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Journal

The Journal of Infectious Diseases, 226(12)

ISSN

0022-1899

Authors

Cunningham, Coleen K

Karron, Ruth A

Muresan, Petronella

et al.

Publication Date

2022-12-13

DOI

10.1093/infdis/jiac253

Peer reviewed

The Journal of Infectious Diseases

MAJOR ARTICLE

Evaluation of Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV/ΔNS2/Δ1313/I1314L and RSV/276 in RSV-Seronegative Children

Coleen K. Cunningham,[1,2,3] Ruth A. Karron,[4] Petronella Muresan,[5] Matthew S. Kelly,[1] Elizabeth J. McFarland,[6] Charlotte Perlowski,[7] Jennifer Libous,[7] Jennifer Oliva,[4] Patrick Jean-Philippe,[8] Jack Moye Jr,[9] Elizabeth Schappell,[4] Emily Barr,[6] Vivian Rexroad,[10] Benjamin Johnston,[11] Ellen G. Chadwick,[12] Mikhaela Cielo,[13] Mary Paul,[14] Jaime G. Deville,[15] Mariam Aziz,[16] Lijuan Yang,[17] Cindy Luongo,[17] Peter L. Collins,[17] and Ursula J. Buchholz,[17] the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) 2018 Study Team

[1]Department of Pediatrics, Duke University Medical Center, Durham, North Carolina, USA; [2]Department of Pediatrics, University of California, Irvine, California, USA; [3]Children's Hospital of Orange County, Orange, California, USA; [4]Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; [5]Statistical and Data Management Center/Frontier Science and Technology Research Foundation, Brookline, Massachusetts, USA; [6]Department of Pediatrics, University of Colorado Anschutz Medical Campus and Children's Hospital Colorado, Aurora, Colorado, USA; [7]FHI 360, Durham, North Carolina, USA; [8]Maternal, Adolescent and Pediatric Research Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA; [9]Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA; [10]Investigational Drug Service Pharmacy, Johns Hopkins Hospital, Baltimore, Maryland, USA; [11]Frontier Science and Technology Research Foundation, Buffalo, New York, USA; [12]Department of Pediatrics, Northwestern University Feinberg School of Medicine and Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, USA; [13]Division of Infectious Diseases, Maternal Child and Adolescent Center, University of Southern California Keck School of Medicine, Los Angeles, California, USA; [14]Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA; [15]David Geffen School of Medicine at University of California, Los Angeles, California, USA; [16]Rush University Medical Center, Cook County Hospital, Chicago, Illinois, USA; and [17]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

Background. This United States-based study compared 2 candidate vaccines: RSV/ΔNS2/Δ1313/I1314L, attenuated by NS2 gene-deletion and temperature-sensitivity mutation in the polymerase gene; and RSV/276, attenuated by M2-2 deletion.

Methods. RSV-seronegative children aged 6–24 months received RSV/ΔNS2/Δ1313/I1314L (10^6 plaque-forming units [PFU]), RSV/276 (10^5 PFU), or placebo intranasally. Participants were monitored for vaccine shedding, reactogenicity, and RSV serum antibodies, and followed over the subsequent RSV season.

Results. Enrollment occurred September 2017 to October 2019. During 28 days postinoculation, upper respiratory illness and/or fever occurred in 64% of RSV/ΔNS2/Δ1313/I1314L, 84% of RSV/276, and 58% of placebo recipients. Symptoms were generally mild. Cough was more common in RSV/276 recipients than RSV/ΔNS2/Δ1313/I1314L (48% vs 12%; $P = .012$) or placebo recipients (17%; $P = .084$). There were no lower respiratory illness or serious adverse events. Eighty-eight and 96% of RSV/ΔNS2/Δ1313/I1314L and RSV/276 recipients were infected with vaccine (shed vaccine and/or had ≥4-fold rises in RSV antibodies). Serum RSV-neutralizing titers and anti-RSV F IgG titers increased ≥4-fold in 60% and 92% of RSV/ΔNS2/Δ1313/I1314L and RSV/276 vaccinees, respectively. Exposure to community RSV during the subsequent winter was associated with strong anamnestic RSV-antibody responses.

Conclusions. Both vaccines had excellent infectivity and were well tolerated. RSV/276 induced an excess of mild cough. Both vaccines were immunogenic and primed for strong anamnestic responses.

Clinical Trials Registration. NCT03227029 and NCT03422237.

Keywords. respiratory syncytial virus; RSV; live-attenuated viral vaccine; neutralizing antibodies; immunogenicity; RNA regulatory protein M2-2.

Respiratory syncytial virus (RSV) is a major cause of morbidity and mortality among infants and children under 5 years of age [1, 2]. Therefore, an effective vaccine could have a significant impact on child health. There is an expanding pipeline of RSV vaccine candidates [3], including live-attenuated candidates [4]. Live-attenuated vaccines administered intranasally induce innate, humoral, and cell-mediated responses at mucosal surfaces [4] in addition to systemic responses. In addition, live-attenuated RSV vaccines express all RSV antigens, including the fusion (F) glycoprotein largely in its prefusion form, resulting in induction of neutralizing antibodies [5, 6]. Expanded understanding of RSV viral pathogenesis [7], together with techniques of reverse genetics [8], allow development of vaccine candidates that elicit strong immune responses while maintaining attenuation [4, 9–15].

One promising attenuation strategy is the deletion of the viral nonstructural protein 2 (NS2), an antagonist of host interferon and apoptosis responses. The most promising NS2 deletion candidate, RSV/ΔNS2/Δ1313/I1314L, also contains a

Received 05 April 2022; editorial decision 16 June 2022; accepted 20 June 2022; published online 23 June 2022

Correspondence: Coleen K. Cunningham, MD, Department of Pediatrics, University of California, Irvine. 333 City Boulevard West, Suite 800, Orange, CA 92868 (ckcunnin@UCI.edu).

The Journal of Infectious Diseases® 2022;226:2069–78

https://doi.org/10.1093/infdis/jiac253

single-codon deletion in the open reading frame (ORF) encoding the polymerase protein L. The resulting candidate is attenuated and moderately temperature sensitive [13, 16]. A second strategy involves deletion of most of the ORF encoding the RNA synthesis regulatory protein M2-2 [10, 17]. Deletion of M2-2 results in increased viral gene transcription and decreased genome replication. The increased gene transcription results in increased synthesis of viral antigens, with the potential for increased immunogenicity; the decreased genome replication results in delayed production of new virus particles, resulting in attenuation. The deletion of most of a viral gene should reduce the potential for deattenuation [17]. In a small previous phase 1 study, a single intranasal dose of 10^6 plaque-forming units (PFU) of RSV/ΔNS2/Δ1313/I1314L was well tolerated and immunogenic in 6 to 24-month-old children, and further clinical evaluation was warranted [13]. RSV/276 has not been previously studied in children; however, the very similar vaccine, MEDI/ΔM2-2, was previous shown to be safe and immunogenic [10]. Herein, we describe the safety, infectivity, and immunogenicity of RSV/ΔNS2/Δ1313/I1314L, evaluated side by side with the M2-2 deletion candidate RSV/276 in RSV-seronegative children aged 6–24 months.

METHODS

Vaccines

RSV/ΔNS2/Δ1313/I1314L and RSV/276 are cDNA derived from RSV subgroup A, strain A2. RSV/ΔNS2/Δ1313/I1314L differs from its wild-type parent (Genbank accession number KT992094) by 2 independent attenuating elements: a 523-nucleotide (nt) deletion of the NS2 gene and an amino acid deletion in the L protein (Δ1313; deletion of S1313), combined with the missense mutation I1314L that increases the phenotypic stability of Δ1313 [16]. RSV/ΔNS2/Δ1313/I1314L also has a 112-nt deletion in the 3′-noncoding region of the SH gene [18]. RSV/276 contains the same 234-nt deletion of the M2-2 ORF as the MEDI/ΔM2-2 vaccine [10, 19] but differs by 2 nt in the 5′-noncoding region of the M2 gene (nt 8198/99; GC in MEDI/ΔM2-2, CG in RSV/276). In addition, RSV/276 differs from recombinant RSV A2 (KT992094) by 19 nt throughout the genome, including 2 coding changes (K51R in the NS2 ORF; T24A in the N ORF). RSV/ΔNS2/Δ1313/I1314L and RSV/276 were recovered from cDNA in Vero cells, and clinical trial material was manufactured (Charles River Laboratories). The RSV/276 vaccine, supplied in Dulbecco's modified Eagle medium with 1 × SPG (sucrose 0.218 M, KH_2PO_4 0.0038 M, K_2HPO_4 0.0072 M, L-glutamic acid 0.0054 M), has a potency of $10^{6.2}$ PFU/mL. The RSV/ΔNS2/Δ1313/I1314L vaccine, supplied in OptiPro SFM with 1 × SPG, has a potency of $10^{7.3}$ PFU/mL. Sequence analysis confirmed identity to the cDNAs from which each was derived. Vaccines were diluted on site to a dose of 10^6 and 10^5 PFU in a 0.5-mL volume for RSV/ΔNS2/Δ1313/I1314L and RSV/276, respectively. The placebo and vaccine diluent was Lactated Ringer's solution. Vaccine or placebo was administered intranasally by drops as a single, 0.5-mL dose divided between nostrils.

Study Design

This randomized (2:2:1 vaccine to vaccine to placebo), double-blind, placebo-controlled study (ClinicalTrials.gov identifiers: NCT03227029/NCT03422237, https://clinicaltrials.gov) was conducted at 12 sites (11 International Maternal Pediatric Adolescent AIDS Clinical Trials [IMPAACT] sites and the Johns Hopkins Center for Immunization Research, Baltimore, MD [CIR]), with accrual between 22 September 2017 and 14 October 2019. Accrual paused during the RSV season (16 October to 31 March at most sites), and participants were followed through the subsequent RSV season. Eligible children were between 6 and <25 months of age, healthy, with no history of lung disease, and RSV seronegative at screening, defined as having a serum RSV 60% plaque reduction neutralizing titer ($PRNT_{60}$) <1:40.

Clinical assessments and nasal washes (NW) were performed on study days 0, 3, 5, 7, 10, 12, 14, 17, and 28 (±1 day), with telephone contact on intervening days. Additional clinical assessments and NW were obtained in the event of respiratory illness (upper respiratory illness [URI]: rhinorrhea, pharyngitis, or hoarseness; cough; acute otitis media [OM]; lower respiratory illness [LRI]); or fever). Some events, including cough, were based on parent report. Events were assigned a grade of 1–4 with 1 denoting a mild event and 4 denoting a serious or life-threatening event. All adverse events and reactogenicity data were collected through day 28. Thereafter, children were monitored until day 56 for serious adverse events (SAE). During the subsequent RSV surveillance period (1 November to 31 March for most sites), families were contacted weekly to determine whether medically attended acute respiratory illnesses (MAARI) occurred, defined as fever, URI, LRI, or OM. Within 3 days of each illness episode, a clinical assessment and NW were obtained to determine if the illness was associated with RSV (RSV-MAARI). Sera to measure RSV antibodies were obtained prior to inoculation, 56 days after inoculation, and before (1–31 October for most sites) and after (1–30 April) the RSV winter surveillance period. For participants enrolled in 2019, the study visit window after the 2019–2020 RSV winter surveillance was extended to 30 September 2020 due to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic.

Written informed consent was obtained from participants' parents/guardians. These studies were approved by each site's institutional review board, conducted in accordance with the principles of the Declaration of Helsinki and Standards of Good Clinical Practice as defined by the International Conference on Harmonization, and monitored by the

independent data safety and monitoring board of the National Institute of Allergy and Infectious Diseases, Division of Clinical Research.

Laboratory Assays

NW from baseline and illness days were tested for common adventitious agents by reverse transcription quantitative polymerase chain reaction (RT-qPCR, Respiratory Pathogens 21 multiplex; Fast-track Diagnostics). Vaccine virus in NW was quantified by immunoplaque assay on Vero cells and by RT-qPCR specific for the RSV matrix (M) gene as previously described [10]. Genetic stability of attenuating elements in vaccine isolates obtained at peak shedding and on the last day of detectable shedding was determined by partial genome sequence analysis as previously described [11, 13].

Serum RSV-$PRNT_{60}$ were determined by complement-enhanced 60% plaque-reduction neutralization assay [20]. Serum immunoglobulin G (IgG) antibody titers to the RSV F glycoprotein (anti-RSV F IgG) were determined by enzyme-linked immunosorbent assay (ELISA) using RSV(A2)F glycoprotein as previously described [10].

Statistical Analysis

Randomized participants who did not receive study product or discontinued without data were excluded from the analysis. One enrolled pair of twins both received the same study product (RSV/276) to reduce the potential cross-contamination that could result if children living together were to receive different products. Both twins were included in the safety analyses to provide a complete safety profile. Percentages of participants who experienced adverse events and exact 90% confidence intervals (CI) were calculated.

Infectivity and immunogenicity analyses included inoculated participants who had data past study entry. Because we determined that twins should not be considered independent observations, 1 child from the set of twins was randomly selected for inclusion in these analyses. Sensitivity analyses using the other sibling supported the overall results (data not shown).

Reciprocal serum $PRNT_{60}$ and anti-RSV F IgG titers were transformed to $\log_2$ values. Although log-transformed, some data deviated from normality; thus, nonparametric methods were used to test for statistical differences. Medians and interquartile ranges (IQR) were used to summarize peak NW titers and serum antibody titers. Mean and standard deviation values were presented to allow descriptive comparisons with other studies (Supplementary Tables 1 and 2). The summaries of vaccine virus shed in NW include only vaccine recipients who were infected with vaccine. Infection was defined as the detection of vaccine virus by immunoplaque assay and/or RT-qPCR and/or a ≥4-fold rise in serum RSV-$PRNT_{60}$ or anti-RSV F IgG titer. One-sided tests were used to compare RSV-$PRNT_{60}$ or anti-RSV F IgG titers for vaccine and placebo recipients; Fisher exact tests were used to compare proportions with ≥4-fold rises in these titers and Wilcoxon rank sum tests were used to compare titers. Analyses were performed using SAS version 9.4 (SAS Institute) and graphs were produced using R software version 3.6.0.

RESULTS

Accrual and Participant Characteristics

The study accrued 65 participants, 26 in each vaccine arm and 13 placebo recipients. Three participants had no data after entry and were taken off study: 2 did not receive study product (1 placebo and 1 RSV/276), and parents of 1 RSV/ΔNS2/Δ1313/I1314L recipient withdrew consent on day 2. All other participants received study product within 3 days of randomization and completed day 28 and 56 evaluations. The distribution of sex, age, ethnicity, and racial characteristics was similar for vaccine and placebo recipients (Table 1).

Of the 62 participants in the safety analysis population, 9 (3 in each arm) did not complete the final, post-RSV season study

Table 1. Baseline Characteristics of Vaccine and Placebo Recipients

Characteristics	Recipients, No. (%)			
	RSV/ΔNS2/Δ1313/I1314L Vaccine (n = 25)	RSV/276 Vaccine (n = 25)	Placebo (n = 12)	Total (n= 62)
Sex, female	13 (52)	7 (28)	9 (75)	29 (47)
Ethnicity				
Hispanic or Latino	9 (36)	9 (36)	3 (25)	21 (34)
Not Hispanic or Latino	15 (60)	16 (64)	9 (75)	40 (65)
Unknown	1 (4)	0 (0)	0 (0)	1 (2)
Race				
African American	7 (28)	7 (28)	6 (50)	20 (32)
White	15 (60)	14 (56)	6 (50)	35 (56)
More than 1 race	2 (8)	2 (8)	0 (0)	4 (6)
Unknown	1 (4)	0 (0)	0 (0)	1 (2)
Residence[a]				
California	3 (12)	6 (24)	2 (17)	11 (18)
Colorado	4 (16)	6 (24)	5 (42)	15 (24)
Tennessee	1 (4)	0 (0)	1 (8)	2 (3)
Illinois	6 (24)	5 (20)	1 (8)	12 (19)
Massachusetts and New York	1 (4)	2 (8)	0 (0)	3 (5)
Maryland	7 (28)	6 (24)	3 (25)	16 (26)
Texas	3 (12)	0 (0)	0 (0)	3 (5)
HIV exposed, uninfected	8 (32)	8 (32)	4 (33)	20 (32)
Age, mo, median (IQR)	13 (7–14)	14 (10–16)	9.0 (7.5–14.5)	12.5 (8.0–15.0)

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; RSV, respiratory syncytial virus.

[a]Of the 12 sites, 3 were in California, 2 each were in New York and Illinois, and 1 each in Texas, Colorado, Massachusetts, Tennessee, and Maryland.

visit due to the coronavirus disease 2019 (COVID-19) pandemic. Among the 53 (82%) participants who completed the final post-RSV surveillance study visit, 5 enrolled in 2019 had the final visit later than the original planned date of 30 April 2020 due to the COVID-19 pandemic. This was deemed to have no effect on outcomes.

Safety and Adverse Events

During the 28 days after inoculation, upper respiratory and/or febrile adverse events occurred often in vaccine and placebo recipients, with 16/25 (64%; 90% CI, 46%–80%) RSV/ΔNS2/Δ1313/I1314L, 21/25 (84%; 90% CI, 67%–94%) RSV/276, and 7/12 (58%; 90% CI, 32%–82%) placebo recipients having 1 or more illness episodes. Most respiratory symptoms were mild (grade 1) (Table 2). There were no LRI or SAE. In the vaccinees with respiratory/febrile illness, other pathogens were often present at the time of illness, including adenoviruses, rhinoviruses, enteroviruses, bocavirus, parainfluenza viruses 1, 3, and 4, or *Mycoplasma pneumoniae*. Among the 16 children in the RSV/ΔNS2/Δ1313/I1314L group with fever or respiratory symptoms, 4 had vaccine virus alone detected (Figure 1), 8 had vaccine virus and another virus detected (yellow bars), and 4 had other respiratory viruses detected but not vaccine virus (hatched bars). Among the 21 children in the RSV/276 group with fever or respiratory symptoms, 12 had vaccine virus alone detected (blue bars), 6 had vaccine virus detected along with another virus (yellow bars), and 3 had no vaccine virus detected (hatched bars); of these, 2 had other viruses and 1 did not have any respiratory pathogens detected. Among the 7 placebo recipients with illness events, 6 had other viruses detected and 1 did not have any respiratory pathogen detected.

Cough was more common in RSV/276 recipients than in RSV/ΔNS2/Δ1313/I1314L recipients (48% vs 12%; $P = .012$) or placebo (17%; $P = .084$) (Table 2). Respiratory symptoms and fever occurred more frequently in the 14 days after inoculation and appeared earlier in RSV/276 recipients compared to those receiving RSV/ΔNS2/Δ1313/I1314L or placebo (Figure 1). Symptoms generally overlapped with vaccine virus detection, suggesting that replication of the candidate vaccines may have been associated with mild URI symptoms in some participants. Nonrespiratory adverse events between days 0 and 28 were mild (grade 1 or 2), not attributed to vaccine, and not different between vaccine recipients and placebo.

Infectivity and Immunogenicity

There were 61 participants included in infectivity analyses after excluding 1 randomly selected twin. Twenty-two of 25 (88%; 90% CI, 72%–97%) RSV/ΔNS2/Δ1313/I1314L recipients, 23/24 (96%; 82%–100%) RSV/276 recipients, and 0/12 (0%; 0%–22%) placebo recipients met the definition of infection with vaccine virus (ie, vaccine shedding and/or 4-fold rise in RSV-serum antibodies). Vaccine virus shedding and median peak virus titers during the first 28 days after inoculation are shown in Table 3 and Figure 2. Shedding of RSV/ΔNS2/Δ1313/I1314L was detected for a median of 10 days (IQR, 8–12 days) by immunoplaque assay and 13 days (12–16 days) by RT-qPCR; for RSV/276, the values were 10 days (7–12 days) by immunoplaque assay and 14 days (11–17 days) by RT-qPCR. Median viral titers were highest on study days 5–7 (Figure 2).

Postinoculation serum antibody responses were assessed on day 56 (Table 4 and Figure 3). Four-fold or greater rises in serum RSV-$PRNT_{60}$ titers and anti-RSV F IgG titers were detected in 15 (60%) RSV/ΔNS2/Δ1313/I1314L vaccinees, 22 (92%) RSV/276 vaccinees, and no placebo recipients ($P < .001$ for each vaccine vs placebo). The frequency of antibody responses in recipients of RSV/ΔNS2/Δ1313/I1314L was lower than in recipients

Table 2. Clinical Assessment During the First 28 Days After Inoculation

Symptoms[b]	Group								
	RSV/ΔNS2/Δ1313/I1314L Recipients (n = 25)			RSV/276 Recipients (n = 25)			Placebo Recipients (n = 12)		
	Grade[a]			Grade[a]			Grade[a]		
	1	2	3	1	2	3	1	2	3
Fever	1 (4)	1 (4)	2 (8)	0 (0)	2 (8)	1 (4)	0 (0)	1 (8)	0 (0)
URI	16 (64)	0 (0)	0 (0)	16 (64)	2 (8)	0 (0)	5 (42)	0 (0)	0 (0)
LRI	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cough	3 (12)	0 (0)	0 (0)	11 (44)	1 (4)	0 (0)	1 (8)	1 (8)	0 (0)
Otitis media	0 (0)	1 (4)	0 (0)	0 (0)	2 (8)	0 (0)	0 (0)	0 (0)	0 (0)
Any respiratory or febrile illness[c]	12 (48)	2 (8)	2 (8)	14 (56)	6 (24)	1 (4)	5 (42)	2 (17)	0 (0)

Data are No. (%).

Abbreviations: LRI, lower respiratory tract illness; URI, upper respiratory tract illness.

[a]Worst disease severity grade: mild is equivalent to grade 1, moderate to grade 2, and severe to grade 3. There were no grade 4 events. A participant was only counted once in each symptom category, based on the worst (highest) grade within that category.

[b]Participants with indicated respiratory or febrile symptoms occurring in the 28 days after inoculation. LRI was defined as wheezing, rhonchi, or rales or a diagnosis of pneumonia or laryngotracheobronchitis (croup). URI was defined as rhinorrhea, pharyngitis, or hoarseness.

[c]A participant was only counted once in this summary row, based on the worst (highest) grade respiratory or febrile symptom reported.

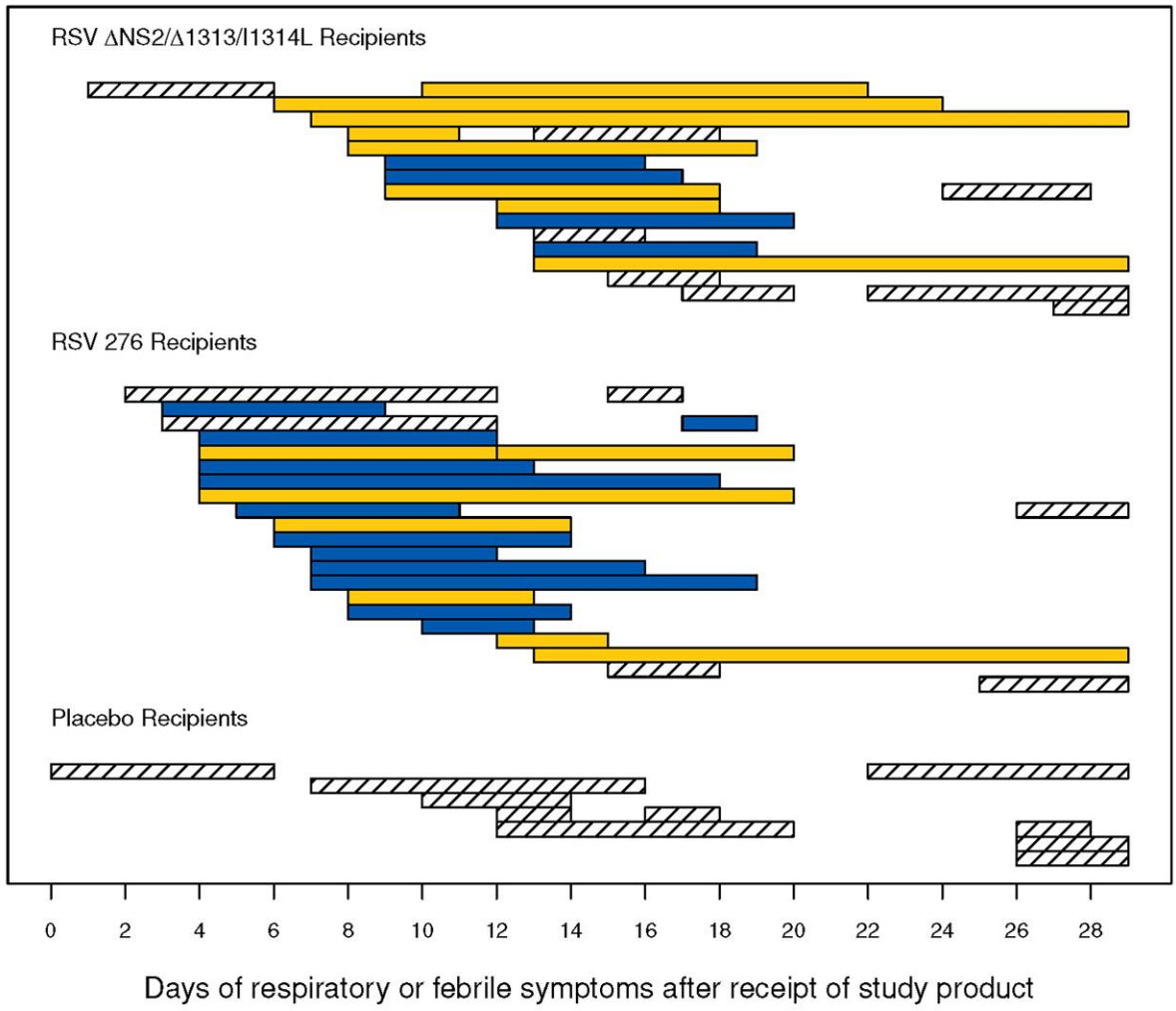

Figure 1. Symptom days in participants with respiratory or febrile illness reported after receipt of vaccine or placebo. Each row represents 1 participant, grouped by candidate vaccine or placebo. Day 0 is day of vaccination. The boxes depict the duration of episodes of respiratory or febrile illness that occurred in the first 28 days after receipt of study product in 16 of 25 (64%) who received RSV/ΔNS2/Δ1313/I1314L vaccine, 21 of 25 (84%) who received RSV/276 vaccine, and 7 of 12 (58%) placebo recipients. Nasal washes (NW) were performed on study days 0, 3, 5, 7, 10, 12, 14, 17, and 28 and in the event of respiratory illness (upper respiratory illness: rhinorrhea, pharyngitis, or hoarseness; cough; acute otitis media; lower respiratory illness) or fever. NW during illnesses were tested for common adventitious agents by reverse-transcription quantitative polymerase chain reaction (RT-qPCR). Vaccine virus in NW was quantified by immunoplaque assay and by RT-qPCR. Blue denotes illness episodes with vaccine virus alone detected in 1 or more days during the illness, yellow denotes vaccine virus plus additional virus(es), and hatch denotes episodes without vaccine virus (either other virus isolated or no viruses).

of RSV/276; however, the RSV/ΔNS2/Δ1313/I1314L arm had a larger number of baseline (preimmunization) RSV-neutralizing antibody titers above the limit of detection (7 in the RSV/ΔNS2/Δ1313/I1314L group vs 5 in the RSV/276 group; Supplementary Figure 1) and 0/7 (RSV/ΔNS2/Δ1313/I1314L) and 3/5 (RSV/276) had a 4-fold rise in antibody titer. This suggests that preexisting RSV serum neutralizing antibodies, even at low levels, might interfere with the ability of RSV/ΔNS2/Δ1313/I1314L to induce detectable RSV antibody responses, even when vaccine shedding occurred (Supplementary Figure 1).

Sequence analyses of the NS2/N and M2-2 genome regions were successful for 19/25 RSV/ΔNS2/Δ1313/I1314L and 23/25 RSV/276 vaccinees, respectively, confirming the stability of the deletions. The Δ1313/I1314L site was successfully sequenced for isolates from 15/25 RSV/ΔNS2/Δ1313/I1314L vaccinees, confirming the stability of this site through the period of shedding. In the remaining vaccinees, shedding was absent or too low to confirm genetic stability of these regions; there was no evidence of genetic instability.

RSV Surveillance

During the RSV season following receipt of vaccine, all-cause MAARI rates were similar between groups (28% [90% CI, 14%–46%] in the RSV/ΔNS2/Δ1313/I1314L group; 44% [90% CI, 27%–62%] in the RSV/276 group; and 25% [90% CI, 7%–53%] in the placebo group). Among those who completed RSV surveillance, 11/22 (50%) RSV/ΔNS2/Δ1313/I1314L vaccinees, 5/21 (24%) RSV/276 vaccinees, and 4/9 (44%) placebo

Table 3. Vaccine Virus Shedding and Peak Virus Titers During the First 28 Days After Inoculation

Group	Shedding Vaccine Virus, % (90% CI)[a]	Peak Viral Titer in NW Specimens, Median (IQR)	
		Plaque Assay, Log_{10} PFU/mL[b]	RT-qPCR, Log_{10} Copies/mL[c]
RSV/ΔNS2/Δ1313/I1314L recipients (n = 25)	88 (72–97)	3.1 (1.8–3.8)	5.1 (4.2–5.4)
RSV/276 recipients (n = 24[d])	92 (76–99)	3.2 (2.8–4.0)	5.8 (5.2–6.4)
Placebo recipients (n = 12)	0	0.5 (0.5–0.5)	1.7 (1.7–1.7)

Abbreviations: CI, confidence interval; IQR, interquartile range; NW, nasal wash; PFU, plaque-forming unit; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

[a]Percentage of children with vaccine virus detected in NW specimens by immunoplaque assay and/or RT-qPCR.

[b]For each child, the individual peak (highest) NW specimen titer, irrespective of day, measured by immunoplaque assay and expressed as log_{10} PFU per milliliter. The lower limit of detection was 0.5 log_{10} PFU/mL.

[c]For each participant, the individual peak (highest) NW specimen titer, irrespective of day, measured by RT-qPCR and expressed as log_{10} copies per milliliter. The lower limit of detection was 1.7 log_{10} copies/mL.

[d]Only 24 participants were included in the viral detection and viral shedding assessment because 1 twin was excluded.

recipients experienced community-acquired RSV infection as determined by either an RSV-associated MAARI and/or ≥4-fold increase in RSV-$PRNT_{60}$ or anti-RSV F IgG titers after RSV surveillance. Of 11 RSV/ΔNS2/Δ1313/I1314L vaccinees with evidence of community-acquired RSV infection, only 2 had an RSV-MAARI: 1 with bronchiolitis, wheezing, pneumonia, and OM (coronavirus HKU and RSV-B); and 1 with OM and URI (RSV-B). Of the 5 RSV/276 vaccinees, 3 had an RSV-MAARI: 2 with bronchiolitis (RSV, not typed); and 1 with URI (RSV-A, coronavirus OC43, and adenovirus). In the placebo group, 2/9 (22%) had an RSV-MAARI or MAALRI reported during surveillance: 1 with cough and OM (RSV-A); and 1 pneumonia (RSV-B and adenovirus). The pre- and post-RSV surveillance serum $PRNT_{60}$ and RSV F IgG titers for RSV/ΔNS2/Δ1313/I1314L and RSV/276 vaccinees and placebo recipients are in Table 4 and Figure 3. Among the participants with wild-type RSV infection, the post-RSV surveillance RSV-$PRNT_{60}$ titers were higher for RSV/ΔNS2/Δ1313/I1314L vaccinees (median, 10.1; IQR,

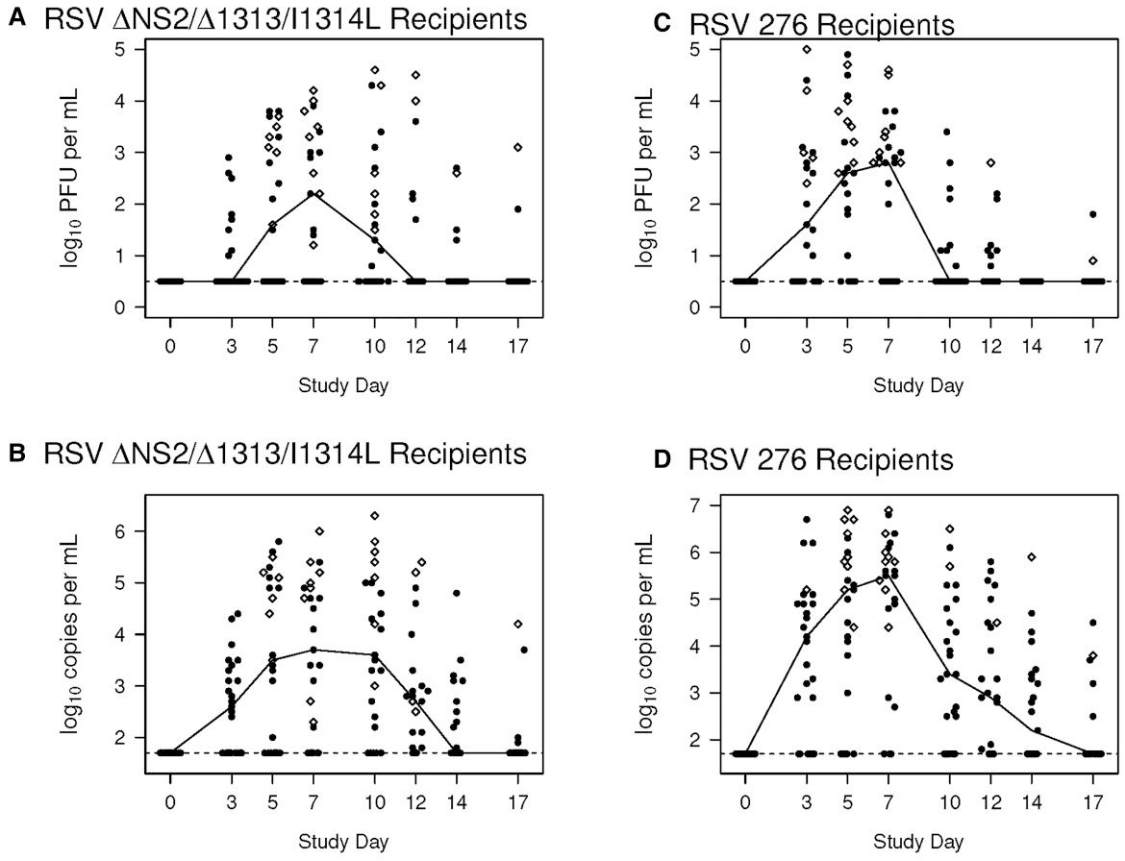

Figure 2. Vaccine virus shed in nasal wash (NW) specimens from vaccinees. Data are for RSV/ΔNS2/Δ1313/I1314L vaccine (*A* and *B*) and RSV/276 vaccine (*C* and *D*). Titers (closed circles and open diamonds) and peak titers (open diamonds) for individual participants are shown along with median titers (solid line). NW specimens were collected from vaccinees during study days 0, 3, 5, 7, 10, 12, 14, 17, and 28 visits (window, indicated study day ± 1 day) after inoculation on day 0, and titers were determined by immunoplaque assay (*A* and *C*) and reverse-transcription quantitative polymerase chain reaction (RT-qPCR) (*B* and *D*). The lower limits of detection (dashed lines) were 0.5 log_{10} plaque-forming units (PFU)/mL and 1.7 log_{10} copies/mL for immunoplaque assay and RT-qPCR, respectively.

Table 4. RSV-Specific Serum Antibody Responses Before and After Inoculation and After RSV Surveillance During the RSV Season, as Defined by the Study[a,b]

	Serum RSV-Neutralizing Antibodies[c]						Serum IgG ELISA RSV F Antibodies[c]					
	Vaccination			RSV Surveillance			Vaccination			RSV Surveillance		
	Median (IQR)[d]			Median (IQR)[d]			Median (IQR)[d]			Median (IQR)[d]		
Group	Before[e]	After[f]	≥4-Fold Rise, No. (%)[g]	Before[a]	After[b]	≥4-Fold Rise, No. (%)[h]	Before[e]	After[f]	≥4-Fold Rise, No. (%)[g]	Before[a]	After[b]	≥4-Fold Rise, No. (%)[h]
RSV/ΔNS2/Δ1313/I1314L recipients (n = 25)	2.3 (2.3-2.3)	5.1 (4.1-6.2)	15 (60)	5.7[i] (3.5-6.4)	7.0[i] (5.8-10.1)	11[i] (50)	7.6 (6.1-8.5)	10.4 (9.3-11.8)	15 (60)	10.4[i] (7.6-11.8)	12.7[i] (11.1-16.6)	11[i] (50)
RSV/276 recipients (n = 24[j])	2.3 (2.3-2.3)	6.7 (6.0-7.8)	22 (92)	6.7[i] (6.1-8.3)	6.4[i] (5.8-7.5)	3[i] (14)	7.2 (6.1-8.6)	12.6 (11.3-13.3)	22 (92)	12.3[i] (11.3-12.6)	12.3[i] (11.5-12.9)	3[i] (14)
Placebo recipients (n = 12)	2.3 (2.3-2.9)	2.3 (2.3-2.3)	0 (0)	2.3[i] (2.3-2.3)	2.3[i] (2.3-5.8)	3[i] (33)	6.8 (5.4-9.5)	6.4 (4.6-7.9)	0 (0)	4.6[i] (4.6-6.5)	4.6[i] (4.6-11.9)	4[i] (44)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; F, fusion; IgG, immunoglobulin G; IQR, interquartile range; $PRNT_{60}$, 60% plaque reduction neutralizing titer; RSV, respiratory syncytial virus.

[a]Before RSV surveillance, collected 1–31 October or on day 56 if collected on or after 1 October.

[b]After RSV surveillance, collected approximately 6–12 months after inoculation (1–30 April except in 5 participants who had visits later due to COVID-19: 1 RSV/ΔNS2/Δ1313/I1314L vaccine recipient had serum collected in June; 2 RSV/276 vaccine recipients had serum collected in September; 2 RSV/276 vaccine recipients had serum collected 1 in June and the other in July).

[c]Serum RSV $PRNT_{60}$ was determined by complement-enhanced 60% plaque reduction neutralization assay; serum IgG titers to RSV F, with ELISA.

[d]Titer results are expressed as median reciprocal log_2 values (with IQRs), determined for all participants in each group. Specimens with titers below the limit of detection were assigned reciprocal titers of 2.3 log_2 ($PRNT_{60}$) and 4.6 log_2 (ELISA).

[e]Before inoculation.

[f]After inoculation, at study day 56.

[g]No. (%) of vaccine and placebo recipients with a ≥4-fold increase between preinoculation and postinoculation antibody titers.

[h]No. (%) of vaccine and placebo recipients with a ≥4-fold increase between pre- and post-RSV surveillance antibody titers.

[i]Three participants had missing data at these time points.

[j]Twenty-four participants were included in these analyses because 1 twin was excluded.

7.7–10.8 log_2) and RSV/276 vaccinees (median, 10.6; IQR, 8.0–11.3 log_2) than for placebo recipients (median, 5.9; IQR, 4.5–6.3 log_2; Supplementary Figure 2), indicating that both vaccines primed for a strong anamnestic response to wild-type RSV that substantially exceeded the primary response to wild-type RSV in placebo recipients.

Assessment of antibody titers in vaccinees who did not have a boosted response after the RSV surveillance period, and thus presumed not to have been infected with wild-type RSV, provided an opportunity to evaluate the durability of the primary response. In the 11 RSV/ΔNS2/Δ1313/I1314L vaccinees who did not acquire wild-type RSV, the pre- and post-RSV surveillance median RSV-$PRNT_{60}$ was minimally changed (median, 6.0 [IQR, 4.6–6.4] vs 5.8 [IQR, 4.7–6.3] log_2). Similar results were observed for their anti-RSV F IgG titers (median, 10.8 [IQR, 9.7–11.7] vs 11.1 [IQR, 9.7–12.0] log_2). In the 16 RSV/276 vaccinees without wild-type RSV, the pre- and post-RSV surveillance median RSV-$PRNT_{60}$ was also minimally changed (median, 7.1 [IQR, 6.2–8.4] vs 6.1 [IQR, 5.6–7.4] log_2). Similar results were observed for their anti-RSV F IgG titer (1 median, 2.4 [IQR, 11.4–12.7] vs 12.2 [IQR, 11.3–12.7] log_2).

DISCUSSION

When administered to RSV-naive children 6–24 months of age, the RSV/ΔNS2/Δ1313/I1314L and RSV/276 candidate vaccines were well tolerated and highly infectious. Fever and mild respiratory symptoms were common in all 3 groups: 64%, 84%, and 58% of recipients of RSV/ΔNS2/Δ1313/I1314L, RSV/276, and placebo, respectively, had 1 or more illness episodes following vaccination. The incidence in the RSV/ΔNS2/Δ1313/I1314L group is similar to the placebo group and is consistent with the high background rates of respiratory/febrile illnesses characteristic of this age group. The incidence of URI symptoms in the RSV/276 group, although mild, was somewhat higher than in the other 2 groups. In particular, cough occurred in 48% of RSV/276 vaccinees, compared to 12% and 17% of RSV/ΔNS2/Δ1313/I1314L and placebo recipients, respectively. It is difficult to fully attribute these symptoms to the vaccines because other respiratory pathogens were often simultaneously detected. However, while 1/7 (14%) placebo recipients had no other respiratory pathogen detected at the time of illness, 4/16 (25%) RSV/ΔNS2/Δ1313/I1314L recipients and 12/21(57%) RSV/276 recipients with respiratory events had vaccine virus detected without detection of other respiratory pathogens, which implicates the vaccine candidates. No LRIs or SAEs were reported.

Vaccination induced neutralizing antibody responses in 92% of participants who received RSV/276, matching or exceeding that observed in previously studied live-attenuated RSV candidates [11–14, 21, 22]. Importantly, vaccinees inoculated with either study product demonstrated anamnestic serum RSV antibody responses following RSV infection, often without

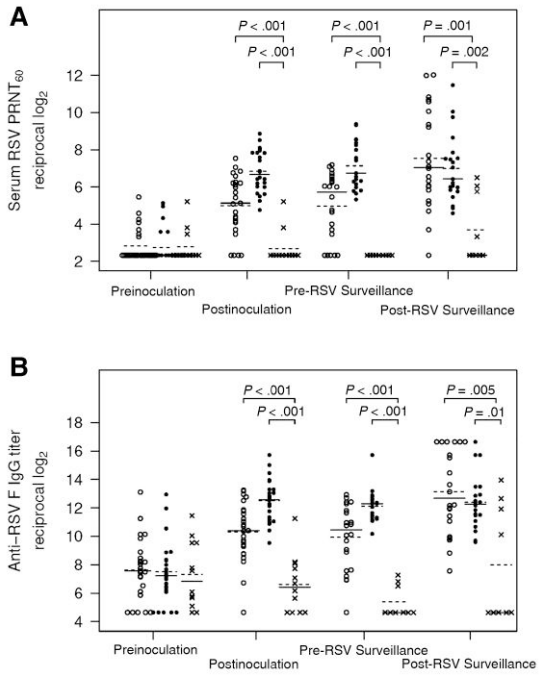

Figure 3. Serum respiratory syncytial virus (RSV) antibody titers in vaccine and placebo recipients. Serum RSV 60% plaque reduction neutralizing titers ($PRNT_{60}$) (*A*) and anti-RSV fusion (F) immunoglobulin G (IgG) titers (*B*) were determined by means of complement enhanced 60% plaque reduction neutralization assay and IgG-specific enzyme-linked immunosorbent assay against purified RSV F protein, respectively, for RSV/ΔNS2/Δ1313/I1314L vaccine (open circles), RSV/276 vaccine (filled circles), and placebo (×'s) recipients in serum samples collected before inoculation (screening), after inoculation (study day 56), before surveillance (October of the enrollment year), and after surveillance (usually April after the RSV season). Titers are expressed as the reciprocal log_2 values. Lines indicate median (solid lines) and mean (dashed lines) values. *P* values were determined by means of Wilcoxon rank sum test. Data for the presurveillance and postsurveillance visits are missing for 3 participants in each treatment group.

associated medically attended illnesses. In those vaccinees without anamnestic responses or other evidence of RSV infection, vaccine-induced antibody responses were durable.

Vaccination induced ≥4-fold rises in serum RSV-neutralizing antibody titers in only 60% of RSV/ΔNS2/Δ1313/I1314L recipients. It is notable that a larger proportion of infants receiving RSV/ΔNS2/Δ1313/I1314L entered the study with higher baseline antibody titers and 6/7 children with higher baseline titers were ≤7 months of age, suggesting that the titers reflected maternal antibodies. These infants accounted for the majority of vaccinees with vaccine shedding without detectable serum antibody responses, suggesting that even low preexisting serum antibody titers might blunt the antibody response. It is also possible that primary antibody responses were present but obscured by the presence of maternal antibodies. The impact of baseline antibody titers appeared to be more pronounced for RSV/ΔNS2/Δ1313/I1314L than RSV/276. In some vaccinees in both arms, we detected a boosting of antibody titers over the RSV season. In most of these vaccinees, post-RSV season titers were substantially higher than those of placebo recipients with evidence of natural RSV infection, suggesting strong anamnestic responses or priming. [15].

Exposure of placebo recipients to community RSV during the surveillance period represents primary infections, and thus their post-RSV surveillance serum antibody titers illustrate primary responses to wild-type RSV infection. It is noteworthy that this median titer, namely 5.9 (IQR, 4.5–6.3) log_2, was similar to primary (day 56) responses to the vaccines, namely 5.1 (IQR, 4.1–6.2) log_2 and 6.7 (IQR, 6.0–7.8) log_2 for RSV/ΔNS2/Δ1313/I1314L and RSV/276, respectively. Thus, in those individuals with serum antibody responses, the vaccines were similar in immunogenicity to wild-type RSV.

In this study, we simultaneously evaluated 2 candidate live-attenuated RSV vaccines. The study was not powered to directly compare the 2 vaccines to each other but to compare each to the placebo and to prespecified infectivity and immunogenicity rates. RSV/276 was anticipated to be a strong candidate due to its similarity to MEDI/ΔM2-2, a candidate previously shown to have excellent immunogenicity despite low viral replication. RSV/276 did achieve the goals for infectivity and immunogenicity, eliciting robust neutralizing antibody responses. However, the relatively high rate of cough and URI after inoculation was unexpected. While the symptoms were mild and well tolerated, that frequency of cough is of potential concern as it might reflect respiratory illness not confined to the upper respiratory tract. However, it is very possible that certain mild URIs, such as grade 1 rhinorrhea, would be acceptable for a live-attenuated RSV vaccine.

Limitations include the small sample size, which precludes firm conclusions regarding rates of vaccine-associated events such as cough. The immune assessment included only the most established correlates of protection against RSV disease, namely serum RSV-neutralizing and anti-RSV F IgG titers. Future studies will include measurements such as serum antibodies specific to the prefusion version of the RSV-fusion protein and measures of cellular and mucosal immunity. Protective efficacy also could not be evaluated. Individuals who had been vaccinated with either vaccine candidate usually did not experience RSV-MAARI upon community exposure, but the sample size of the study was too small to permit definitive conclusions.

In summary, the RSV/ΔNS2/Δ1313/I1314L and RSV/276 vaccines had excellent infectivity, although the infection rate for RSV/ΔNS2/Δ1313/I1314L was somewhat lower than expected based on a previous study [13]. The absence of infection in some recipients of RSV/ΔNS2/Δ1313/I1314L was associated with low levels of preexisting RSV antibodies. In recipients of RSV/ΔNS2/Δ1313/I1314L and RSV/276 who had a serum antibody response, each vaccine seemed as immunogenic as primary infection with wild-type RSV occurring in some placebo recipients during surveillance. Both vaccines were generally well tolerated except that

RSV/276 may have an undesirable rate of cough. For both vaccines, responders demonstrated strong anamnestic serum antibody responses and generally did not experience RSV-MAARI during subsequent wild-type RSV exposure. Further evaluation of the RSV/ΔNS2/Δ1313/I1314L vaccine is warranted.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We especially thank the children and families for their commitment to advancing research in the prevention of RSV. We acknowledge members of the protocol team for their expert contributions, including Jennifer Oliva and Kimberli Wanionek, MS at the Center for Immunization Research Laboratory, Johns Hopkins University; Laboratory Technologist Paul Harding, MS of University of Colorado School of Medicine; Data Managers Frederic Bone, Jared Kneebone, PhD, Linda Marillo, BS, Oswald Dadson, MS, and Andee Fox, MPH at Frontier Science and Technology Research Foundation; Protocol Pharmacist Lynette Purdue, PharmD at Pharmaceutical Affairs Branch, Division of AIDS; Network Laboratory Center Specialist Dale Dayton, RN, CCRA at Children's Hospital of Los Angeles; Westat Clinical Research Associate Scott Watson, BS at Westat, Inc; Staff Scientist Yumiko Matsuoka, PhD, National Institute of Allergy and Infectious Diseases (NIAID); the companion protocol (CIR 321) Medical Monitor Shirley Jankelevich, MD and the CIR 321 Clinical Research Oversight Managers Kelly Cahill, RN, CCRC, RAC, Susan Vogel, RN, BSN, and John Tierney RN, MPM of the Regulatory Compliance and Human Subjects Protection Branch, Division of Clinical Research, NIAID, National Institutes of Health (NIH). Dr Gregory Glenn, Novavax, generously provided the gift of baculovirus-expressed RSV F protein used as ELISA antigen. We thank the members of the NIAID, Division of Clinical Research, Data and Safety Monitoring Board. Finally, we thank the dedicated site investigators and research professionals at the following institutions, in alphabetical order: Ann and Robert H. Lurie Children's Hospital: Jessica D'Angelo, APN; Margaret Ann Sanders, MPH. Boston Medical Center: Ellen R. Cooper, MD; Debra McLaud, RN; Maria Narvaez, MS; Stephen I. Pelton, MD. Johns Hopkins University: Kristi Herbert, CRNP; Karen Loehr, BSN. Jacobi Medical Center Bronx: Andrew Wiznia, MD; Joanna Dobroszycki, MD; Marlene Burey, NP; Raphaelle Auguste, RN. Rush University: Maureen McNichols, RN, CCRC; Ixchell Ortiz-Estes, APN. St Jude Children's Research Hospital: Nehali Patel, MD; Adrienne English, BSN, RN; Jamie Russell-Bell, BSN, RN. SUNY Stony Brook: Sharon Nachman, MD; Christy Beneri, DO; Barsha Chakraborty, BS; Richard Connor, RPh. Texas Children's Hospital: Mariam Pontifes, CCRP; Chivon McMullen, MS, BSN, RN; Shelley Buschur, RN, CNM, ACRN. University of California Los Angeles: Michele F. Carter, BS, RN; Christina S. Shin, PharmD. University of California San Diego: Stephen A. Spector, MD; Anita Darcey, RN; Megan Loughran, BA; Leticia Muttera, PharmD. University of Colorado School of Medicine: Carrie Glenny, RN, BSN, MA; Kacey Navarro, MSN, FNP-C; Shane Curran-Hays, MS; Tina Powell, MS. University of Southern California: Sara Villanueva, LVN; Yvonne Morales, LVN.

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Financial support. This work was supported by NIAID NIH, with cofunding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) NIH, and the National Institute of Mental Health NIH (grant numbers UM1AI068632 [IMPAACT LOC], UM1AI068616 [IMPAACT SDMC], UM1AI106716 [IMPAACT LC], and HHSN275201800001I [NICHD] to the International Maternal Pediatric Adolescent AIDS Clinical Trials [IMPAACT] Network); a cooperative research and development agreement between NIAID, NIH, and Sanofi; the Duke University Center for AIDS Research, an NIH funded program (grant number 5P30 AI064518); and the Colorado Clinical and Translational Science Award from the National Center for Advancing Translational Science (grant number UL1 TR002535). R. A. K. and E. S. were supported by NIAID (grant numbers HHSN272200900010C and 75N93019D00031). C. L., L. Y., P. L. C., and U. J. B. were supported by the Intramural Program of the NIAID.

Potential conflicts of interest. U. J. B., C. L., and P. L. C. are listed as inventors on patents related to live-attenuated RSV vaccines, including vaccines containing genetically stabilized attenuating mutations and received research support and royalties paid by Sanofi. C. K. C. and R. A. K. have served as paid consultants to Sanofi. E. J. M. participated in an advisory board meeting for Sanofi. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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