Phase 1 Study of the Safety and Immunogenicity of a Live, Attenuated Respiratory Syncytial Virus and Parainfluenza Virus Type 3 Vaccine in Seronegative Children

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Background: Respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3) are important causes of lower respiratory tract illness and hospitalization in young children. Currently, there is no licensed vaccine against RSV or PIV3.

Methods: In this randomized, phase 1, double-blind, placebo-controlled, dose-escalating study, 49 healthy RSV/PIV3-seronegative children 6 to <24 months of age were randomized 2:1 to receive 3 doses (at 10^4, 10^5, or 10^6 median tissue culture infective dose [$TCID_{50}$]) of MEDI-534 (a live, attenuated RSV/PIV3 chimeric virus vaccine candidate) or placebo at 2-month intervals. Solicited adverse events (SEs) and unsolicited adverse events (AEs) were recorded during days 0 to 28 after each dose. Nasal wash samples were collected 3 times (days 7–10, 12–18, and 28–34) after each dose and at unscheduled illness visits. Blood for antibody response was collected at baseline and 28 days after each dose. Subjects were followed for 180 days after the last dose or to the end of the RSV season.

Results: Overall, there was no difference in the incidence of SEs and AEs between the RSV/PIV3 vaccine and placebo arms. Runny/stuffy nose was the most commonly reported SE. Medically attended lower respiratory illness rates were balanced between treatment arms, and there was no evidence of enhanced RSV disease or vaccine-related serious AEs. Vaccine virus was detected in most vaccinees on days 7 to 10 after dose 1 in a dose-dependent manner. Seroresponse to RSV and PIV3 was highest in subjects receiving the 10^6 dosage.

Conclusions: The safety profile and vaccine take as measured by shedding and/or seroresponse in this RSV/PIV3-seronegative pediatric population support the continued development of this RSV/PIV3 pediatric vaccine candidate.

Key Words: RSV, parainfluenza virus 3, intranasal vaccine, immunogenicity, adverse effects
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Respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3) are the 2 most common causes of bronchiolitis and pneumonia among infants and young children.[1,2] These viruses cause disease in otherwise healthy infants, making the development of a vaccine a public health priority. Currently, no licensed vaccine exists for the prevention of RSV or PIV3 infection and disease.

Live, attenuated RSV and PIV3 vaccines have been in development for several decades. Intranasal delivery mimics natural infection and offers several advantages, especially for infants and young children, including the potential for induction of both systemic and mucosal immunity.[3] In addition, live viral vaccines have not been associated with enhanced RSV disease, as was seen with studies of an injectable formalin-inactivated RSV vaccine.[4]

One of the approaches to the development of a PIV3 vaccine was intranasal immunization with a live antigenically related animal virus, bovine PIV3 (bPIV3).[5] The bPIV3 virus is approximately 25% related to human PIV3 (hPIV3) by cross-neutralization and induces antibodies that protect against hPIV3 in animal studies, but it is not virulent in humans.[5,6] In previous clinical studies, an intranasal bPIV3 vaccine demonstrated an acceptable safety profile in adults and seropositive children and infants, and it was immunogenic in seronegative children and infants.[7–10] The RSV/PIV3 vaccine candidate (MEDI-534) described in the present study is based on a bPIV3 backbone in which the bPIV3 fusion and hemagglutinin-neuraminidase surface glycoproteins were replaced by those of hPIV3 virus.[11] Additionally, the human RSV F protein was engineered into the genome.[12] Thus, this RSV/PIV3 vaccine delivers antigens thought to be protective against both RSV and hPIV3 and is being developed for the prevention of lower respiratory tract illness in infants.

This RSV/PIV3 vaccine demonstrated an acceptable safety profile in animal models and in phase 1 studies in adults and RSV/PIV3-seropositive children 1 to 9 years of age.[12–15] As anticipated, there was restricted vaccine virus replication and minimal immunogenicity in adults and children who had been previously exposed to both RSV and PIV3.

The purpose of the current study was to evaluate the safety, tolerability, immunogenicity, and viral shedding profiles of 3 dosage levels of the RSV/PIV3 vaccine when administered to RSV- and PIV3-seronegative infants and young children.

SUBJECTS AND METHODS

Study Design

This was a randomized, double-blind, placebo-controlled, dose-escalating, multicenter, phase 1 study of an RSV/PIV3 vac-

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cine candidate (MEDI-534). The institutional review board for each site approved the study protocol and informed consent documents before enrollment. The nature and possible consequences of the study were explained to each child's parent or legal representative, and written informed consent was obtained. This study was registered on www.clinicaltrials.gov (NCT00493285) before subject enrollment and was conducted in accordance with the ethical principles of the Declaration of Helsinki and the International Conference on Harmonisation Guidance for Good Clinical Practice (Topic E6).

The primary objective of this study was to evaluate the safety and tolerability profile of 3 doses of study vaccine administered at 10^4, 10^5, or 10^6 median tissue culture infective dose ($TCID_{50}$) to RSV- and PIV3-seronegative children. Secondary objectives included description of the vaccine virus shedding, immunogenicity, genotypic stability of shed vaccine virus, and incidence of serious RSV disease throughout the study.

Study Population

Study subjects were children 6 to <24 months of age who were RSV- and PIV3-seronegative at baseline as determined by enzyme-linked immunosorbent assay. Each subject was the product of a normal full-term pregnancy (defined as >36 weeks' gestation) and was in good general health. Subjects were excluded for a history of asthma, reactive airway disease, wheezing requiring medication, pulmonary disease, or hospitalization for respiratory illness or mechanical ventilation. Additionally, subjects were excluded for the use of medications other than infrequent over-the-counter medications; receipt of immunosuppressive agents, blood, or immunoglobulin products; receipt of concomitant vaccines within 14 days of dosing for inactivated vaccines or rotavirus vaccines, or within 28 days of dosing for other live viral vaccines. Because the extent of shedding has not been established, subjects living with or attending day care with children <24 months of age or in contact with a pregnant caregiver or an immunocompromised person, healthcare worker, or preschool or day care teacher (for children <6 months of age) were not enrolled.

Study Vaccine

Construction of the MEDI-534 vaccine virus has been previously described.[12] Placebo was visually indistinguishable from vaccine, and both were stored frozen at or below −60°C.

Procedures

Informed consent was obtained from the children's parents or legal guardians before study screening/enrollment. The first subject was enrolled on July 2, 2007, and the last subject's study visit occurred on April 20, 2010. Using a central voice-activated telephone system, eligible subjects were randomized 2:1 (vaccine: placebo) to receive 3 doses of vaccine at 10^4, 10^5, or 10^6 $TCID_{50}$ or placebo at 2-month intervals in a stepwise manner by cohort. Randomization was stratified by age (≤12 vs. >12 months). Dose 1 was administered on day 0, dose 2 was administered 56 ± 8 days after dose 1, and dose 3 was administered 56 ± 8 days after dose 2. Subjects were evaluated 3 times (days 7–10, 12–18, and 28–34) after each dose for the collection of safety data and to obtain nasal wash samples to assess shedding of vaccine virus. Vaccine virus identified in nasal wash samples was quantitated using a fluorescent focus assay (FFA) and evaluated for genotypic stability. Additional nasal wash samples for unscheduled illness visits were obtained as soon as possible after the occurrence of specified symptoms, which included fever ≥100.4°F, cough or runny/stuffy nose for ≥2 consecutive days, and difficulty breathing or any acute respiratory illness within 28 days after dosing. To assess any serious RSV disease, all subjects were monitored for 180 days after the final dose or until the end of the RSV season after vaccination, whichever was later. During the follow-up period, subjects were evaluated for lower respiratory tract symptoms and a nasal wash sample was tested for the presence of vaccine virus or other respiratory viruses. Serum samples for immunogenicity were obtained at baseline before receipt of dose 1 and approximately 28 days after each dose.

Safety

Safety was evaluated by the collection of solicited adverse events (SEs) and unsolicited adverse events (AEs) from day 0 to 28 after each dose. SEs were collected and recorded daily by the parent or legal guardian on a worksheet. SEs included fever (temperature, ≥100.4°F [≥38.0°C], regardless of method), runny and/or stuffy nose, cough, drowsiness, loss of appetite/decreased urine output, irritability/fussiness, oropharyngeal inflammation (laryngitis), and epistaxis. Additionally, medically attended lower respiratory illnesses (MA-LRIs), serious adverse events (SAEs), and significant new medical conditions were collected from day 0 to 180 days after the final dose or until the end of the RSV season, whichever was later. An MA-LRI was defined as a diagnosis of any 1 or more of the following by a healthcare provider: wheezing, pneumonia, croup, rhonchi, rales, bronchitis, bronchiolitis, and apnea. All adverse events were coded (MedDRA version 12.0, the Medical Dictionary for Regulatory Activities terminology, is the international medical terminology developed under the auspices of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use [ICH]) and graded for severity and relationship to study vaccine.

Laboratory Analyses

Immunogenicity Evaluation

A commercial enzyme-linked immunosorbent assay was used to evaluate RSV and PIV3 serostatus for eligibility (kits supplied by Immuno-Biologic Laboratories, Inc, Minneapolis, MN, and Viro-Immun Labor-Diagnostika GmbH, Oberursel, Germany, respectively). The immune response to RSV F was evaluated by measuring the functional serum antibody using a microneutralization assay and an antigenically matched virus. Briefly, 2-fold serial dilutions of heat-inactivated serum samples starting with a 1:5 initial dilution were prepared in virus growth media, and an equal volume of RSV A2 that was engineered to express green fluorescent protein a virus at a known titer was added to each diluted serum sample. After incubation for 1 hour at 37°C, the mixtures were added to confluent Vero cell monolayers grown in 96-well plates. After incubation for 22 hours at 33°C, the number of fluorescence-producing cells (foci) in each well was counted by using a laser imaging counter (IsoCyte, Molecular Devices, Inc, Sunnyvale, CA). The number of foci were determined for negative control wells and each serum dilution. The neutralization titer of a sample was defined as the reciprocal of the respective serum dilution that caused a 50% reduction in the foci count compared with the negative control. The assay was run in duplicate. If the first serum dilution (1:5) did not provide the fluorescent focus unit (FFU) count ≤50% of the input virus, the titer was reported as <5, and a value of 2.5 was imputed for analysis.

The immune response to the PIV3 surface glycoproteins was measured using a hemagglutination inhibition (HAI) assay as previously described.[16,17] Serial 2-fold dilutions of sera were mixed with virus beginning at a dilution of 1:4. After incubation, a suspension of guinea pig erythrocytes was added to the serum/virus dilutions aliquoted into the wells of 96-well plates, and wells were observed for agglutination. End point titers were reported as the reciprocal of the highest dilution of serum that inhibited agglutination. If there was no inhibition at the lowest dilution

(1:4), the titer was recorded as 2 for statistical analysis. The virus used in the HAI assay was the same as the virus strain used in the vaccine,[12] and the positive control serum used in the assay was generated in goat (Veterinary Medical Research and Development, Pullman, WA).

Seroresponse was defined a ≥4-fold increase from baseline in RSV titer by microneutralization assay or PIV3 titer by HAI assay. Data were excluded from their respective immunogenicity analyses after the first confirmed detection of wild-type RSV or PIV3 by reverse-transcription polymerase chain reaction (RT-PCR) in a subject.

Identification of Respiratory and Vaccine Virus

Nasal wash specimens collected on scheduled and unscheduled illness visits were diluted in viral transport medium and shipped at 2°C to 8°C to a central virology laboratory (V-Bio, Inc, St. Louis, MO) within 24 hours of collection, and were assayed for respiratory viruses using tissue culture for virus isolation. Any samples that were determined to be positive for RSV and/or PIV3 at the central virology laboratory were further characterized at the MedImmune Clinical Testing Laboratory (Mountain View, CA). Briefly, viral nucleic acid was isolated from stabilized nasal wash samples, and the detection of wild-type respiratory viruses was performed using real-time quantitative RT-PCR assays that detect type A and B influenza viruses; type A and B RSVs; PIVs 1, 2, and 3; and human metapneumovirus.

Samples positive for RSV and/or PIV3 in culture were also tested for the presence of vaccine virus because culture does not distinguish the vaccine from wild-type hPIV3. Nasal wash samples found to contain vaccine virus in the absence of wild-type virus were subsequently evaluated for genotypic stability as defined by the presence of the RSV F insert using specific primers and probes spanning the bPIV3 N and RSV F gene junctions.

Quantitation of virus shed by vaccine recipients was determined by performing a FFA on nasal wash samples collected from vaccinees. Vero cells were grown to confluence in 96-well plates, and wells were then inoculated in triplicate with 2-fold serial dilutions of virus control or clarified nasal wash sample supernatants. Fluorescent foci, representing infected cells, were enumerated by microscopic observation using appropriate filters. The number of foci per well was then converted to a titer on the assumption that each fluorescent focus corresponds to a single infectious virus particle. Data were reported as an average of 3 replicates as $\log_{10}$ FFU/mL. The limit of quantification (LOQ) was 2.9 FFU/mL.

Statistical Analysis

Sample size was based on clinical rather than statistical considerations. Given the small sample size, analyses were primarily descriptive in nature. Missing data were treated as missing. Vaccine take was defined by recovery of vaccine virus from nasal wash at any time and/or seroconversion. No data were imputed except as described for the immunogenicity values below the LOQ.

For the proportion of subjects with seroresponse, 2-sided 95% confidence intervals were calculated using the Clopper-Pearson exact method. For safety endpoints, the Fisher exact test was used to compare group differences in the incidence rates of SEs and AEs. *P* values were used to screen for differences of potential significance at a 0.05 significance level; thus, no multiplicity adjustment was made.

RESULTS

Subject Disposition and Demographics

A total of 146 subjects were screened for study eligibility, and 101 subjects had a screening blood draw for RSV/PIV3 serostatus. Of the 101 subjects, 60 (59.4%) were both RSV and PIV3 seronegative at screening. In all, 49 subjects were enrolled and randomized into the study at 13 sites in the United States. A total of 19 subjects were enrolled in cohort 1 (10^4 $TCID_{50}$; 13 RSV/PIV3 vaccine, 6 placebo); 15 subjects were enrolled in cohort 2 (10^5 $TCID_{50}$; 9 RSV/PIV3 vaccine, 6 placebo); and 15 subjects were enrolled in cohort 3 (10^6 $TCID_{50}$; 10 RSV/PIV3 vaccine, 5 placebo) (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A957, shows the subject disposition).

The safety population, which comprised subjects who received at least 1 dose and had any safety follow-up, included 100% of both RSV/PIV3 vaccine and placebo recipients. A total of 10 subjects did not receive all 3 doses of study vaccine (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A957, shows the subject disposition). The majority of randomized subjects (79.6%) were between 6 and 12 months of age (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A958, shows the subject demographics and baseline characteristics).

Safety

SEs were common in the 28 days after dosing, with 96.9% of RSV/PIV3 vaccine subjects and 100% of placebo subjects reporting at least 1 SE during the study (data not shown). Overall (after any of the 3 doses), no SEs were reported at a rate ≥10 percentage points higher in the RSV/PIV3 vaccine arm. Because the collected SEs represent events that occur commonly in this pediatric population, SE data were also analyzed for days 0 to 14 after each dose to further examine whether any events may be more likely related to vaccine (Fig., Supplemental Digital Content 3, http://links.lww.com/INF/A959, shows the incidence of solicited events). SEs were reported at the same frequency overall during days 0 to 14 as during days 0 to 28 (96.9% RSV/PIV3 vaccine vs. 100% placebo). Runny/stuffy nose was the only SE that was reported at a higher rate (≥10 percentage points [nonsignificant]) in all RSV/PIV3 vaccine subjects versus all placebo subjects during days 0 to 14 for all doses combined. In addition, runny/stuffy nose was statistically significantly higher in cohort 1 (10^4 $TCID_{50}$) RSV/PIV3 vaccine subjects versus placebo subjects after dose 1 (76.9% vs. 16.7%, $P = 0.04$) but not after doses 2 or 3 or in other cohorts. There did not appear to be a trend toward increasing reactogenicity with increased dosage of RSV/PIV3 vaccine or with subsequent dosing across a cohort.

The majority of SEs were mild or moderate in severity (98.2% in the RSV/PIV3 vaccine group vs. 99.5% in the placebo group). Six subjects in the RSV/PIV3 vaccine group reported severe SEs; 5 reported severe fever (103.2–104.9°F [39.6–40.5°C]), and 1 reported severe laryngitis. One placebo subject reported severe fever as an SE.

AEs were reported frequently in the 28 days after each vaccination (84.4% of RSV/PIV3 vaccine recipients vs. 76.5% of placebo recipients) and, overall, the rates were similar for each cohort (data not shown). The most common AEs in RSV/PIV3 vaccine recipients after dose 1 were teething (21.9% vs. 5.9%), diarrhea (15.6% vs. 11.8%), and otitis media (12.5% vs. 11.8%) in RSV/PIV3 vaccine versus placebo recipients, respectively. The most common AEs in RSV/PIV3 vaccine recipients after dose 2 were teething (14.8% vs. 0%), diarrhea (11.1% vs. 12.5%), and increased body temperature (either measured temperature <100.4°F or parental report that a subject felt warm/hot; 11.1% vs. 12.5%). Similarly, the most common AEs after dose 3 were diarrhea (24.0% vs. 12.5%), teething (16.0% vs. 12.5%), and otitis media (12.0% vs. 0%). There was no observed trend for increasing AEs with subsequent doses or with increasing dosage level.

The majority of AEs occurring through day 28 after dosing were mild or moderate in severity (97.7% of events in the RSV/

PIV3 vaccine group; 98.7% of events in the placebo group). There were 3 severe AEs reported by 2 subjects in the RSV/PIV3 vaccine group: croup and viral gastroenteritis in 1 subject in cohort 1 (10^4 $TCID_{50}$) and otitis media in another subject in cohort 2 (10^5 $TCID_{50}$). There was 1 severe AE of croup reported by a subject in the placebo group. One of the 3 severe AEs in the RSV/PIV3 vaccine group (croup due to PIV1 infection) and the severe AE in the placebo group (croup due to PIV1 infection) were also classified as MA-LRIs and considered possibly related to investigational product by the investigator. All other severe AEs reported within 28 days after dosing in RSV/PIV3 vaccine recipients were considered to be unrelated to study vaccine. No AE was considered to be potentially life-threatening.

No SAEs were reported through the 28-day postdose observation period. Two SAEs were reported outside the primary safety evaluation period during the long-term follow-up phase of the study. One RSV/PIV3 vaccine subject in cohort 1 (10^4 $TCID_{50}$) was hospitalized for streptococcal pharyngitis 94 days after dose 2. One subject in the placebo arm of cohort 2 (10^5 $TCID_{50}$) was hospitalized with bronchiolitis 66 days after dose 3. No SAEs were considered vaccine related. No significant new medical conditions were reported in any subject from receipt of first dose until the end of the study follow-up period.

In all, 13 subjects experienced a total of 18 MA-LRIs from initial dosing until study completion (Table, Supplemental Digital Content 4, http://links.lww.com/INF/A960, shows the MA-LRIs by subject). Of the 18 MA-LRI events, 6 occurred within the primary 28-day postdosing safety period. MA-LRI rates were comparable between both treatment groups (21.9% in MEDI-534 vs. 35.3% in placebo [nonsignificant]). No dosage level relationship was observed, with a similar proportion of subjects reporting MA-LRIs in each cohort. As expected, 72% of the events occurred during the winter respiratory viral season (November–April). No MA-LRIs were associated with vaccine virus shedding.

Vaccine Virus Shedding

Vaccine virus shedding was only detected in RSV/PIV3 vaccine recipients (Fig., Supplemental Digital Content 5, http://links.lww.com/INF/A961, shows the shedding profile in the RSV/PIV3 vaccine group by cohort and dose). Shedding rates were higher in cohorts 2 and 3 than in cohort 1. The majority of shedding was observed in the day 7 to 10 sample window after dose 1 (17 of 29 vaccinated subjects [58.6%]). Only 6 of 31 subjects (19.4%) shed virus in the day 12 to 18 period (1 in cohort 1, 3 in cohort 2, and 2 in cohort 3), whereas no shedding was detected in the latest collection period (days 28–34) in any cohort.

All nasal wash samples containing vaccine virus were determined to be genotypically stable by PCR testing. The quantity of shed virus was determined for 26 samples from 17 RSV/PIV3 vaccine recipients. Of these samples, 19 had results that were less than the LOQ for the assay. The remaining 7 samples with a quantifiable FFA value were all from the day 7 to 10 post–dose 1 collection time point (4 from subjects in cohort 1, 2 from subjects in cohort 2, and 1 from a subject in cohort 3). Peak titers from the 7 samples were similar in all cohorts (4.4 log_{10} FFU/mL in cohort 1, 3.8 log_{10} FFU/mL in cohort 2, and 2.9 log_{10} FFU/mL in cohort 3).

Immunogenicity

In general, a dose response was observed in the seroresponses to both RSV and PIV3 (Table 1). A greater proportion of subjects seroresponded in cohort 3 (10^6 $TCID_{50}$) as compared with the other 2 dosage cohorts. In RSV/PIV3 vaccine recipients, 18.2% of subjects in cohort 1 had a seroresponse against RSV after dose 1, whereas 44.4% developed a response in cohort 3. Similarly, responses after dose 2 were 10% and 55.6%, respectively, for cohorts 1 and 3, whereas responses after dose 3 were 37.5% and 50%, respectively.

The proportion of subjects with a seroresponse to PIV3 was somewhat higher than the proportion with a seroresponse to RSV. After the first dose, a seroresponse was detected in 54.5% of cohort 1 vaccine recipients, which increased to 71.4% in cohort 2 and 80% in cohort 3. After the third dose of vaccine, a seroresponse was detected in 80%, 60%, and 100% of cohort 1, 2, and 3 vaccinees, respectively.

A single placebo subject in cohort 1 had a seroresponse to RSV after dose 3. It is most likely that this subject had a wild-type RSV infection during the course of the study, resulting in the seroresponse; however, no wild-type virus was recovered from this subject.

Vaccine Take (Shedding and/or Seroresponse)

This vaccine was developed to provide protection against 2 viruses using a chimeric vaccine construct; thus, there are a number of ways to evaluate vaccine take (Table 2). Both shedding and immune response are evidence of vaccine's biologic activity. Defining vaccine take as seroresponse or shedding is the more sensitive measure. Using these criteria, all subjects demonstrated vaccine take in the 10^6 cohort. When vaccine take was defined as

TABLE 1. Seroresponse to RSV and PIV3 in Subjects Who Received the RSV/PIV3 Vaccine

	Seroresponse					
	Cohort 1 (10^4 $TCID_{50}$)		Cohort 2 (10^5 $TCID_{50}$)		Cohort 3 (10^6 $TCID_{50}$)	
	n/N (%)	95% CI	n/N (%)	95% CI	n/N (%)	95% CI
RSV						
Dose 1	2/11 (18.2)	2.3–51.8	3/7 (42.9)	9.9–81.6	4/9 (44.4)	13.7–78.8
Dose 2	1/10 (10)	0.3–44.5	1/5 (20.0)	0.5–71.6	5/9 (55.6)	21.2–86.3
Dose 3	3/8 (37.5)	8.5–75.5	1/5 (20.0)	0.5–71.6	4/8 (50.0)	15.7–84.3
PIV3						
Dose 1	6/11 (54.5)	23.4–83.3	5/7 (71.4)	29.0–96.3	8/10 (80.0)	44.4–97.5
Dose 2	6/10 (60.0)	26.2–87.8	4/5 (80.0)	28.4–99.5	7/9 (77.8)	40.0–97.2
Dose 3	8/10 (80.0)	44.4–97.5	3/5 (60.0)	14.7–94.7	8/8 (100)	63.1–100

Seroresponse was defined as a ≥4-fold rise from baseline. Microneutralization assay (RSV) and HAI assay (PIV3) results were excluded from analysis upon demonstration of wild-type RSV/PIV3 shedding. For microneutralization assay analysis, a value of 2.5 was assigned if the result was below the LOQ (<5); and for HAI analysis, a value of 2 was assigned if the result was below the LOQ (<4).

CI indicates confidence interval; RSV, respiratory syncytial virus; PIV3, parainfluenza virus type 3; HAI, hemagglutination inhibition; LOQ, limit of quantification.

TABLE 2. Vaccine Take

Virus to Which Seroresponse Was Detected, Treatment Group	Seroresponse and Vaccine Shedding n/N (%)	Seroresponse or Vaccine Shedding n/N (%)
RSV or PIV3		
10^4 vaccine	6/13 (46.2)	10/13 (76.9)
10^5 vaccine	4/8 (50.0)	8/8 (100.0)
10^6 vaccine	7/10 (70.0)	10/10 (100.0)
RSV and PIV3		
10^4 vaccine	1/13 (7.7)	8/13 (61.5)
10^5 vaccine	2/8 (25.0)	5/8 (62.5)
10^6 vaccine	3/10 (30.0)	10/10 (100.0)

Seroresponse was defined as a ≥4-fold increase from baseline in titer by RSV microneutralization assay/PIV3 hemagglutination inhibition assay. Vaccine shedding was defined as shedding of vaccine-type virus detected in nasal wash samples at any time point.

RSV indicates respiratory syncytial virus; PIV3, parainfluenza virus type 3.

shedding in addition to seroresponse, a dose response was still observed; however, the proportion of subjects was lower (70% vs. 100% for RSV or PIV3, respectively, and 30% vs. 100% for RSV and PIV3, respectively, in the 10^6 cohort).

DISCUSSION

This study is the first to report on the safety, immunogenicity, and viral shedding profiles of this RSV/PIV3 vaccine candidate (MEDI-534) in RSV/PIV3-seronegative young children. Importantly, this study demonstrated biologic evidence of vaccine take in RSV- and PIV3-seronegative children 6 to <24 months of age who received vaccine at 10^4, 10^5, or 10^6 $TCID_{50}$. In general, shedding and seroresponses were dose dependent; vaccine virus shedding was detected in 70% of vaccinees in the 10^6 RSV/PIV3 vaccine group, whereas seroresponse was 50% and 100% to RSV and PIV3, respectively, in this cohort.

The SEs and AEs observed in this study were primarily events that are expected to occur in children aged 6 to <24 months and were evenly distributed between groups. There did not appear to be a trend toward increasing reactogenicity with increased dosage of this RSV/PIV3 vaccine or with subsequent dosing across a cohort. MA-LRIs were not associated with vaccine virus shedding, and no evidence of enhanced RSV disease was present.

Previous studies have also demonstrated acceptable safety and tolerability of other PIV3 and RSV vaccines administered at similar dose levels in seronegative infants (10^4 and 10^5 $TCID_{50}$) and seropositive infants (10^6 $TCID_{50}$).[18–22] Furthermore, previous evaluations of bPIV3, the backbone for the chimeric virus tested in the present study, also revealed an acceptable safety profile.[9,10,22,23]

Vaccine-like virus was detected in nasal wash samples over a short period after dosing and occurred predominately after dose 1. Shedding was detected in the majority of subjects during days 7 to 10 after dose 1, and in more subjects in cohort 2 (62.5%) and cohort 3 (70%) than in cohort 1 (46.2%). Only 2 subjects shed after dose 2 or dose 3. Thus, it appeared that the first dose of the RSV/PIV3 vaccine at each dose level induced responses that restricted vaccine virus replication and shedding after doses 2 and 3. Additionally, the RSV F insert, which was added to the b/h PIV3 genome to create MEDI-534, was present in every sample in which vaccine virus was shed.

In this study, seroresponse and vaccine take were dosage-level dependent. Of the subjects who received dose 3 in the 10^6 cohort, seroresponse was detected in 50% of subjects to RSV and 100% to PIV3 compared with 37.5% (RSV) and 80.0% (PIV3) of subjects who received dose 3 in the 10^4 cohort. Consequently, vaccine take, as measured by the combination of vaccine viral shedding or seroresponse, was seen in a dosage-level–dependent manner and in 100% of subjects in the 10^6 cohort.

There are several important limitations of this study. The primary limitation was the small sample size, which limited the ability to detect uncommon adverse events. However, previous larger studies of the vaccine construct backbone contribute to the overall understanding of the safety of this RSV/PIV3 vaccine candidate, MEDI-534.[9,10,23] The fact that the first nasal wash sample was not obtained until approximately 7 days after dosing may have reduced our ability to detect virus shedding if it occurred earlier and was transient. Shedding has been observed between days 1 and 28 in previous bPIV3 clinical studies, peaking around day 10 after vaccination; differences in assay methods may have resulted in differences in titers detected.[9,10,20,22,23] Additionally, there is no known protective level of anti-PIV3 or -RSV antibody; therefore, although a 4-fold rise from baseline (ie, seroresponse) was used as a marker of immunogenicity, it is not known to be predictive of protection from disease. Thus, although only half of these subjects had a 4-fold rise from baseline in microneutralization antibody titers to RSV at the highest vaccine dose tested, other immune responses not assessed, such as mucosal antibodies or cell-mediated immunity, might protect against RSV disease. All of these subjects demonstrated evidence of vaccine take, confirming adequacy of delivery and of intranasal replication; however, this does not provide evidence of a protective immune response.

In conclusion, this live, attenuated RSV/PIV3 intranasal vaccine, demonstrated acceptable safety, shedding, and immunogenicity profiles in children 6 to <24 months of age who were RSV/PIV3-seronegative at baseline. Three doses administered on a 0-, 2-, and 4-month schedule were infectious and immunogenic in this population. A phase 1/2a study using the same dosages of this RSV/PIV3 vaccine in RSV/PIV3-seronegative children 6 to <24 months old and 2-month-old unscreened infants is currently underway. These data are supportive of continued development of this vaccine candidate for the prevention of lower respiratory tract illness caused by RSV and PIV3 in young children.

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REFERENCES

1. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. *N Engl J Med*. 2009;360:588–598.
2. Weinberg GA, Hall CB, Iwane MK, et al. Parainfluenza virus infection of young children: estimates of the population-based burden of hospitalization. *J Pediatr*. 2009;154:694–699.
3. Durbin AP, Karron RA. Progress in the development of respiratory syncytial virus and parainfluenza virus vaccines [serial online]. *Clin Infect Dis*. 2003;37:1668–1677.
4. Wright PF, Karron RA, Belshe RB, et al. The absence of enhanced disease with wild type respiratory syncytial virus infection occurring after receipt of live, attenuated, respiratory syncytial virus vaccines [serial online]. *Vaccine*. 2007;25:7372–7378.
5. Coelingh KJ, Winter CC, Murphy BR, et al. Conserved epitopes on the hemagglutinin-neuraminidase proteins of human and bovine parainfluenza type 3 viruses: nucleotide sequence analysis of variants selected with monoclonal antibodies. *J Virol*. 1986;60:90–96.

6. van Wyke Coelingh KL, Winter CC, Tierney EL, et al. Attenuation of bovine parainfluenza virus type 3 in nonhuman primates and its ability to confer immunity to human parainfluenza virus type 3 challenge. *Infect Dis*. 1988;157:655–662.
7. Clements ML, Belshe RB, King J, et al. Evaluation of bovine, cold-adapted human, and wild-type human parainfluenza type 3 viruses in adult volunteers and in chimpanzees [serial online]. *J Clin Microbiol*. 1991;29:1175–1182.
8. Karron RA, Wright PF, Newman FK, et al. A live human parainfluenza type 3 virus vaccine is attenuated and immunogenic in healthy infants and children. *J Infect Dis*. 1995;172:1445–1450.
9. Karron RA, Makhene M, Gay K, et al. Evaluation of a live attenuated bovine parainfluenza type 3 vaccine in two- to six-month-old infants [serial online]. *Pediatr Infect Dis J*. 1996;15:650–654.
10. Greenberg DP, Walker RE, Lee MS, et al. A bovine parainfluenza virus type 3 vaccine is safe and immunogenic in early infancy [serial online]. *J Infect Dis*. 2005;191:1116–1122.
11. Haller AA, Miller T, Mitiku M, et al. Expression of the surface glycoproteins of human parainfluenza virus type 3 by bovine parainfluenza virus type 3, a novel attenuated virus vaccine vector [serial online]. *J Virol*. 2000;74:11626–11635.
12. Tang RS, Schickli JH, MacPhail M, et al. Effects of human metapneumovirus and respiratory syncytial virus antigen insertion in two 3′ proximal genome positions of bovine/human parainfluenza virus type 3 on virus replication and immunogenicity [serial online]. *J Virol*. 2003;77:10819–10828.
13. Tang RS, MacPhail M, Schickli JH, et al. Parainfluenza virus type 3 expressing the native or soluble fusion (F) protein of respiratory syncytial virus (RSV) confers protection from RSV infection in African green monkeys [serial online]. *J Virol*. 2004;78:11198–11207.
14. Tang RS, Spaete RR, Thompson MW, et al. Development of a PIV-vectored RSV vaccine: preclinical evaluation of safety, toxicity, and enhanced disease and initial clinical testing in healthy adults. *Vaccine*. 2008;26:6373–6382.
15. Gomez M, Mufson MA, Dubovsky F, et al. Phase-I study MEDI-534, of a live, attenuated intranasal vaccine against respiratory syncytial virus and parainfluenza-3 virus in seropositive children. *Pediatr Infect Dis J*. 2009;28:655–658.
16. Rowe T, Abernathy RA, Hu-Primmer J, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. *J Clin Microbiol*. 1999;37:937–943.
17. World Health Organization. WHO manual on animal influenza: diagnosis and surveillance. Geneva, Switzerland: World Health Organization; 2002.
18. Belshe RB, Newman FK, Anderson EL, et al. Evaluation of combined live, attenuated respiratory syncytial virus and parainfluenza 3 virus vaccines in infants and young children. *J Infect Dis*. 2004;190:2096–2103.
19. Belshe RB, Newman FK, Tsai TF, et al. Phase 2 evaluation of parainfluenza type 3 cold passage mutant 45 live attenuated vaccine in healthy children 6–18 months old. *J Infect Dis*. 2004;189:462–470.
20. Karron RA, Belshe RB, Wright PF, et al. A live human parainfluenza type 3 virus vaccine is attenuated and immunogenic in young infants [serial online]. *Pediatr Infect Dis J*. 2003;22:394–405.
21. Madhi SA, Cutland C, Zhu Y, et al. Transmissibility, infectivity and immunogenicity of a live human parainfluenza type 3 virus vaccine (HPIV3cp45) among susceptible infants and toddlers. *Vaccine*. 2006;24:2432–2439.
22. Lee MS, Greenberg DP, Yeh SH, et al. Antibody responses to bovine parainfluenza virus type 3 (PIV3) vaccination and human PIV3 infection in young infants. *J Infect Dis*. 2001;184:909–913.
23. Karron RA, Wright PF, Hall SL, et al. A live attenuated bovine parainfluenza virus type 3 vaccine is safe, infectious, immunogenic, and phenotypically stable in infants and children [serial online]. *J Infect Dis*. 1995;171:1107–1114.