Vaccine 37 (2019) 2694–2703

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Safety and immunogenicity of a respiratory syncytial virus fusion glycoprotein F subunit vaccine in healthy adults: Results of a phase 1, randomized, observer-blind, controlled, dosage-escalation study

Geert Leroux-Roels [a], Fien De Boever [a], Cathy Maes [a], Thi Lien-Anh Nguyen [b], Sherryl Baker [c,1], Antonio Gonzalez Lopez [c,*]

[a] Centre for Vaccinology, Ghent University Hospital, C. Heymanslaan 10, 9000 Ghent, Belgium
[b] GSK, Avenue Fleming 20, 1300 Wavre, Belgium
[c] GSK, 14200 Shady Grove Rd, Rockville, MD 20850, USA

ARTICLE INFO

Article history:
Received 1 October 2018
Received in revised form 2 April 2019
Accepted 4 April 2019
Available online 12 April 2019

Keywords:
Respiratory syncytial virus
Glycoprotein
Subunit vaccine
Adjuvant
Immunogenicity
Safety

ABSTRACT

Introduction: Respiratory syncytial virus (RSV) is a leading cause of acute lower respiratory tract infections in infants. An investigational vaccine using an engineered recombinant RSV fusion glycoprotein in its post-fusion conformation (RSV F subunit vaccine) has been developed to protect young infants via maternal immunization. This first-in-human, phase I, observer-blind study (NCT02298179) evaluated the safety and immunogenicity of different dosages and formulations of RSV F subunit vaccine in healthy non-pregnant women and men aged 18–45 years.

Methods: Participants were enrolled (1:1:1) in a stepwise dosage-escalation manner into three cohorts to receive RSV F subunit vaccine containing 45 μg, 90 μg and 135 μg of RSV F glycoprotein. Within each cohort, participants were randomized (1:1:1:1) to receive two doses of RSV F subunit vaccine with (aluminum hydroxide or MF59) or without adjuvant, or placebo, ≥28 days apart. Safety (until day 365 post-dose 2), anti-RSV neutralizing antibodies (NAbs) and serum total binding antibodies to RSV F protein (until day 181 post-dose 1) were evaluated.

Results: All formulations were well-tolerated. No vaccine-related serious adverse events were reported. All participants were seropositive for anti-RSV NAbs at baseline, with geometric mean titers (GMTs) ranging from 184 (95% confidence interval [CI]: 127–266) to 380 (95% CI: 272–531). At 28 days post-dose 1, anti-RSV NAb GMTs in vaccine recipients ranged from 893 (95% CI: 702–1,136) to 1,602 (95% CI: 1,243–2,064). No booster effect was observed, but immune responses were maintained above pre-vaccination levels for six months post-dose 1. Ratios of RSV F total binding antibodies fold changes to NAb fold changes ranged from 2.79 to 4.12 at 28 days post-dose 1. The impact of the adjuvant was limited.

Conclusions: A single dose of each formulation of RSV subunit F vaccine was well-tolerated and enhanced preexisting NAb titers through six months of follow-up.

1. Introduction

Although human respiratory syncytial virus (RSV) is a leading cause of respiratory distress and hospitalization in all age groups [1], infants younger than six months are at increased risk for severe RSV diseases, including bronchiolitis, pneumonia, rhinitis and otitis media [2–5]. In 2015, RSV caused an estimated 33.1 million acute lower respiratory tract infections (LRTI) among children younger than 5 years, resulting in 94,600 to 149,400 deaths [5]. Respiratory tract infections caused by RSV impact almost all children by the age of two years [3,6,7] and represent 22% of all episodes of acute LRTIs in young children [8].

Abbreviations: AE, adverse event; CI, confidence interval; GMR, geometric mean ratio; GMT, geometric mean titer; LRTI, lower respiratory tract infections; MF59, 9.75 mg squalene and surfactants; NAb, neutralizing antibody; NOCD, new onset of chronic disease; RSV, respiratory syncytial virus; RSV F, RSV fusion glycoprotein; SAE, serious adverse event.

* Corresponding author.
E-mail addresses: Geert.LerouxRoels@UGent.be (G. Leroux-Roels), Fien.DeBoever@uzgent.be (F. De Boever), Cathy.Maes@uzgent.be (C. Maes), antonio.y.gonzalez@gsk.com (A. Gonzalez Lopez).
[1] Current affiliation: RSC Statistical Consulting, LLC, PO Box 1066, Sparta, NJ 07871, USA.

https://doi.org/10.1016/j.vaccine.2019.04.011
0264-410X/

Oxygen therapy or antiviral ribavirin aerosols may be used for the treatment of severe RSV disease [9]. The neutralizing monoclonal antibody palivizumab is alternatively used for prophylaxis in infants at highest risk for severe infection, but is considered too expensive and impractical for universal use [10]. Despite a consensus on the need of a RSV vaccine [11], there is no licensed product available yet, mainly due to the early age of infection, the capacity of RSV to evade innate immunity, the failure of RSV-induced adaptive immunity to prevent re-infection and the lack of success with candidate vaccines in the elderly [12,13]. The induction of neutralizing antibodies (NAbs) through vaccination may help to protect the population at-risk [12] since the severity of RSV disease is largely determined by the extent of viral replication among other environmental, social, host and viral factors [14].

As maternal antibodies partially protect infants from RSV disease and the accompanying bronchiolitis in their first months of life [15–19], immunization of pregnant women against RSV during the third trimester of gestation has the potential to passively protect young infants from the disease through placental or breast milk transfer of antibodies. Such maternal immunization programs have been proven safe and effective for prevention of neonatal tetanus, pertussis and influenza [20–22].

Eleven proteins are coded by the RSV genome, among which the RSV fusion glycoprotein (RSV F), a surface protein that mediates the fusion between the virus envelope and the target respiratory epithelial cells and is essential to RSV pathogenesis [23,24]. An investigational RSV subunit vaccine (RSV F subunit vaccine) has been developed from an engineered recombinant RSV F glycoprotein in its post-fusion conformation [25]. By immunizing pregnant women during the third trimester of gestation, the use of the RSV F subunit vaccine aims to increase the level of NAbs that are passively transferred to the infants placentally and to protect them from RSV disease during their first months of life.

The purpose of this first-in-human phase I study was to evaluate the safety and immunogenicity of different dosages and formulations of the investigational RSV F subunit vaccine.

2. Methods

2.1. Study design and participants

This phase I, observer-blind, randomized, placebo-controlled, dosage-escalation study was conducted at the Ghent University Hospital, Belgium from December 2014 to March 2017. Healthy non-pregnant women and men aged 18–45 years were enrolled in a 3:1 ratio. More women were enrolled as the RSV F subunit vaccine is intended for use in pregnant women, but men were also included since this was a first-in-human trial and the vaccine may also be tested in the future in other groups of population, such as the elderly.

Participants were enrolled (1:1:1) in a stepwise dosage-escalation manner into three cohorts to receive either 45 μg, 90 μg, or 135 μg of the RSV F subunit vaccine (Fig. 1). Within each cohort, participants were randomized (1:1:1:1) to receive two doses of the RSV F subunit vaccine without adjuvant (non-adjuvanted RSV F subunit groups), with 1 mg aluminum hydroxide (RSV F subunit-Al groups) or with 9.75 mg squalene and surfactants (MF59; RSV F subunit-MF59 groups), or saline placebo (placebo group). The minimum interval between the two doses was 28 days. A Data Monitoring Committee reviewed safety data during scheduled periodic reviews: (1) after enrollment of the first 12 participants in each cohort; (2) seven days after the first dose, before proceeding with enrollment of the remaining participants in that cohort; and (3) after the enrollment of each cohort has been completed, before proceeding to the dosage escalation in the subsequent cohort.

Women of childbearing potential had to practice contraceptive methods from at least two months prior to study entry through at least three weeks after the last study vaccination. Participants with a positive urine pregnancy test prior to study vaccinations or lactating women were excluded from the study. To ensure the participants' safety, each study vaccine recipient becoming pregnant during the study had to be followed-up even if the intended duration of safety follow-up for the study had ended. The exhaustive list of exclusion criteria is provided in Supplement 1.

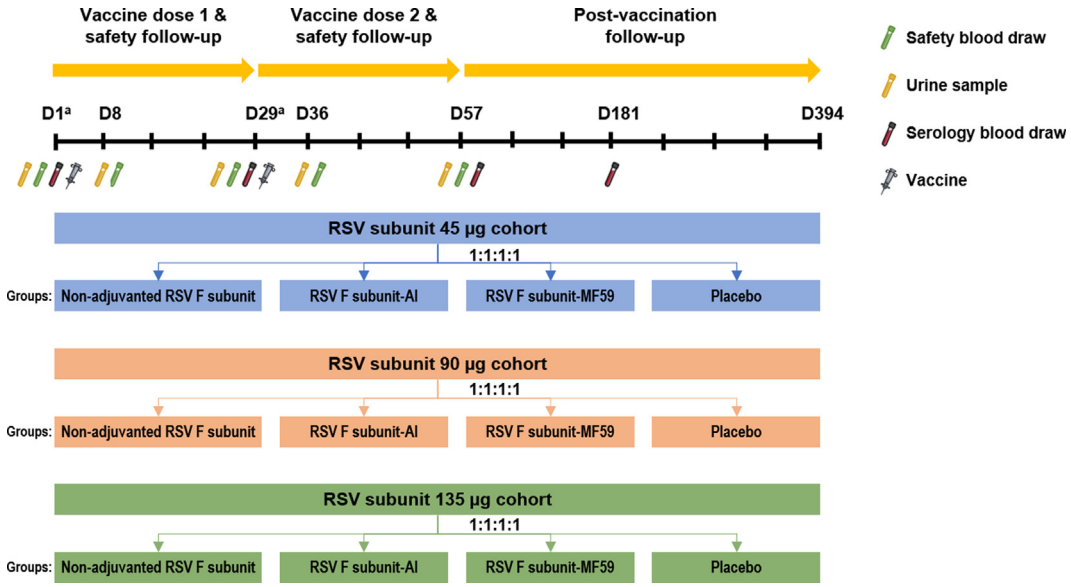

Fig. 1. Study design. Non-adjuvanted RSV F subunit, RSV F subunit-Al, RSV F subunit-MF59, groups receiving the non-adjuvanted, aluminum-adjuvanted and MF59-adjuvanted vaccine formulation, in each cohort; RSV, respiratory syncytial virus; MF59, 9.75 mg squalene and surfactants; D, day. [a]For Day 1 and Day 29 all procedures were performed before vaccination.

The study was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained from each participant prior to enrollment. The study protocol and informed consent/assent forms were reviewed and approved by the Independent Ethics Committee of the Ghent University Hospital. The study is registered at www.clinicaltrials.gov (NCT02298179) and a protocol summary is available at http://www.gsk-clinicalstudyregister.com (study 205219). Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

2.2. Study objectives

The primary objectives were to assess the safety of the RSV F subunit vaccine and to evaluate the serum NAb response to the RSV F subunit vaccine at 28 days after the second dose (Day 57).

The secondary objectives were to further evaluate the immune response to the RSV F subunit vaccine or placebo in terms of NAb titers and serum total binding antibody titers to the RSV F, G and N proteins at baseline (Day 1), 28 days post-dose 1 (Day 29), 28 days post-dose 2 (Day 57) and six months post-dose 1 (Day 181); and to compare the ratio of the serum total binding antibody geometric mean titers (GMTs) to the RSV F protein to the NAb GMTs in RSV F subunit vaccine or placebo recipients at Days 1, 29, 57, and 181. Since serum total binding antibody titers to the RSV F protein and NAb titers are measured by different assay systems and cannot be directly compared, an additional analysis was performed to evaluate the ratios of the RSV F total binding antibody titer fold changes to the NAb titer fold changes.

2.3. Study vaccine

The 3 dosages of the RSV F subunit vaccine evaluated in this study were lyophilized vaccines containing 45 μg, 90 μg or 135 μg of RSV F glycoprotein, each reconstituted either with sterile saline 0.9% NaCl (non-adjuvanted RSV F subunit groups), in sterile saline 0.9% NaCl and 1 mg aluminum hydroxide (RSV F subunit-Al groups) or in sterile saline 0.9% NaCl and the adjuvant MF59 containing 9.75 mg squalene and surfactants (RSV F subunit-MF59 groups). The RSV F subunit vaccine (0.5 mL) or placebo (sterile saline 0.9% NaCl) was injected intramuscularly in the deltoid muscle of the non-dominant arm.

2.4. Safety assessment

Solicited local (injection site induration, swelling, erythema, and pain) and systemic (fever, chills, nausea, myalgia, arthralgia, headache, fatigue, diarrhea, cough, rhinorrhea, and wheezing) symptoms were recorded within seven days, and unsolicited adverse events (AEs) within 28 days after each vaccination. Symptom intensity was graded between 0 and 3, as absent, mild, moderate, and severe.

Serious AEs (SAEs), unsolicited AEs leading to study withdrawal, new onsets of chronic disease (NOCDs; defined as an AE representing a new diagnosis of a chronic medical condition that was not present or suspected in a participant prior to study enrollment) were recorded from study start until study completion.

Hematological and biochemical parameters were measured at Days 1, 8, 29, 36 and 57 (Supplement 2).

2.5. Immunogenicity assessment

Blood was collected from all participants at Days 1, 29, 57 and 181 for serology assays. These consisted of the evaluation of serum anti-RSV NAb titers by a plaque reduction neutralization assay, as previously described [26]. Virus neutralization was performed by incubating a fixed amount of RSV-A (strain A Long, ATCC No. VR-26) with serial dilutions of the test serum. The serum-virus mixture was then transferred onto a monolayer of Vero cells and incubated for three days to allow infection by non-neutralized virus and formation of plaques. Following a fixation step, RSV-infected cells were detected using a primary goat anti-RSV polyclonal antibody directed against viral antigens included in RSV-A and RSV-B, which was obtained in goats immunized with a viral lysate of human RSV isolates, and a secondary anti-goat antibody (IgG) conjugated with fluorescein isothiocyanate, allowing the visualization of plaques by immunofluorescence. The serum neutralizing antibody titer was expressed as the estimated dilution 60 (ED60), which corresponds to the inverse of the interpolated serum dilution that yields a 60% reduction in the number of plaques as compared to the virus control wells. The assay cut-off was set at 8 ED60. Binding antibody titers to RSV F, G (G_a and G_b), and N proteins were measured by a microsphere-based fluorescence multiplex enzyme-linked immunosorbent assay, using Luminex® technology. Due to the use of a distinctly labeled bead type per antigen, antibody levels against RSV F, N, G_a and G_b proteins could be quantified in a single sample volume and assay run by a phytoerythrin-labeled detection antibody. We verified assay specificity using samples from immunization with the RSV F vaccine that elicited antibody responses against RSV F protein only and samples from natural infection that elicited increases in antibody titers against RSV F, G and N proteins.

2.6. Statistical analysis

The target sample size was to enroll 288 healthy adults to obtain 260 evaluable participants, considered as sufficient to provide a descriptive summary of the safety and immunogenicity of the vaccine. This sample size was not driven by statistical assumptions for formal hypothesis testing; therefore, the analyses presented were purely descriptive and no inferential analysis was performed.

The safety analysis was performed in the solicited (for solicited local and systemic symptoms) and the unsolicited (for unsolicited AEs, NOCDs and SAEs) safety sets at each timepoint, which included all participants receiving at least one study vaccination and who provided safety data at the relevant timepoint.

The primary immunogenicity analyses were based on the per-protocol sets for immunogenicity at each timepoint (Fig. 2), which included all participants who received a study vaccination, provided immunogenicity data at the relevant timepoints, and were not excluded prior to unblinding or analysis.

For all treatment groups within each cohort, GMTs of the serum anti-RSV NAbs and of the serum total binding antibodies to each of the RSV F, G and N proteins were computed with their associated 95% confidence intervals (CIs) by exponentiation of the corresponding log-transformed means and 95% CIs. The statistical analyses for GMTs were conducted using an ANCOVA model with dosage and adjuvant as factors and baseline antibody level as covariate. Proportions of participants with a $\geq$4-fold increase in serum anti-RSV NAb titers between baseline and Days 29, 57 and 181 were presented for each treatment group within each cohort, together with their two-sided 95% Clopper-Pearson CIs. For each timepoint, the percentages of seropositive participants for anti-RSV NAbs, the ratios of the serum total binding antibody titers to the RSV F protein to the NAb titers, and the geometric mean ratios (GMRs) of the RSV F total binding antibody titer fold changes to the NAb titer fold changes were also computed with their 95% CIs. Analyses of the ratio of the serum total binding antibody titers to the RSV F protein to the NAb titers used an ANCOVA model with dosage level and adjuvant as factors and the serum NAb titer at

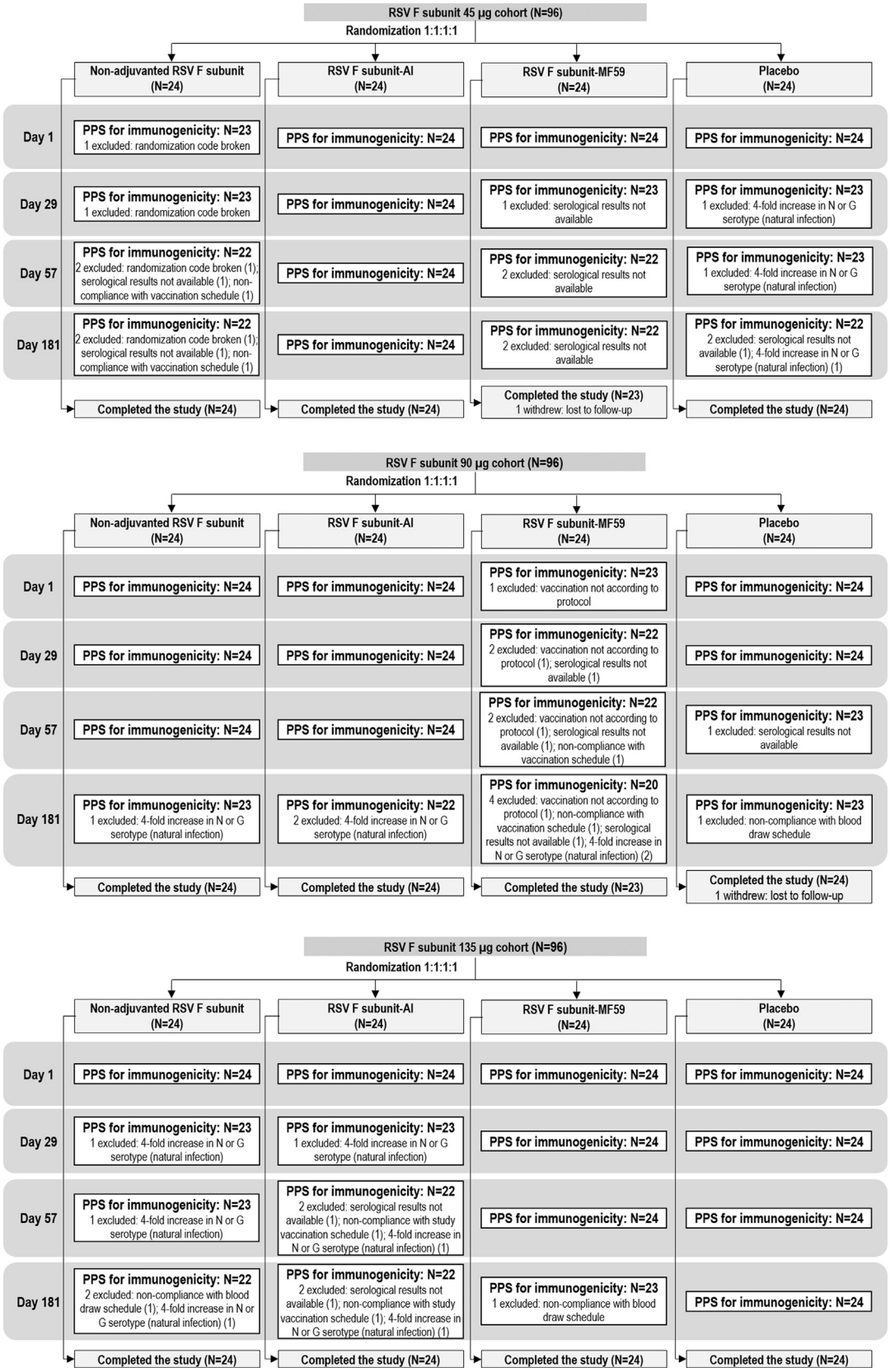

Fig. 2. Flow of participants. Non-adjuvanted RSV F subunit, RSV F subunit-AI, RSV F subunit-MF59, groups receiving the non-adjuvanted, aluminum-adjuvanted and MF59-adjuvanted vaccine formulation, in each cohort; RSV, respiratory syncytial virus; MF59, 9.75 mg squalene and surfactants; N, number of participants; PPS, per-protocol set. Note: A participant could be excluded from the PPS for immunogenicity for more than one reason.

baseline as covariate. Analysis of the GMRs of the RSV F total binding antibody titer fold changes to the NAb titer fold changes used an ANOVA model with dosage group as a fixed factor.

All analyses were performed using SAS version 9.3.

3. Results

3.1. Study population

A total of 288 healthy female and male adults 18–45 years of age were enrolled (Fig. 2). The mean age of the participants ranged from 27.6 to 32.9 years across all groups (Table 1). The proportion of women ranged from 58% to 92% in all groups. Most participants were White/Caucasian (92% to 100%).

3.2. Safety

Until Day 7 post-dose 1, solicited local symptoms were reported by 50–58% (non-adjuvanted RSV F subunit groups), by 79–92% (RSV F subunit-Al groups), by 79–83% (RSV F subunit-MF59 groups) and by 8–21% (placebo groups) of participants. Post-dose 2, these were reported by 61–67% (non-adjuvanted RSV F subunit groups), by 46–54% (RSV F subunit-Al groups), by 57–64% (RSV F subunit-MF59 groups) and by 0–21% (placebo groups) of participants.

Post-dose 1, solicited systemic symptoms were reported by 54–58% (non-adjuvanted RSV F subunit groups), by 58% (RSV F subunit-Al groups), by 58–79% (RSV F subunit-MF59 groups) and by 50–71% (placebo groups) of participants (Table 2). Through Day 7 post-dose 2, these were reported by 33–54% (non-adjuvanted RSV F subunit groups), by 39–58% (RSV F subunit-Al groups), by 41–54% (RSV F subunit-MF59 groups) and by 46–50% (placebo groups) of participants (Table 2).

Any unsolicited AE was reported by 54–71% (no adjuvant RSV F subunit groups), 58–63% (RSV F subunit-Al groups), 58–67% (RSV F subunit-MF59 groups) and 58–71% (placebo) of participants post-dose 1. Unsolicited AEs reported between Day 1 and Day 28 led to withdrawal from the second vaccination for one participant in the non-adjuvanted RSV F subunit group of the 45 μg cohort (urticaria) and one participant in the RSV F subunit-Al group of the 135 μg cohort (otitis media); these participants were excluded from the unsolicited safety set post-dose 2, but did not withdraw from the study. None of these AEs were considered to be possibly related to vaccination. Post-dose 2, 35–54% (non-adjuvanted RSV F subunit groups), 29–50% (RSV F subunit-Al groups), 27–70% (RSV F subunit-MF59 groups) and 42–54% (placebo groups) of participants reported any unsolicited AE.

Among participants receiving the 45 μg dosage, one SAE was reported in the non-adjuvanted RSV F subunit group (acute pancreatitis), one in the RSV F subunit-Al group (cartilage injury) and one in the RSV F subunit-MF59 group (deep vein thrombosis). For recipients of the 90 μg dosage, one SAE was reported in the non-adjuvanted RSV F subunit group (acute pyelonephritis) and four SAEs were reported in the RSV F subunit-MF59 group (Basedow-Graves disease, pyelonephritis, intervertebral disc degeneration and vaginal hemorrhage). Among recipients of the 135 μg dosage, one SAE was reported in the non-adjuvanted RSV F subunit group (head injury) and two SAEs were reported in the RSV F subunit-Al group (anaphylactic shock and depression). No SAEs were considered as probably related to vaccination by the investigators. No participants in the placebo groups reported SAEs. No fatal SAEs were reported. During the entire follow-up, one NOCD was reported in a participant in the RSV F subunit-MF59 group of the 90 μg cohort (Basedow-Graves disease), which was not considered to be possibly related to vaccination.

The hematological and biochemical parameters were within normal ranges in all study groups (data not shown).

3.3. Immunogenicity

All participants were seropositive for anti-RSV NAbs at baseline, with adjusted NAb GMTs ranging from 184 (95% CI: 127–266) to 380 (95% CI: 272–531) across all groups (Fig. 3). Anti-RSV NAb GMTs rose from Day 1 to Day 29 in all groups who received the RSV F subunit vaccine, with the highest levels reached in the RSV F subunit-Al group (NAb GMT: 1602; 4.58-fold increase from baseline and the RSV F subunit-MF59 group (NAb GMT: 1,551; 4.44-fold increase from baseline) of the 135 μg cohort. In the three cohorts, anti-RSV NAb GMTs were slightly lower at one month post-dose 2 (Day 57) compared to one month post-dose 1 (Day 29), with the highest level observed in the RSV F subunit-M59 group of the 135 μg cohort at Day 57 (NAb GMT: 1,316; 3.75-fold increase from baseline). Anti-RSV NAb GMTs decreased further between Day 57 and Day 181. NAb GMTs at Day 181 were slightly higher in the RSV F subunit-Al group (NAb GMT: 788; 2.23-fold increase from baseline) and the RSV F-MF59 group (NAb GMT:

Table 1
Demographic characteristics of the study participants at enrolment (all enrolled set).

	Non-adjuvanted RSV F subunit	RSV F subunit-Al	RSV F subunit-MF59	Placebo
RSV subunit dosage 45 μg	*N = 24*	*N = 24*	*N = 24*	*N = 24*
Mean Age, years (SD)	28.6 (6.8)	27.7 (6.55)	30.2 (7.9)	30.3 (7.62)
Female, n (%)	20 (83)	17 (71)	18 (75)	18 (75)
White – Caucasian European heritage, n (%)	23 (96)	22 (92)	24 (100)	24 (100)
Black-African Heritage/African American, n (%)	0 (0)	1 (4)	0 (0)	0 (0)
Native Hawaiian or other Pacific Islands, n (%)	0 (0)	1 (4)	0 (0)	0 (0)
Other, n (%)	1 (4)	0 (0)	0 (0)	0 (0)
RSV subunit dosage 90 μg	*N = 24*	*N = 24*	*N = 24*	*N = 24*
Mean Age, years (SD)	30.4 (8.31)	31.2 (7.47)	27.6 (6.84)	28.0 (7.3)
Female, n (%)	16 (67)	18 (75)	15 (63)	14 (58)
White – Caucasian/European heritage, n (%)	24 (100)	24 (100)	24 (100)	23 (96)
Black-African Heritage/African American, n (%)	0 (0)	0 (0)	0 (0)	1 (4)
RSV subunit dosage 135 μg	*N = 24*	*N = 24*	*N = 24*	*N = 24*
Mean Age, years (SD)	28.3 (6.8)	29.5 (6.98)	32.9 (8.46)	27.9 (5.86)
Female, n (%)	22 (92)	18 (75)	20 (83)	20 (83)
White – Caucasian/European heritage, n (%)	24 (100)	24 (100)	24 (100)	24 (100)

Non-adjuvanted RSV F subunit, RSV F subunit-Al, RSV F subunit-MF59, groups receiving the non-adjuvanted, aluminum-adjuvanted and MF59-adjuvanted vaccine formulation, in each cohort; RSV, respiratory syncytial virus; SD, standard deviation; MF59, 9.75 mg squalene and surfactants; N, total number of participants; n (%), number (percentage) of participants.

Table 2
Incidence of any, local and systemic solicited symptoms reported in the 7-day interval following vaccination in participants from the cohorts who received the 45 μg, 90 μg and 135 μg dosage of the RSV F subunit vaccine (solicited safety set).

RSV subunit dosage 45 μg									
		Non-adjuvanted RSV F subunit		RSV F subunit-Al		RSV F subunit-MF59		Placebo	
Adverse event	Dose	N	n (%)	N	n (%)	N	n (%)	N	n (%)
Any	Dose 1	24	19 (79%)	24	22 (92%)	24	23 (96%)	24	19 (79%)
	Dose 2	23	18 (78%)	24	19 (79%)	23	17 (74%)	24	12 (50%)
Local	Dose 1	24	14 (58%)	24	21 (88%)	24	19 (79%)	24	2 (8%)
	Dose 2	23	14 (61%)	24	11 (46%)	23	13 (57%)	24	4 (17%)
Systemic	Dose 1	24	14 (58%)	24	14 (58%)	24	15 (63%)	24	17 (71%)
	Dose 2	23	11 (48%)	24	14 (58%)	23	12 (52%)	24	11 (46%)
RSV subunit dosage 90 μg									
		Non-adjuvanted RSV F subunit		RSV F subunit-Al		RSV F subunit-MF59		Placebo	
Adverse event	Dose	N	n (%)	N	n (%)	N	n (%)	N	n (%)
Any	Dose 1	24	20 (83%)	24	21 (88%)	24	22 (92%)	24	18 (75%)
	Dose 2	24	21 (88%)	24	17 (71%)	22	17 (77%)	24	11 (46%)
Local	Dose 1	24	12 (50%)	24	19 (79%)	24	20 (83%)	24	2 (8%)
	Dose 2	24	16 (67%)	24	13 (54%)	22	14 (64%)	24	0 (0%)
Systemic	Dose 1	24	13 (54%)	24	14 (58%)	24	14 (58%)	24	16 (67%)
	Dose 2	24	13 (54%)	24	12 (50%)	22	9 (41%)	24	11 (46%)
RSV subunit dosage 135 μg									
		Non-adjuvanted RSV F subunit		RSV F subunit-Al		RSV F subunit-MF59		Placebo	
Adverse event	Dose	N	n (%)	N	n (%)	N	n (%)	N	n (%)
Any	Dose 1	24	20 (83%)	24	22 (92%)	24	22 (92%)	24	13 (54%)
	Dose 2	24	20 (83%)	23	15 (65%)	24	19 (79%)	24	13 (54%)
Local	Dose 1	24	13 (54%)	24	22 (92%)	24	19 (79%)	24	5 (21%)
	Dose 2	24	16 (67%)	23	12 (52%)	24	15 (63%)	24	5 (21%)
Systemic	Dose 1	24	14 (58%)	24	14 (58%)	24	19 (79%)	24	12 (50%)
	Dose 2	24	8 (33%)	23	9 (39%)	24	13 (54%)	24	12 (50%)

Non-adjuvanted RSV F subunit, RSV F subunit-Al, RSV F subunit-MF59, groups receiving the non-adjuvanted, aluminum-adjuvanted and MF59-adjuvanted vaccine formulation, in each cohort; RSV, respiratory syncytial virus; MF59, 9.75 mg squalene and surfactants; N, number of participants with available safety data; n (%), number (percentage) of participants with given symptoms.

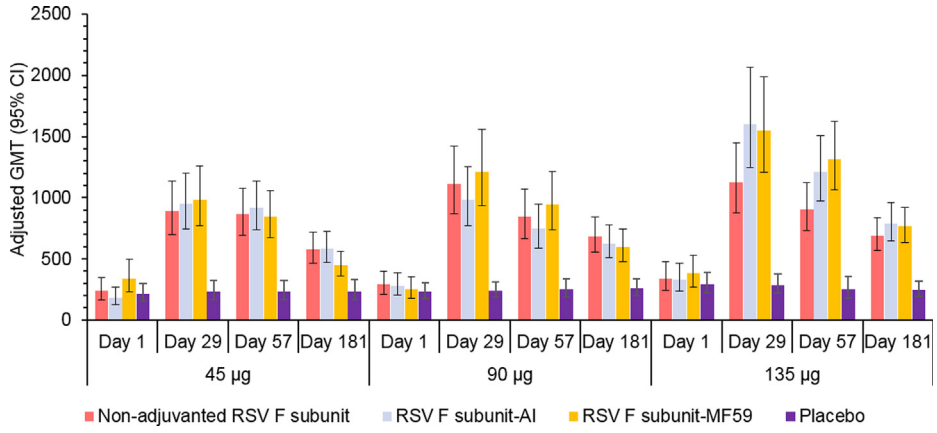

Fig. 3. Neutralizing antibody titers in participants from the cohorts who received the RSV F subunit vaccine containing (A) 45 μg, (B) 90 μg and (C) 135 μg of RSV F (per-protocol set for immunogenicity Day 29, Day 57 and Day 181)*. *Footnote:* Non-adjuvanted RSV F subunit, RSV F subunit-Al, RSV F subunit-MF59, groups receiving the non-adjuvanted, aluminum-adjuvanted and MF59-adjuvanted vaccine formulation, in each cohort; RSV, respiratory syncytial virus; MF59, 9.75 mg squalene and surfactants; GMT, geometric mean titer; 95% CI: 95% confidence interval. *Results are presented for the per-protocol set Day 29 for Days 1 and 29, for the per-protocol set Day 57 for Day 57 and per-protocol set Day 181 for Day 181.

764; 2.16-fold increase from baseline) of the 135 μg cohort than in the other groups. At all timepoints, anti-RSV NAb GMTs were comparable between groups who received the RSV F subunit vaccine with different adjuvants and dosage levels. No increases in anti-RSV NAb GMTs were observed in the participants from the 3 cohorts who received the placebo.

In the participants who received any formulation of the RSV F subunit vaccine, at least 4-fold increases in anti-RSV NAb titers compared with baseline were observed in 25–58% of participants at Day 29, in 17–54% of participants at Day 57, and in 5–32% of participants at Day 181.

All participants had pre-existing antibodies against the RSV F protein, with serum total binding antibody GMTs to the RSV F protein ranging from 671 to 916 across groups at baseline (Table 3). Among the groups who received the RSV F subunit vaccine (any formulation), total binding antibody GMTs to RSV F protein ranged

Table 3
Serum total binding antibody concentrations against RSV proteins F (per-protocol set for immunogenicity Day 29, Day 57 and Day 181).[a]

		Non-adjuvanted RSV F subunit		RSV F subunit-Al		RSV F subunit-MF59		Placebo	
Dosage	Timepoint	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)
45 μg	Day 1	23	671 (470–958)	24	838 (591–1,188)	23	916 (642–1,309)	23	714 (530–961)
	Day 29	23	9,641 (7,964–11,671)	24	11,131 (9,244–13,403)	23	11,639 (9,620–14,080)	23	675 (504–905)
	Day 57	22	8,064 (6,645–9,786)	24	9,707 (8,073–11,672)	22	10,182 (8,395–12,349)	23	757 (541–1,060)
	Day 181	22	4,904 (3,848–6,250)	24	6,584 (5,226–8,294)	22	5,312 (4,171–6,765)	22	690 (506–942)
90 μg	Day 1	24	697 (483–1,005)	24	730 (506–1,053)	22	751 (512–1,101)	24	727 (574–921)
	Day 29	24	13,017 (11,147–15,201)	24	10,694 (9,158–12,488)	22	12,841 (10,921–15,099)	24	684 (515–909)
	Day 57	24	11,470 (9,660–13,618)	24	10,131 (8,533–12,028)	22	11,588 (9,686–13,864)	23	660 (505–864)
	Day 181	23	6,908 (5,627–8,480)	22	6,271 (5,087–7,730)	20	5,824 (4,675–7,256)	23	763 (596–978)
135 μg	Day 1	23	880 (639–1,210)	23	685 (498–942)	24	910 (666–1,244)	24	750 (586–959)
	Day 29	23	13,252 (11,607–15,130)	23	13,514 (11,824–15,446)	24	13,541 (11,890–15,421)	24	851 (617–1,174)
	Day 57	23	11,302 (10,022–12,746)	22	13,483 (11,909–15,264)	24	12,158 (10,806–13,680)	24	791 (596–1,050)
	Day 181	22	6,157 (5,018–7,556)	22	8,651 (7,038–10,635)	23	7,041 (5,765–8,599)	24	833 (663–1,046)

Non-adjuvanted RSV F subunit, RSV F subunit-Al, RSV F subunit-MF59, groups receiving the non-adjuvanted, aluminum-adjuvanted and MF59-adjuvanted vaccine formulation, in each cohort; RSV, respiratory syncytial virus; GMT, geometric mean titers; CI, confidence interval; MF59, 9.75 mg squalene and surfactants; N, number of participants with available data for RSV F serotype.

[a] Results are presented for the per-protocol set Day 29 for Days 1 and 29, for the per-protocol set Day 57 for Day 57 and per-protocol set Day 181 for Day 181.

from 9,641 to 13,541 at one month post-dose 1 (Day 29), and were slightly lower at one month post-dose 2, ranging from 8,064 to 13,483 at Day 57. Total binding antibody GMTs to RSV F protein decreased further between Day 57 and Day 181, ranging from 4,904 to 8,651 at Day 181.

Ratios of RSV F NAb GMTs over total binding antibody GMTs to the RSV F protein ranged from 2.00 to 3.95 at Day 1, from 9.21 to 12.0 at Day 29, from 8.95 to 13.0 at Day 57, and from 8.37 to 13.0 at Day 181 (Fig. 4). The results of a *post-hoc* analysis showed that the GMRs of the RSV F total binding antibody titer fold changes to the NAb titer fold changes ranged from 2.60 to 5.51 across all groups and timepoints in the RSV F subunit cohorts, while the GMRs in the placebo groups were close to 1 at all timepoints (Table 4).

No changes in terms of total binding antibody GMTs to RSV G_a, G_b and N proteins were observed at any timepoint in all groups (data not shown). Of note, data of participants showing 4-fold increases in G or N serotype due to natural infections were excluded from the per-protocol-set for immunogenicity (Fig. 2).

4. Discussion

This first-in-human phase I study showed that various dosages (45 μg, 90 μg and 135 μg of RSV F glycoprotein) and formulations (unadjuvanted or adjuvanted with aluminum or MF59) of the investigational RSV F subunit vaccine had a clinically acceptable safety profile and no safety signal was detected. A single vaccine dose induced increases in NAb titers, which were within the same range for all vaccine formulations. Our study also showed that all formulations induced increases in non-neutralizing serum total binding antibodies, but that no booster effect was observed after the second vaccine dose.

Rates of reported solicited and unsolicited events were similar across all dosages of the RSV F subunit vaccine and for both adjuvants. The absence of impact of the adjuvants on the reactogenicity was an unexpected finding, especially for the MF59 adjuvant, which was previously associated with increased risk of solicited local or systemic adverse reactions compared to non-adjuvanted vaccines [27].

The first dose of the RSV F subunit vaccine elicited 3.21- to 4.58-fold increases in NAb GMTs. Even if the highest levels of NAb GMTs were reached with the vaccine containing the highest dosage of recombinant RSV F glycoprotein, no considerable differences in antibody titers were detected between the various antigen levels (GMTs: 45 μg, 893–983; 90 μg, 981–1,208; 135 μg, 1,123–1,602). The immune responses induced by the RSV F subunit vaccine in our study were comparable to that previously observed with various formulations of another purified recombinant RSV F protein

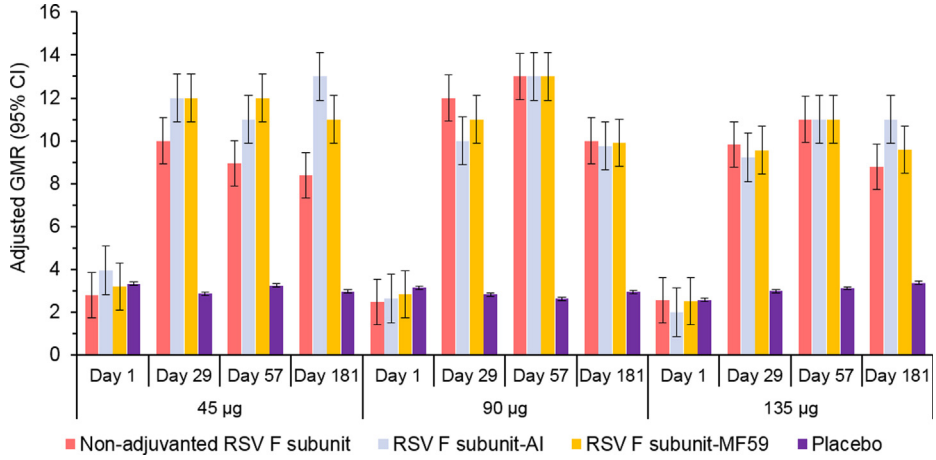

Fig. 4. Ratio of the neutralizing anti-RSV F protein antibody titers over the total binding anti-RSV F protein antibody titers (per-protocol set for immunogenicity Day 29, Day 57 and Day 181)*. *Footnote:* Non-adjuvanted RSV F subunit, RSV F subunit-Al, RSV F subunit-MF59, groups receiving the non-adjuvanted, aluminum-adjuvanted and MF59-adjuvanted vaccine formulation, in each cohort; RSV, respiratory syncytial virus; MF59, 9.75 mg squalene and surfactants; GMR, geometric mean ratio; 95% CI: 95% confidence interval. *Results are presented for the per-protocol set Day 29 for Days 1 and 29, for the per-protocol set Day 57 for Day 57 and per-protocol set Day 181 for Day 181.

Table 4
Geometric mean ratios of the RSV F total binding antibody titer fold changes to the neutralizing antibody titer fold changes (per-protocol set for immunogenicity Day 29, Day 57 and Day 181).[a]

Dosage	Timepoint	Non-adjuvanted RSV F subunit GMR (95% CI)	RSV F subunit-Al GMR (95% CI)	RSV F subunit-MF59 GMR (95% CI)	Placebo GMR (95% CI)
45 μg	Day 29	3.75 (2.62–5.37)	2.79 (1.96–3.97)	4.12 (2.87–5.90)	0.86 (0.75–0.98)
	Day 57	3.12 (2.23–4.37)	2.60 (1.88–3.60)	4.08 (2.91–5.72)	0.98 (0.83–1.15)
	Day 181	2.79 (2.08–3.75)	2.79 (2.10–3.70)	4.01 (2.98–5.39)	0.95 (0.79–1.16)
90 μg	Day 29	4.76 (3.38–6.70)	4.16 (2.95–5.86)	3.71 (2.59–5.31)	0.90 (0.70–1.16)
	Day 57	5.49 (3.79–7.95)	5.18 (3.58–7.49)	4.31 (2.93–6.34)	0.85 (0.68–1.07)
	Day 181	4.10 (2.93–5.76)	3.96 (2.80–5.60)	3.07 (2.14–4.42)	0.96 (0.74–1.25)
135 μg	Day 29	4.63 (3.26–6.57)	4.06 (2.86–5.77)	3.60 (2.55–5.07)	1.16 (0.95–1.42)
	Day 57	4.96 (3.65–6.75)	5.51 (4.02–7.54)	3.79 (2.81–5.12)	1.21 (0.94–1.57)
	Day 181	3.60 (2.77–4.67)	5.29 (4.07–6.88)	3.83 (2.96–4.95)	1.31 (1.00–1.72)

Non-adjuvanted RSV F subunit, RSV F subunit-Al, RSV F subunit-MF59, groups receiving the non-adjuvanted, aluminum-adjuvanted and MF59-adjuvanted vaccine formulation, in each cohort; RSV, respiratory syncytial virus; GMR, geometric mean ratio; CI, confidence interval; MF59, 9.75 mg squalene and surfactants.
[a] Results are presented for the per-protocol set Day 29 for Day 29, for the per-protocol set Day 57 for Day 57 and per-protocol set Day 181 for Day 181.

vaccine, which was engineered to preferentially maintain the prefusion conformation (RSV prefusion F protein) [28], with different formulations of a RSV recombinant F nanoparticle vaccine [29] and with another adjuvanted investigational RSV vaccine based on protein F in its post-fusion conformation [13]. In our study, a low percentage of participants had a post-vaccination 4-fold increase in NAb titers compared with baseline. This observation can probably be explained by the relatively large proportion of participants with high titers at baseline. A strong negative effect of pre-vaccination titers on vaccine responses was previously observed following influenza vaccination [30].

No further increase of immune responses was observed after the second dose of the RSV F subunit vaccine, as similar or slightly lower levels of NAb titers were observed at 28 days after the second compared to the first dose for all formulations. This observation could partially be explained by the high levels of preexisting NAb titers as consequence of previous exposure to RSV during lifetime and the antibody levels achieved with the first vaccine dose [31]. Upon administration of the second vaccine dose, the injected F protein antigen is likely bound by circulating antibodies before it can be recognized and internalized by memory B cells to subsequently acquire the necessary T-cell help [31]. A similar poor booster effect has been previously observed in other studies evaluating various formulations of a RSV recombinant F nanoparticle vaccine in pre-exposed participants [29,32].

A decline in immune responses was seen in all study groups during the 6-month follow-up following vaccination. Although no statistically significant differences across treatment groups were observed, immune responses tended to last longer for participants who received the RSV F subunit 135 μg dosage. The observations likely results from the longer persistence of the antigen on follicular dendritic cells, allowing for a longer stimulation of B cells in the germinal centers, and the less pronounced inhibiting effect of preexisting antibodies [31]. The lack of impact of the adjuvant on the persistence of NAb titers has previously been observed for aluminum-adjuvanted RSV prefusion F protein vaccine formulations [28]. This finding, together with our results, suggests that the saturation level may already be reached with the unadjuvanted formulation of the vaccine.

Besides the increases in NAb titers, which are directed against a very specific part of RSV F protein, the RSV subunit vaccine also induced increases in serum total binding antibody titers to RSV F protein, which are directed against a wide variety of non-neutralizing epitopes of this complex protein. The ratios of the RSV F total binding antibody fold changes to the NAb fold changes were >3 in all groups from the RSV F subunit 90 and 135 μg cohorts at all post-vaccination timepoints, and were >2 in the RSV F subunit 45 μg cohort. In the adjuvanted groups of the RSV F subunit 90 and 135 μg cohorts, there was a slight increase in the GMRs of the RSV F total binding antibody fold changes to the NAb fold changes between Day 29 and Day 57 followed by a decrease at Day 181. These results show that the vaccine also induces non-neutralizing antibodies, which was also observed with a recombinant F nanoparticle vaccine [29], with the RSV prefusion F protein vaccine [26] and with another adjuvanted investigational RSV vaccine based on protein F in its post-fusion conformation [13]. No increase in total binding antibody titers to RSV proteins G and N was observed; however, data from seven participants demonstrating 4-fold increases in antibody titers to RSV proteins G and N due to natural infections were excluded from the per-protocol-set.

The immune responses induced by the different formulations of the investigational RSV F subunit vaccine were similar to those induced by a recombinant F nanoparticle vaccine [29] and another adjuvanted investigational RSV vaccine based on protein F in its post-fusion conformation [13], which both failed to prevent RSV disease in older adults. Recently, antibodies to the RSV F protein in its pre-fusion conformation were shown to have greater neutralizing activity than antibodies to the RSV F protein in its post-fusion conformation [33,34]. In particular, antibodies to the site Ø, which are not induced by the RSV F protein in its post-fusion conformation, are more important than those to the site II, which are induced by the RSV F protein in both its pre-fusion and its post-fusion conformation [34–36]. However, the efficacy of the site II–specific antibodies palivizumab and motavizumab suggest that, at least in young children, there is a concentration of anti-site II antibodies that can protect against RSV disease [34].

This study was limited by its observer-blinded design and the small sample size. No formal conclusion could be drawn since this was an exploratory trial, which was not powered for hypotheses testing between dosage groups. A further limitation was the lack of correlates of protection for the assays used in this study.

5. Conclusions

In summary, this phase 1 study showed that the three increasing dosages of the RSV F subunit vaccine, with or without aluminum or MF59 adjuvant, had a clinically acceptable safety profile. A single dose of the RSV F subunit vaccine induced increases in NAb titers, but no booster effect was observed after the second dose. The immune responses were maintained above pre-vaccination levels for at least six months after the first dose.

A plain language summary contextualizing the results and potential clinical research relevance and impact is displayed in the Focus on Patient Section (Fig. 5).

What is the context?

Infants younger than six months are at particular risk to develop severe, sometimes deadly, respiratory syncytial virus (RSV) disease. Consequently, they should acquire protective immunity as early as possible, ideally at birth or soon after. Maternal vaccination programs consist in immunizing women in the course of their pregnancy. As antibodies are transferred from the mother to the child during pregnancy, maternal vaccination could offer a passive protection to newborns and younger infants.

What is new?

A first-in-human phase I study was conducted in men and non-pregnant women of childbearing age to evaluate the safety and immunogenicity of different dosages and formulations of an investigational RSV vaccine. These were well tolerated by the study participants and one dose of the investigational vaccine increased the neutralizing antibody levels for at least six months, although without a significant effect of a second dose administered at one month after the first.

What is the impact?

This investigational RSV vaccine is able to trigger a well-tolerated antibody immune response lasting for at least six months after the first dose in adults. The results from this study supplement the efforts made in immunology and virology from the past decades aiming to develop an RSV vaccine.

Fig. 5. Focus on the Patient.

Acknowledgements

The authors would like to thank all study participants involved in the trials, as well as Jing Yu (statistical analyses, GSK), Annett Kleinschmidt and Lena Dorothee Schütte (immunogenicity analyses, GSK), and XPE Pharma & Science platform c/o GSK (editorial assistance and manuscript coordination). Claire Verbelen provided medical writing support and Quentin Deraedt coordinated manuscript development and provided editorial support.

Conflict of interest

GLR reports receiving a grant paid to his institution from GSK for the conduct of the study. TLN and AGL are employees of the GSK group of companies. TLN holds shares from GSK as part of her employee remuneration. SB served as paid consultant to GSK for the analysis and interpretation of the data. FDB and CM report no potential conflict of interest.

Role of the funding source

The trial was sponsored by Novartis Vaccines Division. On 02 March 2015, Novartis non-influenza Vaccines Business was acquired by the GlaxoSmithKline group of companies. The Sponsor was involved in all stages of the study conduct and analysis and took charge of all costs associated with developing and publishing this manuscript.

Funding

GlaxoSmithKline Biologicals SA.

Authors' contribution

GLR performed the study, collected and interpreted study data. FDB and CM performed the study and collected study data. TLN collected and interpreted study data. SB interpreted study data. AGL conceived the study, performed the study, collected and interpreted study data.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.04.011.

References

[1] Simoes EA. Respiratory syncytial virus infection. Lancet 1999;354:847–52. https://doi.org/10.1016/S0140-6736(99)80040-3.
[2] Hall CB. Respiratory syncytial virus and parainfluenza virus. N Engl J Med 2001;344:1917–28. https://doi.org/10.1056/NEJM200106213442507.
[3] Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med 2009;360:588–98. https://doi.org/10.1056/NEJMoa0804877.
[4] Iwane MK, Edwards KM, Szilagyi PG, Walker FJ, Griffin MR, Weinberg GA, et al. Population-based surveillance for hospitalizations associated with respiratory syncytial virus, influenza virus, and parainfluenza viruses among young children. Pediatrics 2004;113:1758–64. https://doi.org/10.1542/peds.113.6.1758.
[5] Shi T, McAllister DA, O'Brien KL, Simoes EAF, Madhi SA, Gessner BD, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet 2017;390:946–58. https://doi.org/10.1016/S0140-6736(17)30938-8.
[6] Hogan AB, Glass K, Moore HC, Anderssen RS. Exploring the dynamics of respiratory syncytial virus (RSV) transmission in children. Theor Popul Biol 2016;110:78–85. https://doi.org/10.1016/j.tpb.2016.04.003.
[7] Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. Am J Dis Child 1986;140:543–6. https://doi.org/10.1001/archpedi.1986.02140200053026.
[8] Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010;375:1545–55. https://doi.org/10.1016/S0140-6736(10)60206-1.
[9] Jorquera PA, Tripp RA. Respiratory syncytial virus: prospects for new and emerging therapeutics. Expert Rev Respir Med 2017;11:609–15. https://doi.org/10.1080/17476348.2017.1338567.
[10] Prescott Jr WA, Doloresco F, Brown J, Paladino JA. Cost effectiveness of respiratory syncytial virus prophylaxis: a critical and systematic review.

Pharmacoeconomics 2010;28:279–93. https://doi.org/10.2165/11531860-000000000-00000.
[11] Modjarrad K, Giersing B, Kaslow DC, Smith PG, Moorthy VS, WHO RSV Vaccine Consultation Expert Group. WHO consultation on respiratory syncytial virus vaccine development report from a World Health Organization meeting held on 23-24 March 2015. Vaccine 2016;34:190–7. https://doi.org/10.1016/j.vaccine.2015.05.093.
[12] Graham BS. Biological challenges and technological opportunities for respiratory syncytial virus vaccine development. Immunol Rev 2011;239:149–66. https://doi.org/10.1111/j.1600-065X.2010.00972.x.
[13] Falloon J, Yu J, Esser MT, Villafana T, Yu L, Dubovsky F, et al. An adjuvanted, postfusion F protein-based vaccine did not prevent respiratory syncytial virus illness in older adults. J Infect Dis 2017;216:1362–70. https://doi.org/10.1093/infdis/jix503.
[14] van Drunen Littel-van den Hurk S, Watkiss ER. Pathogenesis of respiratory syncytial virus. Curr Opin Virol 2012;2:300–5. https://doi.org/10.1016/j.coviro.2012.01.008.
[15] Glezen WP, Paredes A, Allison JE, Taber LH, Frank AL. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. J Pediatr 1981;98:708–15. https://doi.org/10.1016/S0022-3476(81)80829-3.
[16] Mazur NI, Horsley N, Englund JA, Nederend M, Magaret A, Kumar A, et al. Breast milk prefusion F IgG as a correlate of protection against respiratory syncytial virus acute respiratory illness. J Infect Dis 2018. https://doi.org/10.1093/infdis/jiy477.
[17] Ochola R, Sande C, Fegan G, Scott PD, Medley GF, Cane PA, et al. The level and duration of RSV-specific maternal IgG in infants in Kilifi Kenya. PLoS ONE 2009;4:e8088. https://doi.org/10.1371/journal.pone.0008088.
[18] Ogilvie MM, Vathenen AS, Radford M, Codd J, Key S. Maternal antibody and respiratory syncytial virus infection in infancy. J Med Virol 1981;7:263–71. https://doi.org/10.1002/jmv.1890070403.
[19] Piedra PA, Jewell AM, Cron SG, Atmar RL, Glezen WP. Correlates of immunity to respiratory syncytial virus (RSV) associated-hospitalization: establishment of minimum protective threshold levels of serum neutralizing antibodies. Vaccine 2003;21:3479–82. https://doi.org/10.1016/S0264-410X(03)00355-4.
[20] Amirthalingam G, Campbell H, Ribeiro S, Fry NK, Ramsay M, Miller E, et al. Sustained effectiveness of the maternal pertussis immunization program in England 3 years following introduction. Clin Infect Dis 2016;63:S236–43. https://doi.org/10.1093/cid/ciw559.
[21] Demicheli V, Barale A, Rivetti A. Vaccines for women to prevent neonatal tetanus. Cochrane Database Syst Rev 2013. https://doi.org/10.1002/14651858.CD002959.pub3. CD002959.
[22] Lambach P, Hombach J, Ortiz JR. A global perspective of maternal influenza immunization. Vaccine 2015;33:6376–9. https://doi.org/10.1016/j.vaccine.2015.08.036.
[23] Gonzalez PA, Bueno SM, Carreno LJ, Riedel CA, Kalergis AM. Respiratory syncytial virus infection and immunity. Rev Med Virol 2012;22:230–44. https://doi.org/10.1002/rmv.1704.
[24] McLellan JS, Ray WC, Peeples ME. Structure and function of respiratory syncytial virus surface glycoproteins. Curr Top Microbiol Immunol 2013;372:83–104. https://doi.org/10.1007/978-3-642-38919-1_4.
[25] Swanson KA, Settembre EC, Shaw CA, Dey AK, Rappuoli R, Mandl CW, et al. Structural basis for immunization with postfusion respiratory syncytial virus fusion F glycoprotein (RSV F) to elicit high neutralizing antibody titers. Proc Natl Acad Sci USA 2011;108:9619–24. https://doi.org/10.1073/pnas.1106536108.
[26] Beran J, Lickliter JD, Schwarz TF, Johnson C, Chu L, Domachowske JB, et al. Safety and immunogenicity of 3 formulations of an investigational respiratory syncytial virus vaccine in nonpregnant women: results from 2 phase 2 trials. J Infect Dis 2018;217:1616–25. https://doi.org/10.1093/infdis/jiy065.
[27] Pellegrini M, Nicolay U, Lindert K, Groth N, Della Cioppa G. MF59-adjuvanted versus non-adjuvanted influenza vaccines: integrated analysis from a large safety database. Vaccine 2009;27:6959–65. https://doi.org/10.1016/j.vaccine.2009.08.101.
[28] Langley JM, Aggarwal N, Toma A, Halperin SA, McNeil SA, Fissette L, et al. A randomized, controlled, observer-blinded phase 1 study of the safety and immunogenicity of a respiratory syncytial virus vaccine with or without alum adjuvant. J Infect Dis 2017;215:24–33. https://doi.org/10.1093/infdis/jiw453.
[29] Glenn GM, Fries LF, Thomas DN, Smith G, Kpamegan E, Lu H, et al. A randomized, blinded, controlled, dose-ranging study of a respiratory syncytial virus recombinant Fusion (F) nanoparticle vaccine in healthy women of childbearing age. J Infect Dis 2016;213:411–22. https://doi.org/10.1093/infdis/jiv406.
[30] Olafsdottir TA, Alexandersson KF, Sveinbjornsson G, Lapini G, Palladino L, Montomoli E, et al. Age and influenza-specific pre-vaccination antibodies strongly affect influenza vaccine responses in the Icelandic population whereas disease and medication have small effects. Front Immunol 2017;8:1872. https://doi.org/10.3389/fimmu.2017.01872.
[31] Zhang Y, Meyer-Hermann M, George LA, Figge MT, Khan M, Goodall M, et al. Germinal center B cells govern their own fate via antibody feedback. J Exp Med 2013;210:457–64. https://doi.org/10.1084/jem.20120150.
[32] Glenn GM, Smith G, Fries L, Raghunandan R, Lu H, Zhou B, et al. Safety and immunogenicity of a Sf9 insect cell-derived respiratory syncytial virus fusion protein nanoparticle vaccine. Vaccine 2013;31:524–32. https://doi.org/10.1016/j.vaccine.2012.11.009.
[33] Capella C, Chaiwatpongsakorn S, Gorrell E, Risch ZA, Ye F, Mertz SE, et al. Prefusion F, postfusion F, G antibodies, and disease severity in infants and young children with acute respiratory syncytial virus infection. J Infect Dis 2017;216:1398–406. https://doi.org/10.1093/infdis/jix489.
[34] Ngwuta JO, Chen M, Modjarrad K, Joyce MG, Kanekiyo M, Kumar A, et al. Prefusion F-specific antibodies determine the magnitude of RSV neutralizing activity in human sera. Sci Transl Med 2015;7. https://doi.org/10.1126/scitranslmed.aac4241. 309ra162.
[35] Widjaja I, Wicht O, Luytjes W, Leenhouts K, Rottier PJM, van Kuppeveld FJM, et al. Characterization of epitope-specific anti-respiratory syncytial virus (anti-RSV) antibody responses after natural infection and after vaccination with formalin-inactivated RSV. J Virol 2016;90:5965–77. https://doi.org/10.1128/JVI.00235-16.
[36] Phung E, Chang LA, Morabito KM, Kanekiyo M, Chen M, Nair D, et al. Epitope-specific serological assays for RSV: conformation matters. Vaccines (Basel) 2019;7. https://doi.org/10.3390/vaccines7010023.