

Efficacy and Safety of 1 and 2 Doses of Live Attenuated Influenza Vaccine in Vaccine-Naive Children

Humberto Bracco Neto, MD, PhD,* Calil K. Farhat, MD,* Miguel Wenceslao Tregnaghi, MD,†
Shabir A. Madhi, MD, PhD,‡ Ahmad Razmpour, PhD,§ Giuseppe Palladino, MD,§ Margaret G. Small, MA,§
William C. Gruber, MD,§ and Bruce D. Forrest, MD, MBA,§ for the D153-P504 LAIV Study Group

Background: We investigated the efficacy and safety of 1 versus 2 doses of live attenuated influenza vaccine (LAIV) in influenza vaccine-naïve children aged 6 to <36 months.

Patients/Methods: Subjects were randomized to 1 of 4 regimens in year 1: 2 doses LAIV, 1 dose LAIV, excipient placebo, or saline placebo. In year 2, LAIV recipients were to receive 1 dose of LAIV and placebo recipients were to receive saline placebo. Because of an unintended treatment allocation error in year 2, 1 block of subjects who were randomized to LAIV received saline placebo and 1 block who were randomized to placebo received LAIV.

Results: In year 1, vaccine efficacy versus placebo among recipients of 2 and 1 doses of LAIV was 73.5% and 57.7%, respectively, against antigenically similar strains. In year 2, absolute efficacy of a single dose of LAIV was 73.6% and 65.2%, respectively, in recipients of 2 and 1 doses of LAIV in year 1. Year 2 efficacy was 57.0% in subjects who received 2 doses of LAIV in year 1 and placebo in year 2. Safety and tolerability of LAIV were consistent with previous studies. Reactogenicity was similar between placebo groups. Seroprevalence rates were significantly higher in the 2-dose versus the 1-dose LAIV group in year 1 and in both LAIV groups versus placebo in years 1 and 2.

Conclusions: One dose of LAIV provided clinically significant protection against influenza in young children previously unvaccinated against influenza; 2 doses provided additional protection. Protection after 2 doses in year 1 persisted through a second season without revaccination. LAIV excipients were not a major contributor to reactogenicity. These benefits provide support for increased use of LAIV in children ≥ 2 years of age.

Key Words: efficacy, influenza, live attenuated influenza vaccine, pediatric, safety

(*Pediatr Infect Dis J* 2009;28: 365–371)

Accepted for publication October 21, 2008.

From the *Federal University of São Paulo, São Paulo, Brazil; †Centro de Desarrollo de Proyectos Avanzados, Hospital Infantil de Córdoba, Córdoba, Argentina; ‡Paediatric Infectious Diseases Research Unit, Chris Hani-Baragwanath Hospital, Johannesburg, South Africa; and §Wyeth Vaccines Research, Pearl River, NY.

Supported by Wyeth Vaccines Research and MedImmune; and consultation/research grants from MedImmune (to H.B.N., C.K.F., M.W.T., and S.A.M.). M.G.S., A.R., G.P., W.C.G., and B.D.F. are employees of Wyeth.

Shabir A. Madhi is currently at Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases, University of the Witwatersrand, Johannesburg, South Africa.

Clinical Trial Registration: ClinicalTrials.gov ID No. NCT00192400.

Address for correspondence: William C. Gruber, MD, Wyeth Vaccines Research, 401 N. Middletown Rd., Pearl River, NY 10965. E-mail: Gruberw@wyeth.com.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

Copyright © 2009 by Lippincott Williams & Wilkins

ISSN: 0891-3668/09/2805-0365

DOI: 10.1097/INF.0b013e31819219b8

Influenza infections are responsible for approximately 20% of excess hospitalizations in young children (<3 years) and approximately 30% of excess outpatient visits in the United States.¹ Acute otitis media (AOM) is a common (15.4 cases per 100 person-months) influenza-related complication among children younger than 4 years of age.²

Beginning with the 2008–2009 influenza season, the Advisory Committee on Immunization Practices of the US Centers for Disease Control and Prevention recommends routine vaccination against influenza for all children 6 months to 18 years of age.³ Influenza vaccines are administered as a 2-dose schedule for previously unvaccinated children aged younger than 9 years, with the doses separated by at least 1 month.³ Several studies have demonstrated that 2 doses of trivalent inactivated vaccine (TIV) are required for meaningful protection against influenza in previously unvaccinated children younger than 8 years of age.^{4–6} The American Academy of Pediatrics' Committee on Infectious Diseases and the Advisory Committee on Immunization Practices recommend that children who received only a single vaccine dose during their first season be given 2 doses of vaccine the following season.³ Given the time constraints associated with annual influenza vaccination, compliance with the recommended 2-dose regimen is less than 50%.^{7–9}

This study was designed to assess prospectively the efficacy, immunogenicity, and safety of 1- and 2-dose regimens of live attenuated influenza vaccine (LAIV) in children aged 6 to <36 months. In addition, the study assessed revaccination with LAIV in a second season and effectiveness of LAIV against AOM and lower respiratory tract infection (LRI). Because upper respiratory symptoms such as nasal congestion and sore throat have been associated with LAIV,¹⁰ this study also attempted to determine whether vaccine excipients such as egg protein³ or hydrolyzed gelatin¹¹ might contribute to reactogenicity by using and comparing saline placebo and excipient placebo formulations.

METHODS

This was a placebo-controlled, multicenter study conducted during the 2001 and 2002 influenza seasons at 35 sites in South Africa, Brazil, and Argentina in accordance with the principles of the Declaration of Helsinki/Somerset West, Republic of South Africa, October 1996. The study protocol and informed consent forms were approved by the independent ethics committee for each center and by the respective country's board of health. For each subject, parents or legal guardians provided written informed consent.

Subjects

We enrolled children 6 to <36 months of age who were in good health. Exclusion criteria in year 1 included any serious chronic disease, immunosuppression or presence of an immunocompromised household member, receipt of any commercial or investigational influenza vaccine before enrollment, a documented history of hypersensitivity to any component of LAIV or placebo,

or any medical condition(s) that in the opinion of the investigator might interfere with interpretation of the study results. In year 2, subjects were required to have been enrolled in year 1 and have received at least 1 prior study dose of vaccine or placebo, with completion of safety and efficacy evaluations during the first season's surveillance phase.

Study Design and Treatments

Subjects were recruited during a period of 6 weeks and randomized (2:2:1:1) to 1 of 4 study groups according to a preprinted randomization allocation list (Fig. 1). Subjects who participated in year 1 and met inclusion criteria were asked to participate in year 2 and receive 1 dose of LAIV or saline placebo (corresponding to the first dose received in year 1) before the second influenza season. Subjects and personnel evaluating vaccine efficacy and safety remained blinded throughout the entire study period. Because of a treatment allocation coding and labeling error in the second season, 683 of 2054 subjects in season 2 received a treatment other than that to which they were randomized, resulting in 2 additional treatment sequences that were unplanned (Fig. 1).

Each dose of LAIV (Wyeth Vaccines, Marietta, PA) was formulated to contain $10^{7\pm 0.5}$ fluorescent focus units of each of the 3 6:2 reassortant influenza virus strains containing the hemagglutinin and neuraminidase antigens of influenza virus strains recommended by the World Health Organization. Vaccine was stored frozen and shipped to the study sites at 2°C to 8°C and stored at that temperature until just before administration. Saline placebo consisted of physiologic saline; excipient placebo was the vaccine excipient alone (sucrose-phosphate-glutamate buffer, arginine, acid-hydrolyzed porcine gelatin, and normal allantoic fluid, in the same concentrations as in LAIV). The total volume of vaccine and both placebos was 0.2 mL administered intranasally (approximately 0.1 mL into each nostril).

Study Evaluations

A daily diary card solicited information for 11 consecutive days after each vaccination (including the day of vaccination) on the following reactogenicity events: axillary temperature, runny nose/nasal congestion, cough, vomiting, decreased activity, decreased appetite, irritability, use of antipyretic medications, and

stomachache. Surveillance for influenza-like illness was based on regular telephone contacts, clinic visits, or home visits, with weekly contacts through the end of the surveillance period. Nasal swab samples were to be obtained within 4 days after onset of illness if at least one of the following occurred: fever, wheezing, shortness of breath, pulmonary congestion, pneumonia, or ear infection; or if at least 2 of the following occurred: runny nose or nasal congestion, sore throat, cough, muscle aches, chills, headache, irritability, decreased activity, or vomiting; or clinical decision of the investigator. Nasal swab specimens were cultured and typed for influenza using standard techniques by laboratories in Brazil, Argentina, and South Africa. Specific strains were identified by standard hemagglutination inhibition assays. Blood samples for assessment of serum antibody titers to each of the vaccine virus strains were collected each year in a subset of subjects before each dose and 35 ± 7 days after the final dose. Samples were shipped to central laboratories in London, UK (year 1) or Cincinnati, OH (year 2). Unsolicited adverse events were collected for 11 days after each dose in year 1 and for 28 days after treatment in year 2. Serious adverse events, including hospitalizations, were monitored through the end of the study.

Study Endpoints

The primary efficacy endpoint was the first episode of culture-confirmed influenza illness caused by community-acquired subtypes antigenically similar (same type, subtype, and serotype) to those contained in the vaccine during year 1. Secondary efficacy endpoints included the first episode of culture-confirmed influenza caused by community-acquired subtypes antigenically similar to those in the vaccine during year 2; the first episode of culture-confirmed influenza caused by any community-acquired subtypes during year 1 and 2; and the first and all episodes of AOM, including any AOM, AOM associated with culture-confirmed influenza virus antigenically similar to a vaccine strain, and AOM associated with fever. LRI endpoints included the incidence of any LRI; the first incidence of any pneumonia, radiographically confirmed pneumonia, bronchitis, bronchiolitis, other LRI, LRI that required hospitalization, hospitalization associated with pneumonia, and hospitalization associated with radiographically confirmed pneumonia. Immunogenicity endpoints included the response for

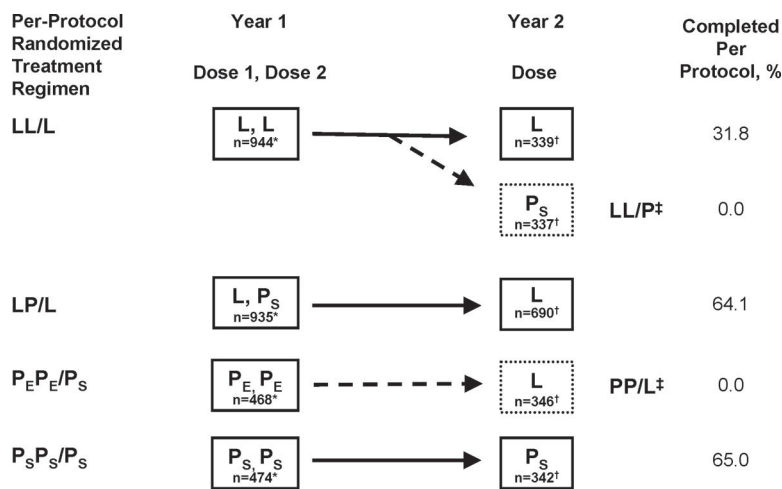


FIGURE 1. Randomized treatment regimen. L indicates live attenuated influenza vaccine; P_E, excipient placebo; P_S, saline placebo. *Per-protocol efficacy population. †As-treated efficacy population. ‡Unintended treatment regimen resulting from year 2 treatment allocation error.

each virus strain and differences in the immunogenicity response after 2 doses compared with 1 dose of LAIV in year 1 and revaccination in year 2. Safety endpoints were reactogenicity events and adverse events.

Statistical Analysis

The per-protocol population consisted of all eligible subjects who received study treatment as randomized, had no major protocol violations, and were followed-up for at least 15 days after the last vaccination each season. The intent-to-treat population consisted of all subjects who received ≥1 dose of study treatment. The evaluable immunogenicity population consisted of per-protocol subjects who had a prevaccination and ≥1 postvaccination determinate assay result in the same season for any single strain. All subjects who received ≥1 dose of study vaccine were evaluated for safety.

Placebo types were pooled in the efficacy and immunogenicity analyses, but each placebo was assessed separately for safety to discern differences. Vaccine efficacy was defined in terms of incidence rates as $1 - I_L/I_P$, (I_L = incidence rate in LAIV recipients; I_P = incidence rate in placebo recipients). Only the first episode of each kind of illness for each subject was considered for efficacy estimates. Ninety-five percent confidence intervals (CIs) were constructed using a binomial distribution. The Andersen-Gill model for multiplicative hazards of recurrent events was used for all estimates and 95% CI for efficacy, with treatment as the only effect, provided that ≥5 placebo cases were reported.

Seroconversion was defined as a ≥4-fold increase in antibody titer relative to that season’s baseline titer for each strain. Baseline seronegativity was defined as a prevaccination antibody titer of ≤1:4. Geometric means of the titers (GMTs) and the proportion of subjects who seroconverted were calculated.

For the analysis of safety, subjects were analyzed using a 2-sided Fisher exact test according to the dose actually received: LAIV, excipient placebo, or saline placebo in year 1 and LAIV or saline placebo in year 2.

RESULTS

Subjects

We enrolled and randomized 3200 children (Fig. 1). Baseline characteristics were similar across treatment groups (Table 1). A total of 2821 subjects (88.2%) completed year 1 without major protocol violations and constituted the year 1 per-protocol population (see Fig. 2A, Supplemental Digital Content 1, <http://links.lww.com/A872>).

and 2202 subjects continued in year 2 (see Fig. 2B, Supplemental Digital Content 1, <http://links.lww.com/A872>). Because of an unintended treatment allocation error in season 2, one treatment group randomized to LAIV–LAIV/LAIV received placebo rather than LAIV (LAIV–LAIV/placebo), and 1 treatment group randomized to placebo–placebo/placebo received LAIV rather than placebo (placebo–placebo/LAIV). As a result, the overall year 2 per-protocol population included 1364 children (42.6%). The number of episodes of culture-confirmed influenza due to matched strains in the per-protocol populations for year 1 is illustrated in Figure 3.

Primary Endpoint

During year 1, the LAIV–LAIV and LAIV–placebo regimens showed efficacy of LAIV against influenza strains antigenically similar to those in the vaccine of 73.5% (95% CI: 63.6–81.0) and 57.7% (95% CI: 44.7–67.9), respectively (Table 2). Relative efficacy for subjects randomized to LAIV–LAIV was significantly higher than that of the LAIV–placebo group (37.3%; 95% CI: 9.5–56.9; Table 2).

Secondary Endpoints

In year 1, the majority of influenza cases were caused by strains antigenically similar to the vaccine strains, and consequently, the efficacies against any community acquired subtypes were similar: 72.0% (95% CI: 61.9–79.8) and 56.3% (95% CI: 43.1–66.7) for the LAIV–LAIV and LAIV–placebo groups, respectively (Table 2).

In the year 2 per-protocol population, the LAIV–LAIV/LAIV and LAIV–placebo/LAIV regimens showed significant efficacy against any strain of influenza virus antigenically similar to those in the vaccine: 73.6% (95% CI: 33.3–91.2) and 65.2% (95% CI: 31.2–82.8) for the LAIV–LAIV and LAIV–placebo groups, respectively (Table 2). There was no statistically significant difference in efficacy between the LAIV–LAIV/LAIV group and the LAIV–placebo/LAIV group. Many cases observed in year 2 were caused by an antigenically dissimilar influenza B strain and efficacy of LAIV–LAIV/LAIV or LAIV–placebo/LAIV against any community-acquired subtype was lower (ie, 46.6% and 46.4%, respectively).

Two doses of LAIV in year 1 (LAIV–LAIV/placebo) provided 57.0% efficacy (95% CI: 6.1–81.7) against antigenically similar strains through a second influenza season without revaccination compared with placebo (placebo–placebo/placebo). Efficacy was 35.3% (95% CI: –0.3, 58.7) and 20.4% (95% CI: –33.6,

TABLE 1. Baseline Demographic Characteristics

	Treatment Group*			
	LL/L n = 1064	LP _S /L n = 1067	P _E P _E /P _S n = 543	P _S P _S /P _S n = 526
Sex, n (%)				
Girls	512 (48.1)	527 (49.4)	266 (49.0)	252 (47.9)
Boys	552 (51.9)	540 (50.6)	277 (51.0)	274 (52.1)
Race/ethnicity†, n (%)				
White	624 (58.6)	615 (57.6)	315 (58.0)	296 (56.3)
Black	215 (20.2)	231 (21.6)	117 (21.5)	129 (24.5)
Hispanic	2 (0.2)	4 (0.4)	0 (0.0)	1 (0.2)
Asian	4 (0.4)	1 (0.1)	1 (0.2)	1 (0.2)
Mixed race/other	219 (20.6)	216 (20.3)	110 (20.3)	99 (18.8)
Age at first vaccination, mo				
Mean (SD)	20.4 (8.5)	20.1 (8.6)	20.6 (8.3)	20.1 (8.3)

*Vaccination regimen shown as year 1 dose 1 dose 2/year 2 dose.

†Race/ethnicity was determined by self-report.

L indicates live attenuated influenza vaccine; P_E, excipient placebo; P_S, saline placebo.

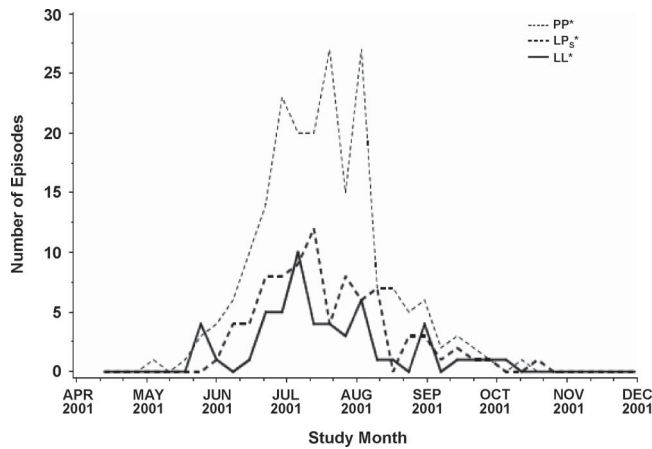


FIGURE 3. Episodes of any culture-confirmed influenza in year 1, per-protocol population. L indicates live attenuated influenza vaccine; P, any placebo. *Vaccination regimen shown as year 1 dose 1 dose 2. PP, n = 942; LP_s, n = 935; LL, n = 944.

52.9) against any community-acquired strain and antigenically dissimilar influenza B strains, respectively. In addition, comparison of placebo–placebo/LAIV and placebo–placebo/placebo in year 2 demonstrated 60.3% (95% CI: 10.9–83.8) efficacy of a single dose of LAIV against matched strains in children previously

unvaccinated against influenza. Against any community-acquired strain and antigenically dissimilar influenza B strains, efficacy was 59.4% (95% CI: 32.3–76.4) and 54.9% (95% CI: 16.6–76.6), respectively.

The LAIV–LAIV regimen in year 1 was significantly effective against the first episode of AOM, the first and all episodes of febrile AOM, and the first and all episodes of influenza-associated AOM caused by strains antigenically similar to those in the vaccine (Table 3). The LAIV–placebo regimen was also significantly effective against the first episode of AOM and the first and all episodes of AOM associated with influenza strains antigenically similar to those in the vaccine (Table 3). The small number of subjects in the LAIV–LAIV/LAIV group during year 2 (n = 338) may have precluded any demonstration of efficacy against AOM; however, in year 2, LAIV–placebo/LAIV (n = 684) was effective against all episodes of AOM and febrile AOM and against the first and all episodes of AOM associated with culture-confirmed influenza (Table 3). The rates of any LRI in year 1 were similar among the 3 randomized treatment groups (LAIV–LAIV, LAIV–placebo, and placebo–placebo), that is, 20.4% (n = 193), 18.8% (n = 176), and 19.0% (n = 179), respectively (see Table 4, Supplemental Digital Content 2, <http://links.lww.com/A873>). The year 1 immunogenicity cohort consisted of 406 subjects of whom 334 (82%) were evaluable (LAIV–LAIV/LAIV, n = 113; LAIV–placebo/LAIV, n = 112; placebo–placebo/placebo, n = 109). In the evaluable immunogenicity population, seroconversion rates ($P \leq 0.003$), GMTs, geometric mean fold rises (GMFRs), and ratios of GMFRs in year 1 were higher among LAIV–LAIV and LAIV–placebo recipients than placebo recipients (see Tables 5 and 6,

TABLE 2. Efficacy of Live Attenuated Influenza Vaccine Against Culture-Confirmed Influenza (Per-Protocol Population)

Year/Influenza Strain	Treatment Group*		
	Efficacy vs. PP/P % (95% CI)	LL/L	LP _s /L
		Relative Efficacy vs. LP _s /L % (95% CI)	Efficacy vs. PP/P % (95% CI)
Year 1, n [†]		944	935
Antigenically similar to those in vaccine [‡]			
Any strain	73.5 (63.6–81.0)	37.3 (9.5–56.9)	57.7 (44.7–67.9)
A/H1	NC	NC	NC
A/H3	72.7 (60.7–81.5)	34.0 (–1.3–57.4)	58.7 (43.4–70.2)
B	81.4 (64.2–91.2)	50.5 (–6.6–78.3)	62.4 (37.8–78.1)
Any subtype			
Any strain	72.0 (61.9–79.8)	36.0 (8.5–55.6)	56.3 (43.1–66.7)
A/H1	NC	NC	NC
A/H3	72.0 (59.8–80.9)	34.5 (0.2–57.5)	57.2 (41.6–69.0)
B	78.7 (60.9–89.3)	46.3 (–9.6–74.9)	60.4 (35.5–76.4)
Year 2, n [‡]		338	684
Antigenically similar to those in vaccine [‡]			
Any strain	73.6 (33.3–91.2)	24.1 (–104.2–75.7)	65.2 (31.2–82.8)
A/H1	94.0 (62.0–99.9)	77.5 (–62.3–99.5)	73.5 (37.2–89.6)
A/H3	49.4 (–253.0–95.4)	–304.7 (–23,778.2–78.9)	87.5 (–26.3–99.7)
B	–102.4 (–2137.1–71.0)	–34.9 (–468.9–72.0)	–50.0 (–1419.7–73.2)
Any subtype			
Any strain	46.6 (14.9–67.2)	0.5 (–57.7–38.5)	46.4 (21.1–63.5)
A/H1	94.0 (62.0–99.9)	79.8 (–42.3–99.5)	70.6 (32.0–88.0)
A/H3	49.4 (–253.0–95.4)	–304.7 (–23,778.2–78.9)	87.5 (–26.3–99.7)
B	24.1 (–28.5–55.7)	–13.8 (–86.1–31.7)	33.3 (–5.7–57.6)

*Vaccination regimen shown as year 1 dose 1 dose 2/year 2 dose.

[†]Number of patients in the population.

[‡]The following strains isolated in this study were considered antigenically similar to those in the year 1 vaccine: A/New Caledonia/20/99-like (A/H1N1), A/Panama/2007/99-like (A/H3N2), B/Yamanashi/166/98-like, and B/Victoria/504/00-like.

[§]The following strains isolated in this study were considered antigenically similar to those in the year 2 vaccine: A/New Caledonia/20/99-like (H1N1), A/Panama/2007/99-like (H3N2), and B/Victoria/504/00-like.

L indicates live attenuated influenza vaccine; NC, not computable; P, any placebo; P_s, saline placebo.

TABLE 3. Efficacy of Live Attenuated Influenza Vaccine Against Acute Otitis Media (Per-Protocol Efficacy Population)

Year/Illness	Treatment Group*	
	LL/L Efficacy vs. PP/P % (95% CI) [†]	LP _s /L Efficacy vs. PP/P % (95% CI) [†]
Year 1, n [‡]	944	934
AOM		
First episode	20.9 (1.7–36.4)	20.5 (1.3–36.1)
All episodes	19.3 (−0.4 – 35.1)	15.2 (−5.4 – 31.7)
Febrile AOM		
First episode	31.5 (8.0–49.2)	19.4 (−7.0 – 39.4)
All episodes	34.5 (12.7–50.9)	20.1 (−5.4 – 39.4)
Influenza-associated AOM [§]		
First episode	73.2 (50.9–86.3)	69.0 (44.8–83.5)
All episodes	73.5 (52.4–85.3)	69.6 (46.9–82.6)
Year 2, n [‡]	338	682
AOM		
First episode	3.3 (−38.2 – 32.5)	26.7 (−1.6 – 46.8)
All episodes	−0.1 (−41.9 – 29.4)	31.0 (5.4–49.7)
Febrile AOM		
First episode	22.3 (−25.1 – 52.2)	33.5 (−1.2 – 56.0)
All episodes	9.6 (−44.0 – 43.3)	33.5 (1.2–55.3)
Influenza-associated AOM [§]		
First episode	59.5 (−147.2 – 96.1)	90.0 (10.4–99.8)
All episodes	59.8 (−106.7 – 92.2)	90.1 (15.0–98.8)

*Vaccination regimen shown as year 1 dose 1 dose 2/year 2 dose.

[†]For all episodes, the estimate and CIs were computed from the Andersen-Gill model with treatment as the only effect. For first episodes, the estimate and CIs were computed from the proportions of cases by the exact conditional binomial, as for influenza.

[‡]Number of patients in the calculation.

[§]Influenza-associated AOM due to strains antigenically similar to those in the vaccine.

L indicates live attenuated influenza vaccine; P, any placebo; P_s, saline placebo; AOM, acute otitis media.

Supplemental Digital Content 3 and 4, <http://links.lww.com/A874> and <http://links.lww.com/A875>). Seroconversion rates and GMFRs after 2 doses of LAIV were significantly higher than after 1 dose (LAIV–LAIV vs. LAIV–placebo, $P \leq 0.037$ for seroconversion rates; $P < 0.001$ for GMFRs).

The year 2 immunogenicity cohort consisted of 861 subjects of whom 524 (61%) were evaluable (LAIV–LAIV/LAIV, $n = 133$; LP_s/L, $n = 265$; PP/P, $n = 126$). In year 2, increases in seroconversion rates and GMTs were noted among the immunogenicity-evaluable population for each LAIV group postvaccination (see Tables 5 and 7, Supplemental Digital Content 3 and 5, <http://links.lww.com/A874> and <http://links.lww.com/A876>). Baseline seronegative patients had higher seroconversion rates than all subjects in both years (see Table 5, Supplemental Digital Content 3, <http://links.lww.com/A874>).

Safety

The most frequent reactogenicity events for all treatment groups after each dose were cough and runny nose/nasal congestion. No significant differences in the incidence of reactogenicity events were noted between excipient placebo and saline placebo recipients (all $P \geq 0.073$; see Table 8, Supplemental Digital Content 6, <http://links.lww.com/A877>). However, comparing across all 3 groups, after dose 1 more saline placebo recipients had cough (58.2%) than excipient placebo recipients (53.9%) and LAIV recipients (50.3%) ($P < 0.004$).

The proportions of subjects experiencing one or more adverse events within 11 days after vaccination were similar among the treatment groups. The incidence for any event among LAIV, excipient placebo, and saline placebo recipients was 27.2% and 29.1%, 28.2% and 27.5%, and 29.0% and 26.7%, after dose 1 and dose 2, respectively. In season 2, the incidence of any event for LAIV or saline placebo recipients was 23.8% for each group.

The most frequently reported adverse events within 11 days after each of the 3 doses were administered were fever, upper respiratory tract infection, rhinitis, and coughing (see Table 9, Supplemental Digital Content 7, <http://links.lww.com/A878>). No statistically significant differences were noted among treatment groups for these events after any dose. The incidence of respiratory adverse events was similar among LAIV, excipient placebo, and saline placebo recipients in year 1 and LAIV and saline placebo recipients in year 2 (17.2%, 17.6%, and 18.6%; and 13.6% and 14.0% in years 1 and 2, respectively). The incidences of bronchitis (1.2%, 1.6%, and 1.7%; 1.2% and 0.5%, respectively) and bronchospasm (1.0%, 1.3%, and 0.8%; 0.7% and 1.2%, respectively) were also similar across treatment groups.

In year 2, the most frequently reported adverse events within 28 days after treatment were upper respiratory tract infection, rhinitis, fever, and coughing. No statistically significant differences were noted between treatment groups for these events. The incidence of respiratory adverse events was similar between LAIV and saline placebo recipients (28.3% and 28.5%, respectively). The incidences of bronchospasm (1.8% and 1.5%, respectively) were also similar between groups, but there was a significant difference in the rate of bronchitis between LAIV and saline placebo recipients (3.1% and 1.6%, respectively; $P = 0.046$).

In year 1, ≥ 1 serious adverse event was reported by 5.0% of LAIV–LAIV recipients, 3.8% of LAIV–placebo recipients, 3.4% of excipient placebo recipients, and 4.1% of saline placebo recipients. In year 2, 1.6% and 2.4% of LAIV and placebo recipients, respectively, reported ≥ 1 serious adverse event. The majority were respiratory events. Serious adverse events considered to be related to study product were reported in 29 subjects; the most frequent were pneumonia, bronchopneumonia, bronchiolitis, and bronchitis. Three deaths were reported; 2 deaths were accidental and 1 death was the result of *Escherichia coli* septicemia diagnosed 18 days after receipt of the second dose of LAIV in year 1. No death was judged to be related to the study product.

DISCUSSION

In children 6 to <36 months of age who were previously unvaccinated against influenza at enrollment, 1 and 2 doses of LAIV provided clinically significant protection against influenza illness. Efficacy of 2 doses was statistically and clinically significantly greater than the efficacy observed after 1 dose. These data confirm observations from 2 previous studies in influenza vaccine-naive young children that demonstrated 60%, 67%, and 89% efficacy versus placebo after a single dose of LAIV.^{12–14} After revaccination with a single dose in year 2, no significant differences in efficacy were found between children who were vaccinated with 1 dose versus 2 doses in the previous year. Given the implementation hurdles of compliance with the recommended 2-dose regimen, clinically significant efficacy against influenza illness after a single dose of LAIV is reassuring.^{12,14} The efficacy of 1 dose of LAIV, ranging from 57.7% (95% CI: 44.7–67.9) to 89% (95% CI: 65–96) in various studies,¹² is comparable with that reported for 2 doses of TIV in children <9 years of age in a recent meta-analysis: 63% (95% CI: 45–70),¹⁵ but 2 doses of LAIV provide increased protection, and efforts to increase 2-dose compliance should continue.

The unintended year 2 treatment error permitted additional analyses. Vaccine protection against viruses antigenically similar to those in the vaccine, predominantly A/H1N1 strains, persisted through a second season without revaccination, with an efficacy of 57.0% (95% CI: 6.1–81.7). This observation is similar to a previous estimate of the persistent efficacy of 2 doses of LAIV through a second season without revaccination, which was 56.2% (95% CI: 30.5–72.7) against predominantly A/H3N2 strains.¹⁴ However, the treatment allocation error also reduced the sample size of 2 protocol-specified cohorts, namely placebo–placebo/placebo and LAIV–LAIV/LAIV. This limited the statistical power to observe differences in the LAIV–LAIV/LAIV cohort, as seen in the season 2 AOM endpoints.

As expected, immunogenicity was demonstrated in subjects receiving LAIV in year 1; subjects receiving LAIV continued to show increases in seroconversion in year 2, regardless of baseline serostatus. These findings support previous results from efficacy field trials of LAIV in children.^{14,16}

Influenza in children is frequently complicated by AOM.^{2,17–19} Effectiveness of TIV in reducing the incidence of AOM associated with influenza has varied depending on the age of subjects studied, the circulating influenza strains, and the influenza attack rates in a given season.^{17,20–22} Similar variability has been reported in previous studies with LAIV.^{12,16,23,24} In particular, LAIV was shown in 1 study to reduce the incidence of febrile AOM by 30%.¹² This study provides the first evidence that LAIV can significantly reduce the overall incidence of AOM, regardless of whether the AOM was associated with a positive influenza culture. Although efficacy against AOM has been shown in this study, a similar effect against all-cause LRI was not observed. Influenza-associated LRI was not analyzed in this study; therefore it is possible that the lack of effect against LRI may be because pathogens other than influenza were more common causes of LRI in this population during the season studied. In a subsequent comparative study of LAIV and inactivated influenza vaccine in children 6 to 59 months of age, LAIV demonstrated a 50.6% ($P = 0.004$) and 45.9% ($P = 0.046$) reduction in influenza-associated AOM and LRI, respectively, compared with the inactivated vaccine.²⁵

LAIV was well tolerated; no significant differences in solicited reactogenicity events were seen between treatment groups. LAIV was not associated with an increased rate of adverse events through day 11 postvaccination. When adverse events were assessed through day 28 postvaccination in year 2, the rate of bronchitis was significantly increased in LAIV recipients, although rates of bronchospasm and any respiratory adverse events were similar between groups. Additionally, no differences in solicited reactogenicity events or other adverse events were seen after either saline or excipient placebo. This suggests that the excipients in LAIV, which include egg protein and acid-hydrolyzed gelatin, do not contribute to reactogenicity in vaccine recipients.

In this study, a single dose of LAIV provided clinically significant protection against influenza in young children previously unvaccinated against influenza and 2 doses provided persistent protection through a second season without revaccination. These benefits, together with the vaccine's safety profile in children 2 years of age and older,²⁵ provide support for increased use of LAIV in children ≥ 2 years of age.

ACKNOWLEDGMENTS

The authors thank the participating children and their parents, the study nurses and coordinators, the clinical testing laboratory staff, the clinical research associates and the scientists at Wyeth and MedImmune; Anita Moradoghli-Haftvani, who

served as clinical project manager for this study; Drs. Maria Zambon and Richard Ward for performing the hemagglutination inhibition laboratory testing; Dr. Ruth Rappaport for nasal wash IgA ELISA testing, virus strain identification by PCR sequencing, and virus genotyping; Dr. Sheau-Mei Cheng for virus genotyping; Drs. Guadalupe Carballal, Celso Granato, and Nigel Blackburn for virus detection and isolation testing; Drs. Vilma Savy, Alan Hampson, Barry D. Schoub, and Nigel Blackburn for virus strain identification by serotyping; Dr. Jonathan B. Skinner for performing statistical analyses; and Christopher S. Ambrose, MD, and Robert E. Walker, MD, for their critical review of the manuscript and insightful comments, and John E. Fincke, PhD, and Gerard P. Johnson, PhD, who provided medical writing and editorial assistance.

The D153-P504 LAIV Study Group—Argentina: Silvia Elena Gonzalez Ayala, Hospital Interzonal de Agudos Especializado en Pediatría, La Plata-Buenos Aires; Rosa Maria Bologna, Hospital Garrahan, Buenos Aires; Liliana Maria Calanni, CEIN Unidad Infectologica, Neuquén; Enrique Vicente Casanueva, Avenida Cabildo, Buenos Aires; Jose Maria Ceriani Cernadas, Hospital Italiano Vacunatorio, Buenos Aires; Eduardo Antonio Chioconci, CEIN Unidad Infectologica, Neuquén; Aida Mercedes Torres de Navajas, FUNCEI, Buenos Aires; Luisa Mariana de Wouters, Hospital Privado de La Comunidad, Mar del Plata; Fernando Claudio Ferrero, Hospital Pedro Elizalde, Buenos Aires; Eduardo Luis Lopez, Hospital de Niños R. Gutierrez, Buenos Aires; Guillermo Fernandez Mac Loughlin, PAIDEIA, Buenos Aires; Raul Oscar Ruvinsky, Departamento Materno-Infantil del Hospital C.G. Durand, Buenos Aires; Jorge Bernardo Tabora, Buenos Aires; Ricardo Augusto Teijeiro, Hospital Ignacio Pirovano, Buenos Aires; Miguel Wenceslao Tregnaghi, Centro de Desarrollo de Proyectos Avanzados Hospital Infantil de Córdoba, Córdoba. *Brazil:* Emilio Carlos Elias Baracat, Hospital de Clinicas da UNICAMP, Campinas; Eitan Naaman Berezin, Irmandade da Santa Casa de Misericórdia de São Paulo, São Paulo; Lúcia Ferro Bricks, Centro de Saúde Escola Prof Samuel B Pessoa—São Paulo, São Paulo; Antônio José Ledo Alves da Cunha, Instituto de Puericultura e Pediatría Martagão Gesteira da Universidade Federal do Rio de Janeiro, Rio de Janeiro; Clóvis Arns da Cunha, Hospital Nossa Senhora das Graças, Curitiba; Themis Reverbel da Silveira, Hospital de Clínicas de Porto Alegre, Porto Alegre; Calil Kairalla Farhat, Federal University of São Paulo, São Paulo; Victor Hugo Campos Lagos, Universidade Federal de Pelotas, Pelotas; Humberto Bracco Neto, Federal University of São Paulo, São Paulo; Priscila Maria de Oliveira Papaiordanou, Hospital de Clínicas da UNICAMP, Campinas; Renato Tetelbom Stein, Hospital São Lucas da Pontifícia Universidade Católica, Porto Alegre; Lily Yin Weckx, Centro de Referência de Imunobiológicos Especiais Universidade Federal de São Paulo, São Paulo. *South Africa:* Susan Annandale, 1 Military Hospital, Pretoria; Shabir A. Madhi, Chris Hani-Baragwanath Hospital, Johannesburg; H. Siebert, Sr Karien Camphor Nursing Clinic, Pretoria; E. J. Smit, Sr Karien Camphor Nursing Clinic, Pretoria; A. D. Steele, MEDUNSA, Pretoria; C. M. Taylor, Clinical Consultants, Potchefstroom; A.E. van der Vyver, University of the Free State, Bloemfontein; Jacobus C. van Dyk, Femina Clinic, Pretoria; Jan. H. Vermeulen, Panorama Mediclinic, Cape Town; Azeem H. Walele, Wynberg, 2 Military Hospital, Cape Town; H. C. Weber, Jan S. Marais Hospital, Bellville Cape Town.

REFERENCES

1. Neuzil KM, Mellen BG, Wright PF, et al. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. *N Engl J Med.* 2000;342:225–231.

2. Loughlin J, Poullos N, Napalkov P, et al. A study of influenza and influenza-related complications among children in a large US health insurance plan database. *Pharmacoeconomics*. 2003;21:273–283.
3. Fiore AE, Shay DK, Broder K, et al. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. *MMWR*. 2008;57:1–60.
4. Neuzil KM, Jackson LA, Nelson J, et al. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naïve 5-8-year-old children. *J Infect Dis*. 2006;194:1032–1039.
5. Ritzwoller DP, Bridges CB, Shetterly S, et al. Effectiveness of the 2003–2004 influenza vaccine among children 6 months to 8 years of age, with 1 vs 2 doses. *Pediatrics*. 2005;116:153–159.
6. Shuler CM, Iwamoto M, Bridges CB, et al. Vaccine effectiveness against medically attended, laboratory-confirmed influenza among children aged 6 to 59 months, 2003–2004. *Pediatrics*. 2007;119:e587–e595.
7. Jackson LA, Neuzil KM, Baggs J, et al. Compliance with the recommendations for 2 doses of trivalent inactivated influenza vaccine in children less than 9 years of age receiving influenza vaccine for the first time: a Vaccine Safety Datalink study. *Pediatrics*. 2006;118:2032–2037.
8. Centers for Disease Control and Prevention. Influenza vaccination coverage among children aged 6–23 months—United States, 2005–06 influenza season. *MMWR*. 2007;56:959–963.
9. Centers for Disease Control and Prevention. Influenza vaccination coverage among children aged 6–59 months—six immunization information system sentinel sites, United States, 2006–07 influenza season. *MMWR*. 2007;56:963–965.
10. Belshe R, Lee MS, Walker RE, et al. Safety, immunogenicity and efficacy of intranasal, live attenuated influenza vaccine. *Expert Rev Vaccines*. 2004;3:643–654.
11. Nakayama T, Onoda K. Vaccine adverse events reported in post-marketing study of the Kitasato Institute from 1994 to 2004. *Vaccine*. 2007;25:570–576.
12. Belshe RB, Mendelman PM, Treanor J, et al. The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine in children. *N Engl J Med*. 1998;338:1405–1412.
13. Halloran ME, Longini IM Jr, Gaglani MJ, et al. Estimating efficacy of trivalent, cold-adapted, influenza virus vaccine (CAIV-T) against influenza A (H1N1) and B using surveillance cultures. *Am J Epidemiol*. 2003;158:305–311.
14. Tam JS, Capeding MR, Lum LC, et al. Efficacy and safety of a live attenuated, cold-adapted influenza vaccine, trivalent against culture-confirmed influenza in young children in Asia. *Pediatr Infect Dis J*. 2007;26:619–628.
15. Zangwill KM, Belshe RB. Safety and efficacy of trivalent inactivated influenza vaccine in young children: a summary for the new era of routine vaccination. *Pediatr Infect Dis J*. 2004;23:189–197.
16. Belshe RB, Gruber WC, Mendelman PM, et al. Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. *J Pediatr*. 2000;136:168–175.
17. Heikkinen T, Thint M, Chonmaitree T. Prevalence of various respiratory viruses in the middle ear during acute otitis media. *N Engl J Med*. 1999;340:260–264.
18. Ruuskanen O, Arola M, Putto-Laurila A, et al. Acute otitis media and respiratory virus infections. *Pediatr Infect Dis J*. 1989;8:94–99.
19. Neuzil KM, Zhu Y, Griffin MR, et al. Burden of inter-pandemic influenza in children younger than 5 years: a 25-year prospective study. *J Infect Dis*. 2002;185:147–152.
20. Clements DA, Langdon L, Bland C, et al. Influenza A vaccine decreases the incidence of otitis media in 6- to 30-month-old children in day care. *Arch Pediatr Adolesc Med*. 1995;149:1113–1117.
21. Hoberman A, Greenberg DP, Paradise JL, et al. Effectiveness of inactivated influenza vaccine in preventing acute otitis media in young children: a randomized controlled trial. *JAMA*. 2003;290:1608–1616.
22. Ozgur SK, Beyazova U, Kemaloglu YK, et al. Effectiveness of inactivated influenza vaccine for prevention of otitis media in children. *Pediatr Infect Dis J*. 2006;25:401–404.
23. Belshe RB, Gruber WC. Prevention of otitis media in children with live attenuated influenza vaccine given intranasally. *Pediatr Infect Dis J*. 2000;19(suppl 5):S66–S71.
24. Vesikari T, Fleming DM, Aristegui JF, et al. Safety, efficacy, and effectiveness of cold-adapted influenza vaccine-trivalent against community-acquired, culture-confirmed influenza in young children attending day care. *Pediatrics*. 2006;118:2298–2312.
25. Belshe RB, Edwards KM, Vesikari T, et al. Comparative safety and efficacy of live attenuated and inactivated influenza vaccines in infants and young children. *N Engl J Med*. 2007;356:685–696.