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A Phase 2 randomized, observer-blind, placebo-controlled, dose-ranging trial of aluminum-adjuvanted respiratory syncytial virus F particle vaccine formulations in healthy women of childbearing age

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ABSTRACT

Objective: Respiratory syncytial virus (RSV) causes significant morbidity and mortality in infants. We are developing an RSV fusion (F) protein nanoparticle vaccine for immunization of third trimester pregnant women to passively protect infants through transfer of RSV-specific maternal antibodies. The present trial was performed to assess the immunogenicity and safety of several formulations of RSV F vaccine in 1-dose or 2-dose schedules.

Methods: Placebo, or vaccine with 60 μg or 120 μg RSV F protein and 0.2, 0.4, or 0.8 mg aluminum, were administered intramuscularly on Days 0 and 28 to healthy women 18–35 years old. Immunogenicity was assessed from Days 0 through 91 based on anti-F IgG and palivizumab-competitive antibody (PCA) by ELISA, and RSV A and B neutralizing antibodies by microneutralization (MN) assay. Solicited adverse events were collected through Day 7 and unsolicited adverse events through Day 91.

Results: All formulations were well-tolerated, with no treatment-related serious adverse events. Anti-F IgG and PCA responses were correlated and increased after both doses, while MN increased significantly only after the first dose, then plateaued. The timeliest and most robust antibody responses followed one dose of 120 μg RSV F protein and 0.4 mg aluminum, but persistence through 91 days was modestly (~25%) superior following two doses of 60 μg RSV F protein and 0.8 mg aluminum. Western blot analysis showed RSV infections in active vaccinees were reduced by 52% overall (p = 0.009 overall) over the Day 0 through 90 period.

Conclusions: RSV F nanoparticle vaccine formulations were well tolerated and immunogenic. The optimal combination of convenience and rapid response for immunization in the third trimester occurred with 120 μg RSV F and 0.4 mg aluminum, which achieved peak immune responses in 14 days and sufficient persistence through 91 days to allow for passive transfer of IgG antibodies to the fetus. NCT01960686.

1. Introduction

Globally, respiratory syncytial virus (RSV) is estimated to cause 64 million infections per annum resulting in 160,000 deaths [1], the latter concentrated primarily in infants. In the United States (US), it is estimated that RSV causes approximately 57,000 hospitalizations of children <5 years old annually, the majority in infants <1 year of age who were previously healthy and predominantly affecting those <6 months of age [2–5]. There is also growing interest in prevention of respiratory infections in pregnancy, including those due to RSV [6]. As one example, administration of seasonal influenza vaccines in pregnancy is now recognized to confer benefits on the pregnant mother, to improve birth outcomes, and to protect the infant [7–9]. Because of the early age at which protection against RSV must be made available to infants, and potential maternal benefits, an RSV vaccine administered during pregnancy is desirable.

The RSV F protein has been shown to be immunogenic, and contains highly conserved virus-neutralizing sites, including antigenic site II [10–12]. Multiple clinical trials have shown that the

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prophylactic use of monoclonal antibodies such as palivizumab and motavizumab, which bind to antigenic site II, reduce RSV hospitalizations in high-risk and healthy term infants [13–15]. RSV F vaccine induces neutralizing antibodies, including antibodies of palivizumab-like specificity, in the cotton rat model and other test species [10–12,16], and confers protection against subsequent RSV challenge in actively or passively immunized animals [17], in a manner similar to palivizumab. RSV F vaccine was well-tolerated and immunogenic in Phase 1 and Phase 2 studies in human adults [18,19] and demonstrated evidence of protection against serologically-detected RSV infection in women of childbearing age [19].

The RSV F nanoparticle vaccine is being developed for use in maternal immunization to provide passive immunity to the infant via transplacental transfer of protective antibodies produced by the mother. This strategy would provide protection to the infant during the most vulnerable period from birth to 4–6 months of age, and especially the first 3 months, when the majority of severe RSV disease occurs and direct, active, infant vaccination would be unlikely to elicit a timely response. The present trial was undertaken in women of childbearing age to evaluate the possibility that providing the total available dose of RSV F protein (i.e., 120 μg) in a 1-dose regimen with varying amounts of an aluminum phosphate adjuvant might provide comparable or better immune responses than those obtained with a 2-dose regimen using 60 μg of antigen per dose and the same aluminum phosphate content in order to select the candidate vaccine to advance within the maternal immunization program.

2. Methods

This was a Phase 2 multi-center, randomized, observer-blind, placebo-controlled trial performed in 10 centers in the US from October 2013 to April 2014. After approval of the protocol by a Central Institutional Review Board, the trial was conducted according to International Conference on Harmonisation guidelines for Good Clinical Practice, and the Declaration of Helsinki. All participants provided written informed consent prior to any trial procedures.

2.1. Trial design

This trial assessed the safety and immunogenicity of six RSV F protein vaccine formulations compared with a reference formulation (2-dose regimen of 60 μg RSV F vaccine with 0.8 mg aluminum as a phosphate salt [$AlPO_4$]) and placebo in healthy, 18–35 year-old non-pregnant and non-lactating women. Three of the four co-primary objectives contrasted Day 56 anti-F IgG levels elicited with the reference formulation against levels elicited by the various test formulations to determine: (i) the lowest aluminum dose which, when mixed with 60 μg RSV F vaccine and administered as a 2-dose regimen, was capable of supporting a response at least comparable to the reference; (ii) whether a 1-dose regimen of 60 μg RSV F vaccine with 1.2 mg of aluminum could perform as well or better than the reference, and; (iii) whether 1-dose regimens of 120 μg RSV F with varying aluminum doses could perform as well or better than the reference, and the lowest aluminum dose which yielded a satisfactory response. The fourth co-primary objective evaluated the safety profiles of each formulation based on solicited and unsolicited adverse event (AE) reports.

Secondary objectives included a description of the magnitude and kinetics of the anti-F IgG, RSV/A and RSV/B neutralizing antibody titers, and palivizumab-competitive antibodies (PCA) responses to the various vaccine formulations and dose regimens. A post-vaccinal area-under-the-curve (AUC) analysis based on the anti-F IgG antibody responses for the period likely to include the majority of deliveries in a third trimester immunization scheme (Days 14–91) was also performed.

Women were randomized into eight treatment groups (A-H) and into two cohorts (I and II) within each group, with stratification by age (18–25 and 26–35 years) and by the presence of a child ≤5 years of age in the subject's household. Cohorts I and II differed only in the timing of blood draws and the days on which in-clinic follow-up safety visits were conducted.

2.2. Test articles

All 120 μg RSV F vaccine formulations contained 240 μg/mL antigen concentrations pre-formulated in 12.5 mM phosphate buffer, pH 6.2, with 0.15 M NaCl, 0.5% histidine, 0.005% polysorbate (PS)80, and 0.4–2.4 mg/mL of aluminum as $AlPO_4$ (AdjuPhos, Brenntag Biosector, Frederikssund, Denmark) [16]. All 60 μg RSV F vaccine formulations contained 120 μg/mL antigen concentrations in the same buffer and 1.6–2.4 mg/mL aluminum as $AlPO_4$. Placebo consisted of 0.9% sodium chloride (APP Pharmaceuticals, LLC, Schaumburg, Illinois, USA). Vaccinations were administered as 0.5 mL intramuscular (IM) injections into the deltoid, using opposite arms for subsequent injections.

2.3. Immunogenicity

Blood samples were collected at Days 0, 14, 28, 56, and 91 for both cohorts; at Days 21, 42, and 63 for Cohort I, and at Days 35, 49, and 77 for Cohort II. Sera were tested in anti-F IgG and PCA ELISAs [16,18,19]; and in microneutralization (MN) assays against the RSV/A Tracy (A2-like virus) and RSV/B (18537) strains [20]. Serologic methods are summarized in the supplementary materials (Supplement S1).

2.4. Safety

All subjects were monitored for 30 min post-dosing for immediate reactions. Subjects recorded the occurrence and severity of solicited local injection site (pain, bruising, redness, and swelling) and systemic (fever, headache, myalgia, arthralgia, fatigue, chills, vomiting, nausea, and diarrhea) reactions for 7 days after each dose. Subjects were queried for the occurrence of all adverse events (AEs) through Day 91, and medically-attended AEs (MAEs), and serious AEs (SAEs) through Day 182. Serum chemistry and hematology parameters were assessed on Day 0 (pre-dosing) and post-dosing on Days 14, 28, and 56.

2.5. Serologic evidence of RSV infection

A *post hoc* blinded evaluation to seek serologic evidence of recent RSV infection was performed using Western blot analyses of sequential sera evaluating antibody responses to non-F RSV proteins, as previously described [19,21].

2.6. Statistical analysis

The Safety population was all subjects who received any test article dose. Immunologic analyses were conducted using the Per Protocol (PP) population, defined as all subjects who received their assigned vaccine or placebo according to protocol, had RSV serology results at Days 0, 28, and 56, and had no major protocol deviations affecting the primary immunogenicity outcomes.

Demographic parameters and baseline characteristics were summarized by treatment group. Continuous variables were presented as mean and standard deviation (SD) for the non-immunogenicity endpoints, and geometric means and their 95%

confidence interval (CI) for the immunogenicity endpoints. Data from Cohort I and II subjects were pooled by treatment groups whenever possible.

The sample size calculation was based on prior experience with the anti-F IgG EU parameter in young women, for which geometric means had shown a $\log_{10}$ standard deviation of 0.2. A sample size of 90 per treatment provided 90% power to detect non-inferiority to the "standard" treatment (Group B) with a $\log_{10}$ margin of −0.097, or −20%., in a one-sided test with a significance level of 0.025. Given that 360 subjects received any 2-dose/60 μg RSV F vaccine formulation and 270 subjects received any 1-dose/120 μg RSV F vaccine formulation, the trial had ∼95% probability of detecting at least one adverse event that occurred with a rate of 0.9%, 1.1%, or 0.5% in recipients of 1-dose/120 μg RSV F, 2-dose/60 μg RSV F, or any RSV F regimen, respectively.

3. Results

3.1. Disposition

A total of 761 women were screened; 720 were enrolled and randomized to 1 of 8 treatment groups (Fig. 1). All 720 subjects received the first assigned treatment on Day 0 and >90% of subjects receive the second assigned treatment on Day 28. Six hundred and thirty-five subjects (88.2%) completed the trial, including 80 to 93% in the various treatment groups (Fig. 1). Discontinuations were mostly due to voluntary withdrawal unrelated to an AE (49% of discontinuations) or lost to follow-up (46% of discontinuations). All subjects were included in the safety population, and 82–94% were included in the PP Population. Reasons for PP exclusion and discontinuations are summarized in Fig. 1.

Demographic characteristics were generally comparable across treatment groups, with the exception that groups B, D, and H contained 5–10% fewer African Americans (Table 1). Mean ages ranged from 26.8 to 27.7, with a minimum–maximum of 18–35 years as per protocol. About 60% of subjects in each group were in the 26–35 age stratum and did not have children ≤5 years of age in the household.

3.2. Immunogenicity

3.2.1. Anti-F IgG responses

Anti-F IgG levels rose rapidly following the single-dose 120 μg RSV F vaccine with 0.2 or 0.4 mg aluminum and peaked 14 days post-vaccination with 11.6- to 12.7-fold increases (respectively) from baseline (Fig. 2A) and approximately 90% of vaccinees attaining seroconversion (i.e., ≥4-fold increase in post-vaccinal titer). A 9.7-fold increase was achieved with the 120 μg RSV F with 0.8 mg aluminum vaccine formulation at Day 14, which was 20% lower. Responses elicited with all 1-dose/120 μg RSV F vaccines persisted for the entire 3-month immunogenicity evaluable period. The 1-dose/60 μg RSV F vaccine with 1.2 mg aluminum demonstrated similar response kinetics, but with a peak increase that was markedly lower in magnitude.

Two-dose regimens with 60 μg RSV F vaccines (Fig. 2B) elicited 7.4- to 8.8-fold increases in anti-F IgG levels 2 to 3 weeks after the first dose, with no significant differences noted among vaccine formulations of differing aluminum content. Anti-F IgG levels increased 8.6- to 12.1-fold resulting in seroconversion of >90% of subjects one month after the second vaccine dose, favoring vaccines with higher aluminum content. Responses elicited with all 2-dose/60 μg RSV F vaccines remained elevated and significantly higher than placebo for the 3-month immunogenicity evaluable period.

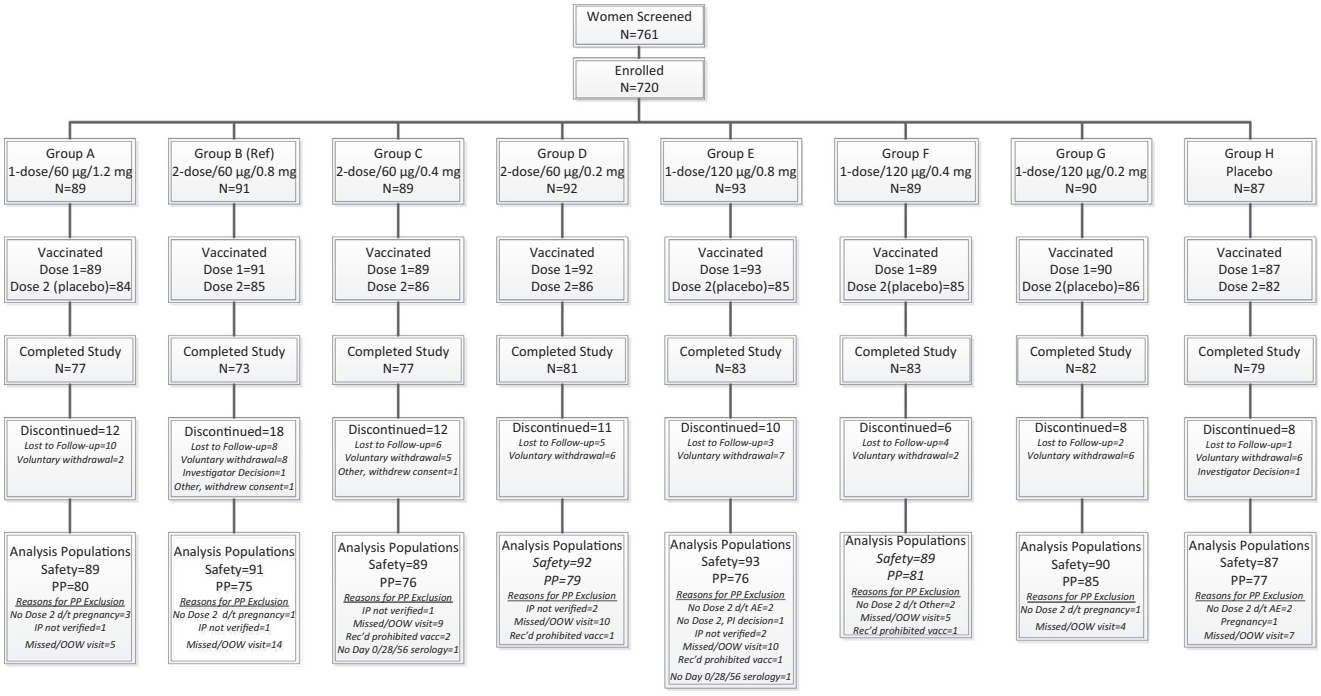

Fig. 1. Enrollment, randomization, and follow-up of trial participants. Note: Treatment group labeling convention: dose-regimen (1- or 2-dose)/ RSV F protein dose (60 or 120 μg)/aluminum phosphate dose (0.2–1.2 mg). Note: Summaries for subjects who did not receive the second test article dose on Day 28 due to an AE or based on the PI's decision include: Group E - One subject developed a series of injection site (erythema, edema, ecchymosis, anesthesia, pruritus, and induration) and systemic (fatigue, muscle pain, and diarrhea) events after the first dose, some with notable durations (i.e., lasting >7 days). A second subject had elevated blood pressure on the day of vaccination. The third subject failed to disclose a current psychiatric history that was exclusionary; Group H - One subject had a grade 3 low hemoglobin count at Day 28 that was unchanged from baseline; another subject had a grade 2 ALT that was ongoing at Day 28. Although repeat testing performed on Day 28 showed the abnormality had spontaneously improved to grade 1, the lab result was not available in time to permit a second vaccination. IP = Investigational product; PP = Per Protocol; OOW = Out-of-window; Ref = Reference formulation.

Table 1
Subject demographics.

Group:	A	B	C	D	E	F	G	H
Regimen:	1-Dose/	2-Dose/	2-Dose/	2-Dose/	1-Dose/	1-Dose/	1-Dose/	Placebo
RSV F Dose:	60 μg	60 μg	60 μg	60 μg	120 μg	120 μg	120 μg	–
$AlPO_4$ Dose:	1.2 mg	0.8 mg	0.4 mg	0.2 mg	0.8 mg	0.4 mg	0.2 mg	–
Safety Population:	N = 89	N = 91	N = 89	N = 92	N = 93	N = 89	N = 90	N = 87
Cohort								
Cohort 1, n (%)	46 (51.7)	49 (53.8)	44 (49.4)	41 (44.6)	42 (45.2)	46 (51.7)	44 (48.9)	48 (55.2)
Cohort 2, n (%)	43 (48.3)	42 (46.2)	45 (50.6)	51 (55.4)	51 (54.8)	43 (48.3)	46 (51.1)	39 (44.8)
Race, n (%)								
White	60 (67.4)	66 (72.5)	60 (67.4)	71 (77.2)	66 (71.0)	59 (66.3)	62 (68.9)	65 (74.7)
Black or African American	23 (25.8)	19 (20.9)	22 (24.7)	15 (16.3)	23 (24.7)	24 (27.0)	23 (25.6)	13 (14.9)
Asian	1 (1.1)	2 (2.2)	3 (3.4)	0 (0.0)	1 (1.1)	5 (5.6)	2 (2.2)	4 (4.6)
American Indian or Alaska Native	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)
Native Hawaiian or Other Pacific Islander	2 (2.2)	1 (1.1)	1 (1.1)	2 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)
Other	3 (3.4)	3 (3.3)	3 (3.4)	4 (4.3)	3 (3.2)	1 (1.1)	3 (3.3)	3 (3.4)
Age (years)								
Mean	27.0	26.8	26.9	26.9	27.5	27.2	27.7	27.3
SD	±4.5	±4.9	±4.7	±5.0	±4.5	±4.4	±4.7	±4.7
Min-Max	19–35	18–35	18–35	18–35	18–35	18–35	18–35	18–35
Height (cm)								
Mean	164.7	163.2	163.1	164.9	164.7	164.5	163.7	162.8
SD	±7.0	±6.7	±6.3	±7.7	±6.7	±6.4	±6.7	±6.8
Weight (kg)								
Mean	77.6	79.1	71.9	78.7	78.1	74.4	76.9	74.0
SD	±21.5	±22.9	±17.2	±21.5	±22.9	±18.7	±21.7	±19.8
Any Child in Home <5 years of age								
Yes	29 (32.6)	35 (38.5)	31 (34.8)	32 (34.8)	35 (37.6)	31 (34.8)	33 (36.7)	31 (35.6)

Peak anti-F IgG GMEUs 14 days after immunization were superior to the reference regimen (2-dose/60 μg with 0.8 mg aluminum) in subjects who received one dose of any of the three 120 μg RSV F formulations, regardless of aluminum content ($p \leq 0.011$ in each case), and in recipients of the 0.4 and 0.2 mg aluminum formulations remained superior for four to five weeks. An area-under-the-curve analysis by treatment group based on anti-F IgG fold-rise from Days 14 to 91 indicated the reference formulation achieved the largest antibody increase integrated over time, followed by the 1-dose/120 μg with 0.4 mg aluminum group (Table S1)]. Notably however, anti-F IgG GMEUs and GMFRs with the 1-dose/120 μg with 0.4 mg aluminum treatment were equal to, or greater than, that of the reference group through Day 42 post-dosing, after which the 2-dose regimen clearly showed an advantage based on the magnitude of the late response to the second dose (Fig. 2A/B).

3.2.2. Palivizumab-competitive antibody IgG ELISA

Despite universal prior exposure to RSV in adults, baseline PCA concentrations were generally below the lower limit of quantitation (33 μg/mL); and no change was observed in the placebo group throughout the trial. Post-vaccinal increases in PCA reflected the same pattern as anti-F IgG, with peak responses of 341–423 μg/mL on Day 14 for the 120 μg 1-dose regimens; 329 μg/mL on Day 21 for the 60 μg, 1-dose regimen; and 292–345 μg/mL on Day 56 for the 60 μg, 2-dose regimens (Fig. 2, Panels C and D). Levels subsequently declined in all groups through Day 91. Like anti-F IgG, over ≥95% of the vaccinated subjects seroconverted for PCA during the trial, with groups F and G (120 μg antigen with 0.4 and 0.2 mg aluminum, respectively) attaining peak levels by Day 14. The post-vaccinal responses of anti-F IgG and PCA correlated strongly (Pearson correlation coefficient 0.936, $p < 0.001$) in active vaccine recipients resulting in a concordance line slope of 0.98 (95% CI: 0.86, 1.01) (Fig. S1B), suggesting that the antibodies binding at or near antigenic site II comprise a significant and consistent portion of the total anti-F IgG pool elicited by the RSV F vaccine. Pre-vaccination levels of anti-F IgG and PCA showed no correlation (Fig. S1A).

3.2.3. Microneutralization activity

Despite the lack of measurable PCA activity before vaccination, neutralizing activity against RSV/A and B strains was substantial in all groups at baseline, indicative of previous RSV exposure and non-site II specific immunity. Baseline levels in the placebo group did not vary through Day 56 (Fig. 3), but MN titers rose in all vaccine groups, approximately doubling at Day 28 against both RSV strains, peaking to similar levels, irrespective of antigen or aluminum dose. Levels in the 2-dose/60 μg antigen groups increased somewhat, but not statistically significantly, after the second dose, and the amplitude of these additional changes corresponded to the aluminum dose. Baseline MN titers were very heterogeneous, and a *post hoc* analysis performed to examine the highest responses attained in groups that received 2-dose and 1-dose regimens (groups 60 μg with 0.8 and 0.4 mg aluminum vs. groups 120 μg with 0.4 and 0.2 mg aluminum, respectively) showed that women entering the trial with the lowest baseline MN titers attained 5-fold and 4-fold increases against RSV/A and B post-vaccination, respectively, at Day 28 (Table S2). As was the case with anti-F IgG and PCA, post-vaccinal RSV/A and RSV/B MN titers were significantly (Pearson correlation co-efficient 0.415 and 0.394 at days 28 and 56 respectively, both $p < 0.001$) positively correlated with each other, and also positively correlated with both anti-F IgG and PCA levels (all correlations, $p < 0.001$). Positive correlations generally persisted at 56 days in both the single-dose and two-dose groups considered individually, although the correlation between PCA levels and RSV/A MN titers was no longer significant for single dose recipients.

3.2.4. Impact of subject age and exposure to young children

There were an insufficient number of non-white subjects (<25 per group), or morbidly obese subjects to allow for meaningful

contrasts based on these demographic variables. Examination of immune responses in the two age strata showed generally similar patterns to the entire population, although baseline RSV/A (but not RSV/B MN titers) were slightly higher in the older group. The 120 μg single dose groups, especially with 0.4 or 0.2 mg of aluminum, gave the best early responses in both age strata. Women with children under 5 years of age in the home, a potential proxy for frequent RSV exposure, had 10 to 33% higher anti-F IgG, PCA, and RSV MN titers at baseline, and these differences were significant for anti-F IgG EU, PCA concentrations, and RSV/A MN titers (all $p < 0.001$); the trend was similar for RSV/B MN but did not attain significance. Conversely, women without children had somewhat larger fold-rises (again significant for anti-F IgG, PCA, and RSV/A MN) in response to vaccine which tended to reduce, but not entirely eliminate, differences based on child exposure post-immunization. Once again, however, the 120 μg single dose

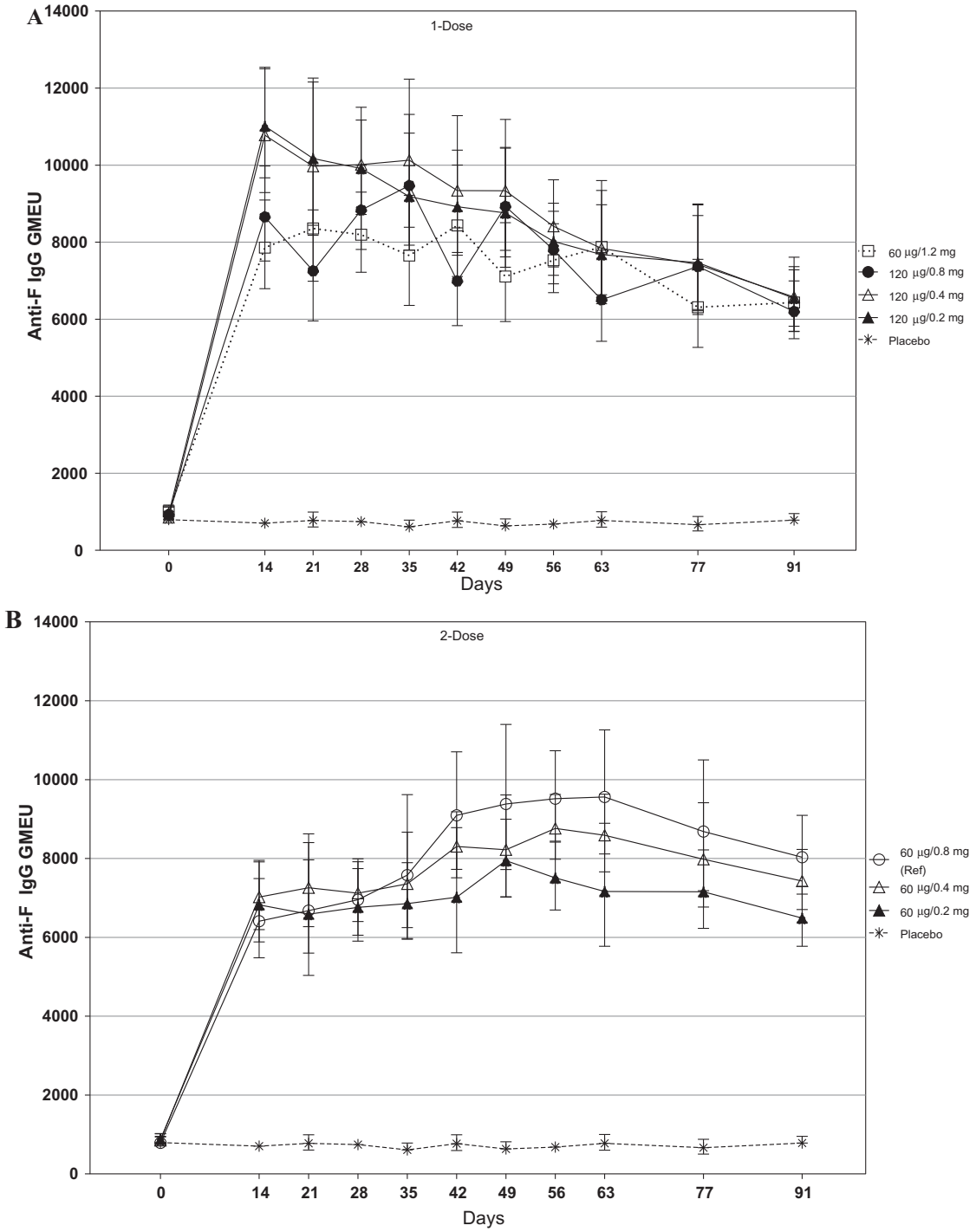

Fig. 2. Kinetics of vaccine-induced anti-F responses. Shown are the anti-F IgG GMEU (Panels A and B) and PCA GMCs (Panels C and D) with 95% CIs (error bars), by treatment group. Panels A and C show the effects of a 1-dose regimen of 120 μg F protein with 0.2–0.8 mg aluminum phosphate or of 60 μg F protein with 1.2 mg aluminum phosphate in groups A, E-G and H (placebo). Panels B and D show the effect of a 2-dose regimen of 60 μg F protein with 0.2–0.8 mg aluminum phosphate in groups B-D and H (placebo). The placebo group is included in all panels; the reference formulation (group B, 2 doses/60 μg with 0.8 mg aluminum) is included with the 2-dose regimens only (Panels B and D).

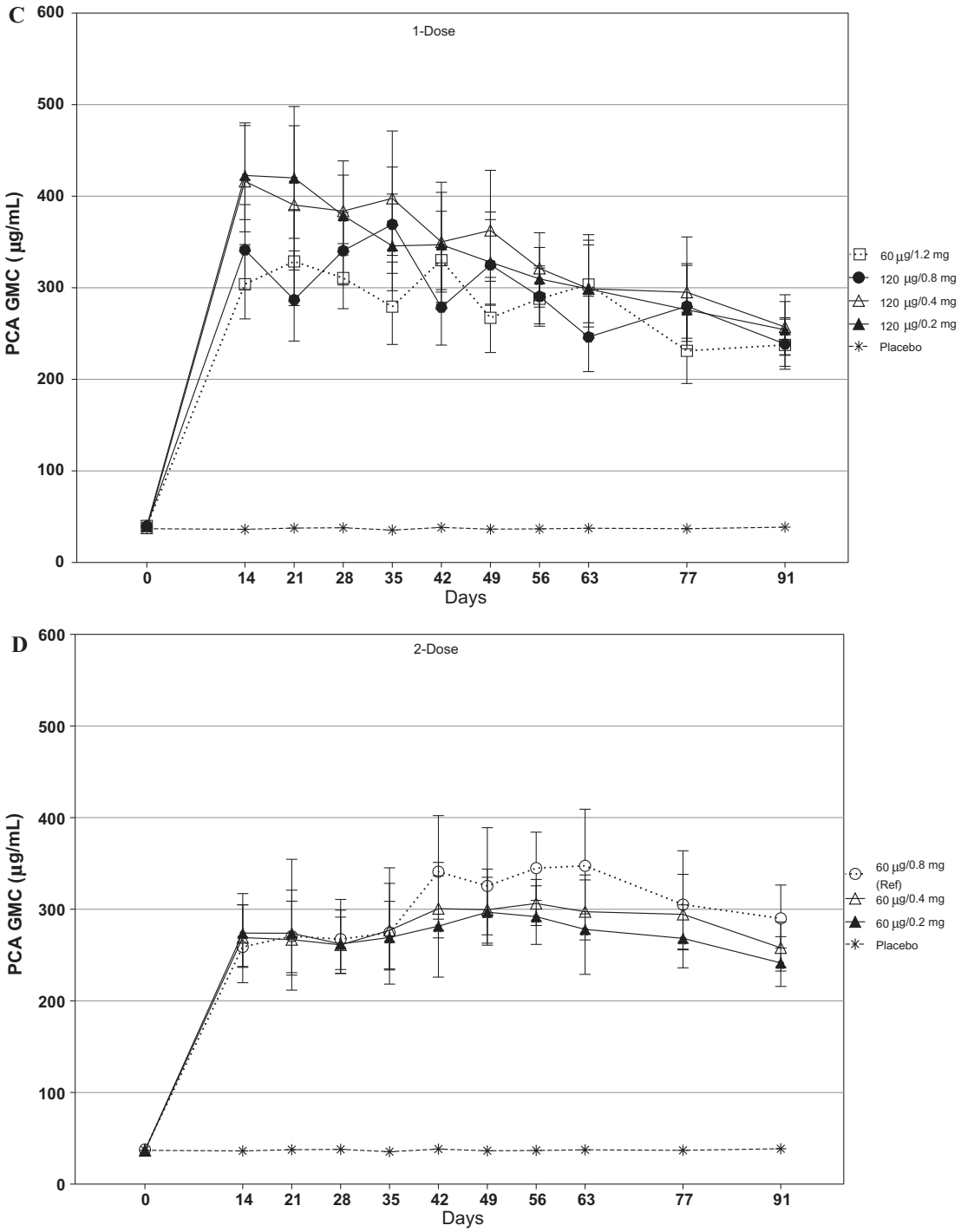

Fig. 2 (*continued*)

groups gave the best early responses in both groups of women, regardless of the presence of children under 5 years in the home.

3.3. Safety

All vaccine formulations were well-tolerated (Table S3). No deaths or treatment-related SAEs were reported.

Unsurprisingly, the active formulations were more reactogenic than placebo, with 74–85% of vaccinated subjects reporting at least one solicited AE compared with 49% of placebo recipients (Table S3 and Fig. 4A and B). The majority of solicited AEs were mild to moderate in severity; overall severe solicited events occurred in 3–7% of vaccinated subjects versus 3% of placebo recipients. Pain was the most frequent local reaction (Table S4), occurring in 55–72% of active vaccinees, compared with 10% of placebo recipients, with no increase in incidence or severity noted after a second dose of active vaccine. Although subtle, local reactogenicity rates were slightly higher with the 120 µg dose compared with the 60 µg dose at equivalent aluminum content. The frequency of solicited systemic AEs was generally comparable across the active vaccine groups (Table S4), with no increase in reactogenicity after the second dose in 2-dose active vaccine groups. Headache, muscle pain, fatigue, nausea, and joint pain were the most frequently reported events; all but nausea were more frequent in vaccinees than pla-

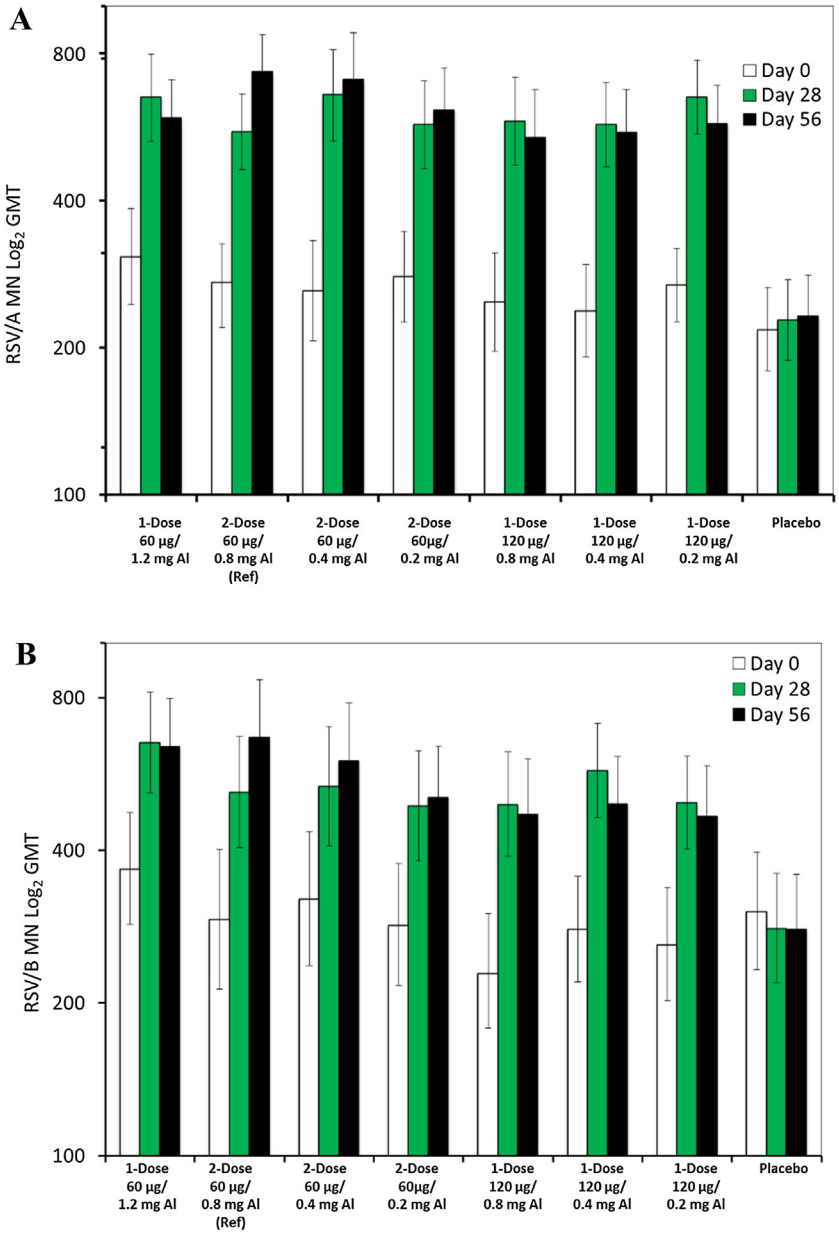

Fig. 3. RSV/A and RSV/B neutralizing antibody responses. Shown are the geometric mean microneutralization titers and associated 95% CIs against RSV/A (Panel A) and RSV/B (Panel B) in the 8 treatment groups at Days 0 (pre-immunization), 28 (four weeks after the first injection) and 56 (four weeks after the second injection).

cebo recipients. Fever (oral temperature >38.0 °C) was an uncommon solicited AE with 14 instances reported in total by 12 subjects (~1.7% overall): 10 instances after active vaccine doses (~1.1% of active doses), of which 4 were described as severe (>38.9 °C); and 4 cases after placebo doses (~0.7% of placebo doses), of which 1 was severe.

Unsolicited AEs were reported by 52–70% of the participants (Table S3). No dose effect was observed for antigen or aluminum content, and only 6.7–17.2% of participants had an AE considered related to their vaccinations. The most commonly reported unsolicited events occurred in active vaccinees and placebo recipients at similar incidences: upper respiratory tract infection (13% vs. 13%), headache (7% vs. 3%), and oropharyngeal pain (5% vs. 3%). Medically-attended AEs, SAEs, and significant new medical illnesses occurred with similar frequency amongst all active vaccine groups and placebo recipients (Table S5). Clinical laboratory assessments failed to reveal any toxicity, other than a small, consistent decrease in hemoglobin levels seen in all groups, including placebo.

Fifteen pregnancies were reported in vaccinated subjects and two in placebo recipients. One ectopic pregnancy in the 1-dose/120 µg with 0.4 mg aluminum group occurred approximately 3 months after vaccination in a subject with prior complicated gynecologic history; one pregnancy in the 1-dose/120 µg with 0.8 mg aluminum was terminated during treatment for benign ovarian teratomas, and three elective terminations were performed in various groups. Two spontaneous first trimester abortions were reported in the vaccine group (excluding the ectopic pregnancy), a rate of 2/14 (14.3%), which is within the range of background rates observed in prospective studies [22–24]. Seven pregnancies, six in the active groups and one in the placebo group, resulted in full-term deliveries of healthy normal newborns. Outcomes are unknown for 2 remaining pregnancies that involved active vaccinees who were lost to follow-up.

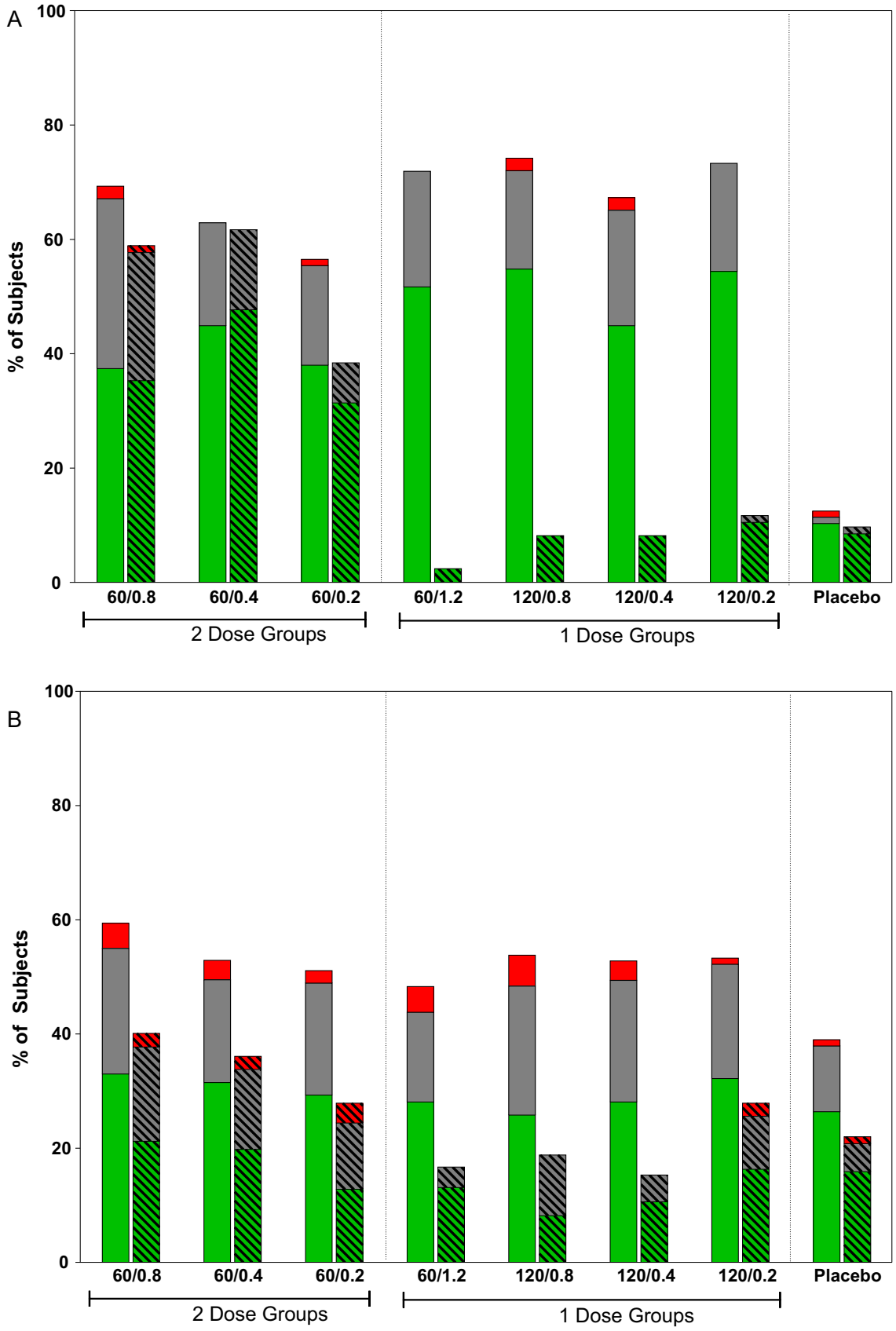

Fig. 4. Summary of subjects reporting local or systemic reactogenicity by treatment group and severity. Percentages are based on the number of subjects that received the first dose on Day 0 (solid bars) and the second dose on Day 28 (stripe bars) of the assigned test article in each group. Overall proportion of subjects reporting local (4A) and systemic (4B) adverse events have been shown by severity (mild [green], moderate [gray], severe [red]). Solicited AEs included those events reported by subjects (via diary or spontaneously) with an onset within each 7-day post-vaccination window. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Western blot analysis

Given that immunizations occurred just prior to the winter virus season, serological evidence of RSV infection was ascertained by Western blot (Fig. 5) in placebo and pooled 1-dose active vaccine recipients in order to seek preliminary evidence of vaccine efficacy. Approximately 10% of subjects in both active and placebo groups had evidence of a recent-past RSV infection at Day 0. Following immunization, serologic evidence of a new RSV infection was reduced by 52% overall (64% at Day 28 and 47% at Day 91; p = 0.009 overall) among active vaccinees. The attack rate and vaccine effect observed appear consistent with our prior studies of young women [18], and suggest that the vaccine may generate protective immunity against serologic-confirmed RSV infection.

4. Discussion

Although the magnitude of the RSV disease burden in healthy infants should drive uptake of an RSV vaccine by mothers in their third trimester, compliance could be significantly hampered by an immunization regimen requiring more than one administration. A single dose regimen of 120 μg RSV F with 0.4 mg of aluminum elicited robust immune responses, with functional activity demonstrated by *in vitro* neutralization assays and competition for palivizumab binding to the F protein, and of magnitude consistent with previous Phase 1 and 2 studies with the RSV F vaccine [19]. The use of 120 μg RSV F in a single administration also matched the immune responses of a two dose regimen with 60 μg RSV F with an almost indistinguishable anti-F IgG AUC (Table S1). Given that third trimester immunization might in some cases occur relatively late in gestation, and that the timing of delivery is always uncertain, the rapidity of the peak response to the 120 μg RSV F with 0.4 mg of aluminum coupled with the favorable one-dose schedule outweighs the slight AUC advantage of a two dose regimen.

While a determination of the clinical relevance of the immune measures will be clarified only in an RCT, the concordance of anti-F IgG to PCA and positive correlation with neutralizing titers made anti-F IgG a rational basis for dose selection. All formulations were well-tolerated, with only injection site pain and transient systemic reactions (headache, muscle ache, fatigue, and joint pain) showing any increase over placebo injections. Induction of fever is undesirable in pregnancy, and in this regard the incidence of fever after active vaccine doses differed from that after placebo by <0.5%. No deaths, no treatment-related SAEs, or significant clinical laboratory variations were observed.

Vaccinating pregnant women may enhance the quantity and quality of maternal RSV antibodies available for transplacental transfer and lead to lower rates or RSV disease in their infants. The ideal vaccine regimen to implement a successful maternal immunization strategy must also consider the optimal gestational age to target for vaccination to ensure an adequate window for both the initial immunogenic response and transplacental transfer of maternal antibodies. A single-dose regimen has both advantages for logistics and compliance because optimally timed *in utero* antibody transfer to the infant has been shown to be enhanced by an extended duration between vaccine administration [25–27]. A single-dose regimen in low-resource countries would be particularly beneficial as women often present for initial prenatal care at an advanced gestational age and have limited numbers prenatal care visits overall.

This is the second trial to demonstrate a decreased rate of serologically-detected RSV infection in RSV F-immunized women of childbearing age [18]. Although clinical respiratory illness outcomes were not collected in this trial, and would not be expected to be severe in healthy young non-pregnant subjects, it is clear that RSV infections can result in serious outcomes (e.g., hospitalization) in pregnant women [28,29]. Although the rates of infection described here and in the previous trial were acquired over only a portion of an RSV season and also might not be reflective of a population with more intense exposure to young children, they

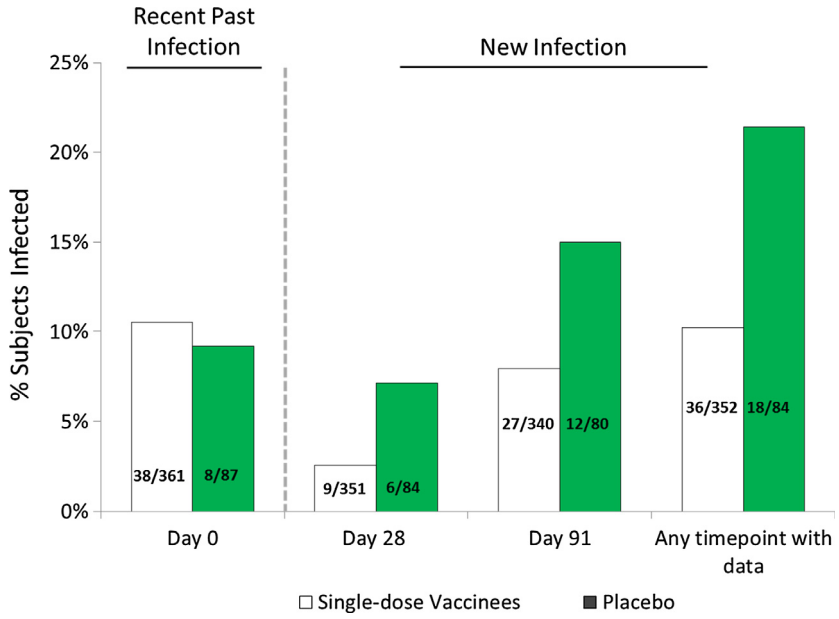

Fig. 5. Serologic determination of RSV infection by Western blot before and during RSV season. Membrane-bound RSV proteins were probed with subject serum samples collected at Days 0, 28, and 91. Color development of bands with molecular weights in kilodalton ranges (K) of 70–90 K, 46–50 K, 39–41 K, 33–35 K, and 28 K correspond to the following RSV proteins: G, F1, nucleoprotein, phosphoprotein, and matrix protein, respectively. A "recent-past RSV infection" profile was characterized by the visualization of 2 or more of these protein bands at enrollment. An "acute RSV infection" profile was characterized by an increase in intensity or the identification of new bands that correspond to 1 or more proteins. Percentages are based on the number of subjects with a serologically-confirmed RSV infection among evaluable subjects with sera collected on Day 28 or Day 91 separately, or on either day (i.e., any timepoint with data). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were consistently above 20% and the vaccine effect approximated a vaccine efficacy of 50%. These data suggest that any randomized control trial in the context of maternal immunization against RSV should include monitoring of PCR-confirmed illness in mothers, known to have complications due to RSV infections [28], as well as infants to better characterize clinical outcomes and to further understand the disease burden and vaccine effect. As has been shown in the context of influenza immunization [8,30], RSV vaccination of pregnant women may demonstrate benefits to the mother, pregnancy outcomes and, and her infant.

Baseline levels of anti-F and RSV-neutralizing antibodies were high in these women indicating prior environmental exposure to RSV/A and B in a demographic that has frequent exposure to young children and consistent with previous observations [18]. All RSV F formulations increased neutralizing antibody levels against both RSV/A and RSV/B strains in vaccinees, independent of a second dose. Importantly, women entering the trial with the lowest RSV-neutralizing antibody titers had higher post-vaccinal fold-increases in their MN titer compared to women who entered the trial with high baseline MN titers. In the setting of maternal immunization, this robust response could extend protection to infants who would otherwise be most vulnerable to RSV disease. The antibodies elicited were also functional, and able to compete with palivizumab for binding to the antigenic site II epitope. While immunizing pregnant women with young families may select for an even higher background exposure to RSV, and therefore modulation of the vaccine response, we find no clear evidence that antibody levels ultimately achieved will be negatively affected in the 35.7% of our subjects with children under 5 in the home.

The single injection of 120 μg RSV F protein with 0.4 mg aluminum phosphate was well-tolerated, elicited robust anti-F IgG antibodies, high PCA responses, and enhanced neutralizing antibody titers, and should improve compliance over a 2-dose regimen. This vaccine is now being tested in 3rd trimester pregnant women (NCT02624947) to evaluate protection of the infant in the first few months of life against clinical disease during the RSV season (primary objective), and to assess the burden of RSV disease and infection in mothers (exploratory objective). The recent safety and efficacy observations in the context of maternal immunization with seasonal influenza [31–36] have provided further impetus to assess the effectiveness of an F vaccine against RSV disease in this setting with potential to benefit both the infant and mother.

Author contributions

L.F.F., G.M.G., D.N.T., E.K., S.P.H., D.J., H.L., and P.A.P made substantial contributions to conception and design, or acquisition of data.

A.A., L.F.F., E.K., G.M.G., S.P.H., D.J., D.N.T., H.L., and P.A.P made substantial contributions to analysis and interpretation of data.

A.A., L.F.F., E.K., G.M.G., S.P.H., D.J., D.N.T., H.L., and P.A.P have been involved in drafting the manuscript or revising it critically for important intellectual content.

Conflicts of interest

Allison August (A.A.), Louis F. Fries (L.F.F.), Gregory M. Glenn (G. M.G.), Somia P. Hickman (S.P.H.), Eloi Kpamegan (E.K.), Dewal Jani (D.J.), Hanxin Lu (H.L.), and D. Nigel Thomas (D.N.T) are either current or former employees of Novavax, and all report holding stock options, restricted shares, or both in Novavax. Pedro A. Piedra (P.A. P.) is an academic collaborator on this trial and his academic institution was contracted for research laboratory testing performed as part of the conduct of this trial.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.05.045.

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