyrogenic.

[0141] In another aspect, the composition can include one or more diluents, preservatives, solubilizers, emulsifiers, and/or adjuvants. For example, the composition can include minor amounts of wetting or emulsifying agents, or pH buffering agents to improve vaccine efficacy. The composition, or various components thereof, can be a solid form, such as a lyophilized powder suitable for reconstitution, a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

[0142] Other components can be included in the disclosed composition, such as delivery vehicles including, but not limited to, aluminum salts, water-in-oil emulsions, biodegradable oil vehicles, oil-in-water emulsions, biodegradable microcapsules, and/or liposomes. In other aspects, the composition can include antibacterial agents such as benzyl alcohol and/or methyl paraben; antioxidants such as ascorbic acid and/or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid and the like, and/or derivatives thereof; buffers such as acetates, citrates, and/or phosphates, and agents for the adjustment of tonicity, such as sodium chloride and/or dextrose.

[0143] Administration of the composition can be systemic or local. Methods of administering a composition include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intravenous and subcutaneous), epidural, and mucosal (e.g., intranasal and oral or pulmonary routes or by suppositories). In some aspects, compositions described herein are administered intramuscularly (IM), intravenously (IV), subcutaneously, transdermally or

intradermally. The disclosed compositions can be administered by any convenient route, for example by infusion and/or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucous, colon, conjunctiva, nasopharynx, oropharynx, vagina, urethra, urinary bladder and intestinal mucosa, etc.) and can be administered together with other biologically active agents. In some aspects, administration intranasally or by way of other mucosal tissues, of the composition can induce an antibody or other immune response that is substantially higher than other routes of administration. In another aspect, administration intranasally or by way of other mucosal tissues, of the compositions disclosed herein can induce an antibody or other immune response at the site of immunization.

[0144] For example, compositions for enhancing an immune response in a subject can include an engineered RSV soluble fusion protein (sF) and an adjuvant comprising glucopyranosyl lipid A (GLA) in an oil-in-water emulsion (stable emulsion [SE]). In one aspect, SE can be a squalene-based stable emulsion. As already noted above, the antigen RSV sF contains known neutralizing epitopes of the RSV fusion protein and can be recombinantly expressed in an appropriate eukaryotic cell line, such as the Chinese Hamster Ovary (CHO) cell line.

[0145] As noted, in some aspects the adjuvant GLA-SE is included in the composition to boost RSV F-specific neutralizing antibodies, RSV F-specific CD4⁺ T-helper (Th) cells, CD8⁺ cytotoxic T-cells, and to stimulate a Th1 cytokine profile in the subject. These elements of the immune response are thought to contribute to preventing RSV infection and limiting RSV replication, thus diminishing RSV disease (Lambert et al, Molecular and Cellular Response Profiles Induced by the TLR4 Agonist-Based Adjuvant Glucopyranosyl Lipid A, *PLoS One*. 2012;7(12):e51618. doi: 10.1371/journal.pone.0051618. Epub 2012 Dec 28.); Malloy et al, Consequences of immature and senescent immune response for infection with respiratory syncytial virus. L. J. Anderson and B. S. Graham (eds.), Challenges and Opportunities for Respiratory Syncytial Virus Vaccines, Current Topics in Microbiology and Immunology 372, doi: 10.1007/978-3-642-38919-1_11, Springer-Verlag Berlin Heidelberg 2013). Older adults have reduced RSV F-specific interferon gamma (IFN γ) T-cell responses compared to younger adults (Cherukuri et al, Adults 65 years old and older have reduced numbers of functional memory T cells to respiratory syncytial virus fusion protein. Clin Vaccine Immunol. 2013;20:239-47. doi: 10.1128/CVI.00580-12). Thus, although the elicitation of

neutralizing antibodies is likely to be an effect of a vaccine, an effective RSV vaccine for adult or elderly subjects should also induce a significant cell-mediated immune response. For instance older adults have antibodies to RSV but are deficient in cellular immunity compared to younger adults. Neutralizing antibody responses (Figures 1A and 1B) and IFN γ ELISPOT assay responses to RSV F protein are depicted for young (20-30 years) and elderly (65-85 years) individuals (Cherukuri et al., *Clin Vaccine Immunol.*, 20(2):239-247, 2013). For this reason, both GLA and SE are included in an aspect of the compositions and methods disclosed herein, because both are contributors to the adjuvant effect previously observed in animal models. For instance, GLA-SE has been shown to up-regulate Th1 chemokines and induce stronger responses than did alum or GLA or SE alone (Coler et al, Development and characterization of synthetic glucopyranosyl lipid adjuvant system as a vaccine adjuvant. *PLoS One.* 2011;6(1):e16333. doi: 10.1371/journal.pone.0016333; Lambert et al, Molecular and cellular response profiles induced by the TLR4 agonist-based adjuvant glucopyranosyl lipid A. *PLoS One.* 2012;7(12):e51618. doi:10.1371/journal.pone.0051618). Therefore, the disclosed compositions are specifically designed to optimize the immune response to RSV sF in adult and/or elderly subjects.

[0146] In one aspect, an engineered RSV sF is included in the disclosed compositions in the post-fusion form, and an adjuvant comprising GLA in an oil-in-water SE is added. Therefore, in one aspect, RSV sF is a sterile, lyophilized component of the composition. In one aspect, the recombinant, sterile lyophilized RSV sF is provided in an amount of 0.14 mg per vial, nominal extractable weight, which is intended for intramuscular injection following reconstitution with sterile water that can also include the GLA-SE adjuvant. GLA-SE adjuvant can be obtained from Immune Design (Seattle, WA) as an emulsion.

[0147] In one aspect, the composition is prepared on site, where the subject is to be injected, by combining the antigen (RSV sF), adjuvant, and adjuvant diluent, into a single vial, which can then be injected into the subject using a syringe. In one aspect, administration of the composition is achieved *via* IM injection.

Methods of Enhancing RSV Immunity

[0148] In response to RSV infection, neutralizing antibodies that target the RSV Fusion (F) and attachment (G) envelope glycoproteins are produced by the infected subject (Hurwitz JL, “Respiratory Syncytial Virus Vaccine Development,” *Expert Rev. Vaccines*, 10:1415-1433, 2011). RSV F-directed neutralization responses are beneficial since, as described above, RSV F glycoprotein is both highly conserved between the RSV A and RSV B strains of the virus, and is essential for fusion of viral and cellular membranes, a prerequisite for virus entry and replication (Maher CF, et al., 2004). Low RSV neutralizing antibody titers in a subject correlates with a higher risk of more severe RSV disease (Lee FE, et al., *Antiviral Res.*, 63:191-196, 2004). While RSV neutralizing antibodies play a significant role in RSV immunity, providing protection to naive subjects upon passive transfer, cellular responses to RSV can substantially contribute to generation of a robust immune response and eventual recovery (Krillov LR, *Expert Opin. Biol. Ther.*, 2:763-769, 2002, and Graham BS, et al., *Pediatr. Res.*, 34:167-172, 1993).

[0149] The balance of RSV antibodies and cellular immunity required to protect against RSV disease in humans can vary depending on infected age group. For example in the elderly, cellular responses are more difficult to induce, more Th2-biased, and wane more rapidly than in young adults (Kumar R and Burns EA, *Expert Rev. Vaccines*, 7:467-479, 2008). RSV-specific T cell responses in particular decline with age in RSV infection (Cusi MG, et al., *Immun. Ageing*, 7:14, 2010). Elderly individuals can still succumb to severe RSV disease despite being seropositive with RSV neutralizing titers of 9-13 log₂ (Walsh EE, et al., *J. Infect. Dis.*, 189:233-238, 2004). The elderly have T cell defects in RSV responsiveness not seen in the young (Cusi MG, et al.), and despite having similar neutralizing antibody titers to young adults (Falsey AR, et al., *J. Med. Virol.*, 59:221-226, 1999), are more susceptible to RSV disease following infection. These observations suggest that an effective RSV vaccine for the elderly is required to boost both neutralizing antibodies and waning RSV specific cell mediated immunity.

[0150] As mentioned above, the elderly tend to have a Th2 bias in their immune response. The cellular immune response of a mammal includes both a T helper 1 (Th1) cellular immune response and a T helper 2 (Th2) cellular immune response. Th1 and Th2 responses are distinguishable on the basis of the cytokine profiles synthesized in each response. Type 1 T cells produce interferon gamma

(IFN- γ), a cytokine implicated in the viral cell-mediated immune response. IFN- γ can therefore be referred to as a “Th1-type cytokine.” Th2 cells selectively produce interleukin 4 (IL-4), interleukin 5 (IL-5) and interleukin 13 (IL-13), which participate in the development of humoral immunity and have a prominent role in immediate-type hypersensitivity. IL-4, IL-5 and IL-13 can also be referred to as “Th2 type cytokines.” A Th1 response can also be identified by the antibody subtype produced in the response. In rodent models, a Th1-biased response has an IgG2a or IgG2b antibody titer that is greater than the IgG1 antibody titer (IgG2a and IgG2b are Th1 subtypes; IgG1 is a Th2 subtype). (Of note, in humans the converse is true; human IgG1 is a Th1 subtype, and human IgG2 is a Th2 subtype, with a Th1-biased response characterized by greater IgG1 antibody titers than IgG2 antibody titers.) In both rodents and humans, a Th1 response is also marked by an increased CD8⁺ T cell response. An imbalance in the Th1/Th2 cytokine immune response, particularly a Th2 bias in the cellular immune response of an animal, can affect pathogenesis of RSV and the severity of the infection, particularly in the lungs. Additionally, a Th2-biased primary immune response has been correlated with RSV enhanced disease (Hurwitz JL, *Expert Rev. Vaccines*, 10:1415-1433, 2011).

[0151] As such, disclosed herein are methods of enhancing RSV immunity that address the problematic immune response observed in adults and the elderly. The compositions disclosed herein can be employed in such methods to treat and/or prevent RSV infection and to enhance RSV immunity in subjects. Compositions comprising RSV sF are useful in these methods because, as noted above, RSV F is essential for productive infection, is highly conserved between RSV groups (A and B), and contains multiple neutralizing epitopes as well as cluster of differentiation CD4⁺ and CD8⁺ T-cell epitopes. Further, immunity to RSV F protein has been shown to inhibit RSV replication in preclinical animal models. Such results are disclosed in WO 2014/168821, the entirety of the disclosure of which is incorporated herein by reference for all purposes. Additionally, higher titers of antibody to RSV F protein correlate with protection against challenge and against both acute respiratory illness and hospitalization caused by RSV in the elderly (Walsh et al, 2004; Falsey and Walsh, Relationship of serum antibody to risk of respiratory syncytial virus infection in elderly adults. *J Infect Dis.* 1998;177(2):463-6; Falsey et al, Comparison of the safety and immunogenicity of 2 respiratory syncytial virus (RSV) vaccines—nonadjuvanted vaccine or vaccine adjuvanted with alum—given concomitantly with influenza vaccine to high-risk elderly individuals. *J*

Infect Dis 2008;198:1317-26. doi: 10.1086/592168; Lee et al Experimental infection of humans with A2 respiratory syncytial virus. Antiviral Res. 2004 Sep;63(3):191-6). RSV F protein is also a clinically validated target, because it is the target of palivizumab (SYNAGIS[®], MedImmune, US), a monoclonal antibody (mAb) with proven efficacy in the prevention of serious lower respiratory tract disease caused by RSV in children at high risk of RSV disease.

[0152] As noted above, adult and elderly human subjects are especially prone to recurrent and persistent RSV infections that can lead to serious medical conditions due to the character of the immunity triggered by RSV infection. As such, in one aspect, the compositions can be administered to subjects at least 60 years old and older. Subjects can be human. Subjects can be about 60 years old, about 65 years old, and/or between about 60 years old and 65 years old. In some aspects, the subject is between about 60 years old and 87 years old. In another aspect, the subject is at least 65 years old.

[0153] In one aspect, the composition is administered as a single, 0.5 mL intramuscular (IM) dose on an annual basis, in light of the known short-lived immune response to wild-type RSV infection (Falsey et al, 2006). For instance, the composition can be administered at about the same time as, or concurrently with, the commonly administered annual influenza vaccine. For instance, in one aspect, the composition is administered concomitantly with a composition intended to generate an immune response against influenza virus. The composition intended to generate an immune response against influenza virus can be administered contralaterally to the composition comprising RSV soluble F protein.

[0154] The human subject can be RSV seropositive and/or have been previously exposed to RSV. The disclosed methods can be performed on populations that are already seropositive for RSV, or those that were infected with RSV prior to being administered the disclosed compositions.

[0155] In one aspect, a method for administering an immunologically effective amount of the present composition(s) containing an immunogenic RSV-F protein to a subject (such as a human) is provided. As noted above in the description of the disclosed compositions, the composition can include an immunogenic RSV-F protein and at least one adjuvant. The RSV-F can be soluble RSV-F (also designated as RSV-sF). As described above for the disclosed composition(s), the adjuvant can be GLA, such as GLA-SE.

[0156] The disclosed methods are designed to elicit an immune response against RSV in a subject, such as a human subject. The immune response can be a humoral and/or cell-mediated response. In one aspect, the composition, when employed by the disclosed methods, is capable of eliciting, and/or does elicit, at least one immune response in subjects administered the composition. In one aspect, the immune response is selected from a T_{H1}-type T lymphocyte response, a T_{H2}-type T lymphocyte response, a cytotoxic T lymphocyte (CTL) response, an antibody response, a cytokine response, a lymphokine response, a chemokine response, and/or an inflammatory response. In one aspect, the methods of administering the disclosed composition(s) are capable of eliciting in a host at least one immune response such as, but not limited to: (a) production of one or a plurality of cytokines, for example, one or more of interferon-gamma (IFN- γ), and/or tumor necrosis factor-alpha (TNF- α), (b) production of one or a plurality of interleukins, for example, one or more of IL-1, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-16, IL-18, and/or IL-23, (c) production of one or more of a plurality of chemokines, for example, MIP-1 α , MIP-1 β , RANTES, CCL4, and/or CCL5, and/or (d) a lymphocyte response, for example, a memory T cell response, a memory B cell response, an effector T cell response, a cytotoxic T cell response, and/or an effector B cell response or combinations thereof.

[0157] In one aspect, the composition is able to provide an immune response that includes production of Th1-type cytokines, such as, but not limited to IFN γ (Th1 biased) as compared to Th2 biased cytokines such as, but not limited to IL-5 and/or IL-4. In one aspect, administration of the composition enhances a Th1 biased cellular immune response in a mammal that has been previously exposed to RSV. In one aspect, the ratio of Th1/Th2 cellular immune response is at least about 1:1, 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, or up to about 2:1. In one aspect, a method of inducing or enhancing a Th1-type F protein specific CD4 or CD8 response is provided.

[0158] Additionally, when the disclosed methods are performed on a subject, the methods can prophylactically result in, or achieve, a protective immune response to RSV infection and/or at least one symptom thereof in a subject, such as a human subject. The disclosed method(s), when used to treat subjects who have not been exposed to, or infected with, RSV, can prevent RSV infection and/or ameliorate the symptoms thereof. As such, treatment of seronegative and seropositive subjects can induce a protective effect against RSV. This protective effect can provide the treated

subject with an immunity to RSV prior to infection, such that upon exposure to, and infection with, RSV after being administered the disclosed compositions, the subject's immune reaction (humoral and cellular) will be sufficiently strong to combat the infection rapidly, for example, immediately, thereby reducing and/or clearing the virus from the subject's system prior to development of symptoms or at least substantially ameliorating such symptoms, and subsequent complications thereof. The disclosed methods can therefore be performed on seronegative or seropositive subjects or those subjects believed to be at risk of contracting RSV infection. In one aspect, the disease is a disease of the respiratory system, for example, a disease is caused by a virus, such as RSV (RSV A and/or RSV B).

[0159] Therefore, disclosed are methods of enhancing RSV immunity in a human subject. In some aspects, the method of enhancing RSV immunity in the subject can result in, without limitation, enhancing a Th1 biased cellular immune response in the subject, inducing neutralizing antibodies against RSV in the subject, reducing RSV viral titers in the subject, inducing an immune response to RSV in the subject, and/or preventing RSV infection in the subject. In some aspects, the enhanced RSV immunity provided to the subject includes an increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells. In other aspects, the enhanced RSV immunity provided to the subject includes an increase in the RSV microneutralizing antibody titer of the subject over a baseline RSV microneutralizing antibody titer. In some aspects, the enhanced immunity is indicated by an increase in the anti-RSV F protein-specific antibody titer in the subject over a baseline anti-RSV F protein-specific antibody titer.

[0160] In another aspect, the enhanced immunity is indicated by an increase in the subject of RSV-specific antibody titer over a baseline RSV-specific antibody titer. Antibody titers can be determined by any number of known methods, Meso Scale Discovery (MSD) 4-plex assay, competitive ELISA (cELISA) using antibodies with specificity for RSV F protein, such as palivizumab, as the competitor antibody, and the like. Such assays are described in more detail, below.

[0161] The baseline level of RSV F-specific T cells can be: (1) the level in the subject prior to administration of the composition, (2) the mean level found in a pool of subjects who have not

received the composition, and/or (3) the mean level found in a pool of subjects administered an equivalent amount of RSV soluble F protein in a non-adjuvanted composition.

[0162] Thus, in the disclosed methods, the enhanced immunity can be characterized as an increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells. In such aspects, the increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells can be at least about one-fold. In other aspects, the increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells can be, for example, at least about one-, two-, three-, four-, five-, six-, seven-, eight-, nine-, ten-, eleven-, twelve-, thirteen-, fourteen-, or as much as fifteen-fold. In one aspect, the increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells can be at least about one- to about two-fold, about one- to about three-fold, about one- to four-fold, about one- to five-fold, about one- to six-fold, above one- to seven-fold, about one- to eight-fold, about one- to nine-fold, about one- to ten-fold, or about one- to fifteen-fold.

[0163] The amount of RSV F-specific T cells can be determined by known methodologies. For instance, a common method for detecting and quantitating T cell number is the ELISPOT. The T cells induced by the disclosed methods are known to secrete interferon- γ (INF- γ). Thus, the number of induced T cells can be detected and quantitated by INF- γ ELISPOT methodologies, such as those described in more detail below in the Examples section.

[0164] In some aspects, for instance when the composition comprises about 20 μ g or about 50 μ g RSV soluble F protein, the increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells can be, for example, about six- to about ten-fold. In another aspect, such as when the composition comprises about 80 μ g RSV soluble F protein, and the subject is more than 69 years old, and the increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells can be, for example, about eight- to ten-fold, about nine- to ten-fold, about four- to six-fold, or about five- to six-fold.

[0165] In embodiments where the enhanced immunity includes an increase in the number and/or response of RSV F-specific T cells over a baseline level of RSV F-specific T cells, the T cell activity can be determined at about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 days, or more after administration of the composition, depending on the amount of composition administered and other

variables specific to the subject. The manner in which enhanced immunity is quantitated can be determined by various known methods, such as by those described above, such as IFN γ ELISPOT assay, Meso Scale Discovery (MSD) 4-plex IgG, microneutralization assays, competitive ELISA, and assays described in further detail below.

[0166] In other aspects, the enhanced RSV immunity provided to the subject includes an increase in the RSV microneutralizing antibody titer of the subject over a baseline RSV microneutralizing antibody titer. Microneutralization antibody titers can be measured by known methods generally as described in the Examples section, below. In such embodiments, the baseline RSV microneutralizing antibody titer can be increased by, for example, at least about one-, about two-, about three-, about four-, about five-, about six-, about seven-, about eight-, about nine-, about ten-fold, or higher. In one aspect, the baseline RSV microneutralizing antibody titer can be increased by, e.g., about one- to two-fold, about one- to three-fold, about one- to three-fold, about one- to four-fold, about one- to five-fold, about one- to six-fold, about one- to seven-fold, about one- to eight-fold, about one- to nine-fold, or about one- to about ten-fold. In one aspect, the baseline RSV microneutralizing antibody titer can be increased by, e.g., about 2.5- to 4.0-fold, about 3.0- to 4.0-fold, or about 3.5- to 4.0-fold.

[0167] In some embodiments, the enhanced immunity is indicated by an increase in the anti-RSV F protein-specific antibody titer in the subject over a baseline anti-RSV F protein-specific antibody titer, such as, for instance, at least about a 1-fold increase, or as much as a 5-fold increase or more. In such embodiments, the subject can be an elderly human of at least 50 years, 55 years, 60 years, 65 years, 70 years, 75 years, 80 years, 85 years, 90 years, or 95 years of age, for example the subject can be between at least about 60 years old and about 87 years old. Further, where the enhanced RSV immunity provided to the subject includes an increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer, the baseline anti-RSV F protein-specific antibody titer can be increased by at least about one-fold, at least about 5- to 25-fold, at least about 5- to 15-fold, or at least about 10- to 15-fold or more.

[0168] When the composition comprises about 80 μ g RSV soluble F protein, the enhanced immunity provided to the subject includes an increase in the anti-RSV F protein-specific antibody

titer of the subject over a baseline anti-RSV F protein-specific antibody titer of at least about 15- to 25-fold, at least about 20- to 25-fold, or at least about 20-fold or more.

[0169] In other aspects, when the disclosed compositions are administered to a human subject that is more than about 69 years old, and when the composition comprises about 80 µg RSV soluble F protein, the enhanced immunity provided to the subject includes an increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer of at least about 5- to 15-fold, at least about 10- to 15-fold, or at least about 10-fold or more.

[0170] In one aspect, the anti-RSV F protein-specific antibodies are IgG antibodies.

[0171] In another aspect, the enhanced immunity is indicated by an increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer as determined by a competitive ELISA (cELISA) using the antibody palivizumab as the competitor antibody, such that the cELISA measures the titer of antibodies with binding characteristics that are sufficient to block binding of palivizumab. Further, in such aspects, where the enhanced RSV immunity provided to the subject includes an increase in the RSV-specific antibody titer of the subject over a baseline RSV-specific antibody titer as measured by cELISA, the baseline RSV-specific antibody titer can be increased by, e.g., at least about 5- to 35-fold, or at least about 10- to 30-fold.

[0172] In some aspects of the disclosed methods, the administered composition comprises about 20 µg or about 50 µg RSV soluble F protein, and the baseline RSV-specific antibody titer is increased by, e.g., at least about 5- to 25-fold, or about 10- to 25-fold. In some aspects, the composition comprises about 50 µg RSV soluble F protein, and the baseline RSV-specific antibody titer can be increased by, e.g., at least about 10- to 25-fold, at least about 15- to 25-fold, or at least about 15- to 20-fold.

[0173] In embodiments where the composition administered to the subject comprises about 80 µg RSV soluble F protein, the baseline RSV-specific antibody titer can be increased by, e.g., at least about 20- to 35-fold, or at least about 25- to 30-fold. In one such embodiment, the human subject is more than about 69 years old, the composition comprises about 80 µg RSV soluble F protein, and the baseline RSV-specific antibody titer can be increased by, e.g., at least about 25- to 35-fold, or at least about 30-fold.

[0174] In other aspects, the human subject is more than about 69 years old, the composition comprises about 80 µg RSV soluble F protein, and the baseline RSV-specific antibody titer is increased by, e.g., at least about 10- to 25-fold, at least about 15- to 20-fold, or at least about 20-fold.

[0175] In one aspect of the disclosed methods, a composition is administered to the subject that comprises about 120 µg RSV soluble F protein. This also includes an adjuvant comprising about 1.0 µg, about 1.5 µg, about 2.0 µg, about 2.5 µg, about 3.0 µg, about 3.5 µg, about 4.0 µg, about 4.5 µg, or about 5.0 µg GLA in a squalene-based stable emulsion. In this aspect, the RSV soluble F protein can be amino acids 1-524 of RSV soluble F protein from human strain A2 lacking a transmembrane domain (SEQ ID NO: 1). In one aspect, the subject is human and is of at least about 60 years of age. For instance, in one aspect, the subject can be an elderly human of at least 50 years, 55 years, 60 years, 65 years, 70 years, 75 years, 80 years, 85 years, 90 years, or 95 years of age, for example the subject can be at least about 60 years old, at least about 65 years old, or about 60 and 65 years old.

[0176] In one aspect of this method, the composition comprises about 120 µg RSV soluble F protein and the composition is administered intramuscularly, though the composition can also be administered by other routes already mentioned above, such as parenteral administration (e.g., intradermal, intravenous and/or subcutaneous), epidural, and/or mucosal (e.g., intranasal and oral or pulmonary routes or by suppositories).

[0177] In some aspects, the RSV soluble F protein can be recombinant RSV soluble F protein and can be produced *in vitro* by Chinese Hamster Ovary (CHO) cells. Methods of recombinantly producing RSV soluble F protein are described above. See also WO 2014/168821, the entirety of which is incorporated herein by reference for all purposes.

[0178] In one aspect, the RSV soluble F protein is resuspended from lyophilized form in the adjuvant. The composition can be administered in a volume of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or as much as 1.0 mL.

[0179] In an additional aspect, the RSV soluble F protein can be administered as a liquid formulation.

[0180] Variations of the disclosed methods include not only the amount of dosage administered, and the route of administration, but also the frequency of administration of the disclosed

compositions. In some embodiments of the disclosed methods, the composition need only be administered annually or less than annually.

[0181] In still other aspects, the composition is administered concomitantly with a composition intended to generate an immune response against influenza virus. The composition intended to generate an immune response against influenza virus can be administered contralaterally to the composition comprising RSV soluble F protein. The disclosed methods can also be used to enhance immunity RSV seropositive subjects, and/or subjects that were previously exposed to RSV.

[0182] In additional aspects of the disclosed methods, the subject is an adult, e.g., an elderly human, and the method of enhancing RSV immunity in the subject can result in enhancing a Th1-biased cellular immune response in the subject, inducing neutralizing antibodies against RSV in the subject, reducing RSV viral titers in the subject, inducing an immune response to RSV in the subject, and/or preventing RSV infection in the subject.

[0183] In such aspects, the adjuvant can be about 2.5 µg GLA in a squalene-based stable emulsion of about 2% (v/v).

[0184] Thus, the disclosed methods can be performed annually, where the subject is administered the disclosed compositions seasonally, optionally at the same time as administration of a composition intended to elicit an immune response to influenza virus. In such methods, the disclosed composition could be administered into the contralateral arm of the subject, but need not be administered in that location. However, in certain aspects, a single dose of the disclosed composition administered annually can provide protection against RSV disease for a minimum of a single RSV season, and perhaps for as long as two, three, or more RSV seasons.

[0185] By "therapeutically effective dose or amount" or "effective amount" is intended an amount of an antibody or vaccine that when administered brings about a positive therapeutic response with respect to treatment of a patient with a disease or condition to be treated.

[0186] This disclosure employs, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. (See, for example, Sambrook et al., ed. (1989) *Molecular Cloning A Laboratory Manual*

(2nd ed.; Cold Spring Harbor Laboratory Press); Sambrook et al., ed. (1992) *Molecular Cloning: A Laboratory Manual*, (Cold Spring Harbor Laboratory, NY); D. N. Glover ed., (1985) *DNA Cloning*, Volumes I and II; Gait, ed. (1984) *Oligonucleotide Synthesis*; Mullis et al. U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1984) *Nucleic Acid Hybridization*; Hames and Higgins, eds. (1984) *Transcription And Translation*; Freshney (1987) *Culture Of Animal Cells* (Alan R. Liss, Inc.); Immobilized Cells And Enzymes (IRL Press) (1986); Perbal (1984) *A Practical Guide To Molecular Cloning*; the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); Miller and Calos eds. (1987) *Gene Transfer Vectors For Mammalian Cells*, (Cold Spring Harbor Laboratory); Wu et al., eds., *Methods In Enzymology*, Vols. 154 and 155; Mayer and Walker, eds. (1987) *Immunochemical Methods In Cell And Molecular Biology* (Academic Press, London); Weir and Blackwell, eds., (1986) *Handbook Of Experimental Immunology*, Volumes I-IV; *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); and in Ausubel et al. (1989) *Current Protocols in Molecular Biology* (John Wiley and Sons, Baltimore, Md.).

[0187] General principles of antibody engineering are set forth in Borrebaeck, ed. (1995) *Antibody Engineering* (2nd ed.; Oxford Univ. Press). General principles of protein engineering are set forth in Rickwood et al., eds. (1995) *Protein Engineering, A Practical Approach* (IRL Press at Oxford Univ. Press, Oxford, Eng.). General principles of antibodies and antibody-hapten binding are set forth in: Nisonoff (1984) *Molecular Immunology* (2nd ed.; Sinauer Associates, Sunderland, Mass.); and Steward (1984) *Antibodies, Their Structure and Function* (Chapman and Hall, New York, N.Y.). Additionally, standard methods in immunology known in the art and not specifically described can be followed as in *Current Protocols in Immunology*, John Wiley & Sons, New York; Stites et al., eds. (1994) *Basic and Clinical Immunology* (8th ed; Appleton & Lange, Norwalk, Conn.) and Mishell and Shiigi (eds) (1980) *Selected Methods in Cellular Immunology* (W.H. Freeman and Co., NY).

[0188] Standard reference works setting forth general principles of immunology include *Current Protocols in Immunology*, John Wiley & Sons, New York; Klein (1982) J., *Immunology: The Science of Self-Nonself Discrimination* (John Wiley & Sons, NY); Kennett et al., eds. (1980) *Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses* (Plenum Press, NY); Campbell (1984) "Monoclonal Antibody Technology" in *Laboratory Techniques in Biochemistry*

and Molecular Biology, ed. Burden et al., (Elsevier, Amsterdam); Goldsby et al., eds. (2000) Kuby Immunology (4th ed.; H. Freeman & Co.); Roitt et al. (2001) Immunology (6th ed.; London: Mosby); Abbas et al. (2005) Cellular and Molecular Immunology (5th ed.; Elsevier Health Sciences Division); Kontermann and Dubel (2001) Antibody Engineering (Springer Verlag); Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Press); Lewin (2003) Genes VIII (Prentice Hall, 2003); Harlow and Lane (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Press); Dieffenbach and Dveksler (2003) PCR Primer (Cold Spring Harbor Press).

[0189] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

[0190] The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1: Clinical Trial Phase 1a Study Design

[0191] In the Phase 1a study, escalating RSV sF doses were assessed by cohort either in the absence of adjuvant (alone) or in the presence of a fixed dose of 2.5 µg GLA in 2% (v/v) SE. The data demonstrate the contribution of increasing doses of RSV sF to immunogenicity and the contribution of adjuvant to both humoral and cellular immunity. A plateau of response was not observed. Although adjuvant contributed to reactogenicity, safety and tolerability were acceptable. Enrollment in the initial Phase 1a study, a randomized, double-blind, placebo-controlled, dose-escalation study, was completed in the target population of adults 60 years of age and older. 360-day safety and immunogenicity data were obtained.

Composition Components

[0192] An RSV soluble F (sF) protein containing amino acids 1-524 of the RSV A2 F sequence and lacking the transmembrane domain (Huang K, et al., "Recombinant respiratory syncytial virus F protein expression is hindered by inefficient nuclear export and mRNA processing," *Virus Genes*, 40:212-221, 2010) was immuno-affinity purified with the RSV-F-specific mAb, palivizumab (MedImmune, Inc.) from the supernatants of stably transfected Chinese Hamster Ovary (CHO) cells. SDS-PAGE and western blot analysis indicated that affinity-purified RSV sF protein was >95%

pure, running under reducing conditions as both a ~50 kD (F1) and ~20 kD (F2) band. Cryoimaging results indicated the sF protein forms both trimers and larger multimers, while ELISA binding studies confirm that it contains intact site A, B, and C neutralizing epitopes. RSV sF was quantified by Bradford assay and used both for immunizations and coating in ELISA assays.

[0193] GLA, SE, and GLA-SE were obtained from Immune Design Corporation (Seattle, WA) and have been previously described (Anderson RC, et al., “Physicochemical characterization and biological activity of synthetic TLR4 agonist formulations,” *Colloids Surf B Biointerfaces*, 75:123-132, 2010). GLA in an aqueous formulation was used at 5 µg per dose. SE is a stabilized squalene-based emulsion with a mean particle size of ~100 nm that was used at a 2% concentration. Except where otherwise noted, GLA-SE was used at a dose of 5 µg GLA in 2% SE. All composition formulations were prepared within 24 hours of inoculation.

Serum IgG, IgG1, IgG2a and IgA ELISA

[0194] RSV-F-specific IgG antibodies were assessed using Meso Scale Discovery (MSD) 4-plex assay, competitive ELISA (cELISA) as described above. High binding 96 well plates were coated with purified RSV sF. After blocking, serial dilutions of serum were added to plates. Bound antibodies were detected using HRP-conjugated goat anti-mouse IgG, IgG1, or IgG2a (Jackson ImmunoResearch, West Grove, PA) and developed with 3,3',5,5'-tetramethylbenzidine (TMB, Sigma, St. Louis, MO). RSV-F-specific IgA antibodies were detected using HRP-conjugated goat anti-mouse IgA (Invitrogen, Grand Island, NY). The signal was amplified using ELAST ELISA amplification Kit (Perkin Elmer, Waltham, MA) and detected with TMB. Absorbance was measured at 450 nm on a SpectraMax plate reader and analyzed using SoftMax Pro (Molecular Devices, Sunnyvale, CA). Titers are reported as log₂ endpoint titers using a cutoff of 3x the mean of the blank wells.

Immunoassay for the Detection of RSV Strain A Specific Neutralizing Antibodies in Human Sera using Vero Cells Infected with GFP-tagged RSV A2 Virus

[0195] The RSV strain A (RSV-A) microneutralization (MN) assay was developed and optimized by the inventors to detect RSV-A specific antibodies in human sera that neutralize virus infection of Vero cells at an optimized cell density. Briefly, an optimized amount of RSV-A2 virus

engineered to express green fluorescent protein upon replication (RSV A2-GFP) was incubated with serially diluted human sera samples, positive control (PC, human pooled sera), or media (virus only control, VC) at room temperature for 60-80 minutes. The virus-sera mixture was overlaid onto the Vero cell monolayer in 96-well plates and incubated for 22 – 24 hours. Plates were imaged on a high-content imaging system (ImageXpress Micro XLS) to detect individual fluorescent Vero cells (foci) that were indicative of virus infection. The foci were enumerated using the imaging software and $\text{Log}_2 \text{IC}_{50}$ titers were determined using a 2-point interpolation from data normalized to the mean VC on each plate. The 2-point interpolation was calculated from the linear regression of two dilution points that surrounded 50% neutralization of virus. This assay was shown to be robust over a 4 year period with multiple analysts and separate laboratories by monitoring the positive control (PC) trending on each assay plate.

[0196] The precision and linearity of the RSV A MN assay were qualified at BioAgilytix Laboratories prior to clinical sample testing. The assay linearity was demonstrated with five positive serum samples with concentrations in the titer range of 4 to 10 $\text{log}_2 \text{IC}_{50}$. These were prepared by spiking positive control sera into negative depleted human serum. The assay precision (repeatability, intermediate and total precision) was evaluated using a separate pooled human sera sample and the five linearity samples; the total assay variability (CV%) ranged from 20.7% to 30.2% for the six samples. Given the observed assay variability of the qualified assay, using a 3-fold rise of MN IC_{50} titer as the seroresponse criteria gives an expected false seroresponse rate due to assay variability of <1% through statistical simulation.

[0197] The PC sample with an expected RSV A MN titer of 9.66 $\text{log}_2 \text{IC}_{50}$ was included with each assay run of clinical sample testing. The control sample testing results demonstrated a total assay variability (CV%) of 23.3% during testing. This is consistent with the total assay variability observed during qualification.

[0198] In the placebo group of the Phase I a study, the false seroresponse rate was 0% using a 3-fold rise in MN IC_{50} titer of post-dose vs pre-dose samples as the seroresponse criteria. This clinically validates the use of a 3-fold rise in MN IC_{50} titers as the seroresponse criteria.

Qualified Immunoassay for the Quantitation of RSV F Peptide Pool-Specific IFN- γ Responses by ELISPOT in Human Peripheral Blood Mononuclear Cells

[0199] Interferon gamma (IFN γ) responses to RSV F peptide pool (RSV Fpp) were measured using an Enzyme Linked Immunosorbent Spot Assay (ELISPOT) that has been developed and qualified in human peripheral blood mononuclear cells (PBMC). Cryopreserved human PBMCs were thawed and plated at an optimized cell number into hydrophobic PVDF membrane microtiter plates pre-coated with anti-human IFN γ . A control donor sample consisting of cryopreserved PBMC from a RSV F-responding donor was included on every plate. Three different stimulants were added to each plate in replicates: mock (0.2% DMSO), Staphylococcal Enterotoxin B (SEB), and RSV Fpp. Mock stimulation was used as non-specific control. SEB is a mitogen that is able to activate T cells to secrete IFN γ . A threshold response to SEB was used as a marker of sample quality. The RSV Fpp consists of overlapping peptides derived from the RSV F protein sequence included in the compositions and is capable of stimulating both CD4⁺ and CD8⁺ T cells. During the stimulation, RSV F-specific memory T cells secreted IFN γ in response to activation by these antigenic peptides. Secreted IFN γ was captured onto the PVDF membrane with anti-human IFN γ . Captured IFN γ was detected with a biotinylated anti-IFN γ antibody, followed by secondary detection antibody [streptavidin-alkaline phosphatase (ALP)]. The spots were visualized using substrate 5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt and nitro-blue tetrazolium chloride, (BCIP/NBT). The chromophore spots were counted using a CTL Immunospot Analyzer and the data was expressed as spot forming counts per million PBMC (SFC/10⁶ PBMC).

[0200] To ensure data quality, acceptance criteria were implemented to determine if the data from an individual sample are valid. Individual sample results were considered valid if the SEB-specific response meets the minimum threshold requirement and the control donor sample responds as expected on each plate. RSV Fpp IFN γ responses for each individual donor can be reported with or without mock subtraction.

[0201] The Lower Limit of Quantification (LLOQ), “the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy,” and precision of the RSV Fpp specific ELISPOT assay were qualified at a commercial laboratory prior to clinical sample testing. The evaluated LLOQ of the RSV Fpp specific response was 33.3 SFC/10⁶ PBMC.

The assay precision (repeatability, intermediate and total precision) was evaluated using PBMC samples from six donors that had RSV Fpp specific IFN γ responses ranging from 59.7 to 543.8 SFC/10⁶ PBMC. The total assay variability (CV%) ranged from 16.3% to 48.0%. Given the observed assay variability of the assay, using a 3-fold rise in the RSV Fpp specific IFN γ response as the cell-mediated immunity (CMI) responder criteria, gives an expected false CMI responder rate due to assay variability of < 5% through statistical simulation.

[0202] The control donor sample with an average RSV Fpp specific IFN γ response level of 192.5 SFC/10⁶ PBMC was included with each assay run of clinical sample testing. The control donor sample testing results demonstrated a total assay variability (CV%) of 32.9% during testing. This is consistent with the total assay variability observed during qualification.

[0203] In the placebo group of the Phase1a study, the false CMI responder rate was 0% using a 3-fold rise in the RSV Fpp specific IFN γ response of post-dose vs pre-dose samples as the CMI responder criteria. This clinically validates the use of a 3-fold rise in the RSV Fpp specific IFN γ response as the CMI responder criteria.

RSV-Specific 4-plex Immunoassay for the Detection of IgG Antibodies Against RSV Antigens F, N, Ga, and Gb in Human Sera Using a Electrochemiluminescence Detection Platform

[0204] The RSV Meso Scale Discovery (MSD) 4-plex IgG assay was developed to simultaneously measure the levels of IgG antibodies specific to four RSV antigens (F, N, Ga and Gb) in human sera samples. The RSV MSD 4-plex IgG assay serves a dual purpose: to detect recent RSV infection (using a composite of three readouts of RSV-N, Ga and Gb), and to assess the immunogenicity of the composition (using the RSV-F readout). The four RSV antigens (F, N, Ga and Gb) were individually spotted onto custom manufactured MSD plates. The 4-plex assay control was prepared from five lots of pooled normal human sera. The 4-plex reference standard was pooled human sera from five individual donors who had high IgG serum titer. Antibodies present in human sera samples, the 4-plex assay control and the 4-plex reference standard were serially diluted and added to the plates. RSV antigen-specific IgG antibodies that bound to their respective RSV antigens were then detected using a goat anti-human IgG antibody containing a labeled reporter (SULFO-TAGTM). Plates were read on the MSD plate reader for electrochemiluminescence. For each antigen, the RSV-specific IgG levels in human sera samples and 4-plex assay control were calculated relative

to the 4-plex reference standard and reported in antibody units per milliliter (Ab units/mL). Results from development experiments showed that RSV antigen-specific antibodies added to the 4-plex plate only bound to their corresponding antigens, demonstrating the specificity of the assay.

[0205] The linear dilutability, relative accuracy, LLOQ, and precision of the MSD 4-plex assay were qualified at Tandem Labs prior to clinical sample testing. Five linearity samples prepared from the reference standard serum, with sample dilution ranges from 1:50 to 1:12800, were used to evaluate assay linear dilutability and relative accuracy. The anti-RSV-F, Ga, Gb and N IgG titer values of reference standards were arbitrarily defined as 100 Ab units/mL. The assay demonstrated linear dilutability and $\pm 25\%$ relative accuracy, with measured values within 25% of the expected values for sample dilutions ranging from 1:100 to 1:10,000 for F, Ga, Gb and N. The evaluated LLOQ values for F, Ga, Gb and N are 0.66 F Ab units/ml, 0.73 Ga Ab units/ml, 0.71 Gb Ab units/ml and 0.80 N Ab units/ml. The assay precision (repeatability, intermediate and total precision) was evaluated using a pooled human serum sample and two donor samples. The total assay variability (CV%) ranged from 7% to 14% for the 3 samples across F, Ga, Gb and N. Given the observed assay variability of the qualified assay, using a 3-fold rise of antibody relative units/mL titer for F, Ga, Gb or N as the seroresponse criteria gives an expected false seroresponse rate due to assay variability of $< 1\%$ through statistical simulation.

[0206] This method allows for the simultaneous detection of recent RSV infection (RSV-N, Ga and Gb readouts) and assessment of immunogenicity elicited by the disclosed compositions (RSV-F readout) in clinical sera samples. The 4-plex assay control sample was included with each assay run of clinical sample testing with expected F, Ga, Gb and N titers of 92.62 F units/mL, 159.17 Ga units/mL, 98.60 Gb units/mL and 145.97 N units/mL respectively. The control sample testing results demonstrated a total assay variability (CV%) of 11.5%, 10.7%, 10.5% and 11.4% for F, Ga, Gb and N, respectively, during testing (data not shown). This is consistent with the total assay variability observed during qualification.

[0207] In the placebo group of the Phase I study, the false seroresponse rate was 0% using a 3-fold rise in anti-F antibody titer of post-dose vs pre-dose samples as the seroresponse criteria. This clinically validates the use of a 3-fold rise in anti-F antibody titers as the seroresponse criteria.

Optimized Immunoassay for the Quantitation of Palivizumab-like Activity in Human Sera using a Palivizumab-competitive Enzyme-Linked Immunosorbent Assay

[0208] Palivizumab is a humanized monoclonal antibody that binds to a highly conserved neutralizing epitope (site A) on the fusion (F) protein of respiratory syncytial virus (RSV). Because palivizumab is an efficacious and proven therapy to prevent severe RSV disease in high-risk infants, the palivizumab-competitive enzyme-linked immunosorbent assay (cELISA) was developed and optimized to evaluate the relative palivizumab-like activity in human sera samples. Briefly, biotin-labeled palivizumab was added to serial dilutions of human sera samples, control (palivizumab in phosphate buffered saline) and reference standard (palivizumab in pooled human sera). The mixture was then added to 96-well plates coated with purified recombinant RSV F antigen and incubated at room temperature for one hour to allow competition for binding to the palivizumab epitope on RSV F. Biotin-labeled palivizumab bound to RSV F was detected by horseradish peroxidase-conjugated streptavidin and the colorimetric substrate 3,3',5,5'-tetramethylbenzidine. The concentration of palivizumab-like activity in each sample was reported in units of microgram per milliliter ($\mu\text{g/mL}$) relative to the reference standard.

[0209] The linearity, accuracy, LLOQ, and precision of the palivizumab cELISA assay were qualified as an exploratory assay prior to clinical sample testing. Six linearity samples with an expected palivizumab-like activity range from 10 to 4000 $\mu\text{g/mL}$ were prepared from a pooled human sera sample spiked with palivizumab. The assay demonstrated linearity and $\pm 20\%$ relative accuracy, with measured values of palivizumab-like activity within 20% of the expected values. The evaluated LLOQ value was 4.55 $\mu\text{g/mL}$ palivizumab-like activity. The assay precision (repeatability, intermediate and total precision) was evaluated using a pooled human sera sample and the 6 linearity samples. The total assay variability (CV%) ranged from 7.4% to 16.3% for the 7 samples. Given the observed assay variability of the qualified assay, using a 3-fold rise of $\mu\text{g/mL}$ palivizumab-like activity titer as the sera-responder criteria gives an expected false seroresponse rate due to assay variability of $< 1\%$ through statistical simulation (data not shown).

[0210] A control sample consisting of 100 $\mu\text{g/mL}$ palivizumab in PBS was included with each assay run of clinical sample testing. The control sample testing results demonstrated a total assay

variability (CV%) of 15.6% during testing. This is consistent with the total assay variability observed during qualification.

[0211] In the placebo group of the Phase I a study, the false seroresponse rate was 0% using a 3-fold rise in palivizumab-like activity titer of post-dose vs pre-dose samples as the seroresponse criteria. This clinically validates the use of a 3-fold rise in palivizumab-like activity titers as the seroresponse criteria.

Statistics

[0212] Data was analyzed using Prism GraphPad software. Data shown is representative of two or more experiments. All data is expressed as arithmetic mean $\pm$ standard error of the mean (SEM). Statistical significance was calculated by One way ANOVA followed by a Tukey post test with a cutoff of $p < 0.05$.

Study Design

[0213] The initial study of the disclosed compositions in humans was a First-Time-In-Human double-blind, randomized, controlled, cohort escalation study evaluating the safety and tolerability of a single ascending IM dose of RSV sF protein alone (20, 50, or 80 μ g) or of the disclosed composition comprising ascending doses of RSV sF (20, 50, or 80 μ g) in combination with 2.5 μ g GLA in 2% (v/v) SE in adults ≥ 60 years of age who were healthy or who had stable, chronic underlying medical conditions other than immunodeficiency or autoimmune disorder (see Table 2).

Table 2: Study Treatment Regimen

Cohort	Number of Subjects	Treatment Regimen
1	24	20 μ g RSV sF (N = 20) or placebo (N = 4) as a single IM dose
1a	24	20 μ g MEDI7510 ^a (N = 20) or placebo (N = 4) as a single IM dose
2	24	50 μ g RSV sF (N = 20) or placebo (N = 4) as a single IM dose
2a	24	50 μ g MEDI7510 ^a (N = 20) or placebo (N = 4) as a single IM dose

Table 2: Study Treatment Regimen

Cohort	Number of Subjects	Treatment Regimen
3	24	80 µg RSV sF (N = 20) or placebo (N = 4) as a single IM dose
3a	24	80 µg MEDI7510 ^a (N = 20) or placebo (N = 4) as a single IM dose

GLA = glucopyranosyl lipid A; IM = intramuscular; RSV = respiratory syncytial virus; SE = stable emulsion; sF = soluble fusion protein; v/v = volume per volume.

^a Dosage given is for the RSV sF component of MEDI7510, which also includes 2.5 µg GLA in 2% (v/v) SE

[0214] The study was unblinded for a planned interim analysis of safety and immunogenicity after all subjects who had completed the Day 91 safety assessment to select doses to be used in the planned Phase 1b study. A total of 146 subjects were enrolled in the study, and 144 were dosed and included in the analysis cohorts. The median age of subjects enrolled was 68.0 years (ranged from 60 to 87 years of age), and 42.4% of subjects were older than 69 years of age. Of the enrolled subjects, 52.8% were male, 34% were Hispanic Latino ethnicity, and 88.9% were White. All subjects were evaluable for safety and immunogenicity.

Example 2: Clinical Trial Phase 1a Study Results

[0215] Samples for humoral immunogenicity assessment were collected from all subjects on Days 1 and 29 as well as on days 61, 91, 181, 271, and 361. Samples for cell-mediated immunity assessment were obtained on Days 1, 8 and 29. Results show that administration of the composition resulted in substantial humoral immune responses as assessed by all assays used: RSV A MN, anti-F IgG, and palivizumab-cELISA for humoral responses. Assay descriptions are provided in Annex 9. Geometric mean titers for RSV microneutralizing antibodies and for RSV F IgG antibodies are presented in Figure 2A, Figure 2B, and Figure 2C, respectively.

[0216] The compositions also resulted in substantial cellular immune response on Day 8 as assessed by IFN γ ELISPOT assay (Figure 1). The response was diminished by Day 29.

[0217] Reversed cumulative distribution of microneutralization titers (log2) at Day 29 post dose as compared to baseline titers for RSV sF (non-adjuvanted), compared to RSV sF (adjuvanted, MEDI7510) administered at 20 µg, 50 µg, or 80 µg, with 2.5 µg GLA in 2% (v/v) SE) is provided in Figure 3.

[0218] Figure 4 provides post baseline titer/count data from all active groups at primary timepoint (Day 8 post dose for ELISPOT assays, and Day 29 post dose for all the other biomarkers) combined. All comparisons between humoral immunogenicity assays have P value <0.001, Pearson Correlation. Comparisons between humoral immunogenicity assays and ELISPOT assay have p value < 0.02, Pearson Correlation, showing that assay results are highly correlated.

[0219] Baseline (BL) titer effects on post-dose fold rise from baseline data are depicted in Figure 5. In this set of data, BL < Median vs $\geq$ Median, by assay. The bar graphs depict the overall fold change in antibody titers at day 29 post dose for microneutralization, RSV sF IgG and competitive ELISA assays, as well as fold change in IFN γ ELISPOT assay at day 8 post dose for RSV sF (non-adjuvanted) compared to RSV sF (adjuvanted, MEDI7510) administered at 20 µg, 50 µg, or 80 µg, with 2.5 µg GLA in 2% (v/v) SE).

[0220] The data provided in Figure 6 show that RSV sF (adjuvanted, MEDI7510) shifts antibody population to high microneutralization titers in an antigen dose-dependent manner. Antibody microneutralization titers are given for RSV sF (non-adjuvanted) compared to RSV sF (adjuvanted, MEDI7510) administered at 20 µg, 50 µg, or 80 µg, with 2.5 µg GLA in 2% (v/v) SE).

[0221] The reversed, cumulative distribution of ELISPOT (SFC/10⁶ PMBC), at Days 8 and 29 post dose, as compared with baseline for RSV sF (non-adjuvanted), and compared to RSV sF (adjuvanted, MEDI7510), administered at 20 µg, 50 µg, or 80 µg, with 2.5 µg GLA in 2% (v/v) SE) is provided in Figure 7.

[0222] Baseline (BL) antibody titer effects on post-dose titer are provided in Figure 8. In this figure, BL < Median vs $\geq$ Median by assay. Data are provided pertaining to the change in response (GMT) at day 29 post dose for microneutralization, RSV sF IgG and competitive ELISA assays, as well as (GMC) change in IFN γ ELISPOT assay at day 8 post dose for RSV sF (non-adjuvanted) compared to RSV sF (adjuvanted, MEDI7510) administered at 20 µg, 50 µg, or 80 µg, with 2.5 µg GLA in 2% (v/v) SE).

[0223] Age effects on post-dose titer for subjects 60–69 years of age as compared with subjects greater than 69 years of age, by assay, is evident from the data provided in Figure 9. Here, the change in response (GMT) at day 29 post dose for microneutralization, RSV sF IgG and competitive ELISA assays, as well as (GMC) change in IFN γ ELISPOT assay at day 8 post dose for RSV sF (non-adjuvanted) compared to RSV sF (adjuvanted, MEDI7510) administered at 20 μ g, 50 μ g, or 80 μ g, with 2.5 μ g GLA in 2% (v/v) SE), indicates that the greatest response in some instances was observed at the highest dose and in the most elderly population of subjects.

[0224] Age effects on post-dose fold rise from baseline is depicted in Figure 10 for subjects of 60–69 years of age as compared with subjects greater than 69 years of age, by Assay. Change in response (GMT) at day 29 post dose for microneutralization, RSV sF IgG and competitive ELISA assays, as well as (GMC) change in IFN γ ELISPOT assay at day 8 post dose for RSV sF (non-adjuvanted), compared to RSV sF (adjuvanted, MEDI7510) administered at 20 μ g, 50 μ g, or 80 μ g, with 2.5 μ g GLA in 2% (v/v) SE), is provided.

[0225] In Figure 11, post-dose (Day 8) RSV F-specific IFN γ ELISPOT data are provided, showing that day 8 post dose counts of RSV sF spot-forming cells per million PBMCs *versus* a baseline count of RSV sF spot-forming cells per million PBMCs there is a general trend of increasing response in a dose-dependent manner.

Example 3: Clinical Trial Phase 1a Study Safety Conclusion

Results show that solicited symptoms, collected Days 1 to 7 after dosing, were increased by inclusion of adjuvant. All solicited symptoms were mild/moderate (Grade 1 or 2) in severity. The most frequent solicited symptoms reported were tenderness and pain at the injection site. Systemic solicited symptoms were uncommon and not clearly increased in subjects who received either RSV sF or the disclosed composition, compared to placebo. In subjects in the 3 cohorts in which escalating doses of RSV sF were administered with adjuvant, the proportion of subjects reporting any injection site tenderness/soreness was 55% to 65%, without RSV sF dose dependence, compared to 4.2% in the placebo group and 5% to 20% in the nonadjuvanted RSV sF alone groups. Similarly, the proportion reporting injection site pain was 40% to 65% in the adjuvanted groups; 4.2% in placebo group, and 0% to 5% in RSV sF alone groups. The majority of these events were mild (Grade 1) in severity, and the longest median (range) duration in adjuvanted groups was 2 (1, 4)

days. RSV sF alone did not contribute to reactogenicity, and there was no dose dependence of site reactions when RSV sF was administered alone or as part of the composition.

[0226] Initial analysis shows no adverse events (AEs) of special interest for an adjuvanted vaccine. The single, potentially autoimmune AE, new onset hypothyroidism, occurred in a subject who received RSV sF without adjuvant. There were no related SAEs: of the 3 SAEs reported, 2 occurred within 28 days of dosing (bladder cancer onset Day 7, staphylococcal abscess onset Day 1) and 1 occurred on Day 86 (cerebrovascular accident). There was no AE pattern of concern.

[0227] Upon initial analysis, subjects reported no withdrawals or deviations with potential effect on outcome and no seroconversion to wild-type antigens were observed. 92% of peripheral blood mononuclear cell (PBMC) specimens were evaluable for F-specific IFN γ ELISPOT. Table 3: Patient Reported Symptoms

	Placebo	RSV sF (unadjuvanted)			Dose of RSV sF in MEDI7510*		
		20 μ g	50 μ g	80 μ g	20 μ g	50 μ g	80 μ g
% subjects	4.2	5	20	10	65	55	65
% Grade 2 (highest reported)	0	0	0	0	10	10	20
Duration, median (Min, Max)	1 (1, 1)	6 (6, 6)	2 (2,2)	1 (1, 1)	2 (1, 3)	2 (1, 4)	2 (1, 3)

[0228] These results indicate an acceptable safety and tolerability profile with a substantial humoral and cellular immune response. At the highest dose of composition tested, 74% of subjects had a significant (≥ 3 -fold) rise in IFN γ ELISPOT counts, 50% of subjects had a significant (≥ 3 -fold) rise in microneutralizing antibodies, and 95% of subjects had post-dose microneutralization antibody titers $\geq 67^{\text{th}}$ percentile of baseline titers. (Figure 1, and Tables 4-7). The data indicate that an adjuvant effect was observed.

Table 4: Dose response in interferon gamma ELISPOT response observed for RSV sF antigen alone or MEDI7510 comprising RSV sF antigen with 2.5 ug of GLA in 2\$ (v/v) SE. Plateau of response to composition is not observed.

Change From Baseline	% Subjects, Day 8					
	20 µg sF	50 µg sF	80 µg sF	20 µg sF +GLA-SE	50 µg sF +GLA-SE	80 µg sF +GLA-SE
	N=16	N=16	N=15	N=16	N=17	N=19
3-Fold Rise	12.5	25	47	19	35	74

Table 5: Significant Shift in Population to High Microneutralization Titers Generated in an Antigen Dose-Dependent Manner. Data are the proportion of subjects whose post-dose response is greater than the 69th percentile of titers at baseline.

Placebo N=24	20 µg sF N=20	50 µg sF N=20	80 µg sF N=19	20 µg sF +GLA-SE N=20	50 µg sF +GLA-SE N=20	80 µg sF +GLA-SE N=20
29.2 (12.6-51.1)	55.0 (31.5-76.9)	70.0 (45.7-88.1)	89.5 (66.9-98.7)	75.0 (50.9-91.3)	84.2 (60.4-96.6)	95.0 (75.1-99.9)

Table 6: Seroresponse: F IgG, cELISA and Microneuts (MN)

Assay	% Subjects With $\geq$ 4-fold Rise From Baseline, Day 29					
	20 µg sF N=20	50 µg sF N=20	80 µg sF N=19	20 µg sF +GLA-SE N=20	50 µg sF +GLA-SE N=20	80 µg sF +GLA-SE N=20
MN	10	5	26	5	11	25
F IgG	65	80	84	90	90	100
cELISA	75	100	79	90	95	100
Assay	% Subjects With $\geq$ 3-fold Rise From Baseline, Day 29					
	20 µg sF N=20	50 µg sF N=20	80 µg sF N=19	20 µg sF +GLA-SE N=20	50 µg sF +GLA-SE N=20	80 µg sF +GLA-SE N=20
MN	15	20	26	25	32	50

F IgG	80	90	90	90	95	100
cELISA	90	100	95	95	95	100

MN = RSV A microneutralization assay; F IgG = anti-F IgG MSD assay; cELISA = palivizumab competitive ELISA assay.

Table 7: Significant Microneutralizing Responses Generated Day 29

Analysis	Placebo N=24	20 µg sF N=20	50 µg sF N=20	80 µg sF N=19	20 µg sF +GLA-SE N=20	50 µg sF +GLA-SE N=20	80 µg sF +GLA-SE N=20
GMTs 95% CI	430 (286- 648)	619 (419- 914)	982 (696- 1385)	1116 (814- 1532)	810 (611-1073)	1183 (813-1721)	1405 (1079- 1829)
Mean	8.75	9.27	9.94	10.12	9.66	10.21	10.46
Log2 95% CI	(8.16- 9.34)	(8.71- 9.84)	(9.44- 10.44)	(9.67- 10.58)	(9.26- 10.07)	(9.67- 10.75)	(10.08- 10.84)

Example 4: Clinical Trial Phase 1b Study

[0229] In the Phase 1b study, a higher fixed dose of RSV sF (120 µg) is administered in combination with one of three different escalating doses of GLA: 1.0, 2.5, or 5.0 µg, all of which are formulated in 2% (v/v) SE. An additional cohort received 80 µg of RSV sF with 2.5 µg of GLA in 2% SE. Subjects are randomized as described in Table 8. Enrollment in the randomized, double-blinded, placebo-controlled Phase 1b study is completed in the target population of adults 60 years of age and older. Three hundred and sixty (360)-day safety, tolerability and immunogenicity data is obtained. A total of 264 subjects are randomized and 261 subjects are dosed in this study. The median age of enrolled subjects is 68 (range 60, 91) years.

[0230] The composition of the administered RSV sF is prepared as indicated above, in Example 1. Safety, tolerability and immunogenicity are assessed according to the methods and assays used in Phase 1a, Example 1, above. Immunoassays for the detection of RSV strain A specific neutralizing antibodies using Vero cells infected with GFP-tagged RSV A2 virus, quantitation of RSV F peptide pool-specific IFN- γ responses by ELISPOT in peripheral blood mononuclear cells, and detection of IgG antibodies against RSV antigens F, N, Ga, Gb using a electrochemiluminescence detection platform, and quantitation of palivizumab-like activity using a palivizumab-competitive ELISA are performed as shown above, in Example 1, Phase 1a. Statistics are also performed as noted above, in Example 1.

Study Design

[0231] The study of the disclosed compositions in humans is a double-blind, randomized, controlled, cohort study evaluating the safety, tolerability and immunogenicity of administering a single IM dose of 120 μ g RSV sF in combination with escalating doses of 1.0, 2.5 or 5.0 μ g GLA in 2% (v/v) SE in adults $\geq$ 60 years of age who are healthy or who have stable, chronic underlying medical conditions other than immunodeficiency or autoimmune disorder (Table 8). An additional cohort receives 80 μ g of RSV sF with 2.5 μ g of GLA in 2% SE.

Table 8: Study Treatment Regimen, Phase 1b

Cohort	Number of Subjects	Treatment Regimen
1	50	120 μ g RSV sF (N = 40) with 1.0 μ g GLA in 2% (v/v) SE, or placebo (N = 10) as a single IM dose
2	95	120 μ g RSV sF (N = 40) with 2.5 μ g GLA in 2% (v/v) + placebo in contralateral arm; 120 μ g RSV sF (N = 40) with 2.5 μ g of GLA in 2% (v/v) SE + inactivated influenza vaccine (IIV) in the contralateral arm, or placebo (N = 15) + IIV in the contralateral arm, all as a single IM dose
3	95	120 μ g RSV sF (N = 40) with 5 μ g GLA in 2% (v/v) + placebo in contralateral arm; 120 μ g RSV sF (N = 40) with 5 μ g of GLA in 2% (v/v) SE + inactivated

Table 8: Study Treatment Regimen, Phase 1b

Cohort	Number of Subjects	Treatment Regimen
		influenza vaccine (IIV) in the contralateral arm, or placebo (N = 15) + IIV in the contralateral arm, all as a single IM dose
4	24	80 ug RSV sF (N = 20) with 2.5 ug GLA in 2% (v/v) SE, or placebo (N = 4)

GLA = glucopyranosyl lipid A; IM = intramuscular; RSV = respiratory syncytial virus; SE = stable emulsion; sF = soluble fusion protein.

[0232] The study provides interim analysis of safety, tolerability and immunogenicity. A total of 264 subjects are randomized and 261 subjects are dosed in this study. The median age of enrolled subjects is 68 (range 60, 91) years.

Example 5: Clinical Trial Phase 2 Study

[0233] In the Phase 2 study, a dose of MEDI7510 is administered simultaneously with a dose of inactivated influenza vaccine (IIV) and is assessed by the experimental design shown in Figure 12. Subjects are randomized 1:1 to receive MEDI7510 + IIV or placebo + IIV in RSV Season 1 of dosing, and subjects who received MEDI7510 + IIV in RSV Season 1 are re-randomized, and blinded in Season 2, as shown in Figure 12, to receive either MEDI7510 + IIV or placebo + IIV. Enrollment in the randomized, double-blind, placebo-controlled Phase 2 study, is completed in the target population of adults 60 years of age and older, as in the Phase 1a study. Safety, tolerability, immunogenicity and efficacy data are obtained. The IIV dose is administered to the contralateral arm of subjects.

[0234] The composition of RSV sF administered is prepared as indicated above, in Example 1. Influenza vaccine dosages (IIV) are obtained from available commercial sources and their manufacture and preparation are as reported elsewhere.

[0235] Efficacy is assessed as the occurrence of acute respiratory syncytial virus-associated respiratory illness (ARA-RI), an endpoint that includes both identification of respiratory illness and

confirmation of temporal association of illness and detection of RSV in respiratory secretions, including nasal swabs or sputum. Laboratory confirmation is performed using an approved, commercial real-time polymerase chain reaction (PCR) assay. Respiratory samples that test positive in PCR assay will be reflexed for RSV A or B subtyping (genotyping) by G gene sequencing. Genotyping of RSV A and RSV B viruses will be based on the sequence variability of the G attachment protein gene. Specifically, the second hypervariable region of the G glycoprotein gene will be compared with those of reference strains representing different RSV A or B genotypes deposited in Genbank for classification of RSV A or B viruses.

[0236]

t of specified style in document. -1 **Definition of Clinical Illness Required for a Diagnosis of ARA-RI**

Upper Respiratory Symptom	Lower Respiratory	Systemic Symptom
Nasal congestion/rhinorrhea (runny or stuffy nose)	Cough	Myalgias or arthralgias (overall body aches or aches in multiple joints)
Sore throat	Dyspnea (shortness of breath)	Fatigue (tiredness)
Earache or ear pain	Sputum (coughing up sputum or phlegm)	Headache
	Wheezing	Decreased appetite

ARA-RI = acute respiratory syncytial virus-associated respiratory illness.

Lay terms in parentheses.

Definition requires a minimum of one symptom from any 2 of the 3 columns (upper respiratory, lower respiratory, and systemic symptom columns) in this table, ie; (a) one symptom from upper respiratory symptom column and one symptom from lower respiratory symptom column, (b) one symptom from upper respiratory symptom column and one symptom from systemic symptom column, or (c) one symptom from lower respiratory column and one from systemic symptom column **AND** laboratory confirmation on at least one sample obtained between Day 1 to Day 8 of illness.

[0237] Safety, tolerability and efficacy are assessed according to the methods and assays used in Phase 1a, Example 1, above. Immunoassays for the detection of RSV strain A specific neutralizing antibodies using Vero cells infected with GFP-tagged RSV A2 virus, quantitation of RSV F peptide pool-specific IFN- γ responses by ELISPOT in peripheral blood mononuclear cells, and detection of IgG antibodies against RSV antigens F, N, Ga, Gb using a electrochemiluminescence detection platform, and quantitation of palivizumab-like activity using a palivizumab-competitive ELISA are performed as shown above, in Example 1, Phase 1a. Statistics are also performed as noted above, in

Example 1. In addition, assays to measure the effect of MEDI7510 on safety and immunogenicity of influenza vaccination are carried out using HAI hemagglutination inhibiting antibody titers.

Study Design

[0238] The study of the disclosed compositions in humans administered concurrently with influenza vaccine is a double-blind, randomized, controlled, cohort study evaluating the efficacy, safety, tolerability and immunogenicity of administering a single IM dose of 120 µg RSV sF in combination with 2.5 µg GLA in 2% (v/v) SE in adults ≥ 60 years of age who are healthy or who have stable, chronic underlying medical conditions other than immunodeficiency or autoimmune disorder (see Table 9).

Table 9: Study Treatment Regimen, Phase 2

Cohort	Number of Subjects	Treatment Regimen
1	950	120 µg IM MEDI7510 ^a dose with contralateral IM IIV dose, Season 1
2	950	placebo IM with contralateral IM IIV dose, Season 1
1a	400	120 µg IM MEDI7510 ^a dose with contralateral IM IIV dose, Season 2
1b	400	placebo IM with contralateral IM IIV dose, Season 2

GLA = glucopyranosyl lipid A; IM = intramuscular; SE = stable emulsion; IIV = inactivated influenza vaccine, standard individual annual dose.

^a Dosage given is for MEDI7510, which includes 2.5 µg GLA in 2% (v/v) SE

In Season 1, subjects are randomized 1:1 (1600 in the Northern Hemisphere and 300 in the Southern Hemisphere) to receive either:

- MEDI7510 (120 µg RSV sF + 2.5 µg GLA in 2% [v/v] SE) as a single IM injection in one arm and IIV as a single IM injection in the contralateral arm
- saline placebo as a single IM injection in one arm and IIV as a single IM injection in the contralateral arm

[0239] A total of 1900 subjects are enrolled in Season 1 of the study, and 800 subjects continue on to Season 2 of the study. The median age of subjects enrolled is about 68.0 years. All subjects are evaluable for safety, tolerability and immunogenicity.

[0240] Season 1 and Season 2 are defined as beginning in the months in which the influenza vaccine is typically administered. Season 1 begins in October for the northern hemisphere, and April for the southern hemisphere. In Season 2, the 800 subjects who received MEDI7510 in the Northern hemisphere are re-randomized to receive either IIV with placebo or IIV with MEDI7510 by the end of October in Season 2, approximately one year after Season 1.

[0241] Efficacy data are collected during the RSV season for the appropriate hemisphere. Safety and immunogenicity follow-up data is obtained for cohorts 1 and 2 for at least 180 days.

[0242] The efficacy of MEDI7510 will be confirmed in a large, controlled, double-blind, randomized Phase 3 study to be conducted in 5,718 subjects with subjects randomized 2:1 to receive MEDI7510 + IIV or placebo + IIV in Season 1 of dosing. (*See Figure 13*) The goal of the Phase 3 study is to provide an adequate assessment of the safety and efficacy of MEDI7510 to permit filing for licensure. The primary objective of Season 2 of the study is to assess the need for annual revaccination to inform recommendations for a booster strategy).

Incorporation by Reference

[0243] All references cited herein, including patents, patent applications, papers, text books and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated herein by reference in their entirety.

Equivalents

[0244] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the disclosed compositions and methods. The foregoing description and Examples detail certain embodiments of the disclosed compositions and methods. It will be appreciated, however, that the disclosed compositions and methods can be practiced in many ways and the

disclosed compositions and methods should be construed in accordance with the appended claims and any equivalents thereof

[0245] The breadth and scope of the disclosure should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

WHAT IS CLAIMED IS:

1. A method of enhancing respiratory syncytial virus (RSV) immunity in a human subject, comprising administering to the human subject a single intramuscular dose of a composition comprising:
about 20 µg, about 50 µg, or about 80 µg RSV soluble F protein, and an adjuvant comprising glucopyranosyl lipid A (GLA) in a squalene-based stable emulsion,
wherein the RSV soluble F protein is amino acids 1-524 of RSV soluble F protein from human strain A2 lacking a transmembrane domain (SEQ ID NO: 1).
2. The method of claim 1, wherein the human subject is at least about 60 years old.
3. The method of claim 1, wherein the enhanced immunity comprises at least a one-fold increase in RSV F-specific T cells over a baseline level of RSV F-specific T cells.
4. The method of claim 1, wherein adjuvant comprises about 2.5 µg glucopyranosyl lipid A (GLA) in a squalene-based stable emulsion of about 2% (v/v).
5. The method of claim 2, wherein the baseline level of RSV F-specific T cells is the level in the subject prior to administration of the composition.
6. The method of claim 2, wherein the baseline level of RSV F-specific T cells is the mean level found in a pool of subjects who have not received the composition.
7. The method of claim 2, wherein the baseline level of RSV F-specific T cells is the mean level found in a pool of subjects administered a non-adjuvanted composition comprising about 20 µg, about 50 µg, or about 80 µg RSV soluble F protein.
8. The method of any one of claims 1 to 7, wherein the subject is between at least about 60 years old and about 87 years old.
9. The method of any one of claims 1 to 8, wherein the enhanced immunity comprises at least a one- to about a ten-fold increase in RSV F-specific T cells over the baseline level of RSV F-specific T cells.
10. The method of claim 9, wherein the enhanced immunity comprises at least a one- to about a seven-fold increase in RSV F-specific T cells over the baseline level of RSV F-specific T cells.

11. The method of claim 10, wherein the enhanced immunity comprises at least a one- to about a three-fold increase in RSV F-specific T cells over the baseline level of RSV F-specific T cells.
12. The method of claim 11, wherein the composition comprises about 20 µg or about 50 µg RSV soluble F protein.
13. The method of claim 9, wherein the enhanced immunity comprises at least a six- to about a ten-fold increase in RSV F-specific T cells over the baseline level of RSV F-specific T cells.
14. The method of claim 13, wherein the composition comprises about 80 µg RSV soluble F protein.
15. The method of claim 14, wherein the subject is about 60 years old to about 69 years old.
16. The method of claim 14, wherein the subject is more than 69 years old, and wherein the enhanced immunity comprises at least an eight- to about a ten-fold increase in RSV F-specific T cells over the baseline level of RSV F-specific T cells.
17. The method of claim 16, wherein the enhanced immunity comprises at least a nine- to about a ten-fold increase in RSV F-specific T cells over the baseline level of RSV F-specific T cells.
18. The method of claim 14, wherein the enhanced immunity comprises at least a four- to about a six-fold increase in RSV F-specific T cells over the baseline level of RSV F-specific T cells.
19. The method of claim 18, wherein the enhanced immunity comprises at least a five- to about a six-fold increase in RSV F-specific T cells over the baseline level of RSV F-specific T cells.
20. The method of any one of claims 1 to 19, wherein the T cell activity is measured at about 8 days after vaccination.
21. The method of any one of claims 1 to 20, wherein the number of T cells is determined by IFN γ ELISPOT assay.
22. The method of claim 1, wherein the enhanced immunity response comprises at least a one-fold increase in the RSV microneutralizing antibody titer of the subject over a baseline RSV microneutralizing antibody titer.
23. The method of claim 22, wherein the baseline RSV microneutralizing antibody titer is the titer in the subject prior to administration of the composition.
24. The method of claim 22, wherein the baseline RSV microneutralizing antibody titer is the geometric mean titer in a pool of subjects who have not received the composition.

25. The method of claim 22, wherein the baseline RSV microneutralizing antibody titer is the geometric mean titer in a pool of subjects administered a non-adjuvanted composition comprising 20 µg, about 50 µg, or about 80 µg RSV soluble F protein.
26. The method of any one of claims 22 to 25, wherein the subject is between at least about 60 years old and about 87 years old.
27. The method of any one of claims 22 to 26, wherein the enhanced immunity comprises at least a one- to about a four-fold increase in the RSV microneutralizing antibody titer of the subject over the baseline RSV microneutralizing antibody titer.
28. The method of claim 27, wherein the enhanced immunity comprises at least a one- to about a three-fold increase in the RSV microneutralizing antibody titer of the subject over the baseline RSV microneutralizing antibody titer.
29. The method of claim 28, wherein the enhanced immunity comprises at least a two- to about a three-fold increase in the RSV microneutralizing antibody titer of the subject over the baseline RSV microneutralizing antibody titer.
30. The method of any one of claims 22 to 26, wherein the composition comprises about 80 µg RSV soluble F protein, and wherein the enhanced immunity comprises at least a 2.5- to about a 4.0-fold increase in the RSV microneutralizing antibody titer of the subject over the baseline RSV microneutralizing antibody titer.
31. The method of claim 30, wherein the subject is more than about 69 years old, and wherein the enhanced immunity comprises at least a 3.0- to about a 4.0-fold increase in the RSV microneutralizing antibody titer of the subject over the baseline RSV microneutralizing antibody titer.
32. The method of claim 31, wherein the enhanced immunity comprises at least a 3.5- to about a 4.0-fold increase in the RSV microneutralizing antibody titer of the subject over the baseline RSV microneutralizing antibody titer.
33. The method of any one of claims 21 to 32, wherein the RSV microneutralizing antibody titer in the subject is determined by microneutralization assay performed about 29 days after vaccination.

34. The method of claim 1, wherein the enhanced immunity comprises at least a 5-fold increase in the anti-RSV F protein-specific antibody titer in the subject over a baseline anti-RSV F protein-specific antibody titer.
35. The method of claim 34, wherein the baseline anti-RSV F protein-specific antibody titer is the titer in the subject prior to administration of the composition.
36. The method of claim 34, wherein the baseline anti-RSV F protein-specific antibody titer is the geometric mean titer in a pool of subjects who have not received the composition.
37. The method of claim 34, wherein the baseline anti-RSV F protein-specific antibody titer is the geometric mean titer in a pool of subjects administered a non-adjuvanted composition comprising 20 µg, about 50 µg, or about 80 µg RSV soluble F protein.
38. The method of any one of claims 34 to 37, wherein the subject is between at least about 60 years old and about 87 years old.
39. The method of any of claims 34 to 38, wherein the enhanced immunity comprises at least a 5- to about a 25-fold increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer level found in a pool of non-immune subjects.
40. The method of claim 39, wherein the enhanced immunity comprises at least a 5- to about a 15-fold increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer.
41. The method of claim 39, wherein the enhanced immunity comprises at least a 10- to about a 15-fold increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer.
42. The method of any one of claims 34 to 38, wherein the composition comprises about 80 µg RSV soluble F protein, and wherein the enhanced immunity comprises at least a 15- to about a 25-fold increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer.
43. The method of claim 42, wherein the enhanced immunity comprises at least a 20- to about a 25-fold increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer.

44. The method of claim 42, wherein the enhanced immunity comprises at least a 20-fold increase in the anti-RSV F protein-specific antibody titer in the subject over a baseline anti-RSV F protein-specific antibody titer.
45. The method of any one of claims 34 to 38, wherein the subject is more than about 69 years old, wherein the composition comprises about 80 µg RSV soluble F protein, and wherein the enhanced immunity comprises at least a 5- to about a 15-fold increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer.
46. The method of claim 45, wherein the enhanced immunity comprises at least a 10- to about a 15-fold increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer.
47. The method of claim 45, wherein the enhanced immunity comprises at least a 10-fold increase in the anti-RSV F protein-specific antibody titer of the subject over a baseline anti-RSV F protein-specific antibody titer.
48. The method of any one of claims 34 to 47, wherein the anti-RSV F protein-specific antibodies are IgG antibodies.
49. The method of any one of claims 34 to 48, wherein the anti-RSV F protein-specific antibody titer in the subject is determined at about 29 days after vaccination.
50. The method of any one of claims 34 to 38, wherein the enhanced immunity comprises at least a 5- to about a 35-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.
51. The method of claim 50, wherein the enhanced immunity comprises at least a 10- to about a 30-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.
52. The method of any one of claims 34 to 38, wherein the composition comprises about 20 µg or about 50 µg RSV soluble F protein, and wherein the enhanced immunity comprises at least a 5- to about a 25-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.

53. The method of claim 52, wherein the enhanced immunity comprises at least a 10- to about a 25-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer level found in a pool of non-immune subjects.

54. The method of any one of claims 34 to 38, wherein the composition comprises about 50 µg RSV soluble F protein, and wherein the enhanced immunity comprises at least a 10- to about a 25-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.

55. The method of claim 54, wherein the enhanced immunity comprises at least a 15- to about a 25-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.

56. The method of claim 54, wherein the enhanced immunity comprises at least a 15- to about a 20-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.

57. The method of any one of claims 34 to 38, wherein the composition comprises about 80 µg RSV soluble F protein, and wherein the enhanced immunity comprises at least a 20- to about a 35-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.

58. The method of claim 57, wherein the enhanced immunity comprises at least a 25- to about a 30-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.

59. The method of any one of claims 34 to 38, wherein the subject is more than about 69 years old, wherein the composition comprises about 80 µg RSV soluble F protein, and wherein the enhanced immunity comprises at least a 25- to about a 35-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.

60. The method of claim 59, wherein the enhanced immunity comprises at least a 30-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.

61. The method of any one of claims 34 to 38, wherein the subject is more than about 69 years old, wherein the composition comprises about 80 µg RSV soluble F protein, and wherein the enhanced immunity comprises at least a 10- to about a 25-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.

62. The method of claim 61, wherein the enhanced immunity comprises at least a 15- to about a 20-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.

63. The method of claim 61, wherein the enhanced immunity comprises at least a 20-fold increase in the RSV-specific antibody titer in the subject over a baseline RSV-specific antibody titer.

64. The method of any of claims 50 to 63, wherein the RSV-specific antibody titer in the subject is determined at about 29 days after vaccination.

65. The method of claim 34, wherein the RSV-specific antibody is an antibody with binding characteristics that are equivalent to palivizumab.

66. A method of inducing an immune response to respiratory syncytial virus (RSV) in a human subject, comprising administering to the human subject a composition comprising: about 120 µg RSV soluble F protein, and an adjuvant comprising about 1.0 µg, about 2.5 µg, or about 5.0 µg glucopyranosyl lipid A (GLA) in a squalene-based stable emulsion, wherein the RSV soluble F protein is amino acids 1-524 of RSV soluble F protein from human strain A2 lacking a transmembrane domain (SEQ ID NO: 1).

67. The method of claim 66, wherein the human subject is at least about 60 years old.

68. The method of claim 66, wherein the adjuvant comprises about 1.0 µg GLA.

69. The method of claim 66, wherein the adjuvant comprises about 2.5 µg GLA.

70. The method of claim 66, wherein the adjuvant comprises about 5.0 µg GLA.

71. The method of claim 66, wherein the GLA is in a squalene-based stable emulsion of about 2% (v/v).

72. The method of any one of claims 66 to 70, wherein the human subject is about 60 years old.

73. The method of any one of claims 66 to 70, wherein the human subject is at least about 65 years old.

74. The method of any one of claims 66 to 70, wherein the human subject is between about 60 and 65 years old.

75. The method of any one of claims 66 to 74, wherein the composition is administered intramuscularly.

76. The method of any one of claims 1 to 75, wherein the RSV soluble F protein is recombinant RSV soluble F protein.
77. The method of claim 76, wherein the RSV soluble F protein is produced *in vitro* by Chinese Hamster Ovary (CHO) cells.
78. The method of any one of claims 1 to 77, wherein the RSV soluble F protein is resuspended from lyophilized form in the adjuvant.
79. The method of any one of claims 1 to 78, wherein the composition is administered in a volume of about 0.5 mL.
80. The method of any one of claims 1 to 79, wherein the composition is administered about annually.
81. The method of any one of claims 1 to 80, wherein the composition is administered concomitantly with a composition intended to generate an immune response against influenza virus.
82. The method of claim 81, wherein the site of administration of the composition for RSV is ipsilateral or contralateral to the site of administration of the composition for influenza.
83. The method of any one of claims 1 to 82, wherein the human subject is RSV seropositive.
84. The method of any one of claims 1 to 83, wherein the human subject has been previously exposed to RSV.
85. The method of any one of claims 1 to 84, wherein the method of enhancing RSV immunity comprises enhancing a Th1 biased cellular immune response in the subject.
86. The method of any one of claims 1 to 85, wherein the method of enhancing RSV immunity comprises inducing neutralizing antibodies against RSV in the subject.
87. The method of any one of claims 1 to 86, wherein the method of enhancing RSV immunity comprises reducing RSV viral titers in the subject.
88. The method of any one of claims 1 to 87, wherein the method of enhancing RSV immunity comprises inducing an immune response to RSV in the subject.
89. The method of any one of claims 1 to 88, wherein the method of enhancing RSV immunity comprises preventing RSV infection in the subject.

1/15

FIG. 1

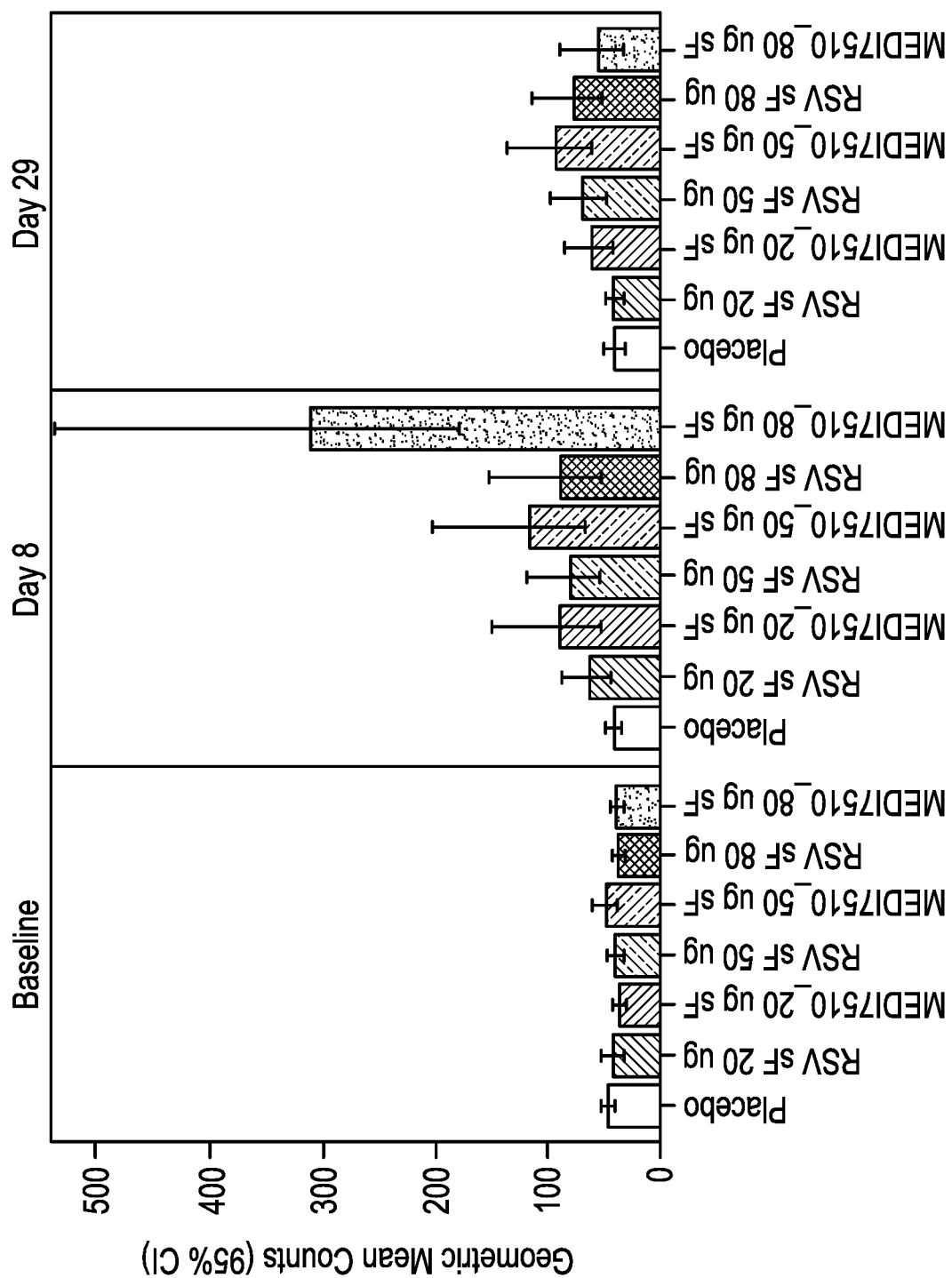


FIG. 2A

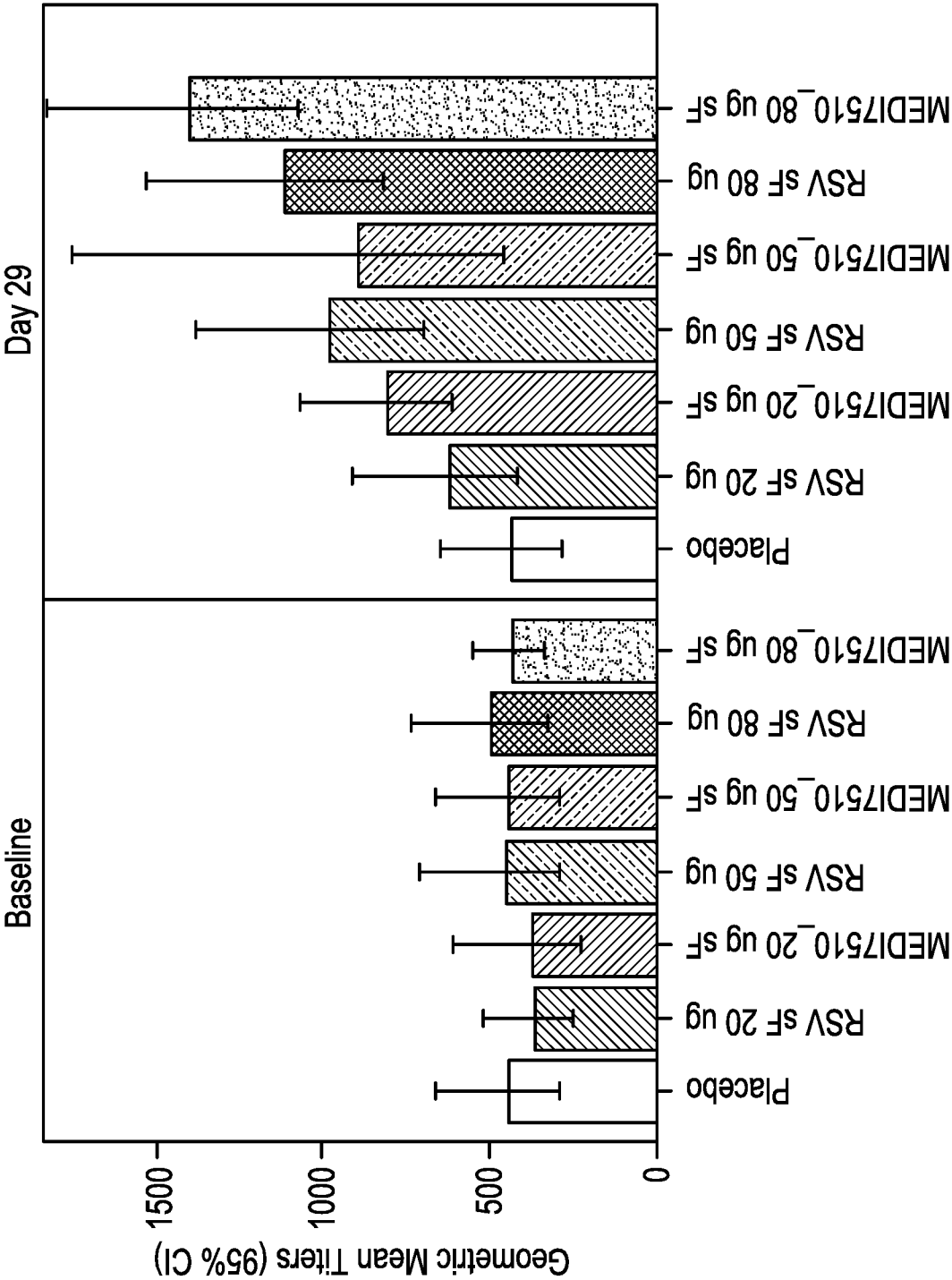
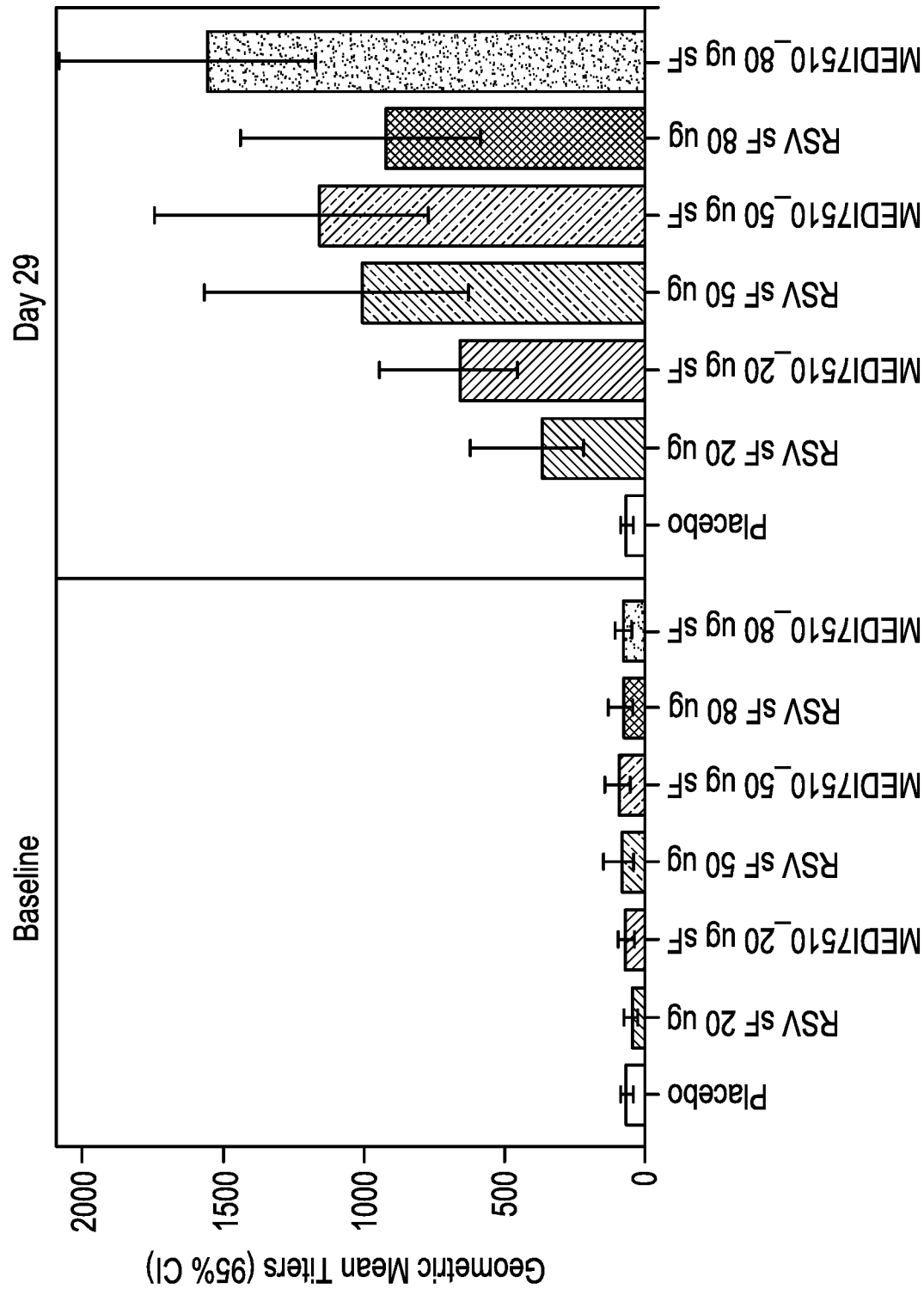
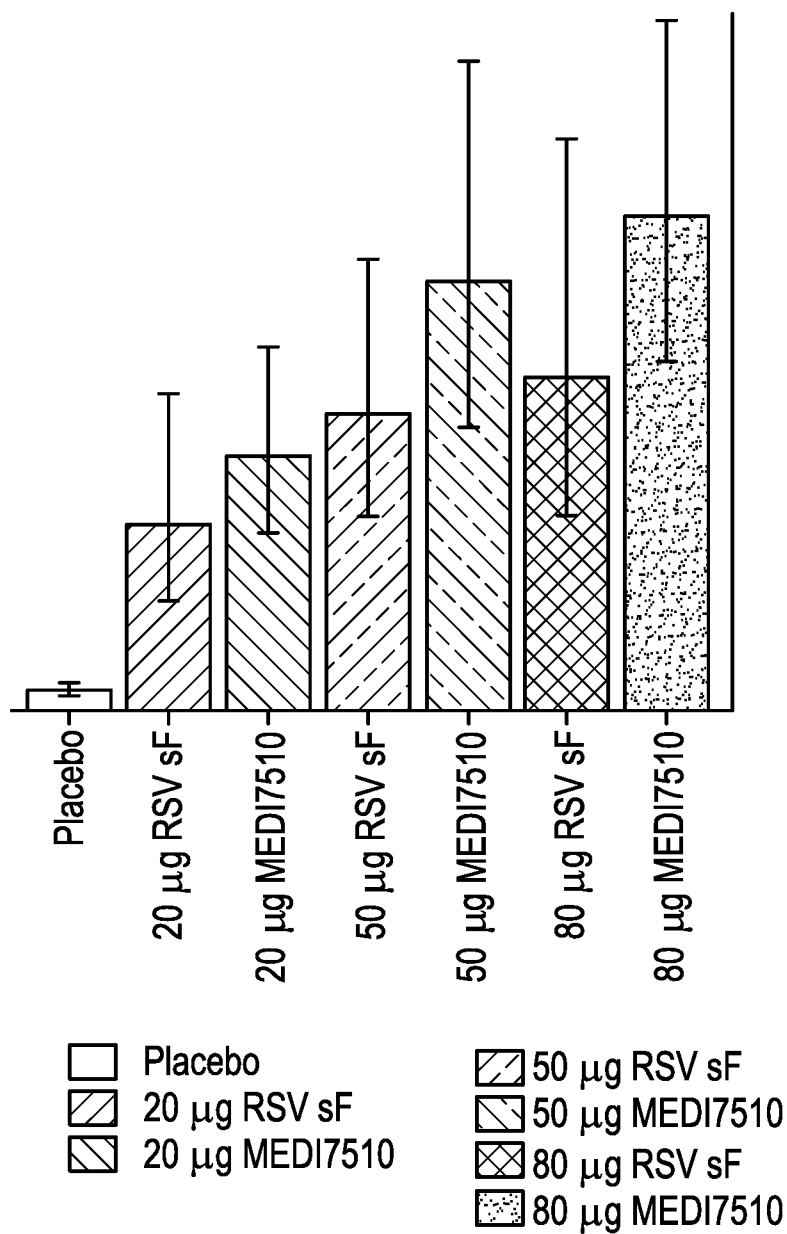


FIG. 2B



4/15

FIG. 2C

Palivizumab competitive
ELISA titers

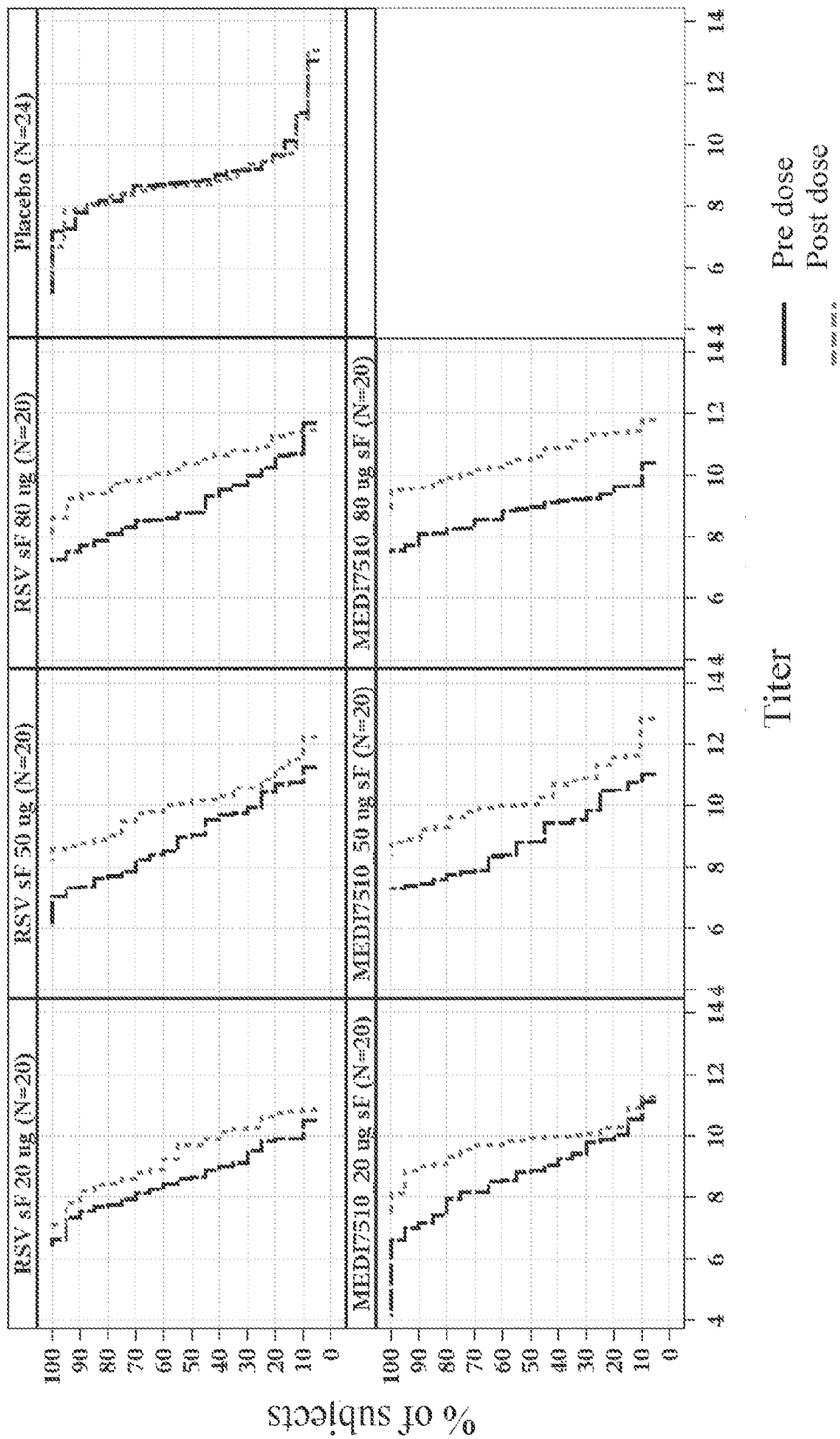


FIGURE 3

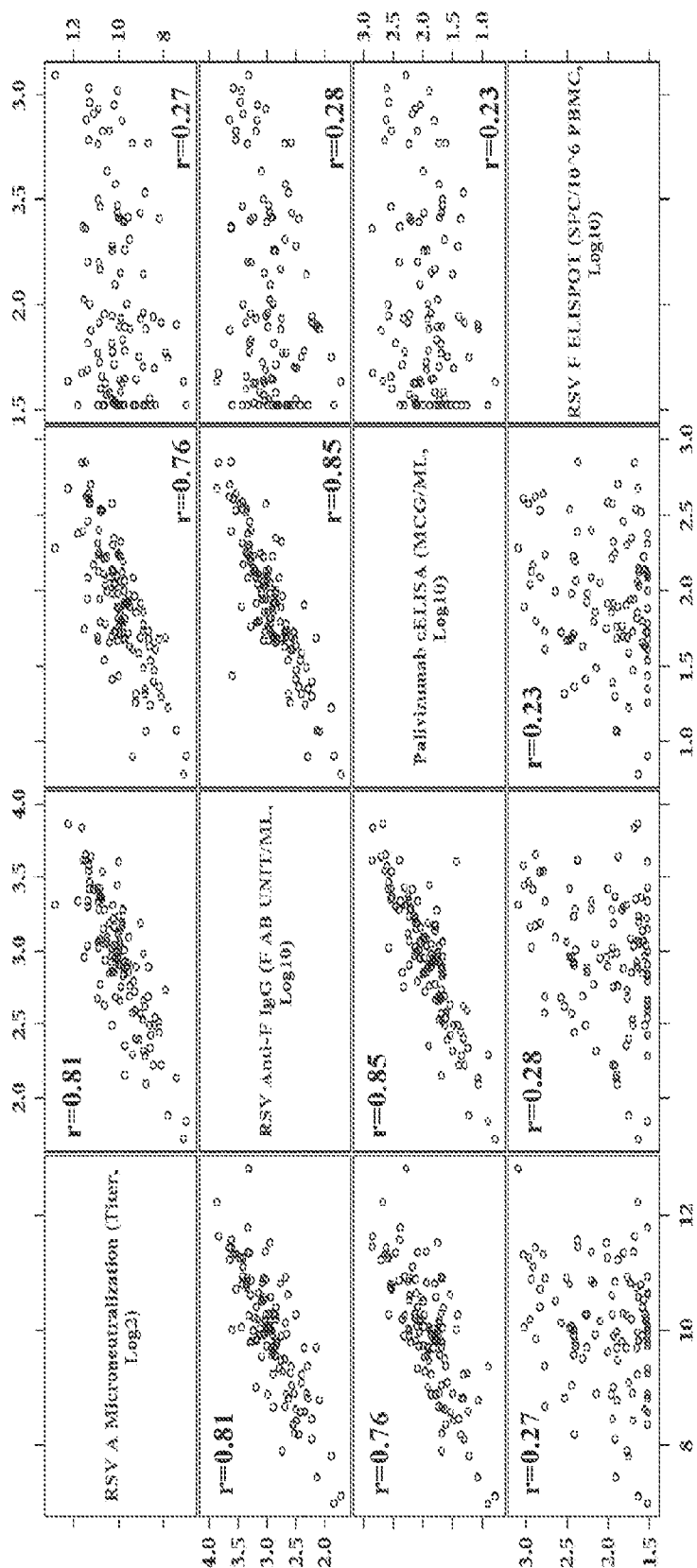


FIGURE 4

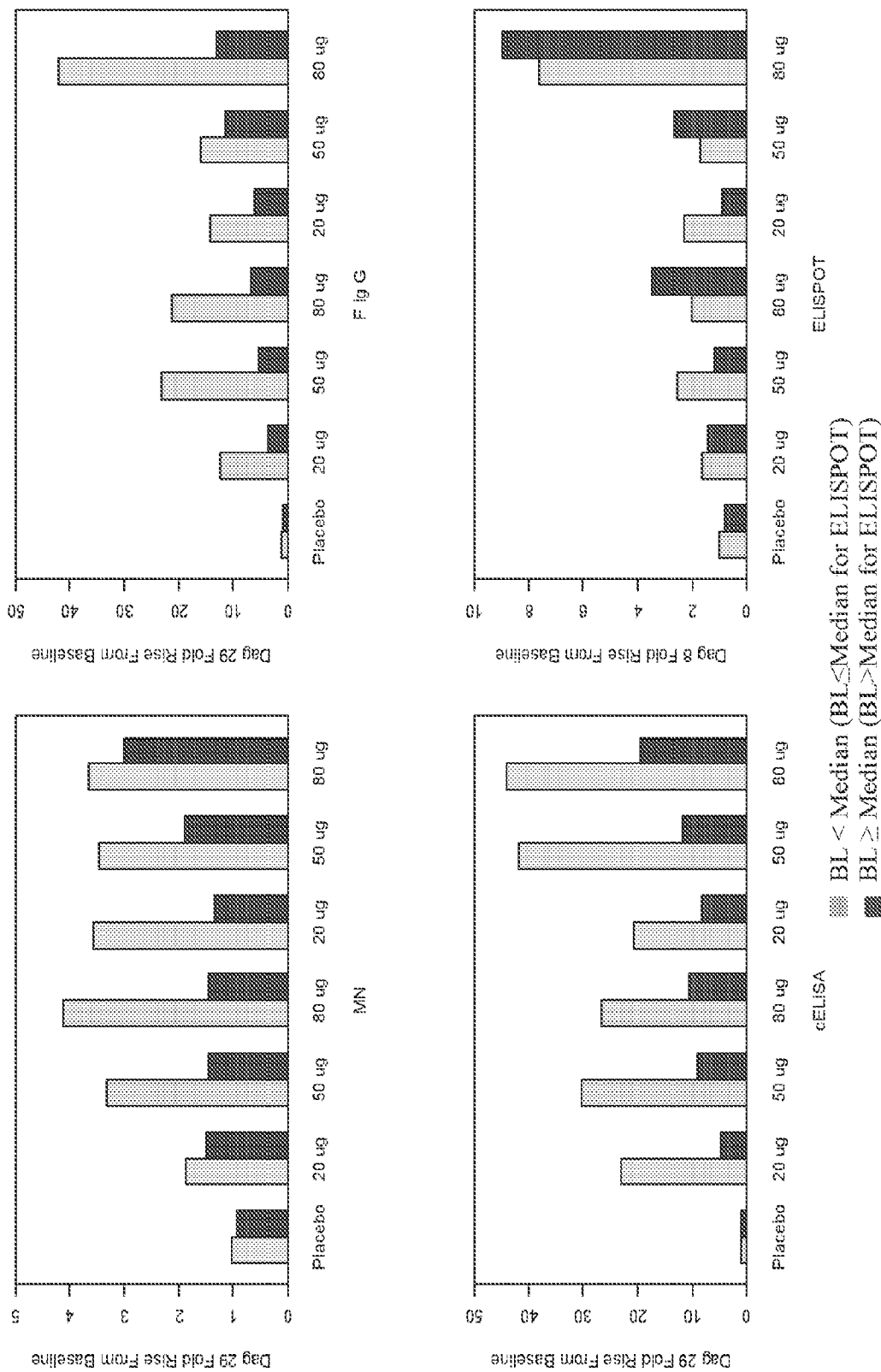


FIGURE 5

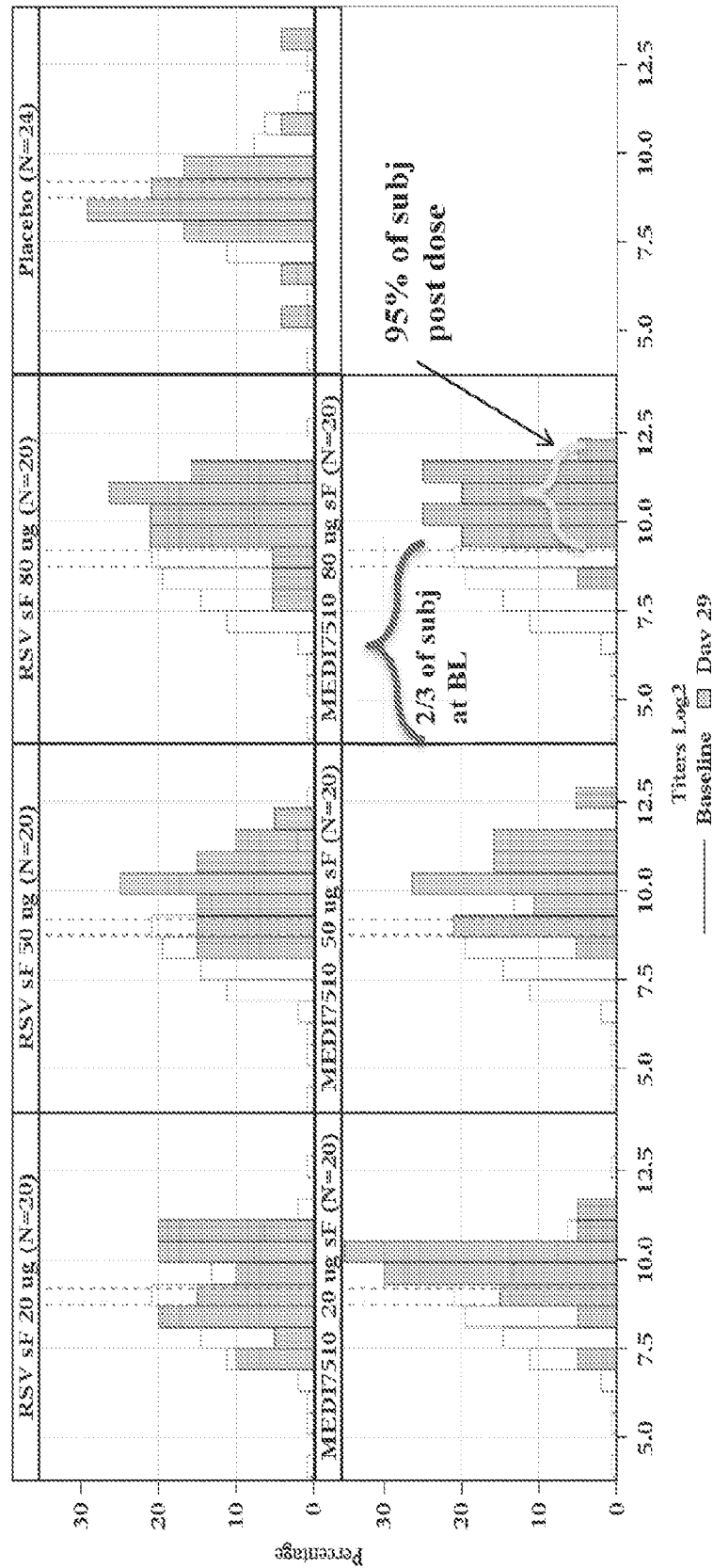


FIGURE 6

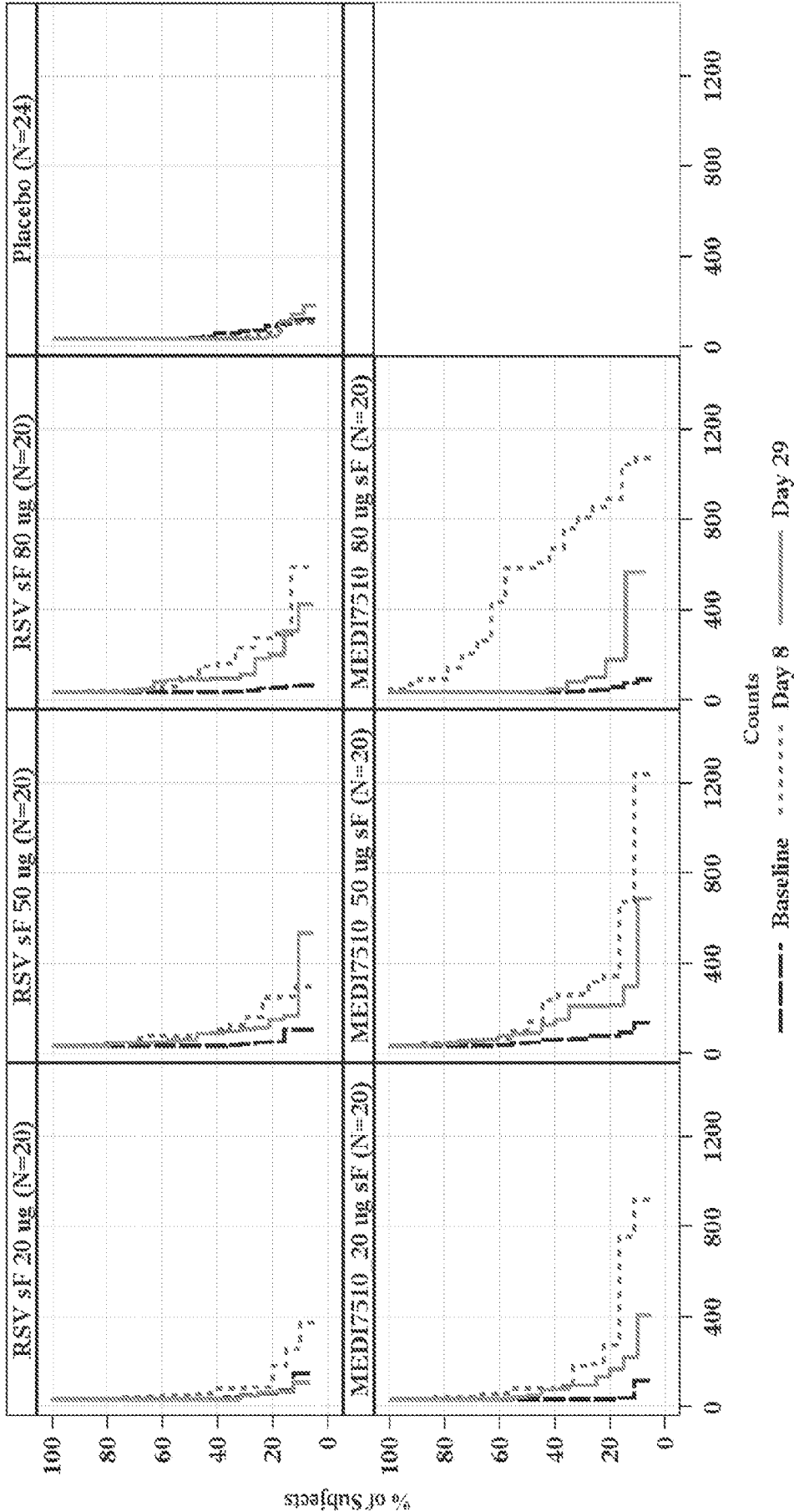


FIGURE 7

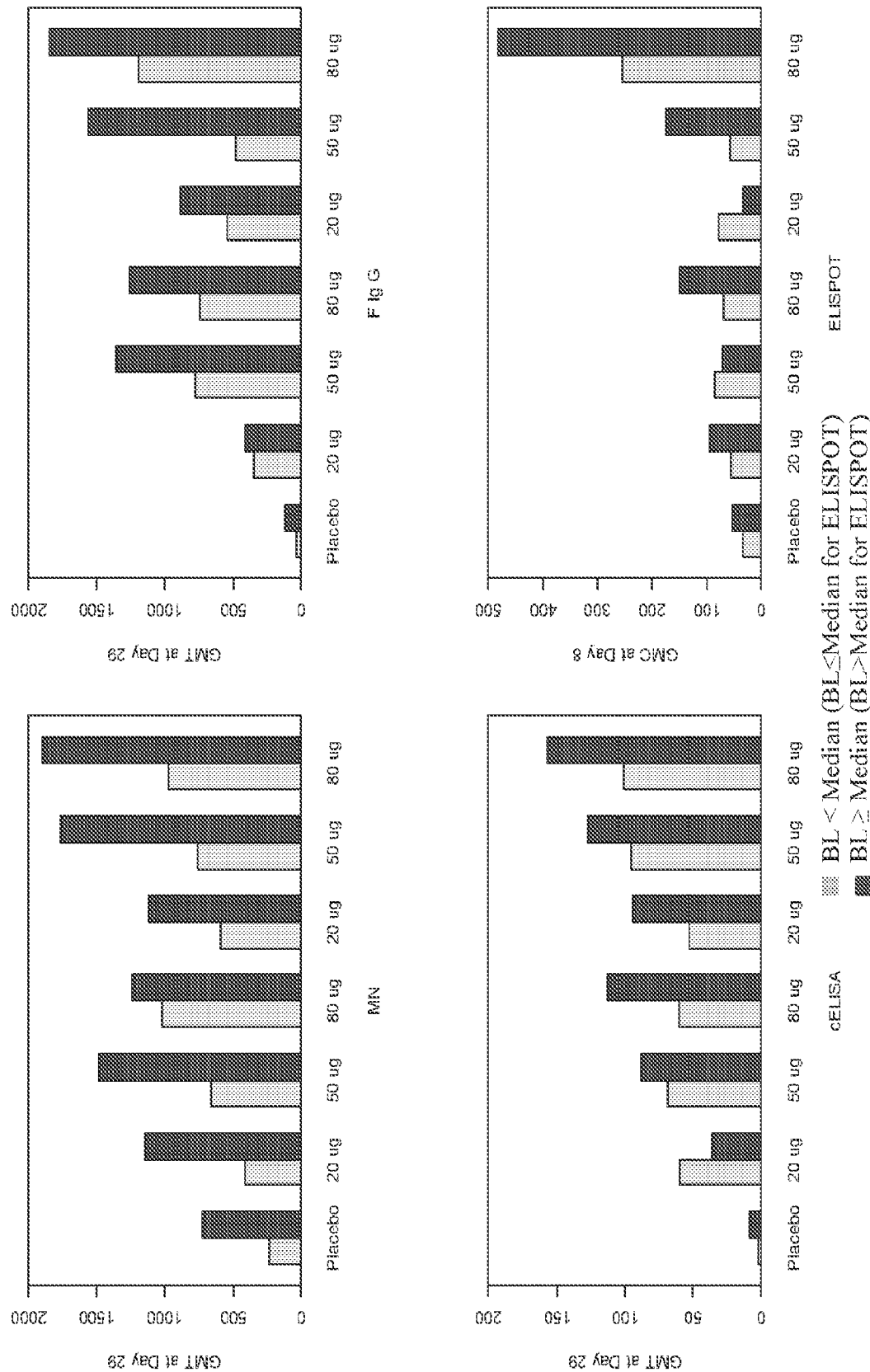


FIGURE 8

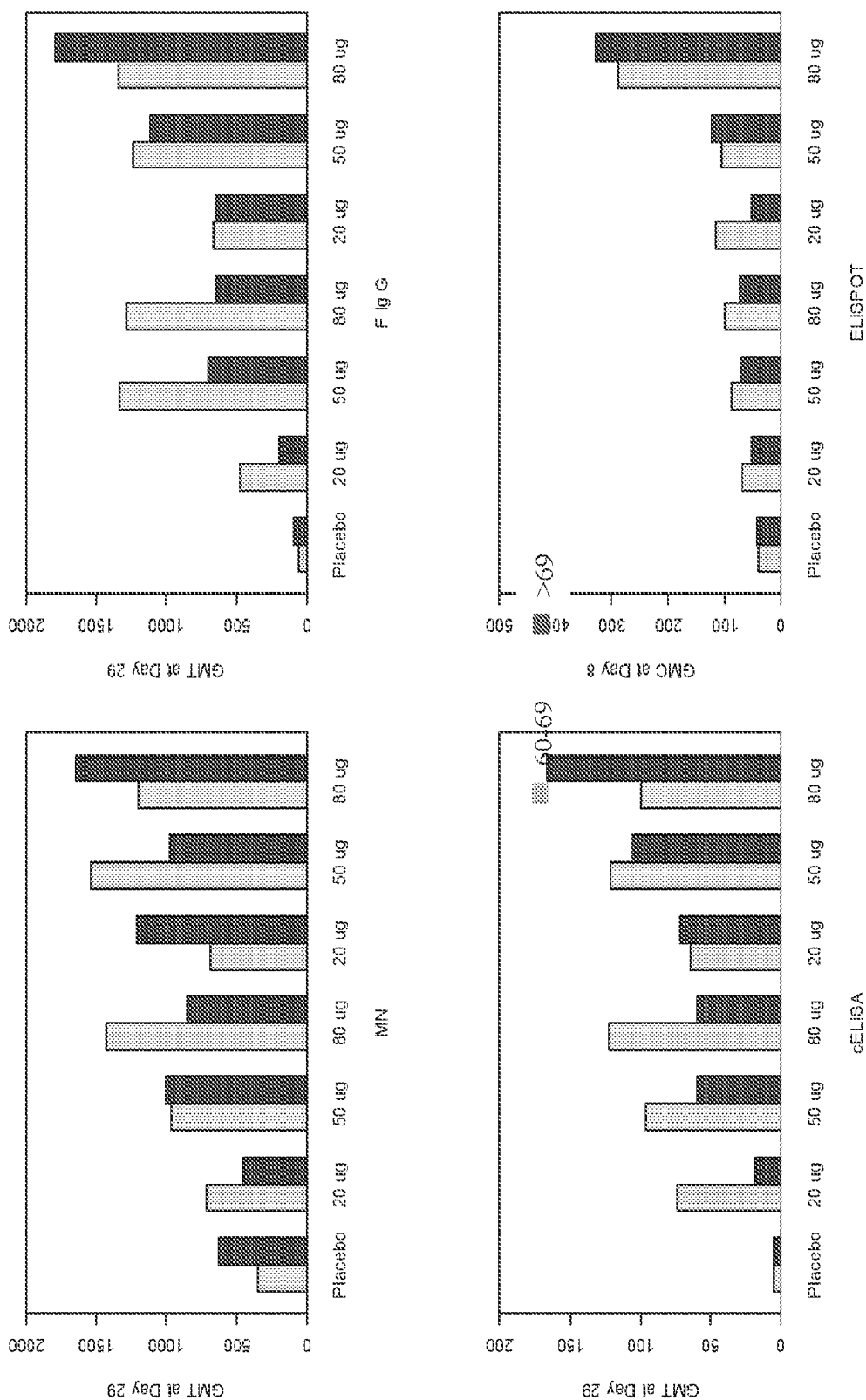


FIGURE 9

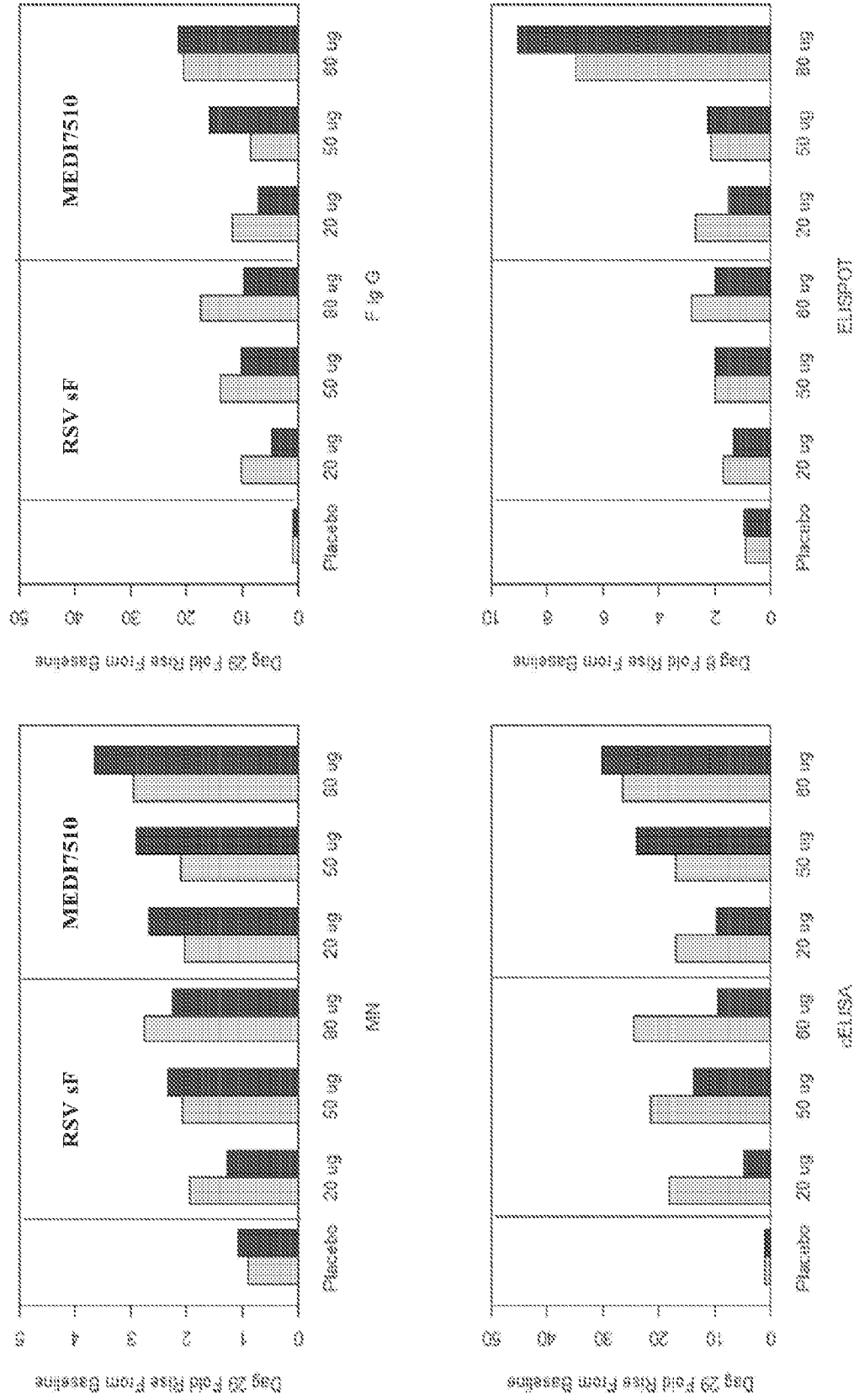


FIGURE 10

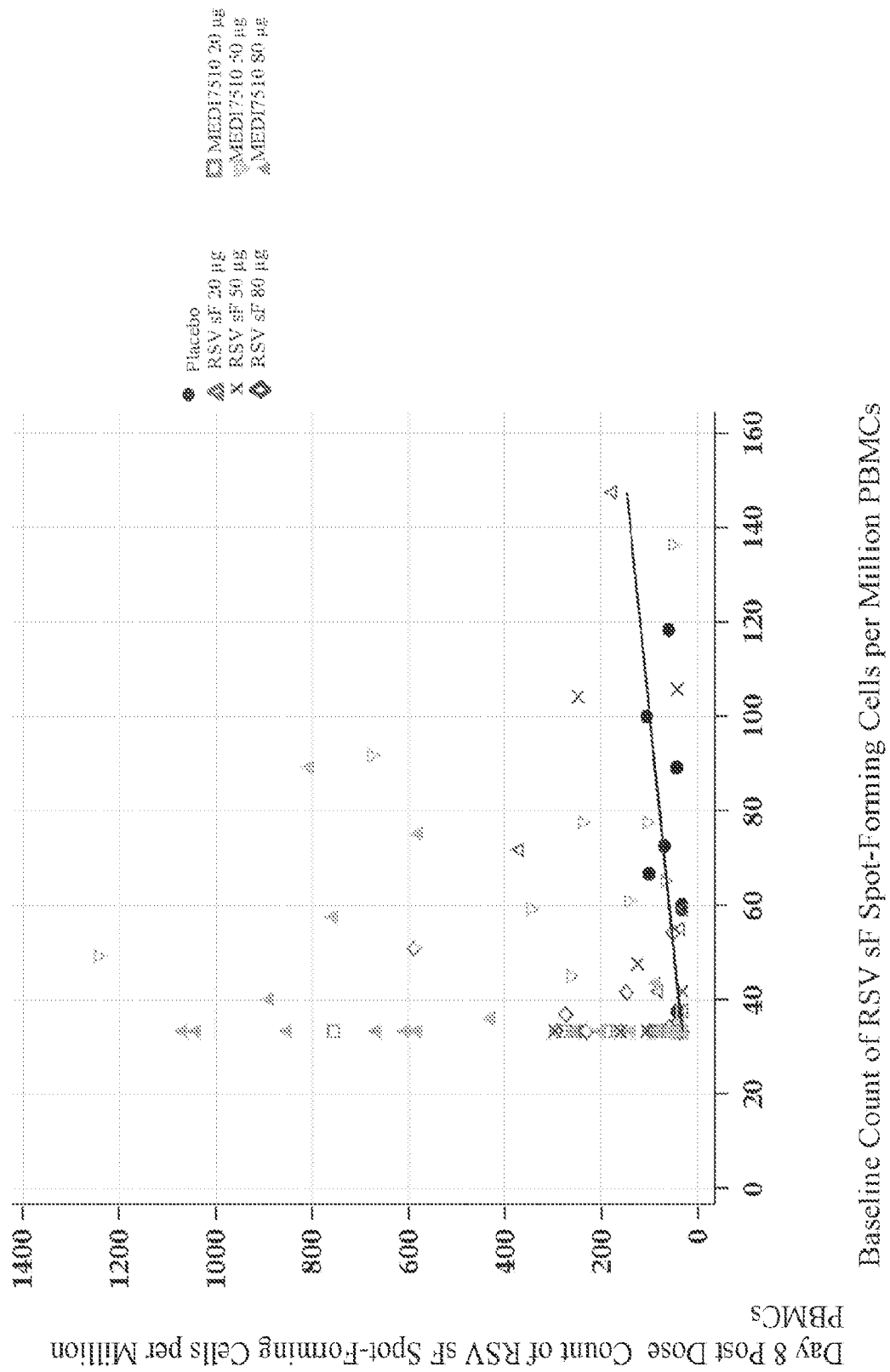


FIGURE 11

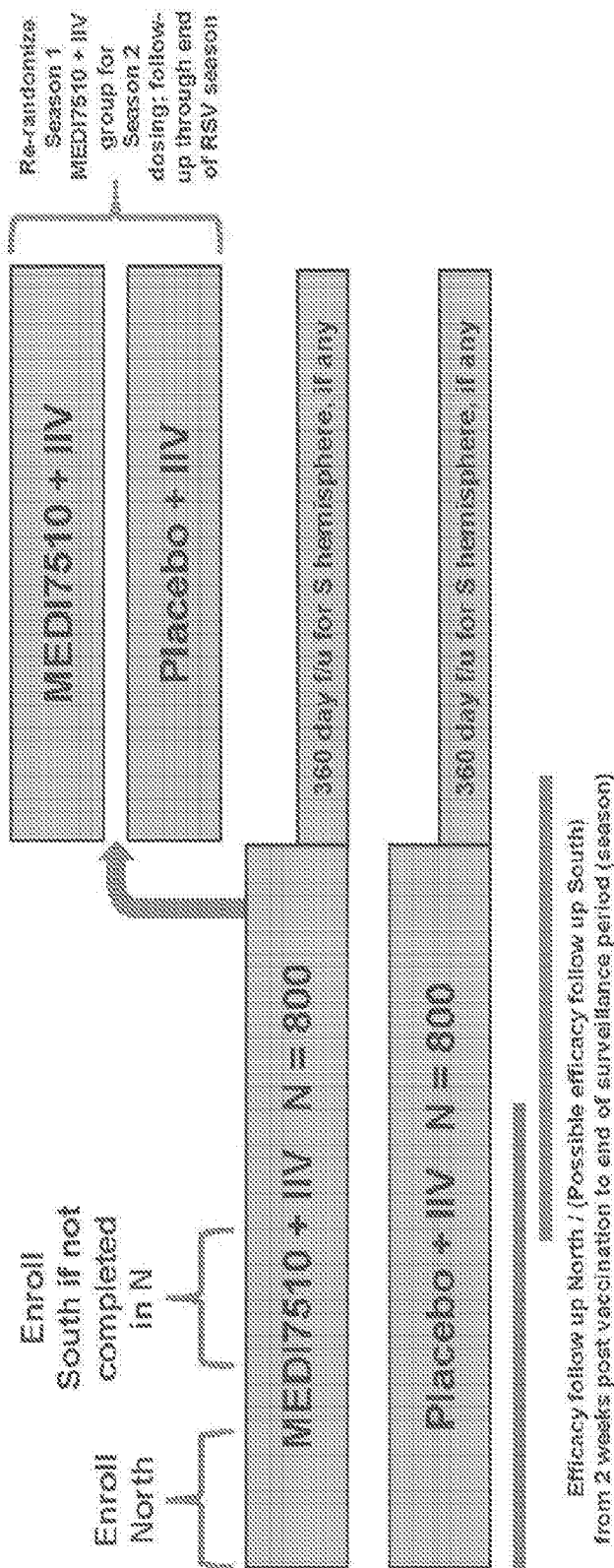
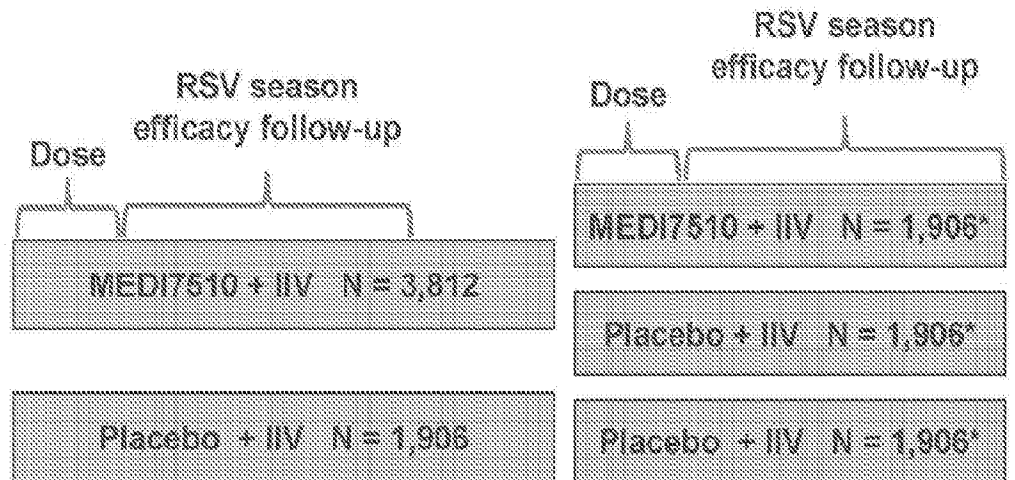


FIGURE 12

FIGURE 13

*actual projected N=1,620 due to 15% attrition

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/020597

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/00, A61K 39/12, A61K 39/155, A61K 39/39; A61P 31/14, A61P 37/04 (2016.01)

CPC - A61K 2039/545, A61K 2039/55505, A61K 2039/55566, A61K 2039/55572; C12N 2760/18534 (2016.05)

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 39/00, A61K 39/12, A61K 39/155, A61K 39/39; A61P 31/14, A61P 37/04 (2016.01)

CPC - A61K 39/12, A61K 39/39, A61K 2039/545, A61K 2039/55505, A61K 2039/55566, A61K 2039/55572; C12N 2760/18534 (2016.05)

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

USPC - 424/211.1; 530/350; 536/23.72 (keyword delimited)

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

PatBase, Google Patents, PubMed.

Search terms used: respiratory syncytial virus "F protein" RSV-F glucopyranosyl lipid A GLA squalene

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/168821 A1 (MEDIMMUNE, LLC) 16 October 2014 (16.10.2014) entire document	1-8, 22-26, 34-38, 65-74
A	WO 2014/198407 A1 (AMVAC AG) 18 December 2014 (18.12.2014) entire document	1-8, 22-26, 34-38, 65-74
A	WO 2013/139911 A1 (CRUCCELL HOLLAND B.V.) 26 September 2013 (26.09.2013) entire document	1-8, 22-26, 34-38, 65-74
A	US 2012/0135028 A1 (BLAIS et al) 31 May 2012 (31.05.2012) entire document	1-8, 22-26, 34-38, 65-74
P, X	LAMBERT et al. "A Novel Respiratory Syncytial Virus (RSV) F Subunit Vaccine Adjuvanted with GLA-SE Elicits Robust Protective TH1-Type Humoral and Cellular Immunity In Rodent Models," PLoS One, 20 March 2015 (20.03.2015), Vol. 10, No. 3, e0119509, Pgs. 1-24. entire document	1-8, 22-26, 34-38, 65-74
P, X	PATTON et al. "Enhanced immunogenicity of a respiratory syncytial virus (RSV) F subunit vaccine formulated with the adjuvant GLA-SE in cynomolgus macaques," Vaccine, 20 July 2015 (20.07.2015), Vol. 33, Iss. 36, Pgs. 4472-4478. entire document	1-8, 22-26, 34-38, 65-74

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

"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

16 May 2016

Date of mailing of the international search report

03 JUN 2016

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
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Authorized officer

Blaine R. Copenheaver

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/020597

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:

- a. ☒ forming part of the international application as filed:
☒ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13*ter*. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*. 1(a)).
☐ on paper or in the form of an image file (Rule 13*ter*. 1(b) and Administrative Instructions, Section 713).
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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs:1-13 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/020597

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.: 9-21, 27-33, 39-64, 75-89
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

Remark on Protest

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