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MAJOR ARTICLE

Safety and Immunogenicity of an Investigational Respiratory Syncytial Virus Vaccine (RSVPreF3) in Mothers and Their Infants: A Phase 2 Randomized Trial

Zourab Bebia,[1] Osvaldo Reyes,[2] Robert Jeanfreau,[3] Anu Kantele,[4] Ruth Graciela De Leon,[5] Marta García Sánchez,[6] Peyman Banooni,[7] Glenn J. Gardener,[8] José Luis Bartha Rasero,[9] Maria Begoña Encinas Pardilla,[10] Joanne M. Langley,[11] Claudio Maañón Di Leo,[12] Elisabeth Botelho-Nevers,[13] Jim Buttery,[14] Helene Laurichesse,[15] Shabir A. Madhi,[16] Adrián Martín García,[17] Thorsten Stanley,[18] Tiphaine Barjat,[19] Rebecca Griffith,[20] Maria Mercedes Castrejón-Alba,[21] Magali de Heusch,[22] Ilse Dieussaert,[23] Melanie Hercor,[22] Patricia Lese,[1] Hui Qian,[1] Antonella N. Tullio,[1] and Ouzama Henry[1,a]

[1]GSK, Rockville, Maryland, USA; [2]International Vaccination Centre, National Network of Researchers of Panama, Panama, Panama; [3]MedPharmics, Metairie, Louisiana, USA; [4]Meilahti Vaccine Research Centre, Inflammation Centre, Helsinki University Hospital and University of Helsinki, Helsinki, Finland; [5]Institute Conmemorativo Gorgas de Estudios de la Salud, Panama, Panama; [6]Hospital Quironsalud Málaga, Málaga, Spain; [7]Matrix Clinical Research, Los Angeles, California, USA; [8]Mater Research Institute, University of Queensland, South Brisbane, Australia; [9]Hospital Universitario La Paz, Madrid, Spain; [10]Hospital Puerta de Hierro Majadahonda, Majadahonda, Spain; [11]Canadian Center for Vaccinology, IWK Health Centre, Nova Scotia Health Authority–Dalhousie University, Halifax, Nova Scotia, Canada; [12]Hospital Costa del Sol, Marbella, Spain; [13]Infectious and Tropical Diseases Department, CIC 1408 INSERM, Centre Hospitalier Universitaire de Saint-Etienne, Saint-Etienne, France; [14]Infection and Immunity Department, Monash Children's Hospital, Melbourne, Victoria, Australia; [15]Centre Hospitalier Universitaire Clermont-Ferrand, Clermont Ferrand, France; [16]South African Medical Research Council Vaccines and Infectious Diseases Analytics Research Unit, Infectious Diseases and Oncology Research Institute, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [17]Department of Obstetrics and Gynecology, Hospital Universitario de Burgos, Burgos, Spain; [18]University of Otago and Wellington Hospital, Wellington, New Zealand; [19]Department of Gynecology and Obstetrics, CIC 1408 INSERM, Centre Hospitalier Universitaire de Saint-Etienne, Saint-Etienne, France; [20]Optimal Clinical Trials, Auckland, New Zealand; [21]GSK, Panama, Panama; [22]GSK, Wavre, Belgium; and [23]GSK, Rixensart, Belgium

Background. In a phase 1/2 study, a maternal respiratory syncytial virus vaccine candidate (RSVPreF3) demonstrated an acceptable safety profile and efficiently increased RSV-specific humoral immune responses in non-pregnant women.

Methods. In this phase 2 observer-blind, placebo-controlled, randomized clinical trial (NCT04126213), the safety of RSVPreF3 (60 or 120 μg), administered during late second or third trimester, was evaluated in 213 18- to 40-year-old healthy pregnant women through 6 months postdelivery and their offspring through infancy; immunogenicity was evaluated through day 43 postdelivery and day 181 postbirth, respectively.

Results. RSVPreF3 was well tolerated. No pregnancy-related or neonatal adverse events of special interest were considered vaccine/placebo related. In the 60 and 120 μg RSVPreF3 groups: (1) neutralizing antibody (nAb) titers in mothers increased 12.7- and 14.9-fold against RSV-A and 10.6- and 13.2-fold against RSV-B, respectively, 1 month postvaccination and remained 8.9–10.0-fold over prevaccination at day 43 postdelivery; (2) nAb titers were consistently higher compared to placebo recipients; (3) placental transfer ratios for anti-RSVPreF3 antibodies at birth were 1.62 and 1.90, respectively, and (4) nAb levels in infants were highest at birth and declined through day 181 postbirth.

Conclusions. RSVPreF3 maternal vaccination had an acceptable safety risk profile and induced robust RSV-specific immune responses with successful antibody transfer to their newborns.

Clinical Trials Registration. NCT04126213.

Keywords. fetal care; humoral immunity; maternal immunization; neonatal care; passive immunization; placental transfer; pregnancy; preterm birth; RSV.

Lay Summary. ***What Is the Context?*** Infants, especially those less than 6 months of age, are at increased risk of lung infection caused by respiratory syncytial virus (RSV). However, this risk could be reduced with maternal vaccination against RSV during pregnancy. A previous clinical trial found that a vaccine candidate (named RSVPreF3) was well tolerated when given to non-pregnant women.

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[a]Current affiliation: Dynavax Technologies, Emeryville, California, USA.

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Correspondence: Z. Bebia, MD, GSK, 14200 Shady Grove Rd, Rockville, MD 20850 (zourab.x.bebia@gsk.com).

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What is New? In pregnant women, RSVPreF3 was also well tolerated. Occurrence of unsolicited adverse events was similar between vaccine and placebo recipients. None of the serious adverse events or events of interest for pregnant women or newborns were considered related to the study intervention. One month after vaccination, mothers who received RSVPreF3 had 11–15 times higher levels of antibodies against RSV than before vaccination. These antibody levels remained similar until 43 days after delivery. In the infants born to mothers vaccinated during pregnancy with RSVPreF3, antibody levels were highest at birth, when levels were higher than in their mothers, and declined through day 181 postbirth.

What Is the Impact? RSVPreF3 had an acceptable safety risk profile in pregnant women and their babies. This vaccine induced potent immune responses against RSV, with maternal antibodies transferred to infants of the vaccinated mothers.

INTRODUCTION

In children <5 years of age, lower respiratory tract infections (LRTI) caused by respiratory syncytial virus (RSV) are associated with a substantial health burden. Globally, RSV infections in this age group lead to more than 3 million hospitalizations and almost 120 000 deaths annually. Of these hospitalizations and in-hospital deaths, 44% and 46%, respectively, occur in infants up to 6 months of age [1].

Currently, there are no licensed vaccines for the prevention of RSV disease. An anti-RSV F monoclonal antibody (palivizumab) is the only licensed prophylactic intervention with established efficacy. However, it is recommended only for infants known to be at highest risk of complicated RSV disease, requires monthly dosing, and its cost hampers broader implementation [2–5]. As nearly half of the burden of severe RSV disease among children is borne by newborns and infants in their first months of life [1, 6, 7], passive immunization through maternal vaccination may be an effective strategy for prevention of severe disease during early infancy. With several other vaccines, such as those against influenza, pertussis, diphtheria, tetanus, and more recently coronavirus (COVID-19), maternal immunization has already proven to have an acceptable safety profile and to be effective in preventing diseases both in mothers and in their infants [8–10].

In a phase 1/2 study, 2 dose levels (60 and 120 µg) of an RSV prefusion protein vaccine candidate (RSVPreF3) demonstrated an acceptable safety profile and efficiently increased preimmunization RSV-specific antibody levels and neutralizing antibody (nAb) titers in non-pregnant women [11]. Studies that investigated other maternal RSV protein vaccines showed an efficient transfer of vaccine-induced RSV-specific antibodies from the mother to the infant [12–14], with passive immunity conferred to the infants [8, 9].

In this phase 2 study, we investigated the safety and immunogenicity of the RSVPreF3 vaccine given to pregnant women. We also evaluated RSV-specific antibody levels and nAb titers in infants born to vaccinated mothers.

METHODS

Study Design and Participants

This phase 2 observer-blind, placebo-controlled, randomized clinical trial was conducted between 5 November 2019 and 14 May 2021 in Australia, Canada, Finland, France, New Zealand, Panama, South Africa, Spain, and the United States. Eligible participants were healthy women aged 18–40 years at enrollment, with a singleton pregnancy and no reported significant congenital malformations or genetic abnormalities of the fetus. The main exclusion criteria were significant complications in the current pregnancy, a prior history of preterm birth, stillbirth, neonatal death, or ≥2 spontaneous abortions, various acute viral infections or tuberculosis, poorly controlled comorbidities, prior receipt of an RSV vaccine, and immunodeficiency/immunosuppression resulting from a disease or medical therapy. The full list of inclusion/exclusion criteria is provided in the Supplementary Material. Eligible infants had to be in the care of their parent(s) or appointed legal guardian and could not participate in other interventional clinical trials concomitantly.

One screening visit and 8 study visits were foreseen for mothers and 6 visits for their infants, including the delivery visit (Figure 1). Study participants were randomized 1:1:1 to receive 60 or 120 µg of unadjuvanted RSVPreF3 vaccine or placebo on study day 1, between $28^{0/7}$ and $33^{6/7}$ weeks of gestation. The algorithm used a minimization procedure accounting for maternal age (≥18 to <35; ≥35 years), gestational age ($28^{0/7}$–$31^{0/7}$; $31^{1/7}$–$33^{6/7}$ weeks), and center at the time of vaccination. In each of the 3 treatment groups, the infants to be born were also randomized 1:1:1 for blood sampling for immunogenicity assessments at newborn visits 2, 3, or 4 (i.e., 1 sample collected from each infant).

Before enrollment, all participants provided written or witnessed/thumb printed informed consent for their own and their infant's participation. Re-consent was also obtained for infants after birth where local regulations required this. The study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice and is registered on ClinicalTrials.gov (NCT04126213). The protocol was reviewed and approved by the relevant independent ethics committees or institutional review boards, and is available at https://www.gsk-studyregister.com (ID 209544). Anonymized individual participant data and study documents can be requested for further research use at https://www.clinicalstudydatarequest.com.

Objectives

The primary safety objectives were the evaluation of the reactogenicity and the safety of a single RSVPreF3 dose, including pregnancy-related and neonatal adverse events of special

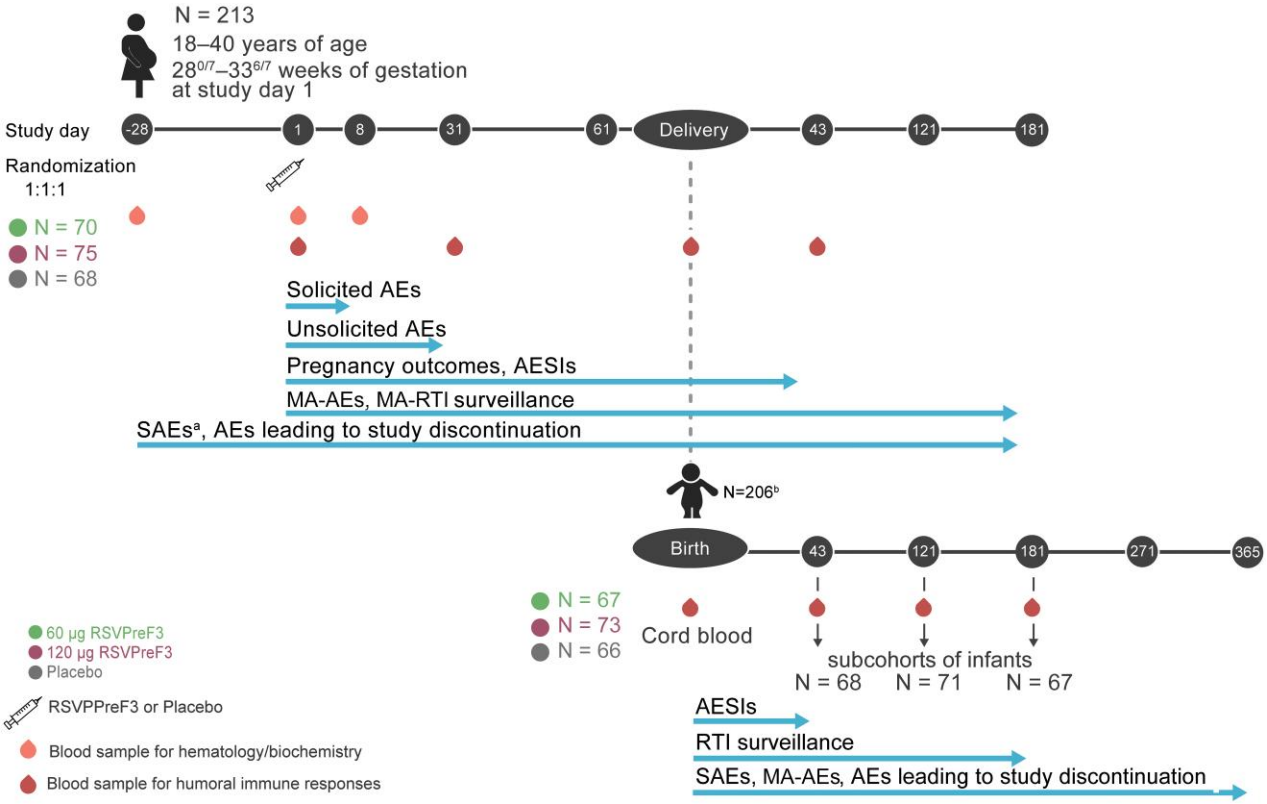

Figure 1. Study design. Abbreviations: AE, adverse event; AESI, adverse event of special interest; MA-AE, medically attended AE; MA-RTI, medically attended respiratory tract infection; N, number of participants; RSVPreF3, respiratory syncytial virus prefusion protein vaccine; RTI, respiratory tract infection; SAE, serious adverse event. [a]Only SAEs related to study participation were recorded from day −28 until vaccination; from vaccination through study end, all SAEs were recorded; [b]207 infants were delivered but 1 woman refused infant participation.

interest (AESI) as well as pregnancy outcomes up to 6 weeks after birth. Secondary safety objectives included the evaluation of safety in vaccinated mothers through 6 months postdelivery and in infants through 1 year, and the occurrence of RSV-associated medically attended respiratory tract infections (MA-RTIs) in mothers as well as RSV-associated hospitalizations and LRTIs in infants through 6 months from birth.

Primary immunogenicity objectives comprised the evaluation of the humoral immune response in terms of anti-RSVPreF3 immunoglobulin G (IgG) antibodies and RSV-A nAbs in mothers at prevaccination, day 31 postvaccination, and delivery, and in their infants at birth (through evaluation of cord blood), and determination of the placental transfer ratio for anti-RSVPreF3 IgG at birth. Evaluation of anti-RSVPreF3 IgG antibodies and RSV-A nAbs in mothers at day 43 postdelivery as well as of RSV-B nAbs at prevaccination, day 31 postvaccination, delivery, and day 43 postdelivery, and evaluation of RSV-B nAbs in infants at birth as well as anti-RSVPreF3 IgG antibodies and RSV-A and RSV-B nAbs in infants at days 43, 121, and 181 postbirth were secondary immunogenicity objectives.

Study Vaccine

The RSVPreF3 antigen is an engineered version of the RSV fusion protein with a previously used sequence [15] stabilized in its prefusion conformation by the introduction of cysteine residues, leading to the formation of a disulfide bond, and by the filling of hydrophobic cavities [16, 17]. Participants received 1 dose of 60 or 120 µg unadjuvanted RSVPreF3 vaccine or a placebo (saline solution) intramuscularly. The vaccine and placebo differed in appearance, and the vaccine also needed reconstitution. Participants were not aware of allocation and the vaccine/placebo was administered by staff not involved in outcome assessment to avoid any potential biases.

Outcomes and Assessments

Assessment of Safety and RTIs

After vaccination, mothers were observed for 60 minutes for any reactions. Solicited (administration site and systemic) events and unsolicited adverse events (AEs) were recorded for 7 and 30 days postvaccination, respectively (Figure 1). The intensity was graded as 0 (none, only for solicited AEs), 1 (mild), 2 (moderate), and 3 (severe). Grade 3 was defined

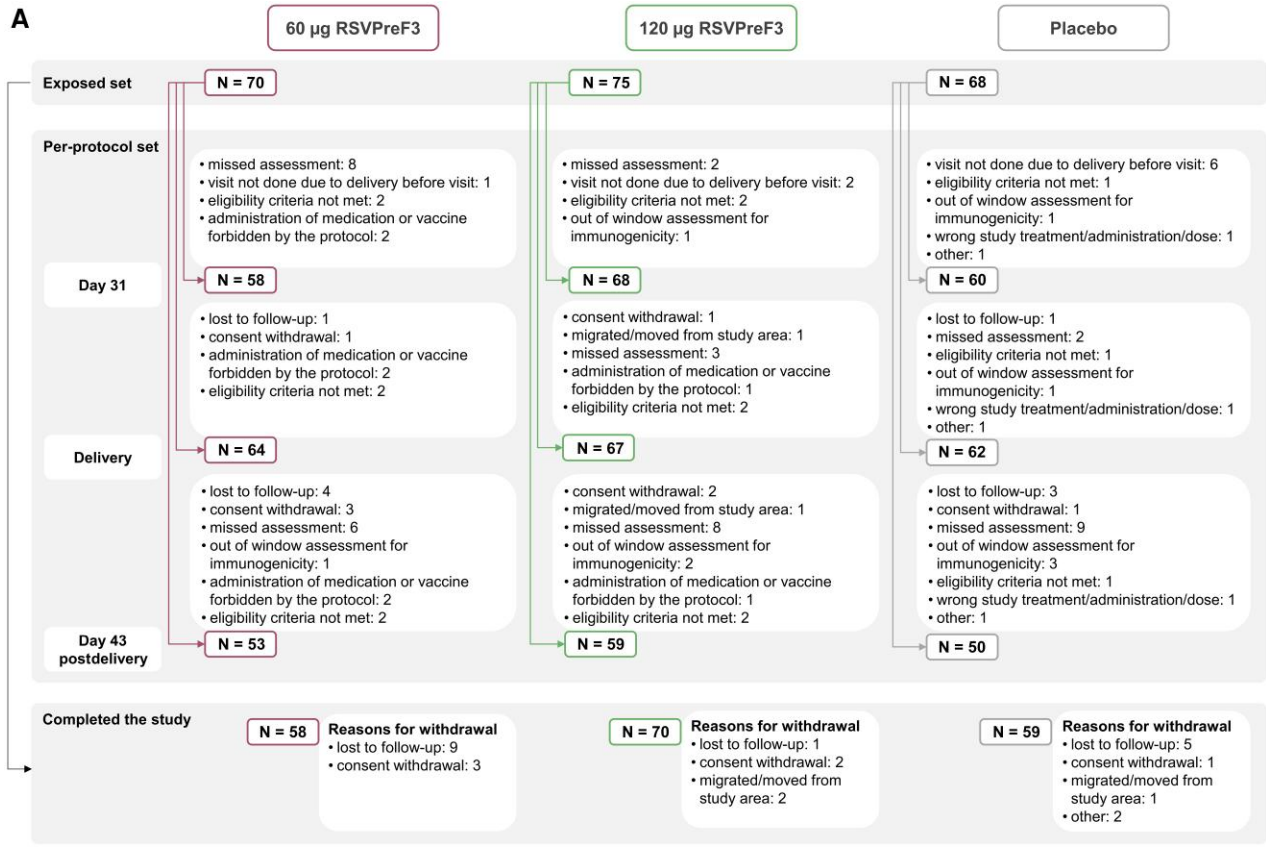

Figure 2. Flow diagram of mothers (*A*) and infants (*B*) participating in the study. Note, some participants were assigned >1 elimination code. Abbreviation: N, number of participants; RSVPreF3, respiratory syncytial virus prefusion protein vaccine.

as significant injection site pain at rest, injection site redness/swelling >100 mm in diameter, fever >39°C, and for all the other AEs preventing normal life or daily activity. The time periods for recording medically attended AEs (MA-AEs), serious AEs (SAEs), AEs leading to discontinuation of the study, pregnancy outcomes, and AESIs (i.e., events affecting the newborn or the pregnancy, comprising adverse pregnancy outcomes as well as pregnancy-related and neonatal AESIs) are shown in Figure 1. AESIs were collected following case definitions from the Global Alignment of Immunization Safety Assessment in Pregnancy [18].

All solicited injection site and systemic events were considered to be causally related to the vaccine/placebo. The causal relatedness to the vaccine/placebo for all other AEs was determined by the blinded investigator.

Hematological and biochemical parameters were measured from blood samples collected at the screening visit and visits on days 1 and 8 (Figure 1). For determining the presence of RSV-A or RSV-B, nasal swabs were collected from mothers in case of MA-RTI (including hospitalization) or from infants in case of RTI episodes and hospitalizations. Surveillance for MA-RTI in mothers was undertaken between the start of the study and day 181 postdelivery, and RTI surveillance in infants was undertaken between birth and day 181 postbirth (Figure 1).

Assessment of Immunogenicity

Maternal immune responses were assessed from blood samples collected at days 1 (prevaccination) and 31, delivery, and day 43 postdelivery. Infant immune responses were assessed from cord blood collected at delivery (or the infant's blood sample collected within 3 days postbirth if cord blood was not obtained) and blood samples collected from randomly selected infants at days 43, 121, or 181 postbirth.

RSV-A and RSV-B nAb titers were determined using an in-house RSV serum neutralization assay, described in greater detail in the Supplementary Material. The assay cut-off was 18 estimated dilution 60 (ED60) for RSV-A and 30 ED60 for RSV-B.

Anti-RSVPreF3 IgG antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) using a cut-off of 25 ELISA units (EU)/mL. Additional details are provided in the Supplementary Material.

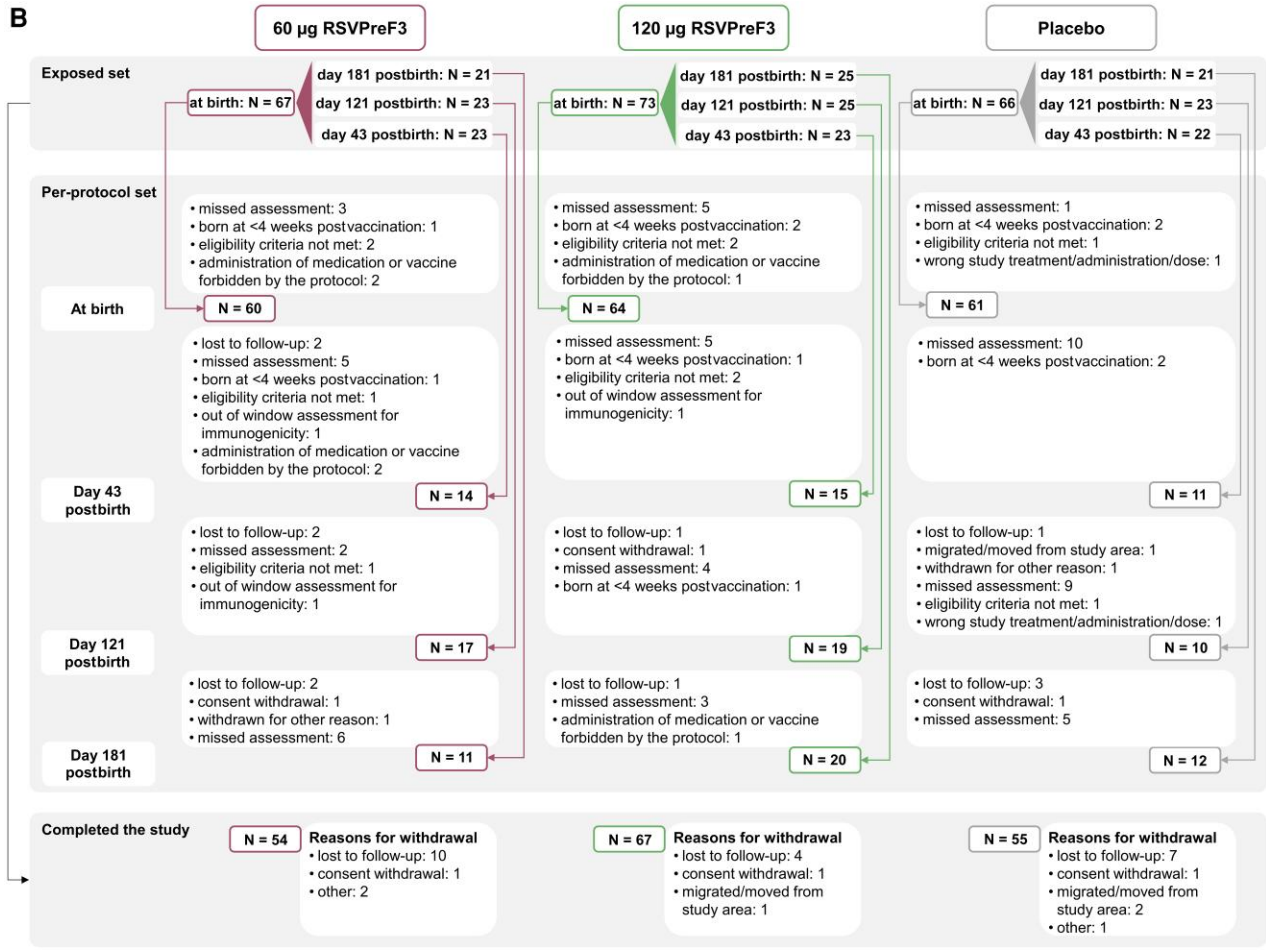

Figure 2. Continued

Statistical Analyses

Sample Size Considerations

In total, up to 300 mothers were planned to be randomized to achieve up to 90 evaluable participants per study group. Additional details are provided in the Supplementary Material.

Safety

Safety was assessed descriptively in the exposed set, comprising mothers who received the study intervention and live-born infants of exposed mothers. Reactogenicity was assessed in mothers from the exposed set with available data. For each applicable safety outcome, the percentage of mothers and/or infants was tabulated along with its exact 2-sided 95% confidence interval (CI).

Immunogenicity

Immunogenicity was assessed in the per-protocol set, comprising mothers who received the study intervention, had postvaccination immunogenicity data, and complied with the requirements of the protocol. Infants included in the per-protocol set were those who were born to exposed mothers ≥4 weeks after post-maternal vaccination, had available postbirth immunogenicity data, and complied with the requirements of the protocol. Geometric mean titers (GMTs) for RSV-A and RSV-B nAbs and geometric mean concentrations (GMCs) for anti-RSVPreF3 IgG antibodies were determined by taking the anti-log of the mean of the log titer transformations. For results below the assay cut-off, an arbitrary value of half the cut-off was considered for the GMT and GMC calculations. To determine fold increases versus pre-vaccination, the geometric mean ratios (GMR) of titers and concentrations were determined. GMTs, GMCs, GMRs, and GMs of placental transfer ratios were calculated with their 2-sided 95% CIs. Additional statistical considerations for the evaluation of immunogenicity are presented in the Supplementary Material. All statistical analyses were performed using the Statistical Analysis Systems Life Science Analytics Framework software.

RESULTS

Study Participants

In total, 328 participants signed the informed consent form, of whom 115 withdrew prior to randomization. Enrollment was

Table 1. Summary of Infant Demography and Baseline Characteristics—Exposed Set

Characteristic	60 µg RSVPreF3 (N = 67)	120 µg RSVPreF3 (N = 73)	Placebo (N = 66)
Gestational age at birth, wk, mean ± SD	39.2 ± 1.2	39.3 ± 1.4	39.2 ± 1.2
Premature birth, ≤37 wk gestational age	2 (3.0)	3 (4.1)	3 (4.5)
Sex			
Male	39 (58.2)	43 (58.9)	29 (43.9)
Female	28 (41.8)	30 (41.1)	37 (56.1)
Race			
American Indian or Alaska Native	0 (0)	1 (1.4)	0 (0)
Asian	0 (0)	0 (0)	1 (1.5)
Black or African American	12 (17.9)	9 (12.3)	9 (13.6)
Native Hawaiian or other Pacific Islander	3 (4.5)	2 (2.7)	1 (1.5)
White	42 (62.7)	49 (67.1)	46 (69.7)
Other	10 (14.9)	11 (15.1)	8 (12.1)
Unknown	0 (0)	1 (1.4)	1 (1.5)
Length, cm, mean ± SD	49.8 ± 2.9	49.7 ± 2.3	50.0 ± 1.9
Weight, kg, mean ± SD	3.2 ± 0.4	3.2 ± 0.5	3.3 ± 0.4
Head circumference, cm, mean ± SD	34.2 ± 1.5	33.9 ± 1.6	34.1 ± 1.7
Breast feeding status at day 43			
Mother breastfeeds and supplements with formula	12 (17.9)	20 (27.4)	15 (22.7)
Mother breastfeeds only	32 (47.8)	37 (50.7)	32 (48.5)
Mother doesn't breastfeed	17 (25.4)	14 (19.2)	15 (22.7)
Unknown	6 (9.0)	2 (2.7)	4 (6.1)
Apgar score at 5 min, mean ± SD	9.0 ± 0.7	9.0 ± 0.7	9.1 ± 0.7
Passive smoking			
Yes	5 (7.5)	7 (9.6)	3 (4.5)
Unknown	62 (92.5)	66 (90.4)	63 (95.5)
Contact with children younger than 6 y			
Yes	35 (52.2)	35 (47.9)	39 (59.1)
No	31 (46.3)	38 (52.1)	25 (37.9)
Unknown	1 (1.5)	0 (0)	2 (3.0)

Data are No. (% of infants in a given category) except where indicated.

Abbreviations: N, maximum number of infants with data; RSVPreF3, respiratory syncytial virus prefusion protein vaccine; SD, standard deviation; wk, week; y, year.

Table 2. Summary of Maternal Demography and Baseline Characteristics—Exposed Set

Characteristic	60 µg RSVPreF3 (N = 70)	120 µg RSVPreF3 (N = 75)	Placebo (N = 68)
Age at vaccination, y, mean ± SD	27.5 ± 5.8	28.2 ± 6.0	28.2 ± 5.8
Age category			
18–<35 y	59 (84.3)	62 (82.7)	56 (82.4)
≥35 y	11 (15.7)	13 (17.3)	12 (17.6)
Gestational age at vaccination, wk			
$<28^{0/7}$	0 (0)	2 (2.7)	1 (1.5)
$28^{0/7}$–$31^{0/7}$	32 (45.7)	32 (42.7)	31 (45.6)
$31^{1/7}$–$33^{6/7}$	37 (52.9)	41 (54.7)	35 (51.5)
$>33^{6/7}$	1 (1.4)	0 (0)	1 (1.5)
Race			
American Indian or Alaska Native	0 (0)	2 (2.7)	0 (0)
Asian	0 (0)	0 (0)	2 (2.9)
Black or African American	12 (17.1)	12 (16.0)	13 (19.1)
Native Hawaiian or other Pacific Islander	3 (4.3)	1 (1.3)	0 (0)
White	45 (64.3)	48 (64.0)	45 (66.2)
Other	10 (14.3)	10 (13.3)	7 (10.3)
Unknown	0 (0)	2 (2.7)	1 (1.5)
Ethnicity			
Hispanic or Latino	21 (30.0)	27 (36.0)	26 (38.2)
Not Hispanic or Latino	47 (67.1)	46 (61.3)	41 (60.3)
Unknown	2 (2.9)	2 (2.7)	1 (1.5)
BMI, kg/m^2, mean ± SD	29.8 ± 5.4	27.4 ± 4.6	28.6 ± 4.6
Time interval between vaccination and delivery, d, mean ± SD	56.6 ± 15.0	57.8 ± 15.8	56.7 ± 15.8
Mode of delivery			
Elective cesarean delivery	1 (1.4)	2 (2.7)	3 (4.4)
Planned cesarean delivery	6 (8.6)	9 (12.0)	3 (4.4)
Unplanned cesarean delivery	6 (8.6)	16 (21.3)	8 (11.8)
Vaginal, instrumental	5 (7.2)	2 (2.7)	6 (8.8)
Vaginal, unassisted	50 (71.4)	44 (58.7)	47 (69.1)
Unknown	2 (2.9)	2 (2.7)	1 (1.5)

Data are No. (% of mothers in a given category) except where indicated.

Abbreviations: BMI, body mass index; d, day; N, maximum number of mothers with data; RSVPreF3, respiratory syncytial virus prefusion protein vaccine; SD, standard deviation; wk, week; y, year.

stopped prematurely due to COVID-19 pandemic-related country/site restrictions. Of the 213 randomized and vaccinated mothers, 187 completed the study (Figure 2*A*). Of the 206 infants included in the exposed set, 176 completed the study (Figure 2*B*). The mean gestational age at birth was 39.2–39.3 weeks across groups, and most infants were breastfed (Table 1). The demographic characteristics of the infants and mothers were similar between groups (Table 1 and Table 2).

Reactogenicity and Safety

Pain was the most frequently reported solicited administration site event (Figure 3). Administration site erythema and swelling were only reported in the RSVPreF3 groups, by ≤6.7% of the mothers. Grade 3 solicited administration site events were reported by at most 1 mother (1.4%) in each vaccine group. The mean duration of any solicited administration site event was ≤4 days.

Fatigue was the most frequently reported solicited systemic event, followed by headache and nausea (Figure 3). Grade 3 fatigue, headache, or nausea was reported by at most 2 mothers (2.7%) in each vaccine group. No instances of solicited fever were reported. The mean duration of any solicited systemic event was ≤2.7 days.

Unsolicited AEs were reported by 30.0%–33.8% of mothers, and grade 3 unsolicited AEs were reported by at most 3 (4.0%) mothers in each group (Figure 4).The most common unsolicited AEs were oropharyngeal pain, reported by 1 (1.4%), 3 (4.0%), and 2 (2.9%) mothers, nasopharyngitis, reported by

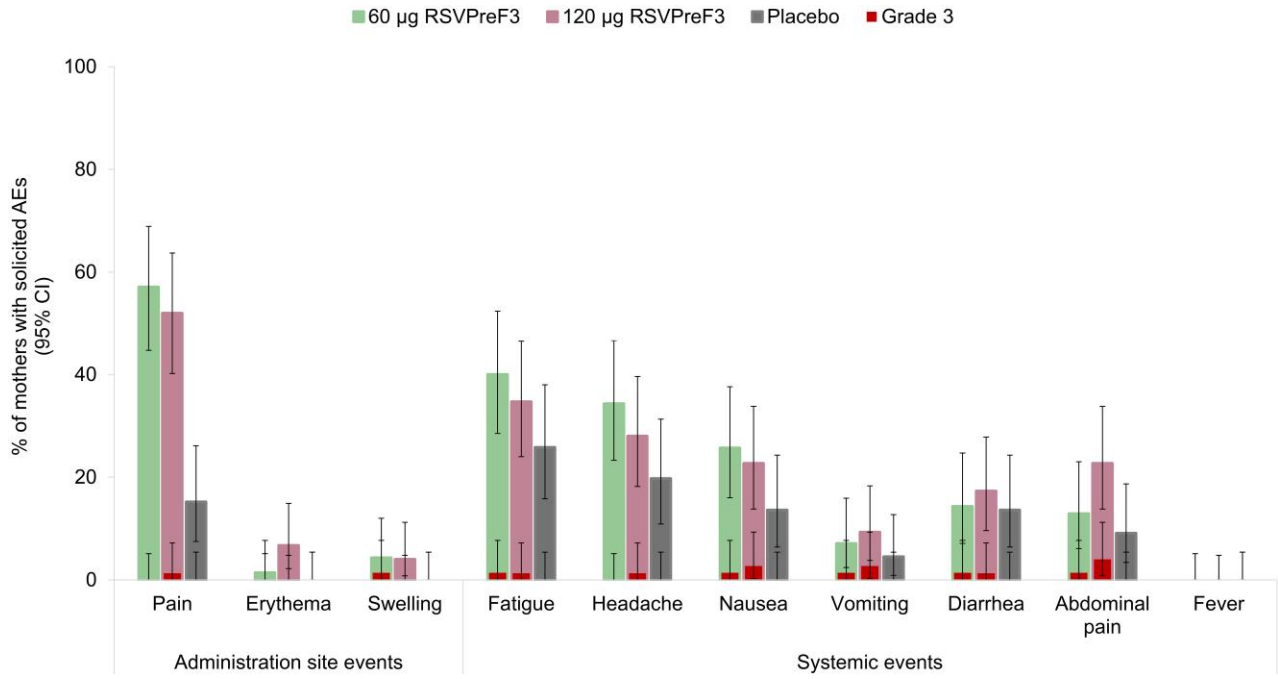

Figure 3. Solicited administration site and systemic events postvaccination. Note, while no solicited fever was reported, there was an instance of fever as part of a serious adverse event of pyelonephritis within 7 days postvaccination. Abbreviations: AE, adverse event; CI, confidence interval; RSVPreF3, respiratory syncytial virus prefusion protein vaccine.

0 (0.0%), 1 (1.3%), and 4 (5.9%) mothers, and urinary tract infections, reported by 2 (2.9%), 1 (1.3%), and 2 (2.9%) mothers in the 60 µg RSVPreF3, 120 µg RSVPreF3, and placebo groups, respectively. Unsolicited AEs assessed as related to the study vaccine/placebo by the investigator were reported by 2 (2.9%), 1 (1.3%), and 1 (1.5%) mothers in the 60 µg RSVPreF3, 120 µg RSVPreF3, and placebo groups, respectively. The most common of these were general disorders and administration site conditions, which included feeling hot, injection site irritation, induration, and malaise. None of the grade 3 unsolicited AEs were assessed as related to the study vaccine/placebo.

SAEs were reported by 22.1%–28.0% of mothers (Figure 4); the most common SAE was fetal distress, reported in 2 (2.9%), 9 (12.0%), and 6 (8.8%) mothers in the 60 µg RSVPreF3, 120 µg RSVPreF3, and placebo groups, respectively. SAEs were reported in 25.4%–31.8% of infants (Figure 4); the most common SAE was congenital naevus, reported in 4 (6.0%), 3 (4.1%), and 4 (6.1%) infants in the 60 µg RSVPreF3, 120 µg RSVPreF3, and placebo groups, respectively. No SAEs were fatal or assessed as related to the study vaccination/placebo, and no mothers or infants withdrew due to an SAE.

MA-AEs were reported by 47.1%–53.3% of mothers (Figure 4). The most common of these was fetal distress syndrome, reported by 4 (5.7%), 8 (10.7%), and 6 (8.8%) mothers in the 60 µg RSVPreF3, 120 µg RSVPreF3, and placebo groups, respectively. MA-AEs were reported in 43.3%–57.5% of infants (Figure 4). The most common of these was an upper respiratory tract infection, reported in 7 (10.4%), 5 (6.8%), and 8 (12.1%) infants in the 60 µg RSVPreF3, 120 µg RSVPreF3, and placebo groups, respectively. None of the MA-AEs were assessed as related to the study vaccine/placebo.

Pregnancy-related AESIs were reported by 17.6%–27.1% of mothers (Figure 4); the most common of these was a nonreassuring fetal status, reported by 8.6%–12.0% of mothers, followed by hypertensive disorders of pregnancy, reported by 7 (10.0%), 4 (5.3%), and 1 (1.5%) mothers in the 60 µg RSVPreF3, 120 µg RSVPreF3, and placebo groups, respectively (Supplementary Table 1). Neonatal AESIs were reported in 14.9%–21.9% of infants (Figure 4); the most common of these was respiratory distress in neonates, reported in 6.0%–6.8% of infants (Supplementary Table 2). None of the pregnancy-related or neonatal AESIs were assessed as related to the study vaccine/placebo.

From vaccination through 6 weeks postdelivery, congenital anomalies were reported in 9 (12.9%), 12 (16.0%), and 11 (16.2%) live births in the 60 µg RSVPreF3, 120 µg RSVPreF3, and placebo groups, respectively. Most anomalies were minor. Three congenital anomalies (all in the 120 µg RSVPreF3 group)—external defects (hypospadias), reported in 2 infants, a functional defect (cardiomegaly) and an internal structural defect (patent duct arteriosus), both in the same infant—were considered as AESIs. One antepartum fetal death/stillbirth with no congenital anomalies was reported in the placebo group. The pregnancy outcomes of 5 mothers who withdrew from the

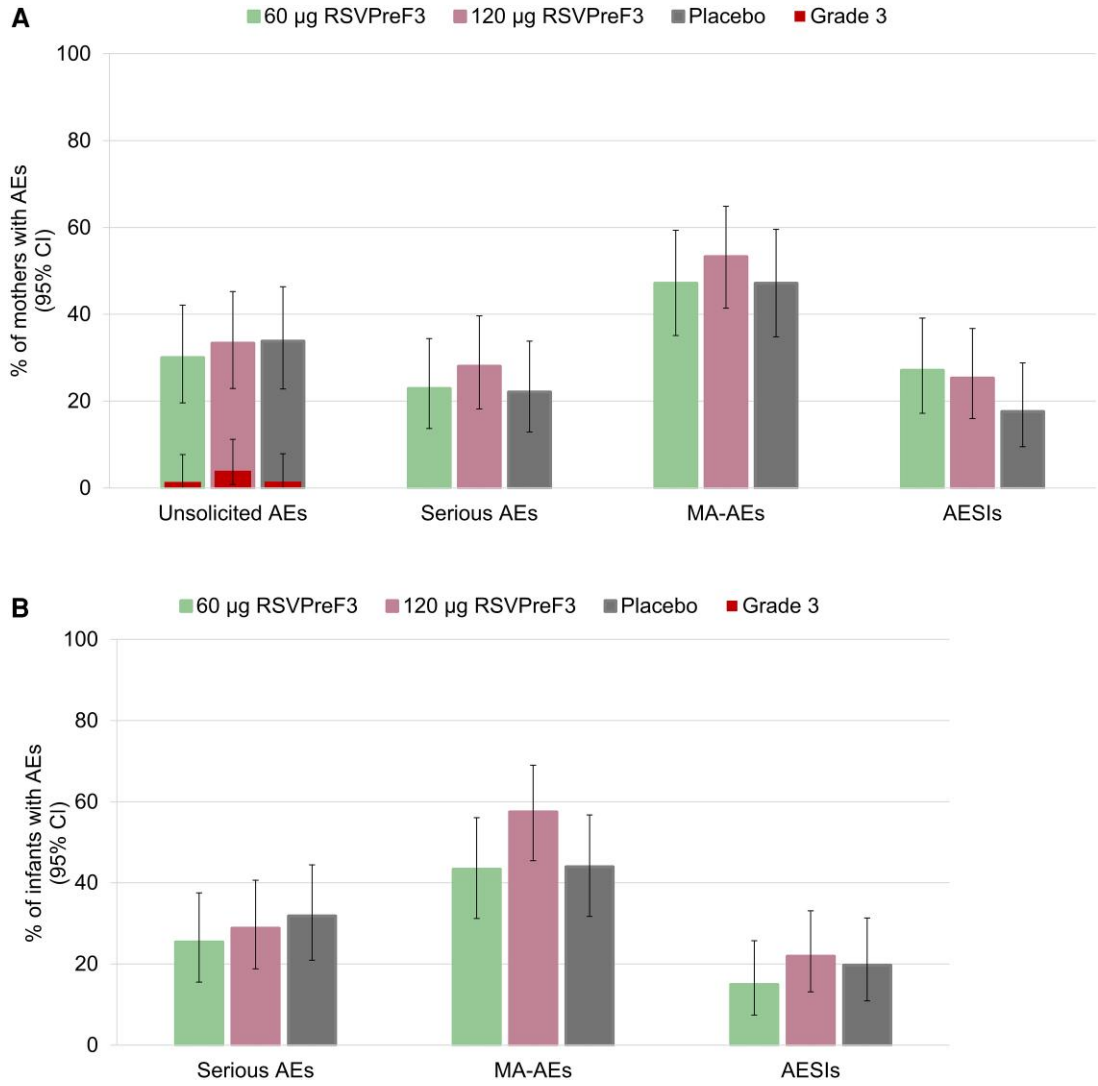

Figure 4. Safety outcomes in mothers (*A*) and infants (*B*). Abbreviations: AE, adverse event; AESI, AE of special interest; CI, confidence interval; MA-AE, medically attended AE; RSVPreF3, respiratory syncytial virus prefusion protein vaccine.

study before delivery remained unknown. Beyond 6 weeks postbirth, 6 congenital anomalies were reported: an abdominal wall anomaly in 1 infant, a congenital arterial malformation in 1 infant, each in the 60 µg RSVPreF3 group, plagiocephaly in 3 infants, phimosis and a preauricular cyst in 1 infant, all in the 120 µg RSVPreF3 group, and a ventricular septal defect in 1 infant in the placebo group.

The hematological and biochemical parameters for most mothers were within normal ranges at baseline. Only 1 mother (from the 60 µg RSVPreF3 group) had increased alanine aminotransferase levels (Food and Drug Administration [FDA] grade 4) and aspartate aminotransferase levels (FDA grade 3) at day 1 and day 8. No trend in the change in hematological and biochemical parameters and no change in hemoglobin levels were observed postvaccination. The laboratory parameters were similar between the groups.

No RSV-associated MA-RTIs or hospitalizations occurred in participating mothers, and no RSV infections were reported in infants during the study. Thus, objectives linked to RSV-RTI/LRTI could not be evaluated.

Immunogenicity

At prevaccination, all mothers were seropositive for RSV-A and RSV-B nAb, and anti-RSVPreF3 IgG (Figure 5). Across groups, prevaccination nAb titers in mothers were 672–736 against RSV-A and 970–1145 against RSV-B (Figure 5). At day 31 postvaccination, nAb titers increased 12.7- and 14.9-fold against RSV-A, and 10.6- and 13.2-fold against RSV-B in the 60 and 120 µg RSVPreF3 groups, respectively. In both groups, nAb titers at day 43 postdelivery remained 8.9–10.0-fold over prevaccination levels. Postvaccination, no increases occurred in placebo recipients. Similar kinetics were also observed for anti-RSVPreF3 IgG levels (Supplementary

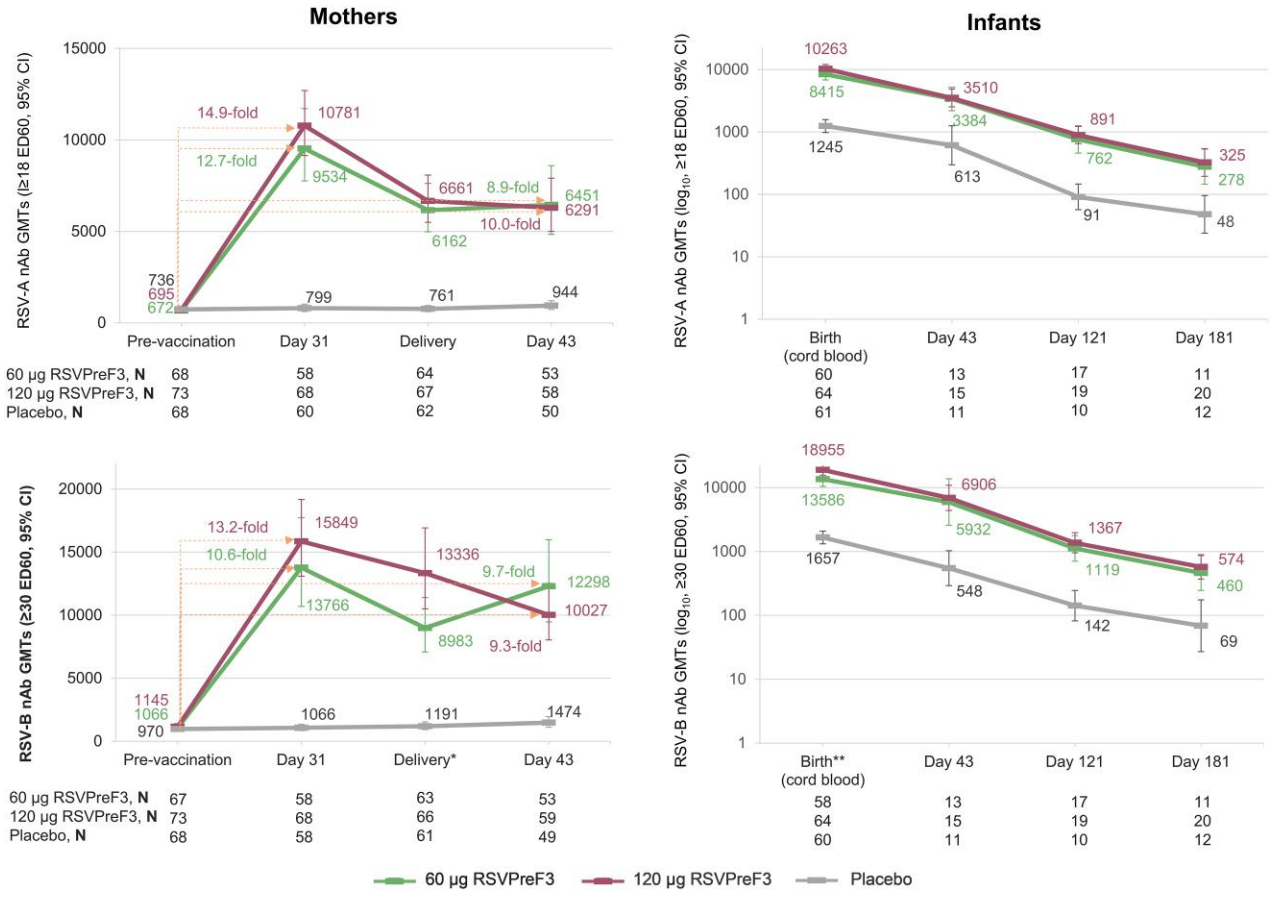

Figure 5. RSV-A and RSV-B nAb GMTs in mothers and infants. *, ** Difference statistically significant between the 60 and 120 µg RSVPreF3 groups (GMT ratios, *1.49 [95% CI, 1.11–2.01] and **1.41 [95% CI, 1.07–1.86]). Abbreviations: CI, confidence interval; ED, estimated dilution; GMT geometric mean titer; N, number of participants; nAb, neutralizing antibody; RSVPreF3, respiratory syncytial virus prefusion protein vaccine.

Figure 1). In the RSVPreF3 groups, the ratios of anti-RSVPreF3 IgG over RSV-A nAb and anti-RSVPreF3 IgG over RSV-B nAb showed some increases from the baseline: the geometric mean ratio of fold increase in vaccine recipients was between 1.06–1.21 and 0.91–1.36, respectively (Supplementary Table 3).

In infants, nAb antibody geometric mean titers against RSV-A and RSV-B, and anti-RSVPreF3 IgG geometric mean concentrations were highest at birth (in the cord blood) and declined thereafter through day 181 postbirth (Figure 5 and Supplementary Figure 1). However, the antibody levels and nAb titers remained higher as compared to the placebo group at all time points. At birth, the geometric means of placental transfer ratios of anti-RSVPreF3 IgG antibodies were 1.62 and 1.90 in the 60 and 120 µg RSVPreF3 groups, respectively.

DISCUSSION

The RSVPreF3 vaccine candidate was developed for active immunization of pregnant women during the late second and third trimester of pregnancy to help protect infants born to vaccinated mothers against RSV. This was the first study of this vaccine candidate to evaluate the reactogenicity, safety, and immunogenicity of a single 60 or 120 µg dose in the target population of healthy pregnant women and their infants. Our findings were in line with those of a previously conducted phase 1/2 study in healthy non-pregnant women [11]: both dose levels demonstrated a favorable safety profile and robust immunogenicity compared to placebo.

The incidence of unsolicited AEs was comparable between vaccine groups and most were of mild or moderate intensity. The reporting rate of solicited events tended to be higher in vaccine than in placebo recipients. The only notable difference in pregnancy-related AESIs observed between the vaccine and placebo groups was in the reporting rate of hypertensive disorders of pregnancy (gestational hypertension and preeclampsia). These events tended to be more frequent in the treatment groups than in the placebo group. Nonetheless, the incidence rate was not dose-dependent and was within the background rate in the general pregnant population [19]. No increase in the rate of preterm labor, preterm birth, or congenital

anomalies was observed in mothers who received the study vaccine compared to the placebo.

For both RSVPreF3 dose levels, robust RSV-A and RSV-B nAb and anti-RSVPreF3 IgG responses were observed in mothers, with a trend towards higher titers/concentrations for the 120 μg as compared to the 60 μg dose level, which reached statistical significance for RSV-B nAb titers at delivery. In mothers, ratios of pre- to postvaccination fold increases in anti-RSVPreF3 IgG concentrations over fold increases in RSV-A or RSV-B nAb titers indicated that increases of RSV-A and RSV-B nAb titers were almost of the same magnitude as for anti-RSVPreF3 IgG up to day 43 postdelivery.

Maternal RSV vaccination is intended to boost the levels of RSV-specific neutralizing antibodies, leading to increased transplacental transport of maternal antibodies to the fetus. Such antibodies are expected to provide protection from RSV illness in infants in the first months of life [20].

The current study demonstrated the successful and efficient placental transfer of maternal antibodies to the fetus. The RSV-A/B nAb and anti-RSVPreF3 antibody levels were higher in the cord blood as compared to maternal circulation at the time of delivery. In addition to placental transfer, maternal antibodies are also passed on to the infant through breast milk [21]. However, analysis of antibody levels in breast milk was not part of this study, and the potential additional benefits the vaccine candidate may provide through breastfeeding remain to be determined in future studies.

These results should be interpreted considering the strengths and limitations of the trial. The study population was intended to be geographically heterogeneous, which was achieved to a certain extent. However, due to the COVID-19–related early stop of enrollment, the number of participants in certain geographic areas was limited and the targeted sample size was not achieved. A post hoc analysis conducted subsequently showed that a sample size of 213 participants would still be sufficient to address the safety and immunogenicity objectives of the study. The COVID-19 pandemic, which coincided with the conduct of this study, was associated with limited transmission of RSV, mostly due to the imposed social restrictions. As a result, no RSV-associated LRTI cases in infants or MA-RTI cases in mothers were recorded in our study despite the close follow-up of infants for worsening RTIs. Hence, we were unable to ascertain any worsening of the RSV disease in vaccinated mothers or their infants.

Overall, the present data supported further evaluation of the 120 μg RSVPreF3 in a phase 3 program. However, on 25 February 2022, the decision was made to stop, as a precautionary measure, enrollment and vaccination in subsequent studies (NCT04605159, NCT04980391, and NCT05229068) following identification of safety signals in NCT04605159. Study participants are closely monitored to ensure they receive the best possible care. Further analyses to better understand safety from these trials are ongoing [22].

CONCLUSIONS

A single dose of maternal RSVPreF3 vaccine administered during the late second or third trimester of pregnancy was well tolerated and demonstrated an acceptable safety profile with respect to pregnancy-related or neonatal AESIs or pregnancy outcomes. The RSVPreF3 vaccine induced robust immune responses in terms of RSV-A and RSV-B nAb and anti-RSVPreF3 IgG, with the 120 μg dose tending to be more immunogenic as compared to the 60 μg dose. Maternal antibodies were successfully transferred to infants and persisted at least until 6 months after birth, with similar kinetics for RSV-A and RSV-B nAb. Overall, these data supported further evaluation of the 120 μg RSVPreF3 formulation in a phase 3 program.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Author contributions. M. B. E. P., J. M. L., S. A. M., Z. B., M. M. C., I. D., O. H., P. L., H. Q., and A. N. T. participated in the conception and design of this study. H. L., C. M. D. L., P. B., T. B., J. L. B. R., J. B., R. G. D. L., M. B. E. P., A. M. G., G. J. G., G. R., R. J. J., A. K., J. M. L., S. A. M., O. R., M. G. S., T. V. S., Z. B., M. M. C., M. d. H., I. D., and M. H. participated in the collection or generation of the study/project data. P. B., T. B., J. B., M. B. E. P., A. M. G., G. J. G., R. J. J., A. K., J. M. L., S. A. M., E. B. N., O. R., and T. V. S. performed the study/project. M. B. E. P., G. J. G., A. K., S. A. M., and O. R. contributed materials/analysis/reagent tools. M. B. E. P., J M. L., S. A. M., Z. B., I. D., O. H., P. L., H. Q., and A. N. T. were involved in the analyses or interpretation of the data. All authors approved the manuscript and attest they meet the ICMJE criteria for authorship.

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