DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

July 11, 2019

***SENT VIA EMAIL***

Allison Lucas
Siri and Glimstad, LLP
200 Park Avenue, Seventeenth Floor
New York, New York 10166
ALucas@sirillp.com

Dear Ms. Lucas:

This letter is our final response to your Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) Freedom of Information Act (FOIA) request of June 11, 2019, assigned #19-00863-FOIA, seeking:

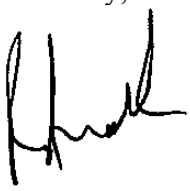
> "…A copy of each clinical trial relied upon by the CDC when recommending the routine use of Fluzone in six-month old babies."

We located 45 pages of responsive records. After a careful review of these pages, no information was withheld from release.

In accordance with the Department's implementing regulations, 45 CFR Part 5, no fees are due for processing request #19-00863-FOIA.

If you need any further assistance or would like to discuss any aspect of the records provided please contact either our FOIA Requester Service Center at 770-488-6399 or our FOIA Public Liaison at 770-488-6277.

Sincerely,



Roger Andoh
CDC/ATSDR FOIA Officer
Office of the Chief Operating Officer
Phone: (770) 488-6399
Fax: (404) 235-1852

#19-00863-FOIA

# Safety and Immunogenicity of an Inactivated Quadrivalent Influenza Vaccine in Children 6 Months through 8 Years of Age

*David P. Greenberg, MD,*† Corwin A. Robertson, MD, MPH,* Victoria A. Landolfi, MS, MBA,* Amitabha Bhaumik, PhD,* Shelly D. Senders, MD,‡ and Michael D. Decker, MD, MPH*§*

**Background:** Strains of 2 distinct influenza B lineages (Victoria and Yamagata) have cocirculated in the United States for over a decade, but trivalent influenza vaccines (TIVs) contain only 1 B-lineage strain. Each season, some or most influenza B disease is caused by the B lineage not represented in that season's TIV. Quadrivalent influenza vaccines (QIVs) containing a strain from each B lineage should resolve this problem.

**Methods:** This was a Phase III, randomized, multicenter trial in the United States among children 6 months to <9 years of age to evaluate the safety and immunogenicity of inactivated QIV compared with inactivated control TIVs containing opposite B-lineage strains. Participants were randomized at a ratio of approximately 4:1:1 to receive QIV, TIV containing a Victoria-lineage B strain or TIV containing a Yamagata-lineage B strain. Sera were collected pre- and 28-days post-final vaccination and safety was assessed for 6 months after the last injection.

**Results:** A total of 4363 participants were enrolled. QIV induced noninferior antibody responses to all A strains and corresponding B strains compared with the control TIVs and superior antibody responses to the noncorresponding B strain in each TIV. Rates of solicited reactions and unsolicited and serious adverse events were similar in all groups.

**Conclusions:** This study demonstrated that QIV is safe and immunogenic among children 6 months to <9 years of age. These findings, along with data from 2 other studies of this QIV in adults, suggest that QIV should offer protection against both B lineages with a safety profile similar to TIV across all ages.

**Key Words:** influenza vaccine, safety, immunogenicity, children

(*Pediatr Infect Dis J* 2014;33:630–636)

Despite widespread availability of vaccines, influenza remains a serious health risk for children in the United States. During the 2008–2009 through 2011–2012 influenza seasons, national laboratory-confirmed influenza hospitalization rates were between 14.2 and 72.8 per 100,000 children ≤ 4 years of age and between 4.2 and 27.3 per 100,000 children 5–17 years of age.[1] During the same period, the total number of pediatric deaths ranged from a low of 26 during the 2011–2012 season to a high of 348 during the 2009–2010 pandemic season.[1]

To help prevent influenza virus infection and disease, the Advisory Committee on Immunization Practices of the US Centers for Disease Control and Prevention recommends routine annual influenza vaccination for all children ≥6 months of age.[2] Seasonal trivalent inactivated influenza vaccines (TIVs) contain 2 influenza A subtype strains, 1 A/H1N1 strain and 1 A/H3N2 strain and 1 influenza B strain, from either the Victoria or Yamagata lineage. These 2 B lineages have circulated globally since the mid-1980s;[3] however, before 2001, only 1 lineage predominated in the United States each season. Since the 2001–2002 season, both B lineages have cocirculated with varying frequencies.[4]

In February of each year, advisory committees of the World Health Organization and US Food and Drug Administration meet to choose which A/H1N1, A/H3N2 and B strains should be included in TIVs for the upcoming influenza season in the Northern Hemisphere. The decision is based on global virologic and epidemiologic surveillance and serologic studies in which circulating strains are tested against ferret and human antibodies. Despite extensive analysis of these and other data, it is difficult to predict, 8–14 months in advance, which B lineage will predominantly circulate in the upcoming influenza season.

The B lineage selected by the World Health Organization and US Food and Drug Administration for vaccine formulation has matched the predominant circulating lineage in only 6 of the past 12 seasons.[5] During this period, influenza B viruses caused a yearly average of approximately 25% of all influenza cases in the United States, with yearly proportions as high as 44%.[5] Even when the predominant B-lineage strain is correctly chosen, because cocirculation occurs annually, some proportion of influenza is caused by an opposite B-lineage strain. For example, during the 2011–2012 season, 51% of circulating B strains were from the Yamagata lineage and 49% were from the Victoria lineage.

While vaccination enhances immunity against the influenza strains contained in TIV, the degree of protection depends in part on how well the vaccine strains match those actually circulating during the influenza season. When the vaccine and circulating A strains are not well-matched or the B strains are of different lineages, the effectiveness of vaccination is reduced.[6] During the 2006–2007 influenza season, among persons ≥9 years of age in Canada, the effectiveness of TIV against a well-matched A/H1N1 strain was 92% [95% confidence interval (CI): 40–91%], whereas the effectiveness of the same TIV, containing a Victoria-lineage B strain, against the circulating Yamagata B-lineage strain was only 19% (95% CI: 112–69%).[7]

Quadrivalent inactivated influenza vaccine (QIV) containing a strain from each of the 2 B lineages, in addition to the standard A/H1N1 and A/H3N2 strains, should resolve the issue of B-lineage mismatch. A modeling study conducted by the US Centers for Disease Control and Prevention found that replacing TIV with QIV has the potential to reduce annual influenza cases, hospitalizations

Accepted for publication December 5, 2013.

From the *Sanofi Pasteur, Swiftwater, PA; †Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA; ‡Senders Pediatrics, Cleveland, OH; and §Department of Preventive Medicine, Vanderbilt University School of Medicine, Nashville, TN.

This study was funded by Sanofi Pasteur, Inc., Swiftwater, PA. D.P.G., C.A.R., V.A.L., A.B., and M.D.D. are employees of Sanofi Pasteur. S.D.S. received a research grant from Sanofi Pasteur to conduct this study. The authors have no other funding or conflicts of interest to disclose.

ClinicalTrials.gov registration number: NCT01240746.

Address for correspondence: David P. Greenberg, MD, 1 Discovery Drive, Swiftwater, PA 18370-0187. E-mail: david.greenberg@sanofipasteur.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 website (www.pidj.com).



ISSN: 0891-3668/14/3306-0630
DOI: 10.1097/INF.0000000000000254

and deaths.[8] Before a manufacturer's QIV can be approved for general use, regulatory authorities must be assured that there are no safety concerns compared with standard-of-care TIVs, and there is no immunologic interference caused by the addition of the second B-lineage strain. We report here the results of a Phase III multicenter study conducted in the United States during the 2010–2011 influenza season to assess the safety and immunogenicity of QIV compared with control TIVs among children 6 months to <9 years of age.

## MATERIALS AND METHODS

### Study Design

This was a Phase III, randomized, observer-blinded, active-controlled, 3-arm, multicenter trial to assess the safety and immunogenicity of QIV compared with control TIVs in a pediatric population (NCT Registry No.: NCT01240746). The primary objective was to demonstrate that for each A and B strain QIV induced noninferior antibody responses compared with those induced by 2 control TIVs, each containing the same A strains and either a Yamagata- or Victoria-lineage B strain. The secondary objective was to demonstrate that for each B strain QIV induced superior antibody responses compared with those of each respective TIV not containing the same B-lineage strain. The study was performed at 69 centers in the United States during the 2010–2011 influenza season. It was approved by all relevant institutional review boards and was carried out in accordance with International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained from parents or legal guardians before children were included in the trial. In addition, for children 7 to <9 years of age, signed assent was obtained. Enrollment took place between November 11, 2010, and June 20, 2012.

### Study Population and Sample Size Calculation

Children included in the study had to be 6 months to <9 years of age and generally in good health. In addition, those who were 6 months to <24 months of age had to be born at full term (≥37 weeks) and with a birth weight ≥2.5 kg (5.5 lbs). Children were excluded if they had a history of allergy to egg proteins, latex or any constituents of the vaccine; a history of serious adverse reactions to any influenza vaccine; received any vaccine in the 4 weeks preceding the first study vaccination (or scheduled between study visits) or influenza vaccine after August 1, 2010; a history of Guillain-Barré syndrome; a known or suspected congenital or acquired immunodeficiency; received immunosuppressive therapy within the preceding 6 months or long-term systemic corticosteroid therapy within the past 3 months; a history of developmental delay, neurologic disorder or seizure disorder; known seropositivity to human immunodeficiency virus, hepatitis B or hepatitis C; or received blood or blood-derived products in the past 3 months.

Enrollment was stratified by age so that approximately half of the participants at each site were 6 months to <36 months of age and half were 3 years to <9 years of age. Enrollment targets were 3340 children in the QIV group and 800 children in each of 2 control TIV groups. This was estimated to yield 95% overall power to demonstrate that the immunogenicity of QIV was noninferior to TIV for all 4 strains and 90% power for each age group separately (6 months to <36 months and 3 years to <9 years) assuming that 90% of those enrolled would be evaluable (approximately 3000 for QIV and 720 for each TIV). This was also estimated to result in at least 99% power to demonstrate immunologic superiority of QIV compared with each respective TIV not containing the corresponding B strain for each age group separately and overall.

### Vaccine Formulation and Administration

QIV contained A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (B Victoria lineage) and B/Florida/04/2006 (B Yamagata lineage) strains. A licensed 2010–2011 formulation of TIV (Fluzone, Sanofi Pasteur, Swiftwater, PA) contained B/Brisbane/60/2008 and an investigational TIV contained B/Florida/04/2006. Each TIV contained the same A/H1N1 and A/H3N2 strains as QIV. Each vaccine was formulated to contain 30 μg hemagglutinin/strain/mL. Doses were provided in prefilled 0.25- or 0.5-mL, single-dose syringes or in 0.5-mL, single-dose vials. Vaccine potency was assessed periodically through a routine stability monitoring program.

Participants were randomized at a ratio of approximately 4:1:1 to be immunized with QIV, licensed TIV or investigational TIV, using a programmed interactive voice response system. Participants were immunized with the appropriate dose of vaccine based on age at the time of enrollment; 0.25 mL for children 6 to <36 months of age and 0.5 mL for children 3 to <9 years of age.[2] Participants received 1 or 2 doses of study vaccine 4 weeks (window, 28–35 days) apart based on their influenza vaccine history, as recommended by the Advisory Committee on Immunization Practices for the 2010–2011 season.[2] All immunizations were administered by intramuscular injection into the anterolateral thigh or the deltoid region. The vaccinees, family members and all site personnel except the vaccination nurse (who did not collect safety data) were blinded to the administered vaccine.

### Hemagglutination Inhibition Assay and Immunogenicity Endpoints

Blood samples were collected on day 0 (prevaccination) and day 28 (window, days 28–35) after the final vaccination. A validated hemagglutination inhibition assay, approved by the Food and Drug Administration for clinical trial testing, was used to quantify antibody titers against study vaccine antigens. Assays were performed by Sanofi Pasteur personnel who were blinded to vaccine assignment. Control and participant sera were incubated with type III neuraminidase to eliminate nonspecific inhibitors. Spontaneous antispecies agglutinins were adsorbed by incubating the sera with a suspension of Turkey red blood cells. Ten 2-fold dilutions (starting at 1:10) of the treated sera were incubated with a previously titrated influenza virus solution at a concentration of 4 hemagglutination units/25 μL. The reported hemagglutination inhibition titer corresponded to the highest serum dilution resulting in complete inhibition of hemagglutination and was determined in 2 independent assay runs.

The titer for each sample was calculated as the geometric mean of the reciprocal of the 2 independent values. The lower limit of quantitation was a titer of 1:10; samples with titers below this level were assigned a titer of 1:5. The seroprotection rate for each group was defined as the percentage of vaccinees with a titer ≥1:40. The seroconversion rate for each group was defined as the percentage of vaccinees with either a prevaccination titer <1:10 and a postvaccination titer ≥1:40 or a prevaccination titer ≥1:10 and a ≥4-fold increase in titer postvaccination.

### Safety Assessments

Solicited injection-site and systemic reactions were recorded for 7 days after each vaccination. Solicited reactions differed by age: for children 6 months to <24 months of age, injection-site tenderness, erythema and swelling; systemic fever, vomiting, abnormal crying, drowsiness, loss of appetite and irritability; for children 2 years to <9 years of age, injection-site pain, erythema and swelling; systemic fever, headache, malaise and myalgia. Unsolicited adverse events (AEs) and serious adverse events (SAEs) were

collected according to International Committee for Harmonization Guideline (E2A) for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. Unsolicited AEs were collected from day 0 to day 28 (window, days 28–35) after the final vaccination. SAEs and AEs of special interest (Guillain-Barré syndrome, Bell's palsy, encephalitis/myelitis, optic neuritis, Stevens-Johnson syndrome, toxic epidermal necrolysis and febrile seizure) were collected for 6 months after the final vaccination.

## Statistical Analyses

All analyses were carried out using SAS version 9.1 or higher (SAS Institute, Cary, NC). Missing and incomplete data were not replaced. Immunogenicity was assessed in all participants who were randomized and received the study or a control vaccine, had a valid postvaccination serology result and completed the study according to protocol (per-protocol analysis set). Noninferiority was assessed for all 4 viral strains in QIV compared with the control TIVs. For comparison of A/H1N1 and A/H3N2 responses, data were pooled among the 2 TIVs. For comparison of B-strain responses, QIV was compared with the respective TIV containing the same B-lineage strain. Noninferiority of geometric mean titers (GMTs) was achieved if the lower limit of the 2-sided 95% CI of the $GMT_{QIV}/GMT_{TIV}$ ratio was > 0.66, and noninferiority of seroconversion rates (SCRs) was achieved if the lower limit of the 2-sided 95% CI of the $SCR_{QIV} - SCR_{TIV}$ difference was >−10%.

Superiority was assessed for each B-lineage strain in QIV compared with each respective TIV not containing the same B-lineage strain. Superiority of GMTs was achieved if the lower limit of the 2-sided 95% CI of the $GMT_{QIV}/GMT_{TIV}$ ratio was >1.5, and superiority of SCRs was achieved if the lower limit of the 2-sided 95% CI of the $SCR_{QIV} - SCR_{TIV}$ difference was >10%.

Safety was assessed in all randomized participants who received a study or control vaccine (safety analysis set) and reported descriptively. The 95% CIs of point estimates were calculated using the normal approximation for quantitative data and the exact binomial distribution (Clopper-Pearson method) for proportions.

# RESULTS

## Disposition and Demographics

A total of 4363 children were randomized, of whom 4348 received study vaccine: 2893 in the QIV group, 734 in the licensed TIV group and 721 in the investigational TIV group (Fig. 1). Approximately three-quarters of the children in each group received a second dose of vaccine, based on Advisory Committee on Immunization Practices recommendations. A total of 350 participants did not complete the vaccination phase of the study (comparable proportion of each group), primarily because of loss to follow up (n = 145), noncompliance (n = 111) and voluntary withdrawal not due to an AE (n = 82). Approximately equal numbers of males and females were enrolled and the mean age and race/ethnic distributions were similar in each group (Table 1).

## Immunogenicity

Prevaccination GMTs were similar across the 3 vaccine groups (see Table, Supplemental Digital Content 1, http://links.

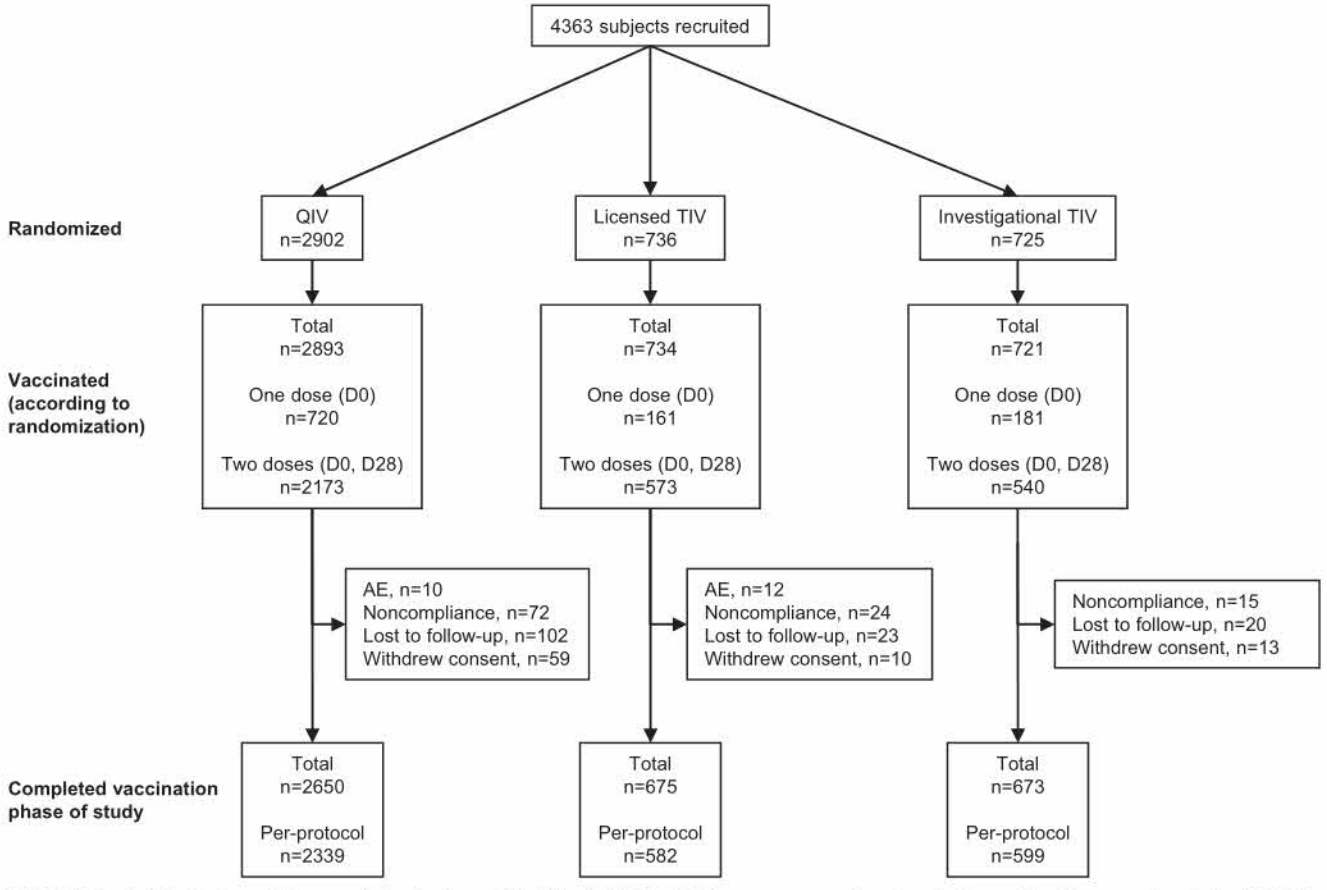



**FIGURE 1.** Subject disposition and study flow. A total of 4363 children were randomized at a ratio of approximately 4:1:1 to receive QIV, licensed TIV or investigational TIV. D0, day 0; D28, day 28; n, number of participants in the group.

**TABLE 1.** Demographics of All Randomized Participants

| | QIV N = 2902 | Licensed TIV N = 736 | Investigational TIV N = 725 |
|---|---|---|---|
| Sex, n (%) | | | |
| Male | 1475 (50.8) | 369 (50.1) | 366 (50.5) |
| Female | 1427 (49.2) | 367 (49.9) | 359 (49.5) |
| Age (months) | | | |
| Mean ± standard deviation | 49.8±29.7 | 49.6±29.0 | 49.6±28.7 |
| Range | 6.0–117.3 | 6.0–107.8 | 6.0–108.0 |
| Race/ethnicity, n (%) | | | |
| American Indian or Alaska Native | 9 (0.3) | 1 (0.1) | 3 (0.4) |
| Asian | 13 (0.4) | 5 (0.7) | 7 (1.0) |
| Black | 595 (20.5) | 147 (20.0) | 139 (19.2) |
| Caucasian | 1693 (58.3) | 433 (58.8) | 417 (57.5) |
| Hispanic | 415 (14.3) | 97 (13.2) | 108 (14.9) |
| Native Hawaiian or Pacific Islander | 2 (0.1) | 0 (0.0) | 2 (0.3) |
| Other | 175 (6.0) | 53 (7.2) | 49 (6.8) |

Values are for participants receiving at least 1 dose of vaccine and are according to the randomization assignment. One child in the QIV group was enrolled at >9 years of age (117.3 months) and she was excluded from the safety and immunogenicity analyses.

N, number of participants in each group; n, number of participants in each group with the characteristic; QIV, quadrivalent influenza vaccine; TIV, trivalent influenza vaccine.

lww.com/INF/B802). For A/H1N1 and A/H3N2, QIV induced postvaccination GMTs and seroconversion rates that were noninferior to those induced by TIV (Table 2). For each B-lineage strain, QIV induced GMTs and seroconversion rates that were noninferior to those induced by TIV containing the same B strain and superior to those induced by TIV not containing the same B strain. Further, all noninferiority and superiority criteria were met for the 2 age subgroups, 6 to <36 months and 3 to <9 years of age (see Tables, Supplemental Digital Content 2, http://links.lww.com/INF/B803: Table A, for 6 months to <36 months of age and Table B, for 3 to <9 years of age). The postvaccination seroprotection rates were similar among the 3 vaccine groups (Table 3).

Some children received investigational TIV in which the antigen content decreased during the study to below a prespecified level (28 μg hemagglutinin /strain/mL), but antibody responses were similar among children administered in-specification (n = 211) and out-of-specification (n = 388) vaccine, and all noninferiority and superiority criteria were met for all participants combined (Table 2) as well as when those who received out-of-specification lots were excluded (see Table, Supplemental Digital Content 3, http://links.lww.com/INF/B804).

## Safety

The proportions of children reporting solicited injection-site and systemic reactions were similar across the vaccine groups (Table 4). The most common reactions among children 6 to <24 months of age were irritability and injection-site tenderness, and the most common reactions among children 2 to <9 years of age were myalgia, malaise and injection-site pain (Fig. 2). In all vaccine groups, most reactions were grade 1 or 2 in intensity, began within 3 days of vaccination and resolved within 3 days of onset.

**TABLE 2.** GMT and Seroconversion Rate Comparisons for QIV Versus TIV Among Subjects 6 Months to <9 Years of Age

| Endpoint | Comparison | Strain | QIV Value | Comparator Vaccine | | GMT Ratio or Seroconversion Rate Difference | Comparison Criteria met? |
|---|---|---|---|---|---|---|---|
| | | | | Vaccine | Value | | |
| GMT | | | | | | GMT ratio | |
| | Noninferiority* | A/H1N1 | 1124 (1060–1192) | Pooled TIV† | 1096 (1008–1192) | 1.03 (0.93–1.14) | Yes |
| | | A/H3N2 | 822 (783–862) | Pooled TIV | 828 (774–887) | 0.99 (0.91–1.08) | Yes |
| | | B/Brisbane | 86.1 (81.8–90.6) | Licensed TIV | 64.3 (58.3–70.9) | 1.34 (1.20–1.50) | Yes |
| | | B/Florida | 61.5 (58.6–64.7) | Investigational TIV | 58.3 (52.6–64.7) | 1.06 (0.94–1.18) | Yes |
| | Superiority‡ | B/Brisbane | 86.1 (81.8–90.6) | Investigational TIV§ | 19.5 (17.4–21.8) | 4.42 (3.94–4.97) | Yes |
| | | B/Florida | 61.5 (58.6–64.7) | Licensed TIV§ | 16.3 (14.8–17.9) | 3.79 (3.39–4.23) | Yes |
| Seroconversion rate (%) | | | | | | Difference in rate | |
| | Noninferiority¶ | A/H1N1 | 92.4 (91.2–93.4) | Pooled TIV | 91.4 (89.7–93.0) | 0.9 (−0.9–3.0) | Yes |
| | | A/H3N2 | 88.0 (86.6–89.3) | Pooled TIV | 84.2 (82.0–86.3) | 3.8 (1.4–6.3) | Yes |
| | | B/Brisbane | 71.8 (69.9–73.6) | Licensed TIV | 61.1 (57.0–65.1) | 10.7 (6.4–15.1) | Yes |
| | | B/Florida | 66.1 (64.1–68.0) | Investigational TIV | 64.0 (60.1–67.9) | 2.0 (−2.2–6.4) | Yes |
| | Superiority‖ | B/Brisbane | 71.8 (69.9–73.6) | Investigational TIV§ | 20.0 (16.9–23.5)‡ | 51.8 (47.9–55.3) | Yes |
| | | B/Florida | 66.1 (64.1–68.0) | Licensed TIV§ | 17.9 (14.9–21.3)‡ | 48.2 (44.3–51.6) | Yes |

Immunogenicity analyses were performed on the per-protocol analysis set: QIV, N = 2339; licensed TIV, N = 582; investigational TIV, N = 599; pooled TIV, N = 1181. Values in parentheses are 95% CIs.

*Noninferiority for GMT was met if the lower limit of the 2-sided 95% CI of the $GMT_{QIV}/GMT_{TIV}$ ratio was >0.66.

†The pooled TIV group includes subjects vaccinated with either licensed TIV or investigational TIV, combined.

‡Superiority for GMT was met if the lower limit of the 2-sided 95% CI of the $GMT_{QIV}/GMT_{TIV}$ ratio was >1.5.

§Investigational TIV did not contain B/Brisbane; licensed TIV did not contain B/Florida.

¶Noninferiority for SCR was met if the lower limit of the 2-sided 95% CI of the $SCR_{QIV} - SCR_{TIV}$ difference was >−10%.

‖Superiority for SCR was met if the lower limit of the 2-sided 95% CI of the $SCR_{QIV} - SCR_{TIV}$ difference was >10%.

**TABLE 3.** Postvaccination Seroprotection Rates Among Subjects 6 Months to <9 Years of Age

| Strain | Seroprotection Rate (%)* | | |
|---|---|---|---|
| | QIV | Licensed TIV | Investigational TIV |
| A/H1N1 | 98.6 (98.1–99.1) | 98.6 (97.3–99.4) | 98.0 (96.5–99.0) |
| A/H3N2 | 99.7 (99.3–99.9) | 99.1 (98.0–99.7) | 99.5 (98.5–99.9) |
| B/Brisbane | 78.6 (76.9–80.3) | 71.9 (68.1–75.6) | 33.7† (29.9–37.7) |
| B/Florida | 71.6 (69.7–73.4) | 29.1† (25.4–33.0) | 69.6 (65.7–73.2) |

Immunogenicity analyses were performed on the per-protocol analysis set: QIV, N = 2339; licensed TIV, N = 582; investigational TIV, N = 599. Values in parentheses are 95% CIs.

*Seroprotection defined as the percentage of vaccinees with a titer ≥1:40.

†Investigational TIV did not contain B/Brisbane; licensed TIV did not contain B/Florida.

**TABLE 4.** Summary of Safety Endpoints for Subjects 6 Months to <9 Years of Age

| Event | QIV n/N (%) | Licensed TIV n/N (%) | Investigational TIV n/N (%) |
|---|---|---|---|
| Solicited reaction | 2107/2745 (76.8) | 523/700 (74.7) | 522/692 (75.4) |
| Injection site (overall) | 1838/2742 (67.0) | 459/699 (65.7) | 449/692 (64.9) |
| Systemic (overall) | 1529/2745 (55.7) | 376/700 (53.7) | 451/692 (65.2) |
| Immediate unsolicited AE* | 7/2892 (0.4) | 2/734 (0.3) | 2/721 (0.3) |
| Unsolicited nonserious AE | 1371/2892 (47.4) | 352/734 (48.0) | 352/721 (48.8) |
| Vaccine related | 177/2892 (6.1) | 50/734 (6.8) | 43/721 (6.0) |
| Grade 3 | 235/2893 (8.1) | 51/734 (6.9) | 67/721 (9.3) |
| Grade 3 vaccine related | 16/2893 (0.6) | 3/734 (0.4) | 2/721 (0.3) |
| SAE | 41/2892 (1.4) | 7/734 (1.0) | 14/721 (1.9) |

Values are for all vaccinees and are according to the vaccine actually received.

*Unsolicited AE occurring ≤20 minutes after vaccination.

n indicates number of participants in the group reporting at least 1 event; N, total number of participants in the group.

In all vaccine groups, <1% of participants reported unsolicited immediate (ie, within 20 minutes of vaccination) AEs, none of which were considered grade 3 in intensity. Proportions of children reporting any vaccine-related or grade 3 nonserious unsolicited AEs were similar for all 3 vaccine groups. The most common nonserious unsolicited AEs were cough, upper respiratory tract infection, fever and vomiting, most of which were grade 1 or 2 in intensity.

Five children receiving QIV (0.2%) discontinued the study because of 1 or more AEs thought to be related to vaccination, including fever, malaise, irritability and abnormal crying, injection-site erythema, swelling and pruritus and hives on face, hands and feet. None of these were considered SAEs. No children receiving a TIV discontinued due to a related AE.

Three SAEs were reported as being related to vaccination by investigators: croup in a 13-month old 3 days after the first dose of QIV, febrile seizure in an 11-month old 8 hours after the second dose of investigational TIV and febrile seizure in a 4-year old 1 day after the first dose of licensed TIV. All resolved and did not result in early discontinuation. One death (not vaccine related) was reported during the study: a case of drowning in a 19-month old that occurred 43 days after the second dose of licensed TIV.

Thirteen participants experienced an AE of special interest, all of which were febrile seizures. Only 2 of these were considered vaccine related and are described above.

## DISCUSSION

Influenza causes substantial illness, complications, hospitalizations and deaths among children.[1,2] For example, during the 2002–2003 and 2003–2004 influenza seasons, laboratory-confirmed influenza accounted for between 50 and 95 clinic visits per 1000 children <5 years of age presenting with acute respiratory tract infection or fever and between 6 and 27 emergency department visits in the same cohort.[9]

Influenza B occurs in persons of all ages, but it appears to affect older children and young adults more than other age groups.[10,11] In addition, type B causes a substantial proportion of influenza-related deaths in the pediatric age group. Based on data provided by US Centers for Disease Control and Prevention, Ambrose and Levin[12] calculated that type B caused some 34% of reported pediatric deaths attributable to influenza during the past 7 influenza seasons (2004–2005 through 2010–2011, excluding the 2009–2010 A/H1N1 pandemic).

TIVs provide substantial protection against influenza illness in children. For example, in a randomized study conducted over a period of 5 seasons among children 1–15 years of age in the United States, TIV reduced symptomatic culture-positive influenza by 77% against A/H3N2 strains and 91% against A/H1N1 strains.[13] In a case-control study conducted in 4 states during the 2010–2011 season among persons presenting to hospitals, emergency departments and medical clinics with laboratory-confirmed influenza illness, the adjusted vaccine effectiveness of TIV was 71% (95% CI, 58–78%) in children 2–8 years of age.[14]

Despite widespread vaccination of children with influenza vaccines, 1 reason that TIV offers suboptimal protection is because it contains a B strain from only 1 of the 2 cocirculating B lineages and often not the most common B-lineage strain in circulation that season. QIV containing a B strain from each lineage offers a promising solution to this problem. In the study reported herein, we showed that QIV was as immunogenic as licensed TIV in children 6 months to < 9 years of age. There was no evidence of immunologic interference as a result of adding the alternate B-lineage strain. In addition, QIV induced superior antibody responses to the B strain not covered by each respective control TIV.

QIV was well-tolerated by the study children and had a safety profile similar to that of licensed TIV, the safety of which has been well-documented.[15–18] As with TIV, injection-site and systemic reactions to QIV were generally mild and short-lived. Of

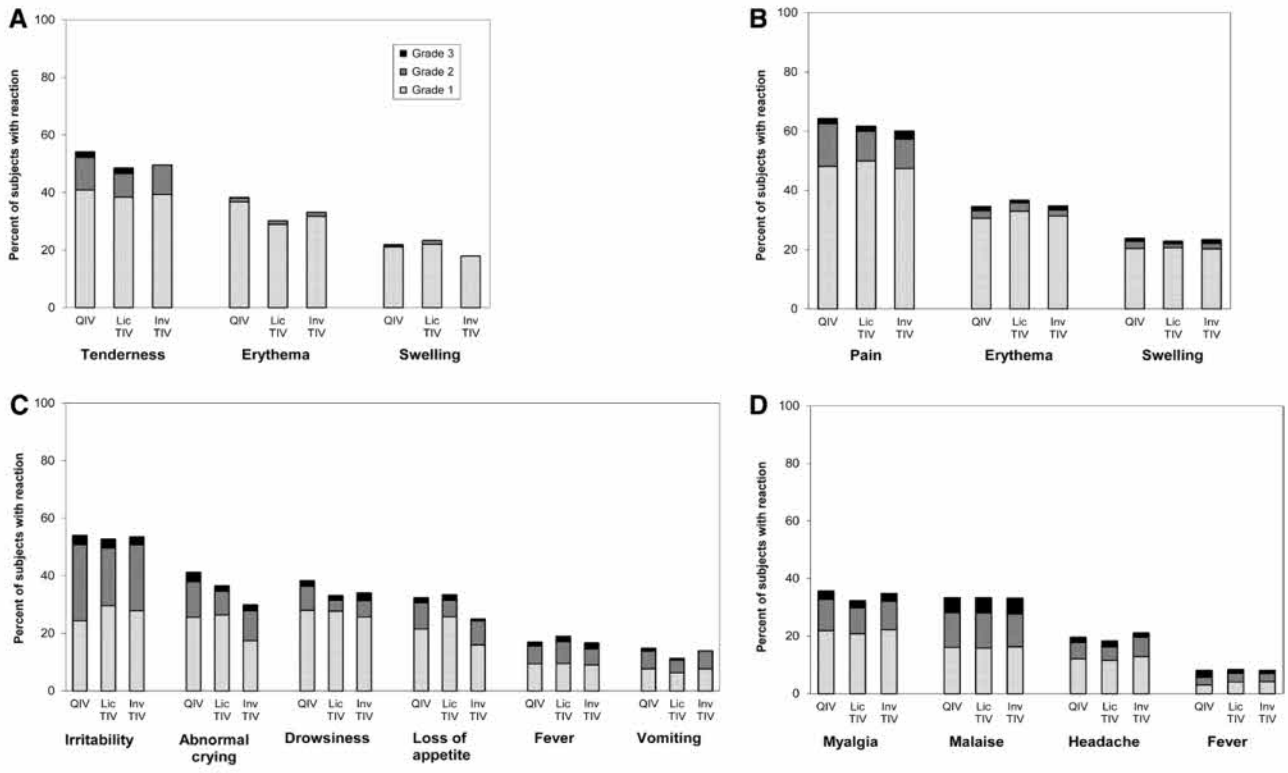



**FIGURE 2.** Solicited adverse reactions. A, B) Solicited injection-site reactions. C, D) Solicited systemic reactions. Panels A and C show reactions in children 6 months to <24 months old and panels B and D show reactions in children 2 years to <9 years old. Safety analyses were performed on the safety analysis set for the vaccine actually received: QIV, N = 2892 (672 were 6 to <24 months of age, 2220 were 2 to <9 years of age); licensed TIV, N = 734 (167 were 6 to <24 months of age, 567 were 2 to <9 years of age); investigational TIV, N = 721 (152 were 6 to <24 months of age, 569 were 2 to <9 years of age). Severity for solicited and unsolicited AEs was recorded as follows. Injection-site tenderness: grade 1 for a minor reaction when injection site is touched, grade 2 for cries and protests when injection site is touched, grade 3 for cries when injected limb is moved or the movement of the injected limb is reduced. Injection-site pain: grade 1 for easily tolerated, grade 2 for sufficiently discomforting to interfere with normal behavior or activities and grade 3 for incapacitating or unable to perform usual activities. Injection-site swelling and erythema: grade 1 for >0 to <25 mm, grade 2 for ≥ 25 to < 50 mm and grade 3 for ≥50 mm. Fever (6 to <24 months of age): grade 1 for ≥100.4°F to ≤101.3°F, grade 2 for >101.3°F to ≤103.1°F and grade 3 for >103.1°F; fever (2 to <9 years of age): grade 1 for ≥100.4°F to ≤101.1°F, grade 2 for ≥101.2°F to ≤102.0°F and grade 3 for ≥102.1°F. Vomiting: grade 1 for 1 episode/24 h, grade 2 for 2–5 episodes/24 h and grade 3 for ≥6 episodes/24 h or requiring parenteral hydration. Abnormal crying: grade 1 for <1 hour, grade 2 for 1–3 hours, grade 3 for >3 hours. Drowsiness: grade 1 for sleepier than usual or less interested in surroundings, grade 2 for not interested in surroundings or did not wake up for a feed/meal and grade 3 for sleeping most of the time or difficult to wake up. Loss of appetite: grade 1 for eating less than normal, grade 2 for missed 1 or 2 feeds/meals completely and grade 3 for refuses ≥3 feeds/meals or refuses most feeds/meals. Irritability: grade 1 for easily consolable, grade 2 for requiring increased attention and grade 3 for inconsolable. Headache, malaise, myalgia and all unsolicited AEs: grade 1 for no interference with activity, grade 2 for some interference with activity and grade 3 for significant interference or prevention of daily activity

note, the rate of fever during the 7 days postvaccination was no higher among QIV recipients than TIV recipients, including in the youngest children 6 to <24 months of age. There was no unusual pattern of unsolicited nonserious or serious AEs. A febrile seizure was reported as related to vaccination in 1 child after licensed TIV and in 1 child after investigational TIV, but none among QIV recipients. Overall, QIV did not present any safety concerns.

The safety and immunogenicity of a quadrivalent live attenuated influenza vaccine (Q/LAIV) have been reported in adults and children.[19,20] In both age groups, Q/LAIV induced noninferior antibody responses compared with trivalent LAIV (T/LAIV) for all corresponding influenza strains. As in our study, the addition of a second B-lineage strain did not interfere with antibody responses. The safety profile of Q/LAIV was comparable to T/LAIV, except for a higher rate of fever after Q/LAIV than after T/LAIV (5.1% vs. 3.1% with fever ≥ 38.0°C and 1.2% vs. 0.3% with fever ≥39.0°C) in the pediatric study.

In a separate study conducted among children 3–17 years of age, the safety profile of another manufacturer's QIV was comparable to 2 control TIVs.[21] Antibody responses to each strain in the QIV were noninferior to those of the same strains in each control TIV and the responses to the B strains in the QIV were superior to those induced by each TIV containing the alternate B-lineage strain.

We have recently published our results of QIV administered to adults ≥18 years of age.[22] As in the pediatric study, QIV induced noninferior antibody responses compared with 2 control TIVs for all corresponding strains and higher responses compared with each TIV that contained the B strain from the opposite lineage. Rates

of solicited and unsolicited AEs were similar between groups and there were no safety concerns. Similar results for QIV versus control TIVs were noted in a third study of QIV in elderly persons 65 years of age and older, which will be published separately.[23]

A limitation of this study was that we restricted enrollment to generally healthy children. Antibody responses would likely be diminished among children with congenital or acquired immunodeficiencies. However, given the similar immune responses shown herein between QIV and the control TIVs, we would expect QIV to perform as well as TIV in high risk populations. We did not evaluate children 9 through 17 years of age, but our data, demonstrating similar immunogenicity and safety profiles between QIV and control TIVs in both younger children and in adults, provide reassurance that QIV would perform comparably among 9- to 17-year olds.

In conclusion, the safety and immunogenicity of QIV was comparable with licensed TIV in a healthy pediatric population. By inducing antibody responses to both B lineages simultaneously, QIV should help overcome the limitations of TIV, namely, its inability to protect against both cocirculating B lineages simultaneously and that it often does not contain the B-lineage strain predominating in a given influenza season.

## ACKNOWLEDGMENTS

*The authors would like to thank the investigators and research staff at the 69 clinical study sites for performing this study and to the many families for their participation. Medical writing assistance in the preparation of this article was provided by Drs. Phillip Leventhal and Kurt Liittschwager of 4Clinics (Paris, France). Support for this study and medical writing assistance was provided by Sanofi Pasteur.*

## REFERENCES

1. Centers for Disease Control. Update: influenza activity - United States, 2011–12 season and composition of the 2012–13 influenza vaccine. *MMWR Morb Mortal Wkly Rep*. 2012;61:414–420.
2. Fiore AE, Uyeki TM, Broder K, et al.; Centers for Disease Control and Prevention (CDC). Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Recomm Rep*. 2010;59(RR-8):1–62.
3. Rota PA, Wallis TR, Harmon MW, et al. Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. *Virology*. 1990;175:59–68.
4. Glezen WP, Schmier JK, Kuehn CM, et al. The burden of influenza B: a structured literature review. *Am J Public Health*. 2013;103:e43–e51.
5. Centers for Disease Control. Past weekly surveillance reports 2013. Available at: http://www.cdc.gov/flu/weekly/pastreports.htm. Accessed January 8, 2013.
6. Belongia EA, Kieke BA, Donahue JG, et al.; Marshfield Influenza Study Group. Effectiveness of inactivated influenza vaccines varied substantially with antigenic match from the 2004–2005 season to the 2006–2007 season. *J Infect Dis*. 2009;199:159–167.
7. Skowronski DM, De Serres G, Dickinson J, et al. Component-specific effectiveness of trivalent influenza vaccine as monitored through a sentinel surveillance network in Canada, 2006-2007. *J Infect Dis*. 2009;199:168–179.
8. Reed C, Meltzer MI, Finelli L, et al. Public health impact of including two lineages of influenza B in a quadrivalent seasonal influenza vaccine. *Vaccine*. 2012;30:1993–1998.
9. Poehling KA, Edwards KM, Weinberg GA, et al.; New Vaccine Surveillance Network. The underrecognized burden of influenza in young children. *N Engl J Med*. 2006;355:31–40.
10. Grant KA, Carville K, Fielding JE, et al. High proportion of influenza B characterises the 2008 influenza season in Victoria. *Commun Dis Intell Q Rep*. 2009;33:328–336.
11. Olson DR, Heffernan RT, Paladini M, et al. Monitoring the impact of influenza by age: emergency department fever and respiratory complaint surveillance in New York City. *PLoS Med*. 2007;4:e247.
12. Ambrose CS, Levin MJ. The rationale for quadrivalent influenza vaccines. *Hum Vaccin Immunother*. 2012;8:81–88.
13. Neuzil KM, Dupont WD, Wright PF, et al. Efficacy of inactivated and cold-adapted vaccines against influenza A infection, 1985 to 1990: the pediatric experience. *Pediatr Infect Dis J*. 2001;20:733–740.
14. Treanor JJ, Talbot HK, Ohmit SE, et al.; US Flu-VE Network. Effectiveness of seasonal influenza vaccines in the United States during a season with circulation of all three vaccine strains. *Clin Infect Dis*. 2012;55:951–959.
15. Mitchell DK, Ruben FL, Gravenstein S. Immunogenicity and safety of inactivated influenza virus vaccine in young children in 2003–2004. *Pediatr Infect Dis J*. 2005;24:925–927.
16. Lina B, Fletcher MA, Valette M, et al. A TritonX-100-split virion influenza vaccine is safe and fulfills the committee for proprietary medicinal products (CPMP) recommendations for the European Community for Immunogenicity, in Children, Adults and the Elderly. *Biologicals*. 2000;28:95–103.
17. Gonzalez M, Pirez MC, Ward E, et al. Safety and immunogenicity of a paediatric presentation of an influenza vaccine. *Arch Dis Child*. 2000;83:488–491.
18. France EK, Glanz JM, Xu S, et al. Safety of the trivalent inactivated influenza vaccine among children: a population-based study. *Arch Pediatr Adolesc Med*. 2004;158:1031–1036.
19. Block SL, Falloon J, Hirschfield JA, et al. Immunogenicity and safety of a quadrivalent live attenuated influenza vaccine in children. *Pediatr Infect Dis J*. 2012;31:745–751.
20. Block SL, Yi T, Sheldon E, et al. A randomized, double-blind noninferiority study of quadrivalent live attenuated influenza vaccine in adults. *Vaccine*. 2011;29:9391–9397.
21. Domachowske JB, Pankow-Culot H, Bautista M, et al. A randomized trial of candidate inactivated quadrivalent influenza vaccine versus trivalent influenza vaccines in children aged 3–17 years. *J Infect Dis*. 2013;207:1878–1887.
22. Greenberg DP, Robertson CA, Noss MJ, et al. Safety and immunogenicity of a quadrivalent inactivated influenza vaccine compared to licensed trivalent inactivated influenza vaccines in adults. *Vaccine*. 2013;31:770–776.
23. Greenberg DP, Robertson C, Talbot HK, et al. Safety and immunogenicity of a quadrivalent inactivated influenza vaccine (QIV) containing two A and two B strains among persons ≥ 65 years of age (Abstract 544, presented). In: *49th Annual Meeting of the Infectious Diseases Society of America (IDSA)*. Boston, MA: Infectious Diseases Society of America; 2011.

OPEN

# Safety and Immunogenicity of a Full-dose, Split-virion, Inactivated, Quadrivalent Influenza Vaccine in Healthy Children 6-35 Months of Age

## *A Randomized Controlled Clinical Trial*

*Corwin A. Robertson, MD, MPH,* Monica Mercer, MD,* Alexandre Selmani, PhD,* Nicola P. Klein, MD, PhD,† Robert Jeanfreau, MD,‡ and David P. Greenberg, MD*§*

**Background:** For children <3 years of age, a half dose of inactivated influenza vaccine (7.5 µg hemagglutinin per strain) has been used for more than 30 years, but several studies indicate that a full dose (15 µg hemagglutinin per strain) can be used in this population without increasing the rate of fever or other reactions. Here, we compare the safety and immunogenicity of full and half doses of quadrivalent, split-virion, inactivated influenza vaccine (IIV4) in children 6–35 months of age.

**Methods:** In this phase IV, randomized, observer-blinded, multi-center study, healthy children 6–35 months of age were randomized 1:1 to be vaccinated with a half or full dose of IIV4 (NCT02915302). The primary objective was to demonstrate that the rate of any fever (≥38.0°C) up to 7 days after a full dose of IIV4 was noninferior to the rate of fever after a half dose.

**Results:** The study included 1950 children. Noninferiority in the rate of fever was demonstrated for the full dose versus the half dose of IIV4 (difference in rate = 0.84%; 95% confidence interval, −2.13% to 3.80%). Solicited reactions and unsolicited adverse events were similar between the dose groups. No vaccine-related serious adverse events were reported. Noninferiority of both hemagglutination inhibition geometric mean titers and seroconversion rates was demonstrated for all 4 vaccine strains for the full dose versus the half dose.

**Conclusions:** In children 6–35 months of age, a full dose of IIV4 was immunogenic and had a safety profile comparable to that of a half dose, with no new safety concerns observed.

**Key Words:** quadrivalent influenza vaccine, children, immunogenicity, safety

(*Pediatr Infect Dis J* 2019;38:323–328)

Accepted for publication October 7, 2018.

From the *Scientific and Medical Affairs, Sanofi Pasteur, Swiftwater, Pennsylvania; †Kaiser Permanente Vaccine Study Center, Oakland, California; ‡MedPharmics, Metairie, Louisiana; and §Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh Pennsylvania.

This work is funded by Sanofi Pasteur. N.P.K. reports grants from Sanofi Pasteur during the conduct of the study and grants from GlaxoSmithKline, Protein Sciences, Pfizer, Merck, Dynavax and MedImmune outside the submitted work. C.A.R., M.M., A.S. and D.P.G. are employees of Sanofi Pasteur. R.J. declares no conflicts of interest. The authors have no other conflicts of interest or funding to disclose.

Address for correspondence: Corwin A. Robertson, MD, MPH, Sanofi Pasteur, Swiftwater, 1 Discovery Drive, Swiftwater, PA 18370. E-mail: corey.robertson@sanofi.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 website (www.pidj.com).



ISSN: 0891-3668/19/3803-0323
DOI: 10.1097/INF.0000000000002227

Young children are at increased risk for influenza virus infection, as well as for severe influenza illness and influenza-related hospitalization.[1,2] Although influenza A has been the focus of most study and prevention efforts, influenza B is now appreciated to be a frequent cause of illness, hospitalization and death.[3] In young children, influenza B is responsible for a disproportionate amount of severe illness and hospitalization.[3,4] Two distinct lineages of influenza B, Victoria and Yamagata, now cocirculate, although their distribution can vary substantially between years and regions.[5]

Because trivalent influenza vaccines contain only a single B-lineage strain, quadrivalent vaccines containing B strains from both lineages have been developed to reduce the risk of influenza illness and its associated morbidity and mortality as immunity to 1 B lineage does not provide adequate protection against the other.[6] A quadrivalent, split-virion, inactivated influenza vaccine (IIV4; Fluzone Quadrivalent; Sanofi Pasteur, Swiftwater, PA) has been available in the United States since 2013 for individuals ≥6 months of age. Clinical trial data indicated that in children 6 months to 8 years of age, IIV4 was as immunogenic as the comparator trivalent inactivated influenza vaccine for each of the 3 shared influenza strains and, despite the additional antigen, the 2 vaccines had a similar safety profile.[7]

For more than 30 years, influenza vaccines for children <3 years to age have contained a half dose of antigen (7.5 µg hemagglutinin per strain)[8] to reduce the risk of fever and febrile convulsions associated with earlier whole-virus influenza vaccines.[9] More recent findings suggest that a full dose (15 µg hemagglutinin per strain) can be used in children <3 years without increased fever or other reactions,[10–13] although this has not yet been established for IIV4, which is currently licensed for use as a half dose in young children. In the current study, we therefore evaluated the safety and immunogenicity of full versus half doses of this IIV4 in healthy children 6–35 months of age. The primary objective was to compare the rates of fever following administration of full and half doses of IIV4.

## MATERIALS AND METHODS

### Study Design

This was a phase IV, randomized, observer-blinded, 2-arm, multi-center study to evaluate the safety and immunogenicity of 2 different doses of IIV4 in healthy children 6–35 months of age. The study was conducted between September 2016 and March 2017 at 38 sites in the United States (ClinicalTrials.gov no. NCT02915302).

### Ethics

The study was approved by the institutional review boards for all sites and conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonisation guidelines for Good Clinical Practice. Before participants were included in the study, written informed consent was provided by their parents or guardians.

## Participants

The study included healthy children 6–35 months of age who had not been vaccinated against influenza during the current season (2016–2017). Children 6–11 months of age had to be born at full term of pregnancy (≥37 weeks) or with a birth weight ≥2.5 kg. Children with moderate or severe acute illness or infection according to the investigator's judgment or febrile illness (temperature ≥100.4°F [38.0°C]) who were otherwise eligible to participate were not enrolled until the illness resolved. Other exclusions are listed in Table, Supplemental Digital Content S1, http://links.lww.com/INF/D336. Enrollment was stratified by age at each site so that approximately equal numbers of children 6–23 and 24–35 months of age would be included.

## Vaccine

In accordance with US Food and Drug Administration guidance for composition of Northern Hemisphere 2016–2017 influenza vaccines, IIV4 (Fluzone Quadrivalent; Sanofi Pasteur) contained the A/California/07/2009 X-179A (H1N1), A/Hong Kong/4801/2014 X-263B (H3N2), B/Brisbane/60/2008 (Victoria lineage) and B/Phuket/3073/2013 (Yamagata lineage) strains. Each half dose (0.25 mL) was formulated to contain 7.5 μg hemagglutinin per strain, and each full dose (0.5 mL) was formulated to contain 15 μg hemagglutinin per strain. Study vaccines were supplied, respectively, in 0.25- or 0.5-mL, prefilled single-dose syringes.

## Study Conduct

Using a preprogrammed interactive response technology system, participants were randomized in a 1:1 ratio to be vaccinated by intramuscular injection with a half or full dose of IIV4. In accordance with US Advisory Committee on Immunization Practices guidance,[8] participants received 2 doses of IIV4 28 days apart (window, 28–35 days) if they had not previously received 2 doses of influenza vaccine. The participant, study site personnel (including investigators) and sponsor's clinical team members involved in the study were blinded to the vaccine dose administered, with the exception of unblinded qualified study staff who administered the vaccine. The unblinded qualified study staff did not collect safety data, and they were instructed to not inform parents or guardians of the dose administered.

## Safety Assessment

For 7 days after each vaccination, parents or guardians took temperature readings each day, measured the size of any local reactions and recorded medications taken for any adverse reactions on a diary card. Investigators or authorized designees interviewed the parents or guardians to collect the information recorded in the diary card. Solicited local reactions consisted of injection-site tenderness, erythema and swelling; solicited systemic reactions consisted of fever, vomiting, abnormal crying, drowsiness, loss of appetite and irritability. Severity gradings for each reaction are provided in Table, Supplemental Digital Content S2, http://links.lww.com/INF/D337. Investigators also collected unsolicited adverse events (AEs) for 28 days after each vaccination and serious AEs (SAEs) up to the end of the trial according to the International Conference on Harmonisation E2A Guideline for Clinical Safety Data Management. SAEs occurring after a participant had completed the study but likely related to the product were also to be recorded.

## Immunogenicity Assessment

Immunogenicity was to be assessed in a planned subset of 1600 participants randomly selected via the interactive response technology system. For the immunogenicity subset, blood samples were collected before the first vaccination and 28 days (window, 28–35 days) after the final vaccination. Hemagglutination inhibition (HAI) titers were measured as described previously.[7] The lower limit of quantitation was set at the reciprocal of the lowest dilution (1:10), and the upper limit of quantitation was set as the highest dilution (1:10,240) used in the assay. Seroconversion was defined as (1) a prevaccination titer <10 and postvaccination titer ≥40; or (2) a prevaccination titer ≥10 and a ≥4-fold increase in postvaccination titer.

## Statistical Analysis

The primary objective was assessed by determining if the rate of any fever (temperature ≥100.4°F [38.0°C]) within 7 days after administration of any full dose of IIV4 was noninferior to the rate of fever after administration of any half dose. All safety endpoints were assessed in all vaccinated participants according to the vaccine received. Noninferiority with respect to fever was considered demonstrated if the upper bound of the 2-sided 95% confidence interval (CI) of the fever rate difference between participants receiving full-dose vaccine and participants receiving half-dose vaccine was <5%. The 95% CI of the rate difference was computed using the Wilson Score method without continuity correction.[14] Assuming a total planned enrollment sample size of approximately 2190 (1095 per group), an attrition rate of 5%, a 1-sided alpha of 2.5%, an expected 14.3% rate of fever,[7] and a noninferiority margin of 5%, the study was powered at approximately 90% to demonstrate noninferiority for fever.

Immunogenicity was assessed according to the vaccine received in all participants in the immunogenicity subset who received at least 1 dose of the study vaccine, had a postvaccination serology result for at least 1 strain and completed the study according to protocol. For immunogenicity and safety variables, the 95% CIs of point estimates were calculated assuming a normal distribution. Geometric means and their 95% CIs were calculated as the anti-log of the mean and 95% of the $\log_{10}$ values. For point estimates of differences in proportions, 95% CIs were calculated using the Wilson Score method without continuity correction or by the exact binomial (Clopper-Pearson) method.

For each strain, noninferiority in the HAI geometric mean titer (GMT) was considered demonstrated if the lower limit of the 2-sided 95% CI of the GMT ratio ($GMT_{full\ dose}/GMT_{half\ dose}$) was >0.667. Noninferiority for seroconversion rates was considered demonstrated if the lower limit of the 2-sided 95% CI for the rate difference for the full dose minus the half dose was >−10%. For the difference in seroconversion rates, the 95% CI of the rate difference was computed using the Wilson Score method without continuity correction. For the 1600 participants who were to be randomly assigned to the immunogenicity subset, assuming an attrition rate of 20%, a 1-sided alpha of 2.5% for each test, the same expected GMTs for each dose group, a standard deviation of log titers against each strain of 0.7 and a noninferiority margin of 0.667, the study power was approximately 97.6% to demonstrate noninferiority for GMTs. Assuming for each strain the same expected seroconversion rates for each vaccine dosing group (90.9% for A/H1N1, 95.4% for A/H3N2, 72% for B/Victoria and 57.5% for B/Yamagata[7]) and a noninferiority margin of 10% for each strain, the planned study power was approximately 93.2% to demonstrate noninferiority for seroconversion rates.

Results of participants receiving exactly 1 dose of IIV4 and those receiving 2 doses were combined in all noninferiority assessments (ie, for fever rate, postvaccination HAI GMTs and seroconversion rates).

Demographic characteristics and safety were assessed in all participants who received at least 1 dose of study vaccine.

Missing data were not imputed. Statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC).

## RESULTS

### Baseline Characteristics and Disposition

The study was conducted between September 23, 2016, and March 6, 2017. Due to lower than expected recruitment, 1950 participants were enrolled in the study instead of the planned 2190 (Fig. 1). The participants were randomly assigned in nearly equal proportions to receive the half dose (n = 955; 49.0%) or full dose of IIV4 (n = 995; 51.0%). Six participants randomized to the full-dose group and 3 randomized to the half-dose group were not vaccinated. In accordance with Advisory Committee on Immunization Practices guidance, a total of 1024 (52.5%) participants received 2 doses of IIV4 (507 [53.1%] in the half-dose group and 517 [52.0%] in the full-dose group). The study was completed by 890 (93.2%) participants in the half-dose group and 917 (92.2%) in the full-dose group. The most common reasons for participant discontinuation were voluntary withdrawal not because of an AE and noncompliance with the protocol. No participants discontinued the study for an AE or SAE considered related to vaccination.

At enrollment, the full- and half-dose groups included nearly equal proportions of males and females (50.6% male in the half-dose group and 50.1% male in the full-dose group). Mean ages and distributions of racial and ethnic origins were also similar between the 2 groups (Table 1).

Immunogenicity was assessed in a randomly selected subset of 715 participants in the half-dose group and 745 in the full-dose group (Fig. 1). Of these, 665 (93.0%) participants in the half-dose group and 682 (91.5%) in the full-dose group completed the study.

### Fever

The difference in rate of fever for the full dose minus the half dose of IIV4 was 0.84% (95% CI, −2.13% to 3.80%) (Table 2). Thus, noninferiority in the rate of fever was demonstrated for the full dose compared with the half dose of IIV4.

### HAI Titers

Of the 1950 participants in the study, 1460 were randomized to the immunogenicity subset. Noninferiority of both HAI GMTs and seroconversion rates was demonstrated for all 4 vaccine strains for the full dose versus the half dose (Table 3). Overall, HAI GMTs and post-/prevaccination GMT ratios were higher for the full dose

**TABLE 1.** Demographic Characteristics

| Characteristics | Half Dose of IIV4 N = 949 | Full Dose of IIV4 N = 992 |
|---|---|---|
| Sex, n (%) | | |
| Male | 480 (50.6) | 497 (50.1) |
| Female | 469 (49.4) | 495 (49.9) |
| Age (months), mean ± standard deviation | 20.4 ± 8.75 | 20.5 ± 8.55 |
| Racial origin, n (%) | | |
| White | 717 (75.6) | 725 (73.1) |
| Black or African-American | 178 (18.8) | 195 (19.7) |
| Asian | 1 (0.1) | 8 (0.8) |
| American Indian or Alaska Native | 9 (0.9) | 10 (1.0) |
| Native Hawaiian or Other Pacific Islander | 4 (0.4) | 5 (0.5) |
| Mixed Origin | 36 (3.8) | 43 (4.3) |
| Missing | 4 (0.4) | 6 (0.6) |
| Ethnicity, n (%) | | |
| Hispanic or Latino | 206 (21.7) | 221 (22.3) |
| Not Hispanic or Latino | 731 (77.0) | 763 (76.9) |
| Missing | 12 (1.3) | 8 (0.8) |

Demographic characteristics were analyzed in all vaccinated participants according to the vaccine received.

**Table 2.** Rates of Fever and Noninferiority Comparison

| Dose Group | Cases of Fever n/N* | % (95% CI) | Noninferior† |
|---|---|---|---|
| Half dose | 101/893 | 11.31 (9.31 to 13.57) | — |
| Full dose | 113/930 | 12.15 (10.12 to 14.42) | — |
| Difference | — | 0.84 (−2.13 to 3.80) | Yes |

Fever was defined as a temperature ≥100.4°F (38.0°C) and was assessed in all vaccinated participants according to the vaccine received.

*The denominator (n) indicates number of cases of fever, and the numerator (N) indicates the number of participants with valid temperature data during the 7 days after vaccination.

†Noninferiority was considered demonstrated if the upper bound of the 2-sided 95% CI of the rate difference between participants receiving the full dose and the half dose of IIV4 was <5%.

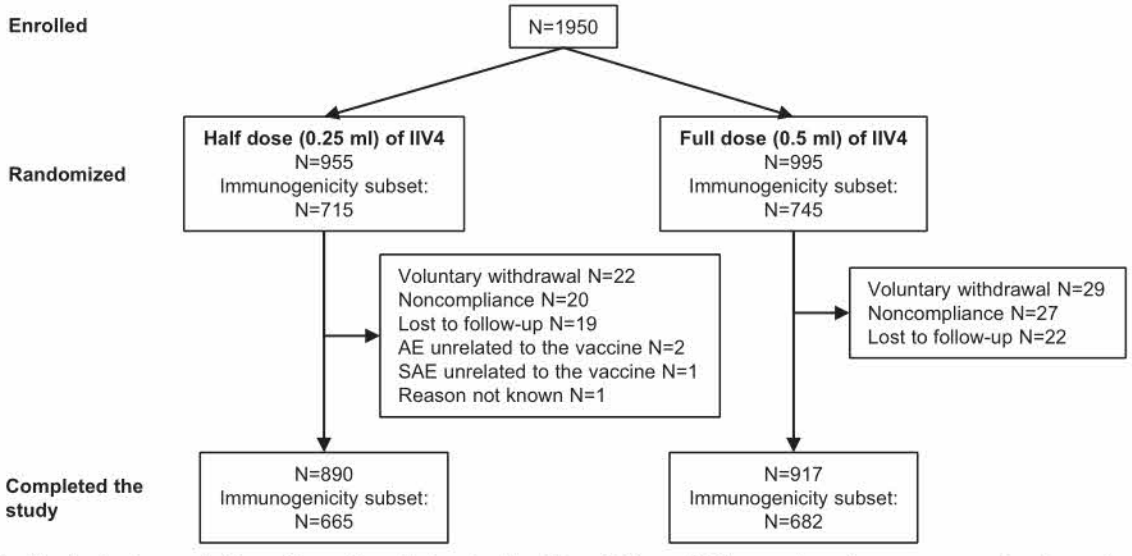



**FIGURE 1.** Study design and disposition of participants. Healthy children 6–35 months of age were randomly assigned 1:1 to receive the half dose or the full dose of IIV4 by intramuscular injection.

**TABLE 3.** Postvaccination Immunogenicity and Noninferiority Comparisons

| Measure | Strain | Half Dose of IIV4 N | Half Dose of IIV4 Value (95% CI) | Full Dose of IIV4 N | Full Dose of IIV4 Value (95% CI) | Comparison (95% CI) | Noninferior? |
|---|---|---|---|---|---|---|---|
| HAI GMT | A(H1N1) | 520 | 214 (185 to 247) | 539 | 310 (271 to 354) | 1.45 (1.19 to 1.77)* | Yes |
| | A(H3N2) | 525 | 221 (191 to 256) | 542 | 332 (290 to 380) | 1.50 (1.23 to 1.83)* | Yes |
| | B/Victoria | 520 | 261 (227 to 299) | 539 | 348 (304 to 398) | 1.33 (1.10 to 1.62)* | Yes |
| | B/Yamagata | 524 | 243 (213 to 277) | 543 | 349 (307 to 397) | 1.44 (1.20 to 1.73)* | Yes |
| Seroconversion rate (%) | A(H1N1) | 470 | 78.9 (75.0 to 82.5) | 483 | 84.1 (80.5 to 87.2) | 5.1 (0.189 to 10.0)† | Yes |
| | A(H3N2) | 475 | 81.9 (78.1 to 85.3) | 487 | 86.2 (82.9 to 89.2) | 4.3 (−0.283 to 8.99)† | Yes |
| | B/Victoria | 470 | 87.2 (83.9 to 90.1) | 483 | 88.6 (85.4 to 91.3) | 1.4 (−2.78 to 5.56)† | Yes |
| | B/Yamagata | 474 | 87.8 (84.5 to 90.6) | 488 | 91.2 (88.3 to 93.5) | 3.4 (−0.465 to 7.36)† | Yes |
| Post- to prevaccination GMT ratio | A(H1N1) | 470 | 9.00 (7.98 to 10.1) | 483 | 14.2 (12.5 to 16.2) | — | — |
| | A(H3N2) | 475 | 11.8 (10.3 to 13.4) | 487 | 16.4 (14.4 to 18.7) | — | — |
| | B/Victoria | 470 | 12.3 (11.0 to 13.9) | 483 | 17.2 (15.3 to 19.4) | — | — |
| | B/Yamagata | 474 | 12.7 (11.3 to 14.1) | 488 | 19.1 (17.0 to 21.5) | — | — |

Immunogenicity was assessed according to the vaccine received in all participants in the immunogenicity subset who received at least 1 dose of the study vaccine, had a valid postvaccination serology result for at least 1 strain and completed the study according to protocol.

*Ratio of HAI GMTs (full dose/half dose). Noninferiority was considered demonstrated if the lower limit of the 2-sided 95% CI was >0.667.

†Difference in seroconversion rate: seroconversion rate for the full dose of IIV4 minus the seroconversion rate for the half dose of IIV4. Noninferiority was considered demonstrated if the lower limit of the 2-sided 95% CI was >−10%.

than for the half dose, as indicated by nonoverlapping 95% CIs. For seroconversion rates, point estimates were higher for the full dose than for the half dose, but 95% CIs overlapped. In analyses stratified by age (6–23 months vs. 24–35 months) and by number of doses received (1 vs. 2 doses)—analyses for which this study was not powered—postvaccination HAI GMTs were generally higher among participants 24–35 months of age and among participants receiving 1 dose of IIV4, with the full dose generally inducing higher HAI GMTs compared with the half dose, regardless of age subgroup or number of doses received (Tables, Supplemental Digital Content S3–S6, http://links.lww.com/INF/D338; http://links.lww.com/INF/D339; http://links.lww.com/INF/D340; http://links.lww.com/INF/D341).

## Solicited Reactions

Proportions of participants reporting any solicited reactions, solicited injection-site reactions and solicited systemic reactions were similar for the full- and half-dose groups (Table 4). In most participants, solicited reactions resolved within 3 days (data not shown). Rates of grade 3 solicited reactions were generally similar for the full and half dose of IIV4 (Table, Supplemental Digital Content S7, http://links.lww.com/INF/D342). The most common grade 3 solicited injection-site reaction was tenderness (1.2% in the full-dose group and 1.7% in the half-dose group), and the most common grade 3 solicited systemic reactions included irritability (4.0% in the full-dose group and 3.6% in the half-dose group) and abnormal crying (2.6% in the full-dose group and 3.1%

**TABLE 4.** Solicited Reactions and Adverse Events

| Event | Half Dose of IIV4 n/N | Half Dose of IIV4 % (95% CI) | Full Dose of IIV4 n/N | Full Dose of IIV4 % (95% CI) |
|---|---|---|---|---|
| Solicited reaction within 7 days | 645/909 | 71.0 (67.9–73.9) | 698/941 | 74.2 (71.3–76.9) |
| Injection site | 480/909 | 52.8 (49.5–56.1) | 533/939 | 56.8 (53.5–60.0) |
| Tenderness | 430/909 | 47.3 (44.0–50.6) | 473/939 | 50.4 (47.1–53.6) |
| Redness | 210/909 | 23.1 (20.4–26.0) | 228/938 | 24.3 (21.6–27.2) |
| Swelling | 117/908 | 12.9 (10.8–15.2) | 138/937 | 14.7 (12.5–17.2) |
| Systemic* | 533/909 | 58.6 (55.4–61.9) | 561/941 | 59.6 (56.4–62.8) |
| Vomiting | 91/908 | 10.0 (8.1–12.2) | 96/941 | 10.2 (8.3–12.3) |
| Abnormal crying | 302/908 | 33.3 (30.2–36.4) | 321/941 | 34.1 (31.1–37.2) |
| Drowsiness | 290/908 | 31.9 (28.9–35.1) | 294/940 | 31.3 (28.3–34.3) |
| Loss of appetite | 248/908 | 27.3 (24.4–30.3) | 266/940 | 28.3 (25.4–31.3) |
| Irritability | 430/908 | 47.4 (44.1–50.7) | 457/940 | 48.6 (45.4–51.9) |
| Immediate unsolicited AE (<30 min) | | | | |
| Any | 2/949 | 0.2 (0.0–0.8) | 0/992 | 0.0 (0.0–0.4) |
| Vaccine related | 1/949 | 0.1 (0.0–0.6) | 0/992 | 0.0 (0.0–0.4) |
| Unsolicited AE within 28 days | | | | |
| Any | 420/949 | 44.3 (41.1–47.5) | 395/992 | 39.8 (36.8–42.9) |
| Vaccine related | 29/949 | 3.1 (2.1–4.4) | 30/992 | 3.0 (2.0–4.3) |
| AE leading to study discontinuation | 3/949 | 0.3 (0.1–0.9) | 0/992 | 0.0 (0.0–0.4) |
| SAE | 5/949 | 0.5 (0.2–1.2) | 5/992 | 0.5 (0.2–1.2) |
| AE of special interest†‡ | 1/949§ | 0.1 (0.0–0.6) | 0/992 | 0.0 (0.0–0.4) |

Safety was assessed in all vaccinated participants according to the vaccine received.

*Fever is displayed in Table 2.

†Included new onset of Guillain-Barré syndrome, encephalitis/myelitis (including transverse myelitis), neuritis (including Bell's palsy, optic neuritis and brachial neuritis), thrombocytopenia, vasculitis, convulsions (including febrile convulsions) and anaphylaxis or other hypersensitivity/allergic reactions.

‡Also included as an SAE.

§Chronic urticaria with onset 3 days after receipt of 1 half dose of IIV4, considered related to vaccination by the investigator.

in the half-dose group). Grade 3 fever was reported for 0.6% (95% CI, 0.2–1.3) of participants who received the half dose and 1.2% (95% CI, 0.6–2.1) who received the full dose. In analyses stratified by age, rates of solicited systemic reactions were generally higher among participants 6–23 months of age who received the full dose compared with participants 24–35 months of age who received the full dose. Within each age subgroup, reactogenicity between the full and half doses was similar (Table, Supplemental Digital Content S8, http://links.lww.com/INF/D343). Among participants receiving 2 doses of IIV4, reactogenicity was generally similar between the first and second doses, irrespective of dosing volume received (Table, Supplemental Digital Content S9, http://links.lww.com/INF/D344).

### Adverse Events

Rates of vaccine-related unsolicited AEs were similar (3.1% for the half-dose group and 3.0% for the full-dose group) (Table 4). Three AEs leading to study discontinuation were reported, all in the half-dose group. None of the AEs leading to study discontinuation were considered related to vaccination.

SAEs were experienced by 5 (0.5%) participants in the half-dose group and 5 (0.5%) in the full-dose group, none of which were considered related to the vaccine. A single AE of special interest (chronic urticaria first appearing 3 days post-vaccination and continuing for >6 weeks) was considered by the investigator to be related to vaccination.

## DISCUSSION

In this study, we showed that in children 6–35 months of age, comparable rates of fever were reported among those receiving a full dose and a half dose of IIV4. Other solicited reactions and unsolicited AEs also occurred at similar rates between for the full and half dose of IIV4, and few cases of grade 3 fever and no cases of febrile convulsion were reported. Thus, no new safety concerns were observed following administration of a full dose of IIV4 in this population. The study also showed that antibody responses induced by the full dose of IIV4 were at least as high as those induced by the half dose, suggesting that a full dose of IIV4 can protect this age group at least as well as a half dose. Of note, 1 dose of IIV4 induced HAI GMTs that were generally higher than those induced by 2 doses, which likely reflects 1-dose participants having been immunologically primed, whereas 2-dose recipients were immunologically naive or relatively naive.

Similar results with respect to the reactogenicity and immunogenicity between full and half doses have been found in several studies, although most have studied trivalent influenza vaccines. Skowronski et al[13] examined the immunogenicity and reactogenicity of 2 full versus 2 half doses of a trivalent inactivated influenza vaccine in previously unimmunized infants (6–11 months of age) and toddlers (12–23 months). They found that the full dose induced higher HAI titers for all 3 vaccine components in infants but not toddlers and that the rate of fever did not increase in either age group. Similarly, Pavia-Ruz et al[12] and Langley et al[11] reported that in children 6–35 months of age, a full dose of trivalent inactivated influenza vaccine induced higher HAI titers than the half dose for all strains without affecting rates of fever or other reactogenicity or safety endpoints. Finally, Jain et al[10] compared the US-licensed standard half-dose IIV4 with an investigational full-dose IIV4 in children 6–35 months of age. Noninferior HAI GMTs and seroconversion rates were demonstrated against all 4 vaccine strains. Also, superior HAI GMTs were demonstrated against both vaccine B strains in children 6–17 months of age and unprimed children 6–35 months of age. As in the other studies, the safety profiles, including the rate of fever, were similar for the 2 vaccines. Thus, all studies to date, including the current one, have indicated that full-dose inactivated influenza vaccines can generally increase HAI antibody titers and can be safely used in young children. Moreover, several studies have demonstrated the effectiveness and safety of full-dose inactivated influenza vaccine in preventing influenza disease in children younger than 3 years of age.[15–17]

This study had some limitations. The study did not assess clinical protection against influenza but rather HAI titers, which are not always predictive of protection[18]; nonetheless, the results suggest that full dose should be at least as effective as the half dose in young children. Another potential limitation is that the planned study size due was not reached to unexpectedly low recruitment. Regardless, the study size was sufficient (ie, power >80%) to demonstrate noninferiority for the primary (rates of fever) and secondary (HAI GMTs and rates of seroconversion) outcomes.

In summary, the results of this study indicate that healthcare providers should be able to safely use the full dose of IIV4 for children 6–35 months of age. As suggested by Jain et al,[10] clinical information should now be sufficient to support using full-dose influenza vaccines for this age group. Indeed, full-dose influenza vaccine has already been recommended for use in children as young as 6 months of age in some countries (eg, Canada, the United Kingdom, Finland and the United States) as part of their national immunization programs.[8,19,20] The ability to use full-dose influenza vaccine should provide healthcare providers with additional flexibility and convenience when vaccinating young children against influenza.

## REFERENCES


1. Fraaij PL, Heikkinen T. Seasonal influenza: the burden of disease in children. *Vaccine*. 2011;29:7524–7528.
2. Iskander M, Booy R, Lambert S. The burden of influenza in children. *Curr Opin Infect Dis*. 2007;20:259–263.
3. Paul Glezen W, Schmier JK, Kuehn CM, et al. The burden of influenza B: a structured literature review. *Am J Public Health*. 2013;103:e43–e51.
4. World Health Organization. Review of the 2010–2011 winter influenza season, northern hemisphere. *Wkly Epidemiol Rec*. 2011;86:222–227.
5. Rota PA, Wallis TR, Harmon MW, et al. Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. *Virology*. 1990;175:59–68.
6. Ambrose CS, Levin MJ. The rationale for quadrivalent influenza vaccines. *Hum Vaccin Immunother*. 2012;8:81–88.
7. Greenberg DP, Robertson CA, Landolfi VA, et al. Safety and immunogenicity of an inactivated quadrivalent influenza vaccine in children 6 months through 8 years of age. *Pediatr Infect Dis J*. 2014;33:630–636.
8. Grohskopf LA, Sokolow LZ, Broder KR, et al. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices - United States, 2017-18 Influenza Season. *MMWR Recomm Rep*. 2017;66:1–20.
9. Wright PF, Dolin R, La Montagne JR. From the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, the Center for Disease Control, and the Bureau of Biologics of the Food and Drug Administration. Summary of clinical trials of influenza vaccines–II. *J Infect Dis*. 1976;134:633–638.
10. Jain VK, Domachowske JB, Wang L, et al. Time to change dosing of inactivated quadrivalent influenza vaccine in young children: evidence from a phase III, randomized, controlled trial. *J Pediatric Infect Dis Soc*. 2017;6:9–19.
11. Langley JM, Vanderkooi OG, Garfield HA, et al. Immunogenicity and safety of 2 dose levels of a thimerosal-free trivalent seasonal influenza vaccine in children aged 6-35 months: a randomized, controlled trial. *J Pediatric Infect Dis Soc*. 2012;1:55–63.
12. Pavia-Ruz N, Angel Rodriguez Weber M, Lau YL, et al. A randomized controlled study to evaluate the immunogenicity of a trivalent inactivated seasonal influenza vaccine at two dosages in children 6 to 35 months of age. *Hum Vaccin Immunother*. 2013;9:1978–1988.
13. Skowronski DM, Hottes TS, Chong M, et al. Randomized controlled trial of dose response to influenza vaccine in children aged 6 to 23 months. *Pediatrics*. 2011;128:e276–e289.

14. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med*. 1998;17:857–872.
15. Heinonen S, Silvennoinen H, Lehtinen P, et al. Effectiveness of inactivated influenza vaccine in children aged 9 months to 3 years: an observational cohort study. *Lancet Infect Dis*. 2011;11:23–29.
16. Nohynek H, Baum U, Syrjanen R, Ikonen N, Sundman J, Jokinen J. Effectiveness of the live attenuated and the inactivated influenza vaccine in two-year-olds - a nationwide cohort study Finland, influenza season 2015/16. *Euro Surveill*. 2016;21:pii=30346.
17. Claeys C, Zaman K, Dbaibo G, et al; Flu4VEC Study Group. Prevention of vaccine-matched and mismatched influenza in children aged 6-35 months: a multinational randomised trial across five influenza seasons. *Lancet Child Adolesc Health*. 2018;2:338–349.
18. Cox RJ. Correlates of protection to influenza virus, where do we go from here? *Hum Vaccin Immunother*. 2013;9:405–408.
19. National Advisory Committee on Immunization (NACI). An Advisory Committee Statement (ACS): statement on seasonal influenza vaccine for 2014–2015. *Can Commun Dis Rep*. 2014;40:1–68.
20. Halasa NB, Gerber MA, Berry AA, et al. Safety and immunogenicity of full-dose trivalent inactivated influenza vaccine (TIV) compared with half-dose TIV administered to children 6 through 35 months of age. *J Pediatric Infect Dis Soc*. 2015;4:214–224.

# Current Abstracts

*Edited by Robert J. Leggiadro, MD*

## Health Risks of Flood Disasters

Paterson DL, Wright H, Harris PNA. *Clin Infect Dis*. 2018;67:1450–1454.

Floods are the most common natural disaster occurring worldwide. Flood waters pose immediate dangers to human health, but also long-term effects resulting from displacement and worsened living conditions. The impact of flood disasters is expected to grow in the future owing to the effects of climate change and population shifts.

Drowning is the most frequent immediate cause of death soon after the onset of flooding. Other acute events include orthopedic injuries and laceration, hypothermia, electrocution and burns from flammable, low-density liquids spreading across the surface of floodwaters. Carbon monoxide poisoning from unventilated electrical generators or cooking implements is also common after floods.

Flood disasters have a significant impact on chronic health conditions, with medication noncompliance because of nonavailability, difficulties with access to health services and the physical workload associated with clean-up and reconstruction being significant issues. The reported impact of any interruption to treatment varies according to the underlying condition, with increased mortality rates after disasters in patients with cardiovascular disease and diabetes. Posttraumatic stress disorder, anxiety and depression are potential mental health consequences for flood victims.

Considerable loss of healthcare infrastructure can be associated with flood disasters, including evacuation of entire tertiary care hospitals. Significant increases in emergency department visits may be recorded for homelessness or inadequate shelter, especially among the elderly.

Trauma is common in patients injured by fast-moving water, trying to escape floodwaters or cleaning up after floods. Cellulitis and deeper skin infections are subsequently common. Cases of cellulitis have been shown to peak 3–4 days after a flooding event and remain above baseline level for up to 3 weeks. Any trauma can introduce pathogenic skin flora into wounds. Therefore, the typical bacterial causes of cellulitis and soft-tissue infections (*Staphylococcus aureus* and *Streptococcus pyogenes*) should be the first considerations when antibiotic therapy is indicated.

However, a number of less commonly encountered water-dwelling organisms (notably *Aeromonas* species) may cause skin infections in patients exposed to flood water. *Vibrio* spp. may be associated with saltwater exposure in the context of storm surges. Infection of contaminated wounds by *Clostridium tetani* may occur in areas of low immunization coverage. In endemic tropical regions, such as Southeast Asia and northern Australia, exposure to soil or water containing *Burkholderia pseudomallei* can result in melioidosis.

Although most soft-tissue infections after floodwater exposure will be bacterial, infections caused by fungal pathogens are well described, and infection may be polymicrobial. Mucormycosis caused by zygomycete fungi may present as a rapidly progressive necrotizing fasciitis with high mortality rates if urgent surgical intervention and antifungal therapy is unavailable or delayed. Nontuberculous mycobacteria may also cause infections after exposure to floodwater, particularly rapid growing *Mycobacterium* species such a *Mycobacterium fortuitum*, *Mycobacterium chelonae* and *Mycobacterium abscessus.*

In reports from the United States and the United Kingdom, acute respiratory infection was the most common infectious disease requiring consultation after flooding. Disruption of housing and overcrowding can increase the risk of transmission of respiratory viral pathogens. Direct contact with floodwater, with immersion, near drowning or aspiration, can lead to inoculation of the lower respiratory tract.

The risk of gastroenteritis after flooding is highest in areas with poor hygiene or an inadequate supply of clean drinking water, although outbreaks of diarrheal diseases are common even in resource-rich areas, particularly if the integrity of sewerage systems is compromised.

Leptospirosis, a spirochetal zoonosis causing an acute febrile illness, has increasingly been recognized as a pathogen associated with flooding and extreme weather events. Outbreaks of leptospirosis have been reported from diverse geographic locations in urban and rural settings, encompassing both the developed and developing world. In endemic areas, mosquito-borne diseases (eg, Japanese encephalitis or dengue fever) may occur at an increased rate after flood events.

*Comment*: Public health preparedness is an essential element in preventing morbidity and mortality associated with flood disasters. Data aggregation analyses can identify communities at greatest risk from flood disasters and can be used to plan cost-efficient preparedness strategies. Prevention of flood disasters rests on flood mitigation schemes and planned removal of populations from flood-prone areas.

Vaccine 30 (2012) 2020–2023

Contents lists available at SciVerse ScienceDirect

Vaccine

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

Brief report

# Febrile seizures after 2010–2011 influenza vaccine in young children, United States: A vaccine safety signal from the vaccine adverse event reporting system

Z. Leroy[a,*], K. Broder[a], D. Menschik[b], T. Shimabukuro[a], D. Martin[b]

[a] Immunization Safety Office, Division of Health Care Quality Promotion, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States
[b] Office of Biostatistics and Epidemiology, Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, MD, United States

ARTICLE INFO

*Article history:*
Received 30 September 2011
Received in revised form 25 October 2011
Accepted 5 December 2011

*Keywords:*
Febrile seizure
Trivalent influenza vaccine
Vaccine safety
Post-marketing surveillance

ABSTRACT

During the 2010–2011 influenza season, the Centers for Disease Control and Prevention and the Food and Drug Administration conducted enhanced vaccine safety monitoring for possible febrile seizures in all trivalent influenza vaccine (TIV) products in the United States using the Vaccine Adverse Event Reporting System (VAERS). We used Empirical Bayesian data mining techniques to assess disproportionate reporting after TIV and reviewed febrile seizure reports in children aged <5 years. On November 23, 2010, the combination of the coding term "febrile convulsion" and the Fluzone® TIV product exceeded a predetermined threshold in the VAERS database. By December 10, we confirmed 43 reports of febrile seizure following TIV in children aged 6–23 months. Clinical features of most reports were consistent with typical uncomplicated febrile seizures, and all children recovered. Further epidemiologic assessment of a possible association between TIV and febrile seizures was undertaken in a separate, population-based vaccine safety monitoring system.

Published by Elsevier Ltd.

## 1. Introduction

The Vaccine Adverse Event Reporting System (VAERS), co-managed by the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA), is the US spontaneous reporting system for adverse events (AEs) after vaccination, and VAERS surveillance for influenza vaccines is implemented annually [1–3]. In 2010, Australia reported a new finding of increased risk of febrile seizures in young children following receipt of 2010 Southern Hemisphere trivalent inactivated influenza vaccine (TIV) from one manufacturer, CSL Biotherapies [4–6]. Because of this finding, CDC and FDA instituted enhanced surveillance in VAERS for febrile seizures after US TIV in children aged <5 years.

## 2. Methods

VAERS accepts reports from healthcare providers, manufacturers, vaccine recipients, and others. Healthcare providers are required to report AEs listed in the VAERS Table of Reportable Events following Vaccination, which does not include febrile seizures after TIV, and are encouraged to report other clinically significant AEs after vaccination [7]. Reported AEs are entered into a database and coded using Medical Dictionary for Regulatory Activities (MedDRA) terms [8]. From July 1, 2010 through December 10, 2010, we used complementary strategies to monitor febrile seizures after US 2010–2011 TIV products: disproportionate reporting analysis and clinical review of individual reports. Foreign reports were excluded. Because VAERS is a routine surveillance program that does not meet the definition of research, it is not subject to institutional review board review and informed consent requirements.

On a bimonthly basis, beginning in October 2010, Empirical Bayesian data mining [9] was conducted to identify AEs reported more frequently than expected following TIV, using published criteria [10]. We evaluated all 2010–2011 influenza vaccine product-specific AE pairs with reporting proportions at least twice that of other vaccines in the VAERS database (i.e., lower bound of the 90% confidence interval of the Empirical Bayesian Geometric Mean [EB05] > 2). The primary analysis required a minimum count of one vaccine-AE combination, and was adjusted for sex, year of initial report receipt, and age group. A secondary age stratified analysis was also conducted using standard pre-specified age groups used at FDA; this analysis was adjusted for sex and year received only. To limit comparisons to other vaccines that might be more similar, we also conducted these analyses using a restricted VAERS database which only included reports following inactivated vaccines (i.e., if a live vaccine was administered with or without inactivated vaccine, these reports were excluded).

DOIs of original articles: 10.1016/j.vaccine.2012.01.027, 10.1016/j.vaccine.2011.12.040
* Corresponding author. Tel.: +1 404 639 2973.
*E-mail address:* ezv6@cdc.gov (Z. Leroy).

0264-410X/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.vaccine.2011.12.042

**Table 1**
Data mining results for reports of febrile convulsion after 2010–2011 Fluzone® in VAERS.

| Date | Number of Reports | EBGM[a] | EB05[b] |
|---|---|---|---|
| November 23, 2010 | | | |
| All ages | 35 | 3.66 | 2.52 |
| Age 0–<18 months[c] | 22 | 4.10 | 2.68 |
| Age 0–<18 months (database restricted to inactivated vaccines) | 15 | 4.08 | 2.50 |
| December 10, 2010 | | | |
| All ages | 41 | 3.36 | 2.44 |
| Age 0–<18 months | 28 | 4.15 | 2.92 |
| Age 0–<18 months (database restricted to inactivated vaccines) | 18 | 3.95 | 2.62 |

[a] Empirical Bayesian Geometric Mean, the point estimate of disproportionality for MedDRA coding term-adverse event combinations.
[b] Lower bound of the 90% confidence interval of the EBGM.
[c] The 11 pre-defined data mining age groups identified by Empirica include (0–<18 months, 18–<54 months, 54 months to <12.5 years, 12.5 years to <16.5 years, 16.5 years to <29.5 years, 29.5 years to <45.5 years, 45.5 years to <64.5 years, 64.5 years to <75.5 years, 75.5 years to <85.5 years, 85.5 years and above, and age unknown). FDA Center for Drug Evaluation and Research epidemiology staff selected these age groups during development of the Empirical Bayesian application which is used for all pharmaceuticals regulated by FDA. The age groups were intended to facilitate examination of product-event combinations in populations such as children, females of childbearing age, and the elderly.

In parallel with VAERS data mining activities, each possible febrile seizure event was identified and reviewed on a daily basis. We conducted automated searches of VAERS reports in children aged <5 years who received 2010–2011 TIV using the MedDRA terms convulsion, grand mal convulsion, status epilepticus, convulsions local and febrile convulsion. In addition, VAERS staff manually reviewed all incoming reports in children aged <5 years after 2010–2011 TIV for potential signs and symptoms of seizures. While VAERS routinely requests medical records for non-manufacturer reports coded as serious[1], during the 2010–2011 influenza season VAERS staff requested medical records for all possible seizures reported to VAERS after TIV in children <5 years. CDC and FDA physicians reviewed each of these VAERS reports and associated records. A febrile seizure was considered verified if a medical provider diagnosed either "febrile seizure" or "seizure" with documented fever ≥100.4°. Level of diagnostic certainty for seizures was classified according to the Brighton Collaboration case definition [11,12].

## 3. Results

In the data mining analysis, disproportionately higher reporting for "febrile convulsion" combined with 2010–2011 Fluzone® was observed for reports received by November 23, 2011, compared with other vaccines in the VAERS database (Table 1). Thirty-five reports with the "febrile convulsion" term were verified after review by a VAERS team physician. In the subsequent data mining analysis for reports received by December 10, 2010, the signal persisted. Forty-one reports with the febrile convulsion code were identified (including the 35 earlier reports); one report did not describe an incident case and was ruled out (Table 1). Of the 40 remaining reports, 33 (83%) were in children aged <2 years. In the age-stratified data mining analysis, the EB05 did not exceed 2.0 in any of the age strata other than 0–<18 months. Similar findings were observed after 2010–2011 Fluzone® when data mining analysis was restricted to only include inactivated vaccines. Taken together, we assessed the clinically relevant age for the signal to be in children 6–23 months. Additionally, disproportionate reporting for febrile seizures was not detected following 2010–2011 TIV products other than Fluzone®.

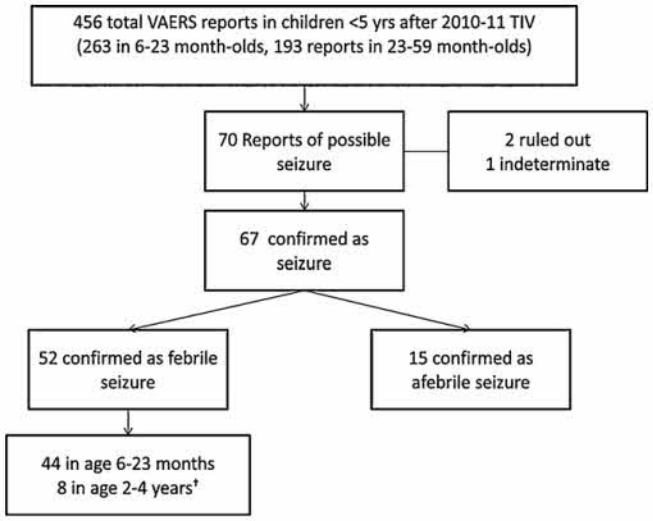



**Fig. 1.** Assessment of febrile seizure reports to VAERS after US 2010–2011 trivalent inactivated influenza vaccine (TIV) 7/01/2010–12/13/2010. †43 of 44 reports in 6–23 months and all reports in 2–4 years were Fluzone®.

For the clinical review component, we identified 456 total reports after 2010–2011 TIV in children aged <5 years. Of these, 70 were assessed as possible seizures and reviewed; medical records were available for all reports. In addition to the 40 reports coded with "febrile convulsion" identified in the data mining analysis, clinical review verified 11 additional reports of febrile seizure after 2010–2011 Fluzone® using the broader search strategy (Fig. 1). Of the 51 confirmed reports, 43 (84%) were in the 6–23 months age group. Most (86%) of the 43 children had onset of febrile seizures on the same day or one day after receipt of Fluzone®. Of 25 subjects with adequate information who had febrile seizure onset within 24 h of Fluzone®, 15 (60%) had onset less than 12 h and 10 (40%) had onset 12–23 h after vaccination.

Among the children aged 6–23 months, 16 (37%) received no other vaccine at the time Fluzone® was administered, while those who received at least one other vaccine concomitantly with Fluzone® received the 13-valent pneumococcal conjugate vaccine most often ($n = 14$) (Table 2). Of 42 children aged 6–23 months with sufficient information, 36 received dose 1 of 2010–2011 Fluzone® before the febrile seizure. Thirty children received medical attention in the emergency department and were discharged home and eight children were hospitalized overnight. Two other children required intensive care unit management for status epilepticus; each had received MMR and other vaccines with Fluzone® and had seizure onset 7–10 days after vaccination. All children recovered. Twenty-one (49%) of 43 reports confirmed as febrile seizure in children 6–23 months by CDC–FDA physician review also met the Brighton case definition for generalized convulsive seizure after Fluzone®, which includes Brighton levels 1–3 (Table 2). Twenty-two (51%) of the reports were classified as Brighton level 4.

## 4. Comment

Rapidly detecting and assessing vaccine safety signals is an important component of US immunization safety monitoring activities [13]. The ability of VAERS staff to perform Empirical Bayesian disproportionate reporting analysis (data mining), while conducting clinical reviews of reports proved useful for a preliminary assessment of the signal for febrile seizures after TIV in young

[1] Resulted in death, life threatening illness, hospitalization, prolongation of hospitalization, or permanent disability.

**Table 2**
Characteristics of 43 confirmed febrile seizure reports to VAERS after 2010–2011 Fluzone® trivalent inactivated influenza vaccine in children aged 6–23 months.

| Characteristic | Finding |
|---|---|
| Median age in months (range) | 12.5 (8–21) |
| Median onset interval in days from vaccination to febrile seizure (range)[a] | 0 (0–10) |
| Male | 20 (47%) |
| Concomitant vaccination[b] | 27 (63%) |
| Clinical course | |
| Hospitalized | 10 (23%) |
| Highest temperature documented | 100.4°–104° F |
| Recovery status | 43 (100%) |
| Medical history[c] | |
| Family history of seizure ($n$ = 21) | 7 (33%) |
| Past history of febrile seizures ($n$ = 39) | 3 (8%) |
| Concurrent illness ($n$ = 38) | 6 (16%) |
| Brighton classification for generalized convulsive seizure | |
| Level 1[d] | 9 (21%) |
| Level 2[e] | 8 (19%) |
| Level 3[f] | 4 (9%) |
| Level 4[g] | 51%) |

[a] Day 0 is day of vaccination. 37 (86%) of subjects had onset at 0–1 days.
[b] Vaccines co-administered: 13-valent pneumococcal conjugate (14), *Haemophilus* influenzae type b (11), Measles-mumps-rubella (8), Varicella (8), Diphtheria-tetanus-acellular pertussis (7), Hepatitis A (8), Hepatitis B (4), Measles-mumps-rubella-varicella (3), Inactivated poliovirus (2).
[c] Among children with sufficient information to document the history.
[d] Level 1: witnessed sudden loss of consciousness and generalized tonic, clonic, tonic-clonic, or atonic motor manifestations.
[e] Level 2: history of unconsciousness and generalized, tonic, clonic, tonic-clonic, or atonic motor manifestations.
[f] Level 3: history of unconsciousness and other generalized motor manifestations.
[g] Level 4: reported generalized convulsive seizure with insufficient evidence to meet the case definition.

children. In recent years, statistical data mining methods have been used to allow rapid identification of possible signals in large spontaneous reporting systems for AEs [14]. Cases of febrile seizures reported to VAERS were identified through FDA data mining that were coded with the MedDRA term "febrile convulsion" combined with Fluzone®. Once the statistical threshold for a vaccine-AE pair is crossed, clinical judgment is required to determine if a safety signal is present. This use of complementary techniques makes VAERS well-suited for immediate response when issues in vaccine safety arise.

Our proactive approach resulted in obtaining all medical documentation, including most vaccination records, for reported cases likely to represent febrile seizures after any influenza vaccine. Clinical review verified that most of the possible seizure reports submitted were febrile seizures. We confirmed that most reports following 2010–2011 TIV in the US were in children aged <2 years, had onset within 0–1 days, and had features that were typical of uncomplicated febrile seizures, which have a good prognosis [15].

Our analyses are subject to several limitations. As a passive surveillance system, VAERS is subject to reporting biases. Moreover, VAERS is not designed to determine if increased risk for an AE after vaccination exists, quantify the level of risk, or assess causality. Based on the recommended immunization schedule, receipt of VAERS reports in young children who received multiple vaccinations is expected [16]. About two-thirds of the children with confirmed febrile seizures had simultaneous vaccination with up to seven vaccines in up to twenty combinations making it difficult to assess the roles, if any, of other recommended vaccines known to be associated with increased febrile seizure risk [17]. Furthermore, some children had evidence of other infections that might have contributed to the febrile seizures. Our enhanced monitoring with differential follow up and coding for febrile convulsions after 2010–2011 TIV may have introduced potential bias as compared to previous years. The application of the Brighton Collaboration case definition for generalized convulsive seizure in VAERS has previously been described [18] and in this case proved challenging, limited by variability of details in medical records. This resulted in over half of the clinically verified febrile seizure reports meeting Level 4 classification in which a seizure has been reported but there is insufficient information documented to meet the Brighton case definition. Lastly, since Fluzone® was the only influenza vaccine recommended for children aged 6–23 months during the US 2010–2011 season, it is unlikely that a signal in this age group with a known risk of febrile seizures would have been detected with any other formulation [5].

Despite the limitations of VAERS and the preliminary nature of the findings, we believed the signal of possible increased risk of febrile seizures following 2010–2011 Fluzone® was important to communicate to the public and the finding was posted on the FDA website in January 2011 [19]. Further assessment of the signal was undertaken and described by the Vaccine Safety Datalink, which is designed to conduct population-based evaluations of AEs following immunization [20].

## Acknowledgements

We would like to thank Drs. Frank Destefano and Claudia Vellozzi for their in-depth review of this manuscript.

In addition, we thank the VAERS Febrile Seizure Assessment Team including Maria Cano, Penina Haber, Paige Lewis, Elaine Miller, and Oidda Museru for CDC; Marthe Bryant, Jenna Lyndly, and Andrea Sutherland for FDA.

*Disclaimer:* The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of HHS, CDC or FDA.

## References

[1] Varricchio F, Iskander J, Destefano F, Ball R, Pless R, Braun MM, et al. Understanding vaccine safety information from the Vaccine Adverse Event Reporting System. Pediatr Infect Dis J 2004;23:287–94.
[2] Vellozzi C, Burwen D, Dobardzic A, Ball R, Walton K, Haber P. Safety of trivalent inactivated influenza vaccines in adults: background for pandemic influenza vaccine safety monitoring. Vaccine 2009;27:2114–20.
[3] Vellozzi C, Broder K, Haber P. Adverse events following influenza A (H1N1) 2009 monovalent vaccines reported to the Vaccine Adverse Event Reporting System, United States, October 1, 2009–January 31, 2010. Vaccine 2010;28:7248–55.
[4] Blyth CC, Currie AJ, Wiertsema SP, Conway N, Kirkham LA, Fuery A, et al. Trivalent influenza vaccine and febrile adverse events in Australia, 2010: clinical features and potential mechanisms. Vaccine 2011;29(32):5107–13.
[5] Investigation into febrile reactions in young children following 2010 seasonal trivalent influenza vaccination. Available at http://www.tga.gov.au/pdf/alerts-medicine-seasonal-flu-100702.pdf.
[6] CDC. Update: Recommendations of the Advisory Committee on Immunization Practices (ACIP) regarding use of CSL Seasonal Influenza Vaccine (Afluria) in the United States during 2010–2011. Available at http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5931a4.htm.
[7] Reportable Events Table. Available at http://vaers.hhs.gov/resources/VAERS_Table_of_Reportable_Events_Following_Vaccination.pdf; 2011 [accessed 23.01.11].
[8] Medical Dictionary for Regulatory Activities Maintenance and Support Services Organization. Available at http://www.meddramsso.com/; 2011 [accessed 26.02.11].
[9] DuMouchel W. Bayesian data mining in large frequency tables, with an application to the FDA spontaneous reporting system. Am Stat 1999;53:177–90.
[10] Szarfman A, Machado SG, O'Neill RT. Use of screening algorithms and computer systems to efficiently signal higher-than-expected combinations of drugs and events in the US FDA's spontaneous reports database. Drug Saf 2002;25(6):381–92.
[11] Bonhoeffer J, Kohl K, Chen R, Duclos P, Heijbel H, Heininger U, et al. The Brighton Collaboration: addressing the need for standardized case definitions of adverse events following immunization (AEFI). Vaccine 2002;21(December (3–4)):298–302.
[12] Bonhoeffer J, Menkes J, Gold MS, de Souza-Brito G, Fisher MC, Halsey N, et al. Generalized convulsive seizure as an adverse event following immunization: case definition and guidelines for data collection, analysis, and presentation.

Brighton Collaboration Seizure Working Group. Vaccine 2004;22(January (5–6)):557–62.
[13] U.S. National Vaccine Plan. Available at http://www.hhs.gov/nvpo/vacc_plan/; 2011 [accessed May 01.05.11].
[14] Report of CIOMS Working Group VIII. Practical Aspects of Signal Detection in Pharmacovigilence. Geneva 2010.
[15] Shinnar S, Glauser T. Febrile seizures. J Child Neurol 2002;17(Supp 1): S44–52.
[16] http://www.cdc.gov/vaccines/recs/schedules/downloads/child/mmwr-child-schedule.pdf.
[17] Barlow WE, Davis RL, Glasser JW, Rhodes PH, Thompson RS, Mullooly JP, et al. The risk of seizures after receipt of whole-cell pertussis or measles, mumps, and rubella vaccine. N Engl J Med 2001;345:656–61.
[18] Kohl KS, Magnus M, Ball R, Halsey N, Shadomy S, Farley TA. Applicability, reliability, sensitivity, and specificity of six Brighton Collaboration standardized case definitions for adverse events following immunization. Vaccine 2008;26(November (50)):6349–60.
[19] FDA. Fluzone Vaccine Safety: FDA and CDC Update on Fluzone Influenza Vaccine and VAERS Reports of Febrile Seizures in Children, January 20, 2011. Available at http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/VaccineSafety/ucm240037.htm; 2011 [accessed 23.01.11].
[20] Tse A, Tseng HF, Greene S, Vellozzi C, Lee G, on behalf of the VSD Rapid Cycle Analysis Influenza Working Group. Identification and evaluation of risk for seizures in children following trivalent inactivated influenza vaccine in the Vaccine Safety Datalink Project, 2010–2011. Vaccine 2011 [concurrent submission].

Vaccine 30 (2012) 2024–2031

Contents lists available at SciVerse ScienceDirect

Vaccine

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

# Signal identification and evaluation for risk of febrile seizures in children following trivalent inactivated influenza vaccine in the Vaccine Safety Datalink Project, 2010–2011

Alison Tse[a,*], Hung Fu Tseng[b], Sharon K. Greene[a], Claudia Vellozzi[c], Grace M. Lee[a,d], On behalf of the VSD Rapid Cycle Analysis Influenza Working Group[1]

[a] Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, MA, United States
[b] Department of Research and Evaluation, Southern California Kaiser Permanente, Pasadena, CA, United States
[c] Immunization Safety Office, Division of Healthcare Quality and Promotion, Centers for Disease Control and Prevention, Atlanta, GA, United States
[d] Division of Infectious Diseases & Department of Laboratory Medicine, Children's Hospital Boston, Boston, MA, United States

ARTICLE INFO

*Article history:*
Received 3 October 2011
Received in revised form
12 December 2011
Accepted 6 January 2012

*Keywords:*
Trivalent inactivated influenza vaccine
Vaccine safety
Vaccine Safety Datalink Project
Febrile seizures

ABSTRACT

In fall 2010 in the southern hemisphere, an increased risk of febrile seizures was noted in young children in Australia in the 24 h after receipt of trivalent inactivated influenza vaccine (TIV) manufactured by CSL Biotherapies. Although the CSL TIV vaccine was not recommended for use in young children in the US, during the 2010–2011 influenza season near real-time surveillance was conducted for febrile seizures in the 0–1 days following first dose TIV in a cohort of 206,174 vaccinated children ages 6 through 59 months in the Vaccine Safety Datalink Project. On a weekly basis, surveillance was conducted with the primary approach of a self-controlled risk interval design and the secondary approach of a current vs. historical vaccinee design. Sequential statistical methods were employed to account for repeated analyses of accumulating data. Signals for seizures based on computerized data were identified in mid November 2010 using a current vs. historical design and in late December 2010 using a self-controlled risk interval design. Further signal evaluation was conducted with chart-confirmed febrile seizure cases using only data from the primary approach (i.e. self-controlled risk interval design). The magnitude of the incidence rate ratio and risk difference comparing risk of seizures in the 0–1 days vs. 14–20 days following TIV differed by receipt of concomitant 13-valent pneumococcal conjugate vaccine (PCV13). Among children 6–59 months of age, the incidence rate ratio (IRR) for TIV adjusted for concomitant PCV13 was 2.4 (95% CI 1.2, 4.7) while the IRR for PCV13 adjusted for concomitant TIV was 2.5 (95% CI 1.3, 4.7). The IRR for concomitant TIV and PCV13 was 5.9 (95% CI 3.1, 11.3). Risk difference estimates varied by age due to the varying baseline risk for seizures in young children, with the highest estimates occurring at 16 months (12.5 per 100,000 doses for TIV without concomitant PCV13, 13.7 per 100,000 doses for PCV13 without concomitant TIV, and 44.9 per 100,000 doses for concomitant TIV and PCV13) and the lowest estimates occurring at 59 months (1.1 per 100,000 doses for TIV without concomitant PCV13, 1.2 per 100,000 doses for PCV13 without concomitant TIV, and 4.0 per 100,000 doses for concomitant TIV and PCV13). Incidence rate ratio and risk difference estimates were lower for children receiving TIV without concomitant PCV13 or PCV13 without concomitant TIV. Because of the importance of preventing influenza and pneumococcal infections and associated complications, our findings should be placed in a benefit–risk framework to ensure that population health benefits are maximized.



DOIs of original articles: 10.1016/j.vaccine.2011.12.040, 10.1016/j.vaccine.2011.12.042
* Corresponding author at: Harvard Pilgrim Health Care Institute, 133 Brookline Avenue, 6th floor, Boston, MA 02215, United States. Tel.: +1 617 509 9978; fax: +1 617 859 8112.
[1] **VSD Rapid Cycle Analysis Influenza Working Group (Group authors)**—Centers for Disease Control and Prevention: Eric Weintraub, Natalie McCarthy, Jerome Tokars, Frank DeStefano, Karen Broder; Group Health Cooperative: Lisa Jackson, Jennifer Nelson; Harvard Pilgrim Health Care Instiute: Martin Kulldorff, Melisa Rett, Tracy Lieu, Lingling Li; HealthPartners Research Foundation: James D. Nordin; Kaiser Permanente Colorado: Jason Glanz, Simon Hambidge, Matthew F. Daley; Marshfield Clinic Research Foundation: Edward A. Belongia, Stephanie Irving; Kaiser Permanente Northern California: Nicola Klein, Roger Baxter; Kaiser Permanente Northwest: Allison Naleway; Southern California Kaiser Permanente: S. Michael Marcy, Steven J. Jacobsen.

0264-410X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2012.01.027

## 1. Introduction

In fall 2010 in the southern hemisphere, an increased risk of febrile seizures was noted in children younger than 5 years of age in Australia in the 24 h following trivalent inactivated influenza vaccine (TIV) manufactured by CSL Biotherapies (Fluvax®, Fluvax Junior®) [1], leading to a recommendation in the U.S. to avoid use of the CSL vaccine in children ages 6 months to 8 years [2]. In the Vaccine Safety Datalink (VSD) Project, which conducts active surveillance for pre-specified adverse events in a well-defined cohort of children and adults, several prior studies of influenza vaccines, which differed from the 2010–2011 formulation, did not suggest an elevated risk of seizures in the 0–7, 0–2, or 1–3 days following influenza vaccination (0 being the day of vaccination) [3–6]. However, because the majority of febrile seizures following TIV manufactured by CSL occurred within 24 h of vaccination, the VSD reexamined its risk interval definition for seizures following influenza vaccines and conducted surveillance for seizures in a shorter risk interval in children ages 6 months through 4 years and 5 through 17 years during the 2010–2011 influenza season. Here we describe the details regarding a signal for seizures following TIV that was identified in children under 5 years of age in sequential monitoring during the 2010–2011 influenza season in the U.S. and the subsequent evaluation conducted to verify the signal.

## 2. Methods

### 2.1. Study population

The CDC-sponsored VSD is a collaboration between 10 medical care organizations (MCO), encompassing data on 9.2 million members annually [7–10]. Among other activities, the VSD monitors potential associations between specific vaccines and pre-specified adverse events using weekly updated data and sequential statistical analysis [4,6,11–14]. Participating MCOs provide computerized weekly aggregate data on demographics, immunizations and medical encounters, including International Classification of Diseases, Ninth Revision (ICD-9) diagnosis codes. Eight MCOs participated during the 2010–2011 influenza season: Group Health Cooperative (Seattle, WA), Kaiser Permanente Colorado (Denver, CO), Kaiser Permanente Northwest (Portland, OR), Harvard Vanguard Medical Associates, and Harvard Pilgrim Health Care (Boston, MA), HealthPartners (Minneapolis-St. Paul, MN), Northern California Kaiser Permanente (Oakland, CA), Southern California Kaiser Permanente (Pasadena, CA) and Marshfield Clinic (Marshfield, WI). Institutional review boards at CDC and each VSD site approved the study and agreed that informed consent was not required.

### 2.2. Seizure definition

Seizures were identified in computerized data with ICD-9 codes 780.3, 780.31, 780.32, and 780.39 occurring in the emergency department (ED) and inpatient settings. The definition was based on prior work demonstrating a high positive predictive value (PPV) for seizure cases following 7-valent pneumococcal conjugate vaccine (PCV7) [15], measles, mumps, and rubella (MMR), and measles, mumps, and rubella-varicella vaccines (MMRV) in the VSD [14], ranging from 81% to 94% for the ED and inpatient settings combined. Codes in the outpatient setting were excluded because they commonly represent management of pre-existing seizure disorders [15]. To avoid including follow-up visits for prior seizure episodes, we only included cases that were the first seizure event in a 6-month period.

### 2.3. Study design for signal identification

For the primary approach, we used the self-controlled risk interval design, which implicitly controls for measured and unmeasured confounders that do not vary over time, such as gender and the presence of chronic health conditions [4,6,16,17]. On a weekly basis, the self-controlled risk interval design was used to test the null hypothesis of equal risk of seizures following 1st dose TIV in a risk interval of 0–1 days post-vaccination (0 being the same day of vaccination) compared with a control interval of 14–15 days post-vaccination. A post-vaccination control interval minimized biases that would be introduced with a pre-vaccination control interval if children were more likely vaccinated during periods of relative health [3] or due to pre-existing seizure disorders [18]. The use of a 14–15-day control interval avoided overlap with the known elevated risk of seizure in the 5–12 days following MMR and MMRV vaccination [14,19,20].

A current vs. historical design that incorporated a historical comparison group was also used as a secondary surveillance approach because the self-controlled risk interval design may lack power early during weekly surveillance when few events will have occurred [21]. In the current vs. historical design, the cumulative number of seizures 0–1 days following TIV during the 2010–2011 season was compared with the number expected based on the rate in TIV vaccinees from historical seasons (i.e. 2005–2006 through 2009–2010), stratified by age and site. Though the current vs. historical design may have more statistical power, a disadvantage is that it may be subject to bias due to differences in confounders between current and historical vaccinees and changes in diagnostic coding over time.

### 2.4. Statistical analysis for signal identification

Sequential statistical methods, which have been developed to address repeated interim testing throughout surveillance, were used [4,6,11,21,22]. We used the binomial-based maximized sequential probability ratio test (MaxSPRT) for the self-controlled risk interval design [21] and the Poisson-based conditional MaxSPRT (CMaxSPRT) for the current vs. historical design [22]. The MaxSPRT uses a one-sided composite alternative hypothesis of excess risk in the exposed post-vaccination interval, while the CMaxSPRT is an extension of the MaxSPRT that adjusts for uncertainty in expected counts when historical data are sparse.

The timeliness of data accrual varies widely depending on whether data are derived from electronic medical record systems or medical claims. To avoid potential bias, adjustments for data lags were incorporated using previously published methods [6,23]. Surveillance for the study ended when (1) the log likelihood ratio (LLR) test statistic exceeded the critical value leading to a statistical signal at $\alpha = 0.01$, (2) the total number of AEs reached a pre-specified upper limit, or (3) the end of the surveillance period on February 5, 2011 was reached.

### 2.5. Signal evaluation

Though statistical signals identified during sequential monitoring do not necessarily represent a true increase in risk, they indicate a need for further study [10]. An evaluation of potential causes of the statistical signal for seizures was conducted using the self-controlled risk interval design to compare the risk of seizures in the risk (0–1 days) vs. control interval (14–20 days) for the first seizures event in a 42-day period. Relative to the first in 6-month criterion used in signal detection, the first in 42-day period criterion used in signal evaluation increased the number of potential cases captured, thus enhancing sensitivity. A longer control interval was used in signal evaluation to provide more

**Table 1**
Criteria for diagnostic certainty of generalized seizures [24].[a]

| Level of diagnostic certainty | Required data |
|---|---|
| Brighton Level 1 | Witnessed sudden loss of consciousness[b] AND generalized[c] tonic[d], clonic[e], tonic–clonic[f], or atonic[g,h] motor manifestations |
| Brighton Level 2 | History of unconsciousness[b,i] AND generalized[c] tonic[d], clonic[e], tonic–clonic[f], or atonic[g,h] motor manifestations |
| Brighton Level 3 | History of unconsciousness[b,i] AND other generalized motor manifestations[j] |
| VSD Level 4A[a] | Witnessed sudden loss of consciousness[b] AND other generalized motor manifestations[j] |
| VSD Level 4B[a] | Altered state of consciousness[k] AND generalized[c] tonic[d], clonic[e], tonic–clonic[f], or atonic[g,h] motor manifestations |
| VSD Level 4C[a] | Altered state of consciousness[k] AND other generalized motor manifestations[j] |
| VSD Level 4D[a] | Clinician diagnosis of seizures |

[a] Modified criteria for inclusion of seizure cases.
[b] Unconsciousness includes unreactive to verbal and painful stimuli.
[c] Synonymous: bilateral, more than minimal muscle involvement.
[d] A sustained increase in muscle contraction lasting a few seconds to a few minutes.
[e] Sudden, brief (<100 ms) involuntary contractions of the same muscle groups, regularly repetitive at a frequency of about two to three contraction(s).
[f] A sequence consisting of a tonic followed by a clonic phase.
[g] A sudden loss of tone in postural muscles, often preceded by a myoclonic jerk and precipitated by hyperventilation.
[h] In the absence of hypotonic hyporesponsive episode (as defined by Brighton Collaboration), syncope, and myoclonic jerks.
[i] The sudden loss of consciousness was not observed, but the patient was found unconscious.
[j] Other generalized motor manifestations include less specific descriptions such as shaking, trembling, shivering and quivering.
[k] Altered state of consciousness may include eyes rolled back, cyanosis, not responsive, unresponsive, unable to wake up, post-ictal state, sleepy or drowsy, confused, lethargic, listless, drooling/foaming at mouth, incontinent of stool or urine if previously continent.

stable estimates of baseline risk of seizures. In addition, medical record reviews of cases in the risk and control intervals following TIV in the 2010–2011 influenza season were conducted.

#### 2.5.1. Medical record reviews

Trained abstractors at each site used a standardized chart review form to extract information on the presence of motor manifestations, loss of consciousness, fever, prior history of seizure, family history of seizures, concomitant event or illness triggering seizure, and medications. A pediatrician (GML) blinded to exposure status further classified and adjudicated seizure events using a modified version of the Brighton Collaboration criteria for seizures [24] (Table 1) and classified the presence of fever using the Brighton Collaboration criterion of a measured temperature of ≥38 °C [25]. The modified version of the Brighton Collaboration criteria for seizures included additional levels of diagnostic certainty developed by the VSD for this study. Though medical records often lack the level of detail necessary to identify Brighton criteria of loss of consciousness and/or motor manifestations, they often contain further information that can be used to distinguish between additional degrees of certainty covered by the VSD levels [15].

Febrile seizure cases were defined as those that met Brighton Collaboration or VSD criteria for seizures and that also had evidence of fever in association with the seizure event in the medical record, as indicated by (1) a recorded temperature of ≥38 °C within 24 h prior of the seizure, (2) parent report of fever, or (3) a clinician's diagnosis of febrile seizure.

#### 2.5.2. Incidence rate ratio estimates

We used conditional Poisson regression to estimate incidence rate ratios (IRR, a measure of relative risk) for febrile seizures following TIV. Because only cases contribute information to the self-controlled risk interval design, vaccinees who did not experience a seizure event in the risk or control interval were not analyzed. Vaccines co-administered with TIV could potentially be confounders and/or effect modifiers. Because 13-valent pneumococcal conjugate vaccine (PCV13) was one of the two most common vaccines administered concomitantly with TIV after its licensure in February 2010 [26], we also collected computerized data on the number of seizures in risk and control intervals following PCV13 to assess confounding or effect modification due to concomitant administration [27]. Since chart review data were not available for seizures following PCV13 without concomitant TIV and the positive predictive value of febrile seizures was high, we used computerized data for seizure cases following PCV13 without concomitant TIV. Data were not available at the time of this assessment to evaluate the contribution of diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP), which was as likely to be co-administered with TIV as PCV13. However, DTaP was not associated with seizures in a prior VSD study [28].

In additional analyses, we explored whether the effects of TIV and PCV13 on risk of seizures were multiplicative by including an interaction term between TIV and PCV13. To explore whether the IRR of febrile seizures following TIV or PCV13 differed by age (6–23 months vs. 24–59 months), we added interactions of TIV and PCV13 with age into the model.

#### 2.5.3. Risk difference estimates

We estimated the risk difference (RD, i.e. attributable risk) comparing the risk of seizures in the risk vs. control intervals following TIV without concomitant PCV13, TIV with concomitant PCV13 and PCV13 without concomitant TIV. The rate difference, expressed in cases per person day, was first calculated for each vaccine group using the formula (IRR-1) × p0, where p0 is the baseline rate of febrile seizures per day in the entire VSD population estimated using computerized data and positive predictive value estimates for ICD9 seizure codes for children ages 6–23 and 24–59 months obtained from the present study and a separate VSD study of risk of febrile seizures following MMR and MMRV vaccination [14]. We then multiplied the rate difference by the length of the risk interval (2 days) to estimate the risk difference for number of excess cases per dose administered. The confidence interval for the RD was calculated using Monte Carlo simulations by generating 1 million realizations of the IRR and the two PPVs independently from the estimated asymptotic distributions, calculating the corresponding RD, and constructing the confidence interval for the RD using the corresponding percentiles. For descriptive purposes, we also estimated the RD for each vaccine group by age in months because the baseline rate of febrile seizures is known to vary with age [29].

## 3. Results

### 3.1. Signal identification

From August 1, 2010 to February 5, 2011, first dose TIV was administered to 206,174 children ages 6–59 months (Table 2) and to 384,098 children ages 5–17 years. During the week of November 14, 2010 in the third week of surveillance, a statistical signal for

**Table 2**
Number of 1st trivalent inactivated influenza vaccine (TIV) doses administered to children ages 6–59 months by select characteristics, Vaccine Safety Datalink, August 1, 2010 to February 5, 2011.

| | 1st TIV doses ($N$ = 206,174) | |
|---|---|---|
| VSD site | | |
| A | 80,288 | (39%) |
| B | 77,664 | (38%) |
| 6 other sites, combined | 48,222 | (23%) |
| Age | | |
| 6–11 months | 40,375 | (20%) |
| 12–15 months | 24,474 | (12%) |
| 16–23 months | 36,203 | (18%) |
| 24–59 months | 105,122 | (51%) |
| Sex | | |
| Male | 106,647 | (52%) |
| Female | 99,525 | (48%) |
| Unknown | 2 | (0%) |
| Manufacturer | | |
| Sanofi-Pasteur | 156,800 | (76%) |
| GlaxoSmithKline | 13,646 | (7%) |
| Novartis | 10,335 | (5%) |
| Other | 6009 | (3%) |
| Missing | 19,384 | (9%) |
| Vaccines administered concomitantly with TIV | | |
| 13-valent pneumococcal conjugate vaccine (PCV13)[a,b] | 57,197 | (28%) |
| Vaccines other than PCV13 | 37,364 | (18%) |
| None (i.e. TIV only) | 111,613 | (54%) |

[a] With or without vaccines other than PCV13.
[b] 10,838 children (5% of children receiving 1st TIV doses) received TIV + PCV13 without other vaccines.

seizures in children 6–59 months was identified using the current vs. historical CMaxSPRT approach, corresponding to a relative risk of 3.3 based on 12 observed vs. 3.6 expected cases (Fig. 1a). Six weeks later, during the week of December 26, 2010, a statistical signal was also detected in children ages 6–59 months using the self-controlled risk interval binomial-based MaxSPRT approach, with a relative risk of 5.7 based on 17 cases in the 0–1 day risk interval and 3 cases in the 14–15 day control interval (Fig. 1b). No statistical signals were identified for children ages 5–17 years during surveillance.

### 3.2. Signal evaluation

#### 3.2.1. Medical record reviews

A total of 32 potential cases in the risk interval (i.e. 0–1 days) and 34 potential cases in the control interval (i.e. 14–20 days) were identified in computerized data. Of the 30 potential cases in the risk interval that had medical records available for review, 27 (90%) cases met Brighton criteria level 1 ($n$ = 2) or VSD criteria levels 4A ($n$ = 1), 4B ($n$ = 3), 4C ($n$ = 7), or 4D ($n$ = 14) for generalized seizures following TIV. Twenty-five seizure cases (83%) in the risk interval were categorized as having a febrile seizure, though only 21 (70%) met the Brighton criterion for documented fever of ≥38 °C. Additional clinical characteristics of chart confirmed cases in the risk and control intervals are described in Table 3. Of the 31 potential cases available for review in the control interval, 26 (84%) met Brighton criteria level 1 ($n$ = 2) or VSD criteria levels 4A ($n$ = 1), 4B ($n$ = 5), 4C ($n$ = 7), or 4D ($n$ = 11), 22 (71%) also had associated fever, and 20 (65%) met the Brighton criterion for fever. The 25 and 22 chart confirmed febrile seizure cases that had occurred in the risk and control intervals following TIV were included in all regression models.

#### 3.2.2. Incidence rate ratios for febrile seizures in the 2010–2011 season

*3.2.2.1. Primary analysis.* Unadjusted for concomitant vaccination, the IRR for febrile seizures in the 0–1 days following TIV compared with an unexposed control interval was 4.0 (95% CI 2.1, 6.2) (Table 4A). With adjustment for same day PCV13 vaccination (Table 4B), the association between TIV and febrile seizures was attenuated but still indicated an increased risk following TIV (IRR 2.4, 95% CI 1.2, 4.7). Similarly, adjusted for concomitant TIV vaccination, the IRR for seizures following PCV13 vaccination was 2.5 (95% CI 1.3, 4.7). Based on the model that did not allow for interaction between TIV and PCV13, receipt of TIV and PCV13 on the same day was associated with 5.9 (95% CI 3.1, 11.3) times the risk of febrile seizures in the 0–1 days following vaccination compared with an unexposed control interval.

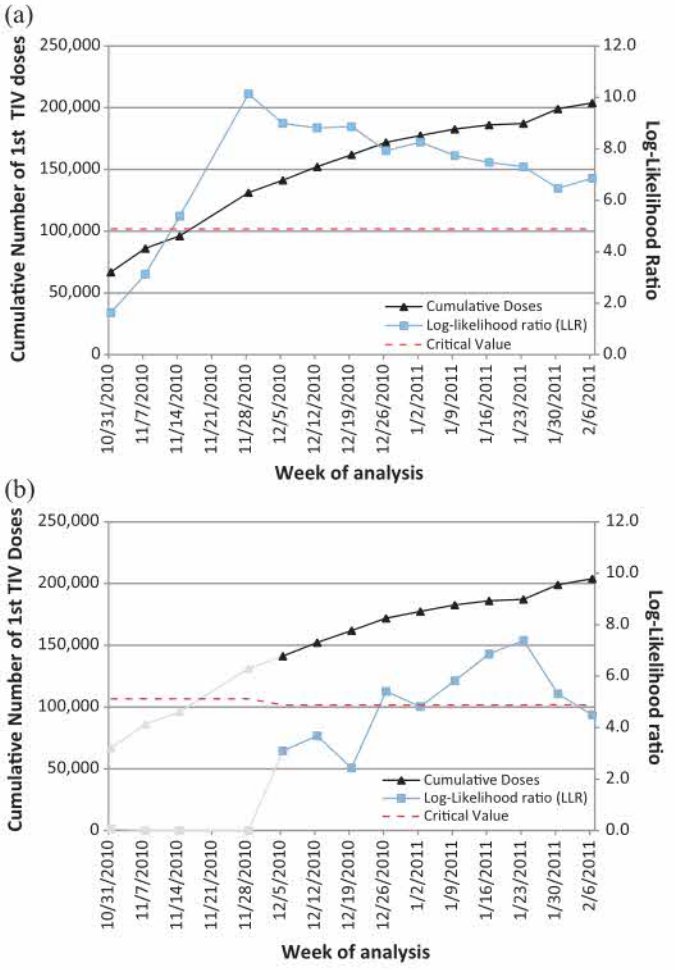



**Fig. 1.** Log-likelihood ratio during prospective surveillance for seizures following 1st dose trivalent inactivated influenza vaccine (TIV) in children ages 6–59 months for (a) current vs. historical and (b) self-controlled risk interval designs in the Vaccine Safety Datalink, August 1, 2010 to February 5, 2011. Critical value thresholds for signal identification are shown by the dashed lines. Control interval definition for self-controlled risk interval design was changed from 7–8 days to 14–15 days post vaccination beginning the week of analysis of 12/5/2010 to avoid overlap with the known increased risk of seizures in the 5–12 days following MMR and MMRV.

*3.2.2.2. Secondary analysis.* When included in the model, the interaction term between TIV and PCV13 was not significant (coefficient for TIV × PCV13 interaction 1.0, 95% CI 0.3, 4.2, $p$-value 0.96). In exploratory analyses evaluating whether the IRR for TIV or PCV13 varied by age (6–23 months vs. 24–59 months), the estimated IRRs for febrile seizures in children 24–59 months following TIV (IRR 1.6, 95% CI 0.5, 5.2) or PCV13 (IRR 1.4, 95% CI 0.2, 8.3) appeared to be lower than the corresponding IRRs in children ages 6–23 months for TIV (IRR 3.0, 95% CI 1.2, 7.4) or PCV13 (IRR 2.5, 95% CI 1.2, 5.0). However, the confidence intervals were wide and the $p$-values for tests of differences in IRR by age were not significant ($p$ 0.41 for TIV, $p$ 0.35 for PCV13).

**Table 3**
Clinical characteristics of chart-confirmed febrile seizure cases occurring in the risk interval (0–1 days) and control interval (14–20 days) following 1st dose trivalent inactivated influenza vaccine (TIV), Vaccine Safety Datalink, August 1, 2010 to February 5, 2011.

| | Cases in risk interval (*N* = 25) | | Cases in control interval (*N* = 22) | |
|---|---|---|---|---|
| Clinical characteristic | *N* | (%) | *N* | (%) |
| Setting of diagnosis | | | | |
| Emergency department | 21 | (84%) | 22 | (100%) |
| Inpatient | 4 | (16%) | 0 | (0%) |
| Previous history of seizures | | | | |
| Yes | 8 | (32%) | 7 | (32%) |
| No | 14 | (56%) | 11 | (50%) |
| Missing | 3 | (12%) | 4 | (18%) |
| Family history of seizures | | | | |
| Yes | 5 | (20%) | 4 | (18%) |
| No | 6 | (24%) | 10 | (45%) |
| Missing | 14 | (56%) | 8 | (36%) |
| Current illness | | | | |
| Upper respiratory illness | 5 | (20%) | 9 | (41%) |
| Urinary tract infection | 1 | (4%) | 2 | (9%) |
| Received concomitant 13-valent pneumococcal conjugate vaccine (PCV13)[a] | | | | |
| Yes | 17 | (68%) | 10 | (45%) |
| No | 8 | (32%) | 12 | (55%) |
| Received concomitant diphtheria, tetanus, and acellular pertussis (DTaP)[a] | | | | |
| Yes | 17 | (68%) | 11 | (50%) |
| No | 8 | (32%) | 11 | (50%) |

[a] 13 cases in the risk interval (48%) and 7 cases in the control interval (32%) received PCV13 and DTaP vaccines concomitantly with TIV.

### *3.2.3. Risk difference estimates for febrile seizures in the 2010–2011 season*

When average RDs were calculated over the entire age range of 6–59 months, the RD was highest in those who received concomitant TIV and PCV13 (average RD per 100,000 doses 17.5, 95% CI 7.4, 36.6) and was lower in children who received TIV without concomitant PCV13 (average RD per 100,000 doses 4.9, 95% CI 0.7, 13.2) or PCV13 without concomitant TIV (average RD per 100,000 doses 5.3, 95% CI 1.2, 13.1). The sum of the average RDs for febrile seizures following TIV without concomitant PCV13 and PCV13 without concomitant TIV (i.e. 4.9 + 5.3 = 10.2 per 100,000 vaccinees) was less than the average RD for TIV with concomitant PCV13 (17.5 per 100,000 doses). Because the background rate of seizures varies considerably with age, increasing from 6 to 16 months followed by a decline from 17 to 59 months, the RDs also varied substantially as a function of age (Fig. 2), with the highest estimates occurring at 16 months and the lowest estimates occurring at 59 months.

## 4. Discussion

During surveillance for potential adverse events in the 2010–2011 influenza season in the VSD, we preliminarily identified an elevated risk of seizures following 1st dose TIV for children ages 6–59 months in mid-November 2010 using computerized data. Upon further investigation, TIV and PCV13 were each associated with an increased risk of febrile seizures independent of concomitant receipt of the other. Importantly, the risk differences were highest following receipt of concomitant TIV and PCV13, in comparison to TIV without concomitant PCV13, or PCV13 without concomitant TIV. Furthermore, the risk differences varied substantially as a function of age, with the highest estimates occurring at 16 months (e.g. 45 per 100,000 doses for TIV with concomitant PCV13) and the lowest estimates occurring at 59 months (e.g. 4 per 100,000 doses for TIV with concomitant PCV13). The sum of the risk differences for TIV without concomitant PCV13 and PCV13 without concomitant TIV appeared to be smaller than the risk difference for concomitant TIV and PCV13, which may suggest that separate day TIV and PCV13 vaccination is associated with an overall smaller risk of febrile seizures when compared with concomitant vaccination. However, caution in interpreting this particular finding is warranted because a formal statistical analysis comparing the risk of seizures in same day vs. separate day TIV and PCV13 vaccinees was not performed.

**Table 4**
Incidence rate ratio (IRR) estimates for febrile seizures in children 6–59 months of age for (A) 1st dose trivalent inactivated influenza vaccine (TIV) and (B) 1st dose TIV and any dose of PCV13, self-controlled risk interval design, Vaccine Safety Datalink, August 1, 2010 to February 5, 2011.

| | Number in risk interval | Number in control interval | IRR (95% CI) for 6–59-month-old children |
|---|---|---|---|
| (A) Model 1 | | | |
| TIV | 25 | 22 | 4.0 (2.1, 6.2) |
| (B) Model 2[a] | | | |
| TIV | 25 | 22 | 2.4 (1.2, 4.7) |
| PCV13 | 29 | 27 | 2.5 (1.3, 4.7) |

[a] Among concomitant TIV + PCV13 vaccinees, the IRR of seizures was 5.9 (95% CI 3.1, 11.3) based on model 2.

Febrile seizures are not uncommon in childhood, affecting 2–5% of children under 5 years of age [29–31] with an estimated background incidence of 240–480 per 100,000 person-years [32,33]. The baseline risk for febrile seizures is known to vary with age, increasing approximately 3-fold from 6 to 16 months of age, followed by a less steep decline of approximately 5-fold from 17 to 59 months of age [28,29]. In addition to age, genetics [34,35], co-morbidities (e.g. pre-term birth, fetal growth retardation) [36,37], or environmental risk factors (e.g. in utero exposure to smoking, antihistamine use) [29,38] may further elevate the risk for seizures following febrile illness, regardless of cause. Of the currently U.S. licensed childhood vaccines, measles containing vaccines have also been associated with febrile seizures in young children. Post-licensure studies have estimated that 25–38 excess febrile seizure cases occur per 100,000 children receiving MMR vaccine when compared with no MMR vaccine [19,39]. Furthermore, first dose MMRV vaccine has been associated with 38–43 excess febrile seizure cases per 100,000 children when compared with simultaneous administration of MMR and varicella vaccines [14,20].

An increased risk of febrile seizures following TIV had not been found prior to the 2010–2011 influenza season in the U.S. [3–6]. Although the VSD used the shorter risk interval of 0–1 days during the 2010–2011 season based on the Australian signal following CSL manufactured vaccines [1], our findings are unlikely to be due to a change in the risk interval definition (i.e. from 0–7 to 0–1 days), since re-evaluation of data from prior seasons using the 0–1 day risk interval did not result in similar elevations in the risk for seizures [40]. While the vaccine used in the 2010–2011 influenza season in the northern hemisphere included the same strains as those used during the 2010 influenza season in the southern hemisphere the elevated risk of seizures following TIV in Australia was limited to CSL manufactured products [1], in contrast to the U.S., where CSL vaccines were not recommended for use in children younger than 9 years of age in the U.S. during the 2010–2011 season [2]. In the VSD during the 2009–2010 influenza season, no increase in risk of seizures was found following H1N1 monovalent inactivated influenza vaccine [6], which is antigenically equivalent to one of three strains included in the 2010–2011 formulation [41]. Thus, it remains unclear whether the new association between influenza vaccines and febrile seizures in the U.S. may be attributed to the strains used in the 2010–2011 vaccine, to concomitant TIV and PCV13 vaccination, or both.

Our findings should be interpreted in the context of the benefits of vaccination in preventing influenza and pneumococcal-related morbidity. The majority of seizures in children is precipitated by febrile illnesses, which are often caused by infections. Influenza

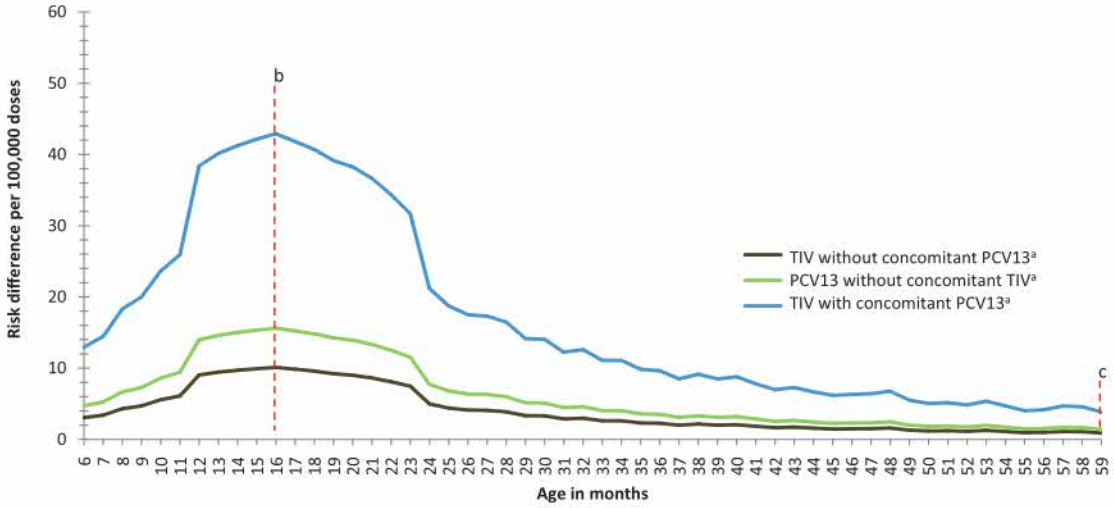



**Fig. 2.** Risk difference estimates for febrile seizures following 1st dose trivalent inactivated influenza vaccine (TIV) stratified by receipt of concomitant 13-valent pneumococcal conjugate vaccine (PCV13) and following any dose of PCV13 without concomitant TIV by age in months, self-controlled risk interval design in the Vaccine Safety Datalink, August 1, 2010 to February 5, 2011. Vaccines may have been received concomitantly with non-TIV, non-PCV13 vaccines. The peak risk difference occurred at 16 months, when the RD was 12.5 per 100,000 doses (1 per 7,980 doses) for TIV without concomitant PCV13, 13.7 per 100,000 doses (1 per 7,293 doses) for PCV13 without concomitant TIV, and 44.9 per 100,000 doses (1 per 2,225 doses) for concomitant TIV and PCV13. The nadir risk difference occurred at 59 months, when the RD was 1.1 per 100,000 doses (1 per 88,495 doses) for TIV without concomitant PCV13, 1.2 per 100,000 doses (1 per 81,300 doses) for PCV13 without concomitant TIV, and 4.0 per 100,000 doses (1 per 24,752 doses) for concomitant TIV and PCV13.

type A and B infections have been associated with febrile seizures, causing up to a quarter of febrile seizure related hospitalizations in children. Conversely, up to 20% of children admitted to the hospital with influenza have febrile seizures [42–46]. Pneumococcal and influenza vaccination may prevent other non-neurologic complications that may also result in hospitalizations or emergency department visits [47–53]. Furthermore, the timing of administration of influenza and pneumococcal vaccines is critical since both influenza and pneumococcal-related morbidity and mortality are known to follow seasonal patterns with peaks typically occurring during the fall and winter seasons [54–56]. The potential for missed vaccination opportunities and the risk for vaccine-preventable diseases in young children are key considerations in decisions regarding timing of vaccination. As of October 2011, the Advisory Committee on Immunization Practices continues to recommend influenza and pneumococcal vaccination using the existing schedule [57]. In order to communicate these findings to providers and the public, the Vaccine Information Statement for TIV has been updated for the 2011–2012 season to include information about the potential for an increased risk of febrile seizures following co-administration of TIV and PCV13 in young children [58].

Limitations are worth noting. Chief among these is that while we estimated incidence rate ratios by age group, the estimates for children 24–59 months of age were imprecise. To estimate risk differences, we thus assumed that incidence rate ratios were constant across all ages, which may have resulted in overestimates of risk differences in older children and underestimates of risk differences in younger children, or vice versa. We did not assess the confounding or synergistic role of concomitant vaccines other than PCV13 (e.g. DTaP); a prior study did not find an association between DTaP and seizures [28], but confounding or effect modification by concomitant administration of DTaP with either PCV13 or the 2010–2011 TIV formulation has not yet been studied. Furthermore, we did not exclude cases noted to have concurrent infections in our analysis due to limited information about attributable causes. In order to minimize bias introduced by time varying confounders (e.g. age and seasonality) [29,33,59,60], we used a control interval that was close in time to the risk interval. In this study, we limited case finding to seizure visits in the inpatient and ED settings among influenza vaccinees, in order to provide an efficient and timely evaluation of the risk for febrile seizures, particularly since the PPV for seizures in the clinic setting has been previously shown to be low [15]. However, this implies that our estimates of the overall burden (i.e. attributable risk) of febrile seizures may be conservative since children with febrile seizures may not always receive care in an inpatient or ED setting. We were unable to review medical records for seizure cases that occurred following PCV13 without TIV vaccination. However, because the positive predictive value for febrile seizures following vaccination was reasonably high in this and prior VSD studies, we believe that any potential bias from misclassification of seizures would be limited [14]. Finally, we did not assess the relative risk of seizures following second dose TIV or PCV13 by dose number.

The present study has several strengths. The VSD is a large collaboration of MCOs which utilizes computerized data on a weekly basis to conduct surveillance, leading to an efficient system that can rapidly evaluate safety concerns that have been previously identified in other surveillance systems and case reports. The ability to conduct fairly rapid medical record reviews enables further evaluation of signals in a timely fashion. Compared with reporting systems that require clinicians or caregivers to voluntarily submit reports of potential adverse events, the VSD conducts surveillance using data from electronic medical records and medical claims in a well-defined population, thus making it less susceptible to biases from underreporting and lack of appropriate comparison groups [61,62]. Moreover, the use of the self-controlled risk interval design avoided bias due to confounding by factors that do not vary over relatively short periods of time, including underlying chronic health conditions.

In summary, an elevated risk of febrile seizures in the 0–1 days following first dose TIV was identified during the 2010–2011 influenza season in children ages 6–59 months in a large U.S. cohort. Among children 6–59 months of age, the IRRs of febrile seizures were elevated for both TIV adjusted for concomitant PCV13 and for PCV13 adjusted for concomitant TIV. The magnitude, in terms of risk difference, was dependent on age and receipt of concomitant PCV13 vaccine, with the highest estimates occurring at 16 months and the lowest estimates occurring at 59 months. As the same three

strains in the 2010–2011 influenza vaccine have been included in the 2011–2012 influenza vaccine [56], further monitoring in the VSD will be conducted for seizures as more doses are administered. Results should be placed in a benefit–risk framework to aid decision making by policymakers to maximize population health benefits.

## Acknowledgements

We gratefully acknowledge our colleagues Dr. Richard Platt and Dr. W. Katherine Yih at the Harvard Pilgrim Health Care Institute (HPHCI). We also thank our programmer analysts, Ruihua Yin and Robert Jin, and data manager Rich Fox for the VSD RCA Coordinating Center at HPHCI. For their advice and support of our work in the Vaccine Safety Datalink, we thank our Harvard Vanguard Medical Associates colleagues, Dr. Benjamin Kruskal and Dr. Thomas Sequist. We sincerely thank our project managers, research associates, programmer analysts, and statisticians from participating VSD sites, including Tricia Kennedy (HPHCI), Patti Benson (Group Health Cooperative [GHC]), Leslie Kuckler (HealthPartners Research Foundation [HPM]), Jill Mesa (Kaiser Permanente Northwest [NWK]), Deanna Cole (Marshfield Clinic Research Foundation [MFC]), Tara Johnson (MFC), JoAnne Shoup (Kaiser Permanente Colorado [KPC]), Lina Sy (Kaiser Permanente Southern California [SCK]), Dr. Lei Qian (SCK), Sungching Glenn (SCK), Amy Liu (SCK), Paula Ray (Kaiser Permanente Northern California [NCK]), Bruce Fireman (NCK), and Ned Lewis (NCK). We would also like to thank our CDC and VSD colleagues Julianne Gee (CDC), Dr. Jonathan Duffy (CDC), Dr. Tom Shimabukuro (CDC), Dr. David McClure (KPC), and Dr. Saad Omer (Kaiser Permanente Georgia) for their valuable advice throughout influenza RCA surveillance.

*Conflict of interest statement:* Dr. Baxter has received research funding from GSK, Merck, Pfizer, Sanofi Pasteur, and Novartis. Dr. Jackson has received research funding from Pfizer and Novartis and travel support from Pfizer. Dr. Jacobsen has received research funding from Merck Research Laboratories and has served as an unpaid consultant to Merck Research Laboratories. Dr. Klein has received research funding from GSK, Merck, Pfizer, Sanofi Pasteur and Novartis. Dr. Nelson has served as a statistical consultant to GSK. Dr. Tseng has received research funding from Merck for other vaccine studies.

*Funding:* This work was supported by a subcontract with America's Health Insurance Plans (AHIP) under contract 200-2002-00732 from the Centers for Disease Control and Prevention (CDC). The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of CDC. Preliminary findings from data included in this manuscript have been previously presented to federal advisory committees for vaccines and posted on the CDC and FDA websites for public health purposes. Data were also presented during the National Immunization Conference, Washington, DC, 2011 and the International Conference on Pharmacepidemiology (ICPE), Chicago, IL, 2011, which included a published abstract. These data have not been previously published as a manuscript.

## References

[1] Blyth CC, Currie AJ, Wiertsema SP, Conway N, Kirkham LA, Fuery A, et al. Trivalent influenza vaccine and febrile adverse events in Australia, 2010: clinical features and potential mechanisms. Vaccine 2011;(June).
[2] Update Recommendations of the Advisory Committee on Immunization Practices (ACIP) regarding use of CSL seasonal influenza vaccine (Afluria) in the United States during 2010–11. MMWR Morb Mortal Wkly Rep 2010;59(August (31)):989–92.
[3] Hambidge SJ, Glanz JM, France EK, McClure D, Xu S, Yamasaki K, et al. Safety of trivalent inactivated influenza vaccine in children 6 to 23 months old. JAMA 2006;296(October (16)):1990–7.
[4] Greene SK, Kulldorff M, Lewis EM, Li R, Yin R, Weintraub ES, et al. Near real-time surveillance for influenza vaccine safety: proof-of-concept in the Vaccine Safety Datalink Project. Am J Epidemiol 2010;171(January (2)):177–88.
[5] Glanz JM, Newcomer SR, Hambidge SJ, Daley MF, Narwaney KJ, Xu S, et al. The safety of trivalent inactivated influenza vaccine in children ages 24 to 59 months. Arch Pediatr Adolesc Med 2011;165(8):749–55.
[6] Lee GM, Greene SK, Weintraub ES, Baggs J, Kulldorff M, Fireman BH, et al. H1N1 and seasonal influenza vaccine safety in the Vaccine Safety Datalink Project. Am J Prev Med 2011;41(2):121–8.
[7] Chen RT, DeStefano F, Davis RL, Jackson LA, Thompson RS, Mullooly JP, et al. The Vaccine Safety Datalink: immunization research in health maintenance organizations in the USA. Bull World Health Organ 2000;78(2):186–94.
[8] DeStefano F. The Vaccine Safety Datalink project. Pharmacoepidemiol Drug Saf 2001;10(August–September (5)):403–6.
[9] Baggs J, Gee J, Lewis E, Fowler G, Benson P, Lieu T, et al. The Vaccine Safety Datalink: a model for monitoring immunization safety. Pediatrics 2011;127(May (Suppl. 1)):S45–53.
[10] Yih WK, Kulldorff M, Fireman BH, Shui IM, Lewis EM, Klein NP, et al. Active surveillance for adverse events: the experience of the Vaccine Safety Datalink project. Pediatrics 2011;127(May (Suppl. 1)):S54–64.
[11] Lieu TA, Kulldorff M, Davis RL, Lewis EM, Weintraub E, Yih K, et al. Real-time vaccine safety surveillance for the early detection of adverse events. Med Care 2007;45(October (10 Suppl. 2)):S89–95.
[12] Yih WK, Nordin JD, Kulldorff M, Lewis E, Lieu TA, Shi P, et al. An assessment of the safety of adolescent and adult tetanus-diphtheria-acellular pertussis (Tdap) vaccine, using active surveillance for adverse events in the Vaccine Safety Datalink. Vaccine 2009;27(July (32)):4257–62.
[13] Belongia EA, Irving SA, Shui IM, Kulldorff M, Lewis E, Yin R, et al. Real-time surveillance to assess risk of intussusception and other adverse events after pentavalent, bovine-derived rotavirus vaccine. Pediatr Infect Dis J 2010;29(January (1)):1–5.
[14] Klein NP, Fireman B, Yih WK, Lewis E, Kulldorff M, Ray P, et al. Measles-mumps-rubella-varicella combination vaccine and the risk of febrile seizures. Pediatrics 2010;126(July (1)):e1–8.
[15] Shui IM, Shi P, Dutta-Linn MM, Weintraub ES, Hambidge SJ, Nordin JD, et al. Predictive value of seizure ICD-9 codes for vaccine safety research. Vaccine 2009;27(August (39)):5307–12.
[16] Klein NP, Hansen J, Lewis E, Lyon L, Nguyen B, Black S, et al. Post-marketing safety evaluation of a tetanus toxoid, reduced diphtheria toxoid and 3-component acellular pertussis vaccine administered to a cohort of adolescents in a United States health maintenance organization. Pediatr Infect Dis J 2010;29(July (7)):613–7.
[17] Kramarz P, DeStefano F, Gargiullo PM, Davis RL, Chen RT, Mullooly JP, et al. Does influenza vaccination exacerbate asthma? Analysis of a large cohort of children with asthma. Vaccine Safety Datalink Team. Arch Fam Med 2000;9(July (7)):617–23.
[18] Keren R, Zaoutis TE, Bridges CB, Herrera G, Watson BM, Wheeler AB, et al. Neurological and neuromuscular disease as a risk factor for respiratory failure in children hospitalized with influenza infection. JAMA 2005;294(November (17)):2188–94.
[19] Farrington P, Pugh S, Colville A, Flower A, Nash J, Morgan-Capner P, et al. A new method for active surveillance of adverse events from diphtheria/tetanus/pertussis and measles/mumps/rubella vaccines. Lancet 1995;345(March (8949)):567–9.
[20] Jacobsen SJ, Ackerson BK, Sy LS, Tran TN, Jones TL, Yao JF, et al. Observational safety study of febrile convulsion following first dose MMRV vaccination in a managed care setting. Vaccine 2009;27(July (34)):4656–61.
[21] Kulldorff M, Davis RL, Kolczakâ€M, Lewis E, Lieu T, Platt R. A maximized sequential probability ratio test for drug and vaccine safety surveillance. Seq Anal: Des Methods Appl 2011;30(1):58–78.
[22] Li L, Kulldorff M. A conditional maximized sequential probability ratio test for pharmacovigilance. Stat Med 2010;29(2):284–95.
[23] Greene SK, Kulldorff M, Yin R, Yih WK, Lieu TA, Weintraub ES, et al. Near real-time vaccine safety surveillance with partially accrued data. Pharmacoepidemiol Drug Saf 2011;(April).
[24] Bonhoeffer J, Menkes J, Gold MS, de Souza-Brito G, Fisher MC, Halsey N, et al. Generalized convulsive seizure as an adverse event following immunization: case definition and guidelines for data collection, analysis, and presentation. Vaccine 2004;22(January (5–6)):557–62.
[25] Marcy MS, Kohl KS, Dagan R, Nalin D, Blum M, Jones MC, et al. Fever as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. Vaccine 2004;22(January (5–6)):551–6.
[26] Licensure of a 13-valent pneumococcal conjugate vaccine (PCV13) and recommendations for use among children—Advisory Committee on Immunization Practices (ACIP), 2010. MMWR Morb Mortal Wkly Rep 2010;59(March (9)):258–61.
[27] Nuorti JP, Whitney CG. Prevention of pneumococcal disease among infants and children—use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine—recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2010;59(December (RR-11)):1–18.
[28] Huang WT, Gargiullo PM, Broder KR, Weintraub ES, Iskander JK, Klein NP, et al. Lack of association between acellular pertussis vaccine and seizures in early childhood. Pediatrics 2010;126(August (2)):263–9.
[29] Vestergaard M, Christensen J. Register-based studies on febrile seizures in Denmark. Brain Dev 2009;31(May (5)):372–7.
[30] Febrile seizures: clinical practice guideline for the long-term management of the child with simple febrile seizures. Pediatrics 2008;121(January (6)):1281–6.

[31] Verity CM, Golding J. Risk of epilepsy after febrile convulsions: a national cohort study. BMJ 1991;303(November (6814)):1373–6.
[32] Verburgh ME, Bruijnzeels MA, van der Wouden JC, van Suijlekom-Smit LW, van der Velden J, Hoes AW, et al. Incidence of febrile seizures in The Netherlands. Neuroepidemiology 1992;11(4–6):169–72.
[33] van Zeijl JH, Mullaart RA, Borm GF, Galama JM. Recurrence of febrile seizures in the respiratory season is associated with influenza A. J Pediatr 2004;145(December (6)):800–5.
[34] Kjeldsen MJ, Kyvik KO, Friis ML, Christensen K. Genetic and environmental factors in febrile seizures: a Danish population-based twin study. Epilepsy Res 2002;51(September (1–2)):167–77.
[35] Offringa M, Bossuyt PM, Lubsen J, Ellenberg JH, Nelson KB, Knudsen FU, et al. Risk factors for seizure recurrence in children with febrile seizures: a pooled analysis of individual patient data from five studies. J Pediatr 1994;124(April (4)):574–84.
[36] Herrgard EA, Karvonen M, Luoma L, Saavalainen P, Maatta S, Laukkanen E, et al. Increased number of febrile seizures in children born very preterm: relation of neonatal, febrile and epileptic seizures and neurological dysfunction to seizure outcome at 16 years of age. Seizure 2006;15(December (8)):590–7.
[37] Visser AM, Jaddoe VW, Hofman A, Moll HA, Steegers EA, Tiemeier H, et al. Fetal growth retardation and risk of febrile seizures. Pediatrics 2010;126(October (4)):e919–25.
[38] Takano T, Sakaue Y, Sokoda T, Sawai C, Akabori S, Maruo Y, et al. Seizure susceptibility due to antihistamines in febrile seizures. Pediatr Neurol 2010;42(April (4)):277–9.
[39] Barlow WE, Davis RL, Glasser JW, Rhodes PH, Thompson RS, Mullooly JP, et al. The risk of seizures after receipt of whole-cell pertussis or measles, mumps, and rubella vaccine. N Engl J Med 2001;345(August (9)):656–61.
[40] Advisory Committee on Immunization Practice. Presentation slides February 2011 and June 2011 meeting [Accessed October 1, 2011]; Available from: http://www.cdc.gov/vaccines/recs/acip/meetings.htm#slides.
[41] Update: influenza activity—United States, August 30, 2009-March 27, 2010, and composition of the 2010–11 influenza vaccine. MMWR Morb Mortal Wkly Rep 2010;59(April (14)):423–30.
[42] Chiu SS, Tse CY, Lau YL, Peiris M. Influenza A infection is an important cause of febrile seizures. Pediatrics 2001;108(October (4)):E63.
[43] Newland JG, Laurich VM, Rosenquist AW, Heydon K, Licht DJ, Keren R, et al. Neurologic complications in children hospitalized with influenza: characteristics, incidence, and risk factors. J Pediatr 2007;150(March (3)):306–10.
[44] Chung B, Wong V. Relationship between five common viruses and febrile seizure in children. Arch Dis Child 2007;92(July (7)):589–93.
[45] Kwong KL, Lam SY, Que TL, Wong SN. Influenza A and febrile seizures in childhood. Pediatr Neurol 2006;35(December (6)):395–9.
[46] Landau YE, Grisaru-Soen G, Reif S, Fattal-Valevski A. Pediatric neurologic complications associated with influenza A H1N1. Pediatr Neurol 2011;44(January (1)):47–51.
[47] Molinari NA, Ortega-Sanchez IR, Messonnier ML, Thompson WW, Wortley PM, Weintraub E, et al. The annual impact of seasonal influenza in the US: measuring disease burden and costs. Vaccine 2007;25(June (27)):5086–96.
[48] Weycker D, Edelsberg J, Halloran ME, Longini Jr IM, Nizam A, Ciuryla V, et al. Population-wide benefits of routine vaccination of children against influenza. Vaccine 2005;23(January (10)):1284–93.
[49] Neuzil KM, Mellen BG, Wright PF, Mitchel Jr EF, Griffin MR. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. N Engl J Med 2000;342(January (4)):225–31.
[50] Neuzil KM, Wright PF, Mitchel Jr EF, Griffin MR. The burden of influenza illness in children with asthma and other chronic medical conditions. J Pediatr 2000;137(December (6)):856–64.
[51] Huang SS, Johnson KM, Ray GT, Wroe P, Lieu TA, Moore MR, et al. Healthcare utilization and cost of pneumococcal disease in the United States. Vaccine 2011;29(April (18)):3398–412.
[52] Rubin JL, McGarry LJ, Strutton DR, Klugman KP, Pelton SI, Gilmore KE, et al. Public health and economic impact of the 13-valent pneumococcal conjugate vaccine (PCV13) in the United States. Vaccine 2010;28(November (48)):7634–43.
[53] Shea KM, Weycker D, Stevenson AE, Strutton DR, Pelton SI. Modeling the decline in pneumococcal acute otitis media following the introduction of pneumococcal conjugate vaccines in the US. Vaccine 2011;29(October (45)):8042–8.
[54] Dowell SF, Whitney CG, Wright C, Rose Jr CE, Schuchat A. Seasonal patterns of invasive pneumococcal disease. Emerg Infect Dis 2003;9(May (5)):573–9.
[55] Zangwill KM, Vadheim CM, Vannier AM, Hemenway LS, Greenberg DP, Ward JI. Epidemiology of invasive pneumococcal disease in southern California: implications for the design and conduct of a pneumococcal conjugate vaccine efficacy trial. J Infect Dis 1996;174(October (4)):752–9.
[56] Update: influenza activity—United States, 2010–11 season, and composition of the 2011–12 influenza vaccine. MMWR Morb Mortal Wkly Rep 2011;60(June (21)):705–12.
[57] Advisory Committee on Immunization Practice. Presentation slides October 2011 meeting [Accessed December 1, 2011]; Available from: http://www.cdc.gov/vaccines/recs/acip/downloads/mtg-slides-oct11/Kroger-Gen-Recs.pdf.
[58] Vaccine information statement: inactivated influenza vaccine 2011–12 [Accessed October 1, 2011]; Available from: http://www.cdc.gov/vaccines/pubs/vis/downloads/vis-flu.pdf.
[59] Manfredini R, Vergine G, Boari B, Faggioli R, Borgna-Pignatti C. Circadian and seasonal variation of first febrile seizures. J Pediatr 2004;145(December (6)):838–9.
[60] Polkinghorne BG, Muscatello DJ, Macintyre CR, Lawrence GL, Middleton PM, Torvaldsen S. Relationship between the population incidence of febrile convulsions in young children in Sydney, Australia and seasonal epidemics of influenza and respiratory syncytial virus, 2003–2010: a time series analysis. BMC Infect Dis 2011;11:291.
[61] Rosenthal S, Chen R. The reporting sensitivities of two passive surveillance systems for vaccine adverse events. Am J Public Health 1995;85(December (12)):1706–9.
[62] Varricchio F, Iskander J, Destefano F, Ball R, Pless R, Braun MM, et al. Understanding vaccine safety information from the Vaccine Adverse Event Reporting System. Pediatr Infect Dis J 2004;23(April (4)):287–94.

*Journal of the Pediatric Infectious Diseases Society*

ORIGINAL ARTICLE

# Time to Change Dosing of Inactivated Quadrivalent Influenza Vaccine in Young Children: Evidence From a Phase III, Randomized, Controlled Trial

**Varsha K. Jain,[1,a] Joseph B. Domachowske,[2] Long Wang,[1,b] Opokua Ofori-Anyinam,[3] Miguel A. Rodríguez-Weber,[4] Michael L. Leonardi,[5] Nicola P. Klein,[6] Gary Schlichter,[7] Robert Jeanfreau,[8] Byron L. Haney,[9] Laurence Chu,[10] Jo-Ann S. Harris,[11] Kwabena O. Sarpong,[12] Amanda C. Micucio,[13] Jyoti Soni,[14] Vijayalakshmi Chandrasekaran,[1,c] Ping Li,[1,d] and Bruce L. Innis [1]**

[1]GSK Vaccines, King of Prussia, Pennsylvania; [2]SUNY Upstate Medical University, Syracuse, New York; [3]GSK Vaccines, Wavre, Belgium; [4]Instituto Nacional de Pediatría de México, Mexico City; [5]Palmetto Pediatrics, Charleston, South Carolina; [6]Kaiser Permanente Vaccine Study Center, Oakland, California; [7]Jean Brown Research, Salt Lake City, Utah; [8]MedPharmics, Metairie, Louisiana; [9]Family Health Care of Ellensburg, Ellensburg and Pacific Northwest University, Yakima, Washington; [10]Benchmark Research, Austin, Texas; [11]Stormont Vail Health, Topeka, Kansas; [12]Sealy Center for Vaccine Development University of Texas Medical Branch, Galveston; [13]Sidney Kimmel Medical College of Thomas Jefferson University, Philadelphia, Pennsylvania; [14]GlaxoSmithKline Pharmaceuticals Ltd, Bangalore, India

***Background.*** Children under 3 years of age may benefit from a double-dose of inactivated quadrivalent influenza vaccine (IIV4) instead of the standard-dose.

***Methods.*** We compared the only United States-licensed standard-dose IIV4 (0.25 mL, 7.5 μg hemagglutinin per influenza strain) versus double-dose IIV4 manufactured by a different process (0.5 mL, 15 μg per strain) in a phase III, randomized, observer-blind trial in children 6–35 months of age (NCT02242643). The primary objective was to demonstrate immunogenic noninferiority of the double-dose for all vaccine strains 28 days after last vaccination. Immunogenic superiority of the double-dose was evaluated post hoc. Immunogenicity was assessed in the per-protocol cohort (N = 2041), and safety was assessed in the intent-to-treat cohort (N = 2424).

***Results.*** Immunogenic noninferiority of double-dose versus standard-dose IIV4 was demonstrated in terms of geometric mean titer (GMT) ratio and seroconversion rate difference. Superior immunogenicity against both vaccine B strains was observed with double-dose IIV4 in children 6–17 months of age (GMT ratio = 1.89, 95% confidence interval [CI] = 1.64–2.17, B/Yamagata; GMT ratio = 2.13, 95% CI = 1.82–2.50, B/Victoria) and in unprimed children of any age (GMT ratio = 1.85, 95% CI = 1.59–2.13, B/Yamagata; GMT ratio = 2.04, 95% CI = 1.79–2.33, B/Victoria). Safety and reactogenicity, including fever, were similar despite the higher antigen content and volume of the double-dose IIV4. There were no attributable serious adverse events.

***Conclusions.*** Double-dose IIV4 may improve protection against influenza B in some young children and simplifies annual influenza vaccination by allowing the same vaccine dose to be used for all eligible children and adults.

**Keywords.** children; double-dose; inactivated quadrivalent influenza vaccine.

Influenza has a high incidence and burden in children [1–4]. In particular, influenza B is reported to cause a disproportionate number of influenza-related deaths in children [5]. Routine vaccination of children against influenza is recommended in the United States [6] and other countries. Quadrivalent influenza vaccines containing 2 influenza A strains and 2 influenza B strains are increasingly used in vaccination programs to replace trivalent vaccines.

Received 31 March 2016; editorial decision 4 October 2016; accepted 10 October 2016; published online January 6, 2017.

[a]Present Affiliation: Bill and Melinda Gates Foundation, Seattle, Washington.

[b]Present Affiliation: Merck Research Laboratory, North Wales, Pennsylvania.

[c]Present Affiliation: GSK Pharmaceuticals, King of Prussia, Pennsylvania.

[d]Present Affiliation: Pfizer VRD, Collegeville, Pennsylvania.

Correspondence: B. L. Innis, MD, GSK Vaccines, King of Prussia, PA 19406 (bruce.2.innis@gsk.com).

**Journal of the Pediatric Infectious Diseases Society 2017;6(1):9–19**



DOI: 10.1093/jpids/piw068

Inactivated influenza vaccines (IIVs) are administered to adults and children from 3 years of age at a dose of 0.5 mL, containing 15 μg of hemagglutinin (HA) per virus strain. In children under 3 years of age, the United States-licensed standard-dose is 0.25 mL, containing 7.5 μg of HA per virus strain. Both the 15 μg and 7.5 μg doses are available for this age group in some countries, including Canada, Brazil, Mexico, Finland, and the United Kingdom. The 7.5 μg dose was introduced in the 1970s to reduce reactogenicity, including febrile convulsions, associated with the whole virus vaccines available at the time [7–11]. However, young children mount a variable immune response to the 7.5 μg dose [12–14]. Currently available split virus vaccines are better tolerated than whole virus vaccines [10, 15, 16], questioning the practice of using the 7.5 μg dose with IIVs.

The inactivated quadrivalent influenza vaccine (IIV4) manufactured in Quebec, Canada by GSK Vaccines is licensed at a double-dose (15 μg per antigen) for children from 6 months of age in Canada and Mexico, but it is currently only licensed

for children 3 years of age and older in the United States. The only IIV4 licensed for use in children 6–35 months of age in the United States is Sanofi Pasteur's Fluzone Quadrivalent in a standard-dose (7.5 μg per antigen). No other IIV is approved in the United States in this age group either because immunogenic noninferiority to Fluzone could not be demonstrated [17, 18] or because of excessive reactogenicity [19, 20].

If the double-dose vaccine could be administered in young children without adverse effects on tolerability, this age group may benefit from potentially improved immunogenicity. In this study, we describe a phase III study that compared the safety and immunogenicity of a double-dose IIV4 manufactured by GSK Vaccines with the United States-approved standard-dose IIV4 in children 6–35 months of age.

## METHODS

This was a phase III, randomized, controlled, observer-blind, multicenter trial in children 6–35 months of age (ClinicalTrials.gov Identifier NCT02242643). The trial was approved by independent ethics committees or institutional review boards, conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines, ICH Harmonised Tripartite guideline for pediatric populations, and US regulatory requirements. Parents or legally acceptable representatives provided written informed consent.

### Participants, Vaccines, and Study Design

Children in stable health were recruited in the United States and Mexico during the 2014–15 influenza season (Supplementary Appendix). The double-dose IIV4 (GSK Vaccines, Quebec, Canada) contained 15 μg HA of each of the 4 strains: A/California/7/2009 (A/H1N1), A/Texas/50/2012 (A/H3N2), B/Brisbane/60/2008 (B/Victoria), and B/Massachusetts/2/2012 (B/Yamagata). The standard-dose IIV4 (Fluzone Quadrivalent; Sanofi Pasteur, Swiftwater, PA) contained 7.5 μg of HA of each of the same strains.

Children were randomized 1:1 to double-dose or standard-dose IIV4. Allocation to a study group at the investigator site was performed using an internet-based randomization system (SBIR). The randomization algorithm used a minimization procedure to balance the composition of treatment groups, accounting for age (6–17 and 18–35 months), center, and influenza vaccine priming status. The study aimed to enroll 40%–50% of children in the 6–17 months age group. Children were considered vaccine-primed if they had received 2 or more doses of influenza vaccine since July 1, 2010 or at least 1 dose of the

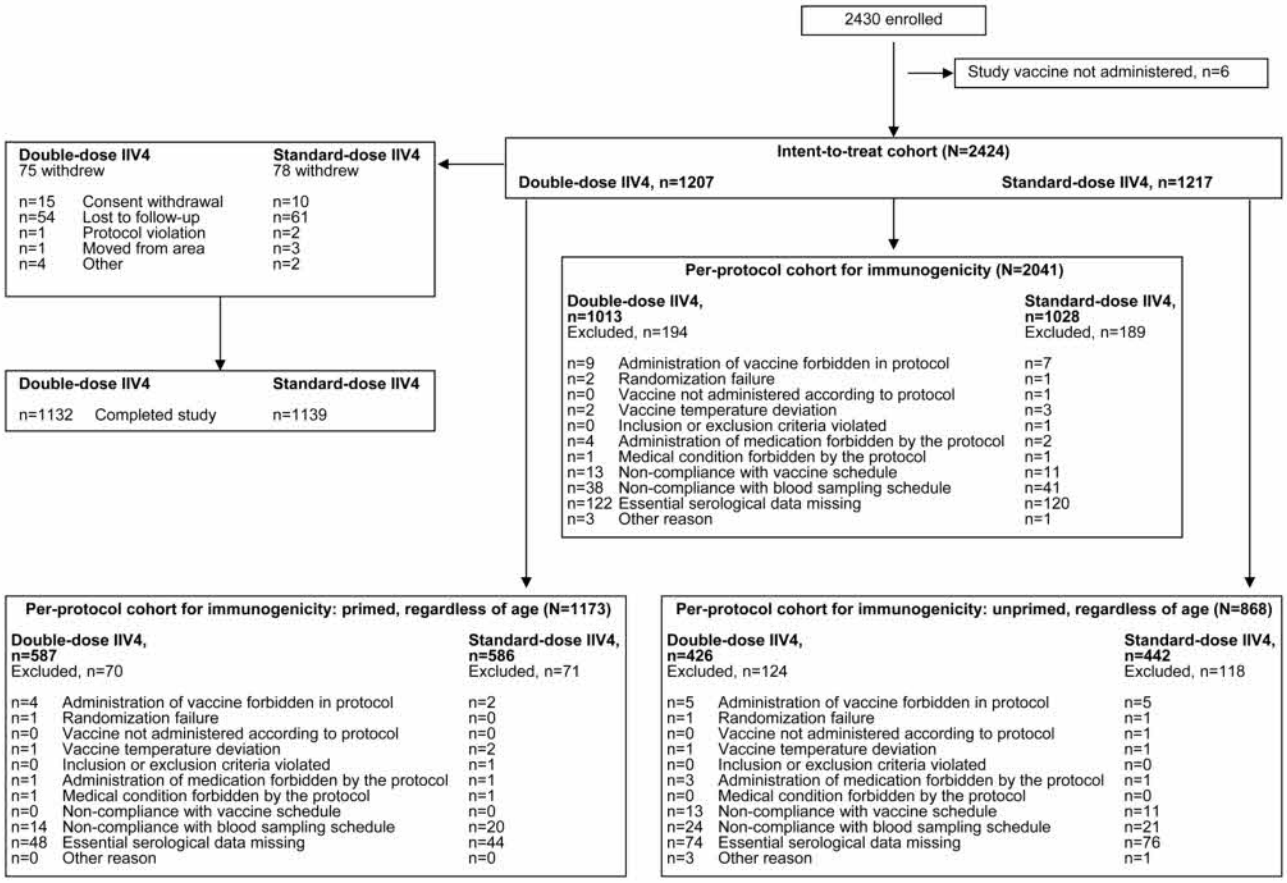



**Figure 1.** Participant disposition.

**Table 1. Participant Demographics (Per-Protocol Cohort)**

| Characteristic | All Children (Regardless Of Priming Status, 6–35 Months) | | Primed Children (6–35 Months) | | Unprimed Children (6–35 Months) | |
|---|---|---|---|---|---|---|
| | Double-Dose IIV4 N = 1013 | Standard-Dose IIV4 N = 1028 | Double-Dose IIV4 N = 587 | Standard-Dose IIV4 N = 586 | Double-Dose IIV4 N = 426 | Standard-Dose IIV4 N = 442 |
| Age at first vaccination, months, mean (SD) | 19.7 (8.7) | 19.9 (8.9) | 24.5 (6.2) | 24.8 (6.1) | 13.1 (7.2) | 13.5 (7.9) |
| Age 6–17 months, n (%) | 400 (39.5) | 401 (39.0) | 74 (12.6) | 69 (11.8) | 326 (76.5) | 332 (75.1) |
| <12 months, n (%) | 213 (21.0) | 226 (22.0) | 0 | 0 | 213 (50.0) | 226 (51.1) |
| Age 18–35 months, n (%) | 613 (60.5) | 627 (61.0) | 513 (87.4) | 517 (88.2) | 100 (23.5) | 110 (24.9) |
| Female, n (%) | 462 (45.6) | 496 (48.2) | 264 (45.0) | 283 (48.3) | 198 (46.5) | 213 (48.2) |
| Geographic ancestry, n (%) | | | | | | |
| Caucasian/European | 647 (63.9) | 667 (64.9) | 393 (67.0) | 400 (68.3) | 254 (59.6) | 267 (60.4) |
| African/African American | 143 (14.1) | 140 (13.6) | 89 (15.2) | 78 (13.3) | 54 (12.7) | 62 (14.0) |
| American Indian or Alaskan Native | 23 (2.3) | 18 (1.8) | 15 (2.6) | 13 (2.2) | 8 (1.9) | 5 (1.1) |
| South East Asian | 17 (1.7) | 20 (1.9) | 11 (1.9) | 14 (2.4) | 6 (1.4) | 6 (1.4) |
| Other | 183 (18.1) | 183 (17.8) | 79 (13.5) | 81 (13.8) | 104 (24.4) | 102 (23.1) |

Abbreviatioins: IIV4, inactivated quadrivalent influenza vaccine; N, number of participants included in analysis; n, number of participants in stated category; SD, standard deviation.

2013–14 influenza vaccine. Vaccine-primed children received a single dose on day 0. Vaccine-unprimed children received 1 dose on day 0 and another on day 28.

#### Study Endpoints

Blood for serologic testing was obtained on days 0 and 28 from primed children and on days 0 and 56 from unprimed children. The following parameters were derived from hemagglutination inhibition (HI) titers: (1) geometric mean titer (GMT), (2) seroconversion rate (SCR), (3) seroprotection rate (SPR), and (4) mean geometric increase (MGI). Seroconversion rate was defined as the percentage of participants with either (1) prevaccination reciprocal HI titer <1:10 and a postvaccination reciprocal titer ≥1:40 or (2) prevaccination reciprocal titer ≥1:10 and at least a 4-fold increase in postvaccination reciprocal titer. Seroprotection rate was defined as the percentage of participants who attained reciprocal HI titers of ≥1:40. Mean geometric increase was defined as the geometric mean of the within-subject ratios of the postvaccination/prevaccination reciprocal HI titer.

Parents recorded solicited injection site and general symptoms on the day of vaccination and for the next 6 days. Spontaneously reported symptoms were recorded until 28 days after vaccination. Serious adverse events (SAEs), potential immune-mediated diseases, and medically attended adverse events were recorded until the final study contact on day 180. Monitoring for febrile seizures was carried out throughout the study.

#### Study Objectives

The primary objective was to demonstrate immunogenic noninferiority of the double-dose versus the standard-dose IIV4 28 days after completion of the vaccination course. Noninferiority criteria were met if, for each of the 4 vaccine strains, the upper limit of the 95% confidence interval (CI) of the GMT ratio (standard-dose/double-dose) was ≤1.5 and the upper limit of the 95% CI of the difference in SCR (standard-dose minus double-dose) was ≤10%.

If the primary objective was achieved, the secondary objective was to evaluate whether double-dose IIV4 produced an immune response against each of the vaccine strains that met Center for Biologics Evaluation and Research (CBER) criteria, ie, the lower limit of the 95% CI of the SCR was ≥40% and the lower limit of the 95% CI of the SPR was ≥70%. Additional secondary objectives were to (1) evaluate GMT, SPR, SCR, and MGI at 28 days after completion of the vaccination course, (2) describe the safety and reactogenicity of the vaccines, and (3) evaluate the relative risk of fever with double-dose versus standard-dose during the 2-day postvaccination period.

A post hoc evaluation was conducted to compare the immune response of the double-dose versus the standard-dose using CBER criteria conventionally applied to establish vaccine lot-to-lot consistency. Immunogenic superiority of the double-dose was concluded if the lower limit of the 95% CI of the GMT ratio (double-dose/standard-dose) was >1.5 and the lower limit of the 95% CI of the difference in SCR (double-dose minus standard-dose) was >10%.

#### Statistics

Enrollment of 1200 children per group (1020 evaluable subjects assuming an attrition rate of 15%) was planned to allow a global statistical power of 99% for the primary objective evaluation. The immunogenicity analysis was based on the per-protocol cohort and the safety analysis was based on the intent-to-treat cohort (Supplementary Appendix). Subgroup analyses

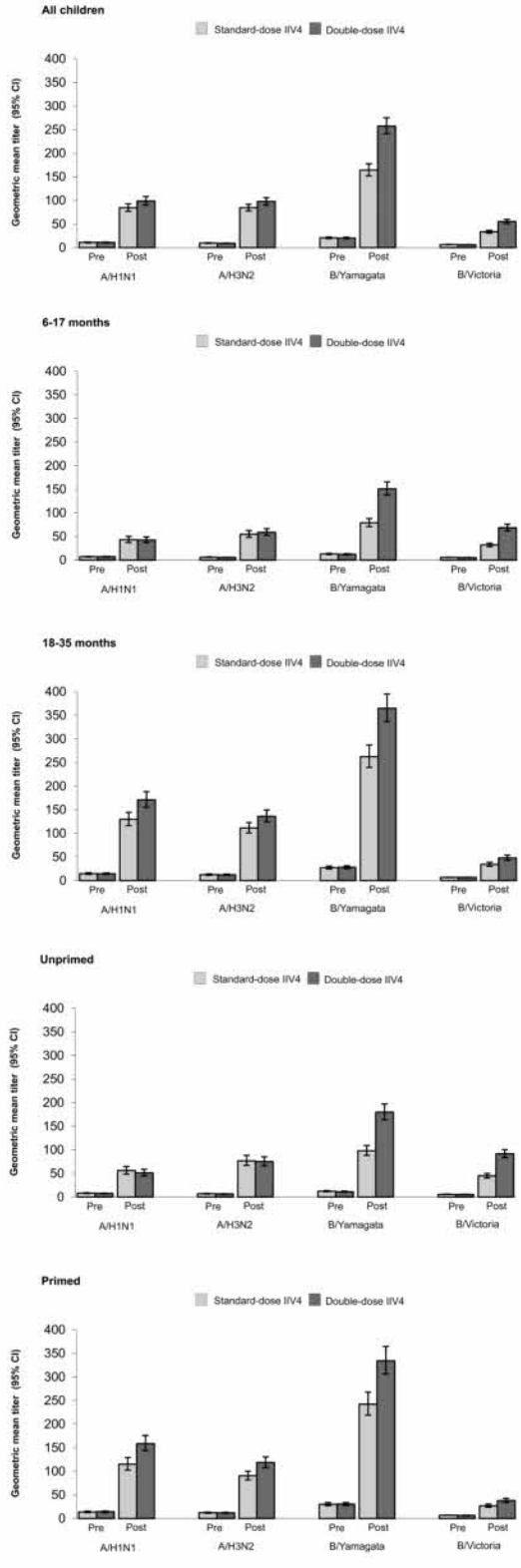



**Figure 2.** Geometric mean titer for all vaccine strains in all children 6–35 months of age regardless of priming status and in each subgroup pre-vaccination and 28 days after completion of vaccination series (per-protocol cohort). CI, confidence interval; IIV4, inactivated quadrivalent influenza vaccine.

according to age and priming status were conducted on the per-protocol cohort.

The overall type I error for the study was 5%. If the primary objective was met, the secondary objective of CBER criteria evaluation was tested to provide supportive evidence of immunogenicity. Calculation of 95% CIs is described in the Supplementary Appendix. The group GMT ratio was computed using an analysis of covariance model on the log-transformed titers. Analyses of immunogenicity excluded participants with missing or nonevaluable measurements at the postvaccination time point. Study power was calculated using PASS 2005 (Supplementary Appendix).

## RESULTS

A total of 2424 and 2041 children were included in the intent-to-treat cohort and per-protocol cohort, respectively (Figure 1). Demographics were similar in both vaccine groups (Table 1). In the per-protocol cohort, 57.5% of children were vaccine-primed; mean age was 24.6 and 13.3 months for primed and unprimed children, respectively. Other demographic characteristics were similar in primed and unprimed children (Table 1).

### Immunogenicity

Both vaccines were immunogenic against all vaccine strains in terms of GMT values (Figure 2). Immunogenic noninferiority of the double-dose IIV4 versus the standard-dose IIV4 was demonstrated for all vaccine strains (Figure 3). Seroconversion rate, SPR, and MGI values were higher in the double-dose group compared with the standard-dose group in the whole study population (6–35 months of age, regardless of priming status; Table 2). The lower limit of the 95% CI for SCR was ≥40% for the double-dose IIV4 against all vaccine strains (Table 2), meeting CBER criteria for demonstration of adequate immunogenicity. For SPR, the lower limit of the 95% CI was ≥70% for all strains except B/Victoria (Table 2).

Immunogenicity was higher in the double-dose group compared with the standard-dose group, particularly against vaccine B strains in children 6–17 months of age and unprimed children (Table 3; Figure 2). When the unprimed group was further evaluated by age, it could be seen that the main difference between vaccines occurred in children 6–17 months of age. These observations prompted us to perform the post hoc evaluation comparing the immune response elicited by the vaccines in the whole study population and according to age group and priming status. The analysis indicated superior immunogenicity of the double-dose IIV4 against both vaccine B strains in children 6–17 months of age and all unprimed children. In children 6–17 months of age, the GMT ratio was 1.89 (95% CI, 1.64–2.17) for B/Yamagata and 2.13 (95% CI, 1.82–2.50) for B/Victoria (Figure 4 and Supplementary Table 1).

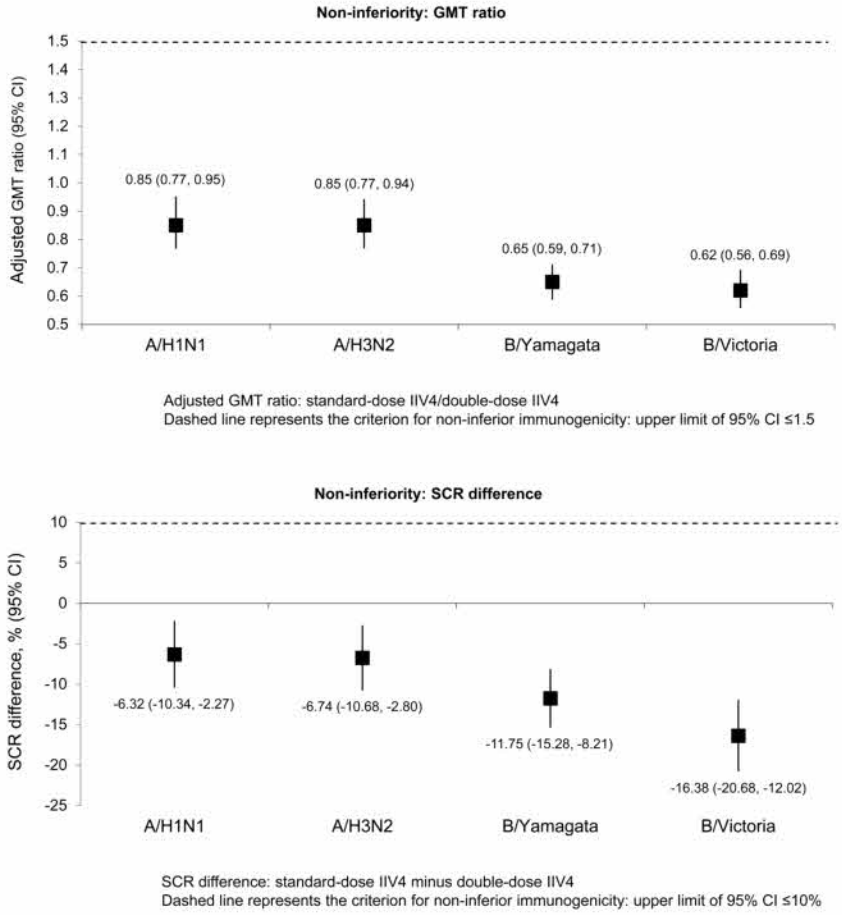



**Figure 3.** Noninferiority of the double-dose versus the standard-dose in all children 6–35 months of age regardless of priming status: geometric mean titer (GMT) ratio and difference in seroconversion rate (SCR) at 28 days after completion of vaccination series (per-protocol cohort). CI, confidence interval; IIV4, inactivated quadrivalent influenza vaccine.

**Table 2. Immunogenicity Against Each Vaccine Strain at 28 Days After Completion of Vaccination Series in All Children 6–35 Months of Age Regardless of Priming Status (Per-Protocol Cohort)**

| | A/H1N1 | | A/H3N2 | | B/Yamagata | | B/Victoria | |
|---|---|---|---|---|---|---|---|---|
| Endpoint | N | Value | N | Value | N | Value | N | Value |
| GMT, 1/DIL (95% CI) | | | | | | | | |
| Double-dose | 1013 | 98.8 (90.3–108.2) | 1013 | 97.7 (90.3–105.7) | 1013 | 257.5 (240.9–275.3) | 1013 | 55.1 (50.8–59.8) |
| Standard-dose | 1028 | 84.4 (76.9–92.6) | 1028 | 84.3 (77.6–91.6) | 1028 | 164.2 (151.8–177.6) | 1028 | 33.4 (30.6–36.4) |
| SCR, % (95% CI) | | | | | | | | |
| Double-dose | 972 | 73.7 (70.8–76.4) | 972 | 76.1 (73.3–78.8) | 974 | 85.5 (83.2–87.7) | 973 | 64.9 (61.8–67.9) |
| Standard-dose | 980 | 67.3 (64.3–70.3) | 980 | 69.4 (66.4–72.3) | 980 | 73.8 (70.9–76.5) | 980 | 48.5 (45.3–51.6) |
| SPR, % (95% CI) | | | | | | | | |
| Double-dose | 1013 | 80.4 (77.8–82.8) | 1013 | 82.2 (79.7–84.5) | 1013 | 97.0 (95.8–98.0) | 1013 | 66.0 (63.0–69.0) |
| Standard-dose | 1028 | 75.4 (72.6–78.0) | 1028 | 77.8 (75.2–80.3) | 1028 | 88.6 (86.5–90.5) | 1028 | 49.8 (46.7–52.9) |
| MGI (95% CI) | | | | | | | | |
| Double-dose | 972 | 9.0 (8.4–9.7) | 972 | 10.7 (10.0–11.6) | 974 | 12.7 (11.7–13.7) | 973 | 8.7 (8.1–9.4) |
| Standard-dose | 980 | 7.7 (7.1–8.3) | 980 | 8.9 (8.2–9.7) | 980 | 8.1 (7.5–8.8) | 980 | 5.4 (5.0–5.8) |

Abbreviations: CI, confidence interval; DIL, dilution; GMT, geometric mean titer; MGI, mean geometric increase; N, number of participants included in analysis; SCR, seroconversion rate; SPR, seroprotection rate.

**Table 3. Comparison of Immunogenicity of the Double-Dose Versus the Standard-Dose According to Age and Priming Status at 28 Days After Completion of Vaccination Series (Per-Protocol Cohort)**

| | A/H1N1 | | A/H3N2 | | B/Yamagata | | B/Victoria | |
|---|---|---|---|---|---|---|---|---|
| Endpoint | N | Value | N | Value | N | Value | N | Value |
| **6–17 months (regardless of priming status)** | | | | | | | | |
| GMT, 1/DIL (95% CI) | | | | | | | | |
| Double-dose | 400 | 42.7 (37.1–49.0) | 400 | 58.9 (52.2–66.4) | 400 | 151.0 (137.4–165.9) | 400 | 68.7 (61.8–76.3) |
| Standard-dose | 401 | 43.2 (37.3–50.0) | 401 | 54.8 (47.9–62.7) | 401 | 79.1 (70.9–88.1) | 401 | 31.9 (28.4–35.7) |
| SPR, % (95% CI) | | | | | | | | |
| Double dose | 400 | 61.3 (56.3–66.1) | 400 | 70.3 (65.5–74.7) | 400 | 94.3 (91.5–96.3) | 400 | 78.3 (73.9–82.2) |
| Standard-dose | 401 | 59.9 (54.9–64.7) | 401 | 67.8 (63.0–72.4) | 401 | 77.6 (73.2–81.5) | 401 | 51.4 (46.4–56.4) |
| SCR, % (95% CI) | | | | | | | | |
| Double-dose | 376 | 58.5 (53.3–63.5) | 376 | 69.1 (64.2–73.8) | 376 | 79.5 (75.1–83.5) | 376 | 77.4 (72.8–81.5) |
| Standard-dose | 375 | 57.6 (52.4–62.7) | 375 | 66.7 (61.6–71.4) | 375 | 61.9 (56.7–66.8) | 375 | 50.4 (45.2–55.6) |
| MGI (95% CI) | | | | | | | | |
| Double-dose | 376 | 6.0 (5.3–6.8) | 376 | 10.2 (9.0–11.6) | 376 | 12.3 (10.7–14.3) | 376 | 12.3 (11.0–13.8) |
| Standard-dose | 375 | 6.1 (5.2–7.1) | 375 | 8.8 (7.7–10.2) | 375 | 6.1 (5.3–7.0) | 375 | 5.7 (5.1–6.4) |
| **18–35 months (regardless of priming status)** | | | | | | | | |
| GMT, 1/DIL (95% CI) | | | | | | | | |
| Double-dose | 613 | 170.9 (155.2–188.3) | 613 | 136.0 (123.7–149.6) | 613 | 364.8 (336.7–395.3) | 613 | 47.8 (42.6–53.6) |
| Standard-dose | 627 | 129.6 (116.3–144.3) | 627 | 111.1 (100.6–122.7) | 627 | 262.1 (239.3–287.1) | 627 | 34.4 (30.4–38.8) |
| SPR, % (95% CI) | | | | | | | | |
| Double-dose | 613 | 92.8 (90.5–94.7) | 613 | 90.0 (87.4–92.3) | 613 | 98.9 (97.7–99.5) | 613 | 58.1 (54.1–62.0) |
| Standard-dose | 627 | 85.3 (82.3–88.0) | 627 | 84.2 (81.1–87.0) | 627 | 95.7 (93.8–97.1) | 627 | 48.8 (44.8–52.8) |
| SCR, % (95% CI) | | | | | | | | |
| Double-dose | 596 | 83.2 (80.0–86.1) | 596 | 80.5 (77.1–83.6) | 598 | 89.3 (86.5–91.7) | 597 | 57.0 (52.9–61.0) |
| Standard-dose | 605 | 73.4 (69.7–76.9) | 605 | 71.1 (67.3–74.7) | 605 | 81.2 (77.8–84.2) | 605 | 47.3 (43.2–51.3) |
| MGI (95% CI) | | | | | | | | |
| Double-dose | 596 | 11.7 (10.7–12.8) | 596 | 11.1 (10.1–12.1) | 598 | 12.9 (11.8–14.0) | 597 | 7.0 (6.4–7.7) |
| Standard-dose | 605 | 8.9 (8.1–9.8) | 605 | 9.0 (8.2–9.9) | 605 | 9.7 (8.9–10.6) | 605 | 5.2 (4.7–5.7) |
| **Primed (regardless of age)** | | | | | | | | |
| GMT, 1/DIL (95% CI) | | | | | | | | |
| Double-dose | 587 | 158.8 (143.3–176.0) | 587 | 118.4 (107.5–130.3) | 587 | 334.3 (306.4–364.7) | 587 | 38.1 (34.0–42.8) |
| Standard-dose | 586 | 115.0 (102.6–128.9) | 586 | 90.4 (81.8–100.0) | 586 | 242.2 (219.1–267.7) | 586 | 26.7 (23.6–30.3) |
| SPR, % (95% CI) | | | | | | | | |
| Double-dose | 587 | 90.6 (88.0–92.9) | 587 | 87.2 (84.2–89.8) | 587 | 98.1 (96.7–99.1) | 587 | 49.4 (45.3–53.5) |
| Standard-dose | 586 | 82.1 (78.7–85.1) | 586 | 80.2 (76.7–83.4) | 586 | 93.7 (91.4–95.5) | 586 | 40.1 (36.1–44.2) |
| SCR, % (95% CI) | | | | | | | | |
| Double-dose | 570 | 80.5 (77.0–83.7) | 570 | 77.9 (74.3–81.2) | 572 | 86.5 (83.5–89.2) | 571 | 48.0 (43.8–52.2) |
| Standard-dose | 563 | 70.3 (66.4–74.1) | 563 | 67.1 (63.1–71.0) | 563 | 78.0 (74.3–81.3) | 563 | 38.4 (34.3–42.5) |
| MGI (95% CI) | | | | | | | | |
| Double-dose | 570 | 10.9 (10.0–12.0) | 570 | 10.0 (9.1–10.9) | 572 | 10.7 (9.9–11.6) | 571 | 5.6 (5.1–6.1) |
| Standard-dose | 563 | 8.5 (7.7–9.3) | 563 | 7.6 (6.9–8.3) | 563 | 8.2 (7.6–8.9) | 563 | 4.0 (3.6–4.4) |
| **Unprimed (regardless of age)** | | | | | | | | |
| GMT, 1/DIL (95% CI) | | | | | | | | |
| Double-dose | 426 | 51.4 (44.7–59.1) | 426 | 75.0 (66.0–85.3) | 426 | 179.8 (163.7–197.4) | 426 | 91.7 (83.8–100.3) |
| Standard-dose | 442 | 56.0 (48.4–64.8) | 442 | 76.8 (66.9–88.3) | 442 | 98.1 (88.1–109.3) | 442 | 44.8 (40.1–50.0) |
| SPR, % (95% CI) | | | | | | | | |
| Double-dose | 426 | 66.2 (61.5–70.7) | 426 | 75.4 (71.0–79.4) | 426 | 95.5 (93.1–97.3) | 426 | 89.0 (85.6–91.8) |
| Standard-dose | 442 | 66.5 (61.9–70.9) | 442 | 74.7 (70.3–78.7) | 442 | 81.9 (78.0–85.4) | 442 | 62.7 (58.0–67.2) |
| SCR, % (95% CI) | | | | | | | | |
| Double-dose | 402 | 63.9 (59.0–68.6) | 402 | 73.6 (69.0–77.9) | 402 | 84.1 (80.1–87.5) | 402 | 88.8 (85.3–91.7) |
| Standard-dose | 417 | 63.3 (58.5–67.9) | 417 | 72.4 (67.9–76.7) | 417 | 68.1 (63.4–72.6) | 417 | 62.1 (57.3–66.8) |
| MGI (95% CI) | | | | | | | | |
| Double-dose | 402 | 6.9 (6.1–7.8) | 402 | 11.8 (10.4–13.4) | 402 | 16.0 (13.9–18.5) | 402 | 16.2 (14.8–17.8) |
| Standard-dose | 417 | 6.8 (5.9–7.8) | 417 | 11.2 (9.7–12.9) | 417 | 8.0 (6.9–9.3) | 417 | 8.0 (7.2–8.8) |
| **Unprimed (6–17 months)** | | | | | | | | |
| GMT, 1/DIL (95% CI) | | | | | | | | |
| Double-dose | 326 | 36.2 (31.3–41.9) | 326 | 56.7 (49.7–64.8) | 326 | 146.8 (132.5–162.7) | 326 | 84.6 (76.7–93.3) |
| Standard-dose | 332 | 38.0 (32.5–44.4) | 332 | 54.1 (46.6–62.7) | 332 | 71.1 (63.6–79.4) | 332 | 35.5 (31.5–40.0) |

**Table 3. Continued**

| | A/H1N1 | | A/H3N2 | | B/Yamagata | | B/Victoria | |
|---|---|---|---|---|---|---|---|---|
| Endpoint | N | Value | N | Value | N | Value | N | Value |
| SPR, % (95% CI) | | | | | | | | |
| Double-dose | 326 | 57.4 (51.8–62.8) | 326 | 68.7 (63.4–73.7) | 326 | 94.2 (91.0–96.5) | 326 | 87.4 (83.3–90.8) |
| Standard-dose | 332 | 57.2 (51.7–62.6) | 332 | 68.4 (63.1–73.3) | 332 | 75.9 (70.9–80.4) | 332 | 55.1 (49.6–60.6) |
| SCR, % (95% CI) | | | | | | | | |
| Double-dose | 304 | 54.9 (49.2–60.6) | 304 | 68.4 (62.9–73.6) | 304 | 79.3 (74.3–83.7) | 304 | 87.2 (82.9–90.7) |
| Standard-dose | 309 | 55.0 (49.3–60.7) | 309 | 68.0 (62.4–73.1) | 309 | 58.9 (53.2–64.4) | 309 | 54.4 (48.6–60.0) |
| MGI (95% CI) | | | | | | | | |
| Double-dose | 304 | 5.5 (4.7–6.3) | 304 | 10.3 (8.9–11.9) | 304 | 12.8 (10.7–15.1) | 304 | 15.7 (14.0–17.6) |
| Standard-dose | 309 | 5.5 (4.6–6.5) | 309 | 9.1 (7.7–10.6) | 309 | 5.6 (4.7–6.6) | 309 | 6.5 (5.8–7.3) |
| **Unprimed (18–35 months)** | | | | | | | | |
| GMT, 1/DIL (95% CI) | | | | | | | | |
| Double-dose | 100 | 161.7 (125.9–207.7) | 100 | 187.0 (143.6–243.6) | 100 | 347.7 (296.3–407.9) | 100 | 119.2 (96.8–146.7) |
| Standard-dose | 110 | 180.9 (141.0–232.1) | 110 | 222.0 (173.3–284.4) | 110 | 260.0 (217.5–310.8) | 110 | 90.5 (73.2–111.8) |
| SPR, % (95% CI) | | | | | | | | |
| Double-dose | 100 | 95.0 (88.7–98.4) | 100 | 97.0 (91.5–99.4) | 100 | 100 (96.4–100) | 100 | 94.0 (87.4–97.8) |
| Standard-dose | 110 | 94.5 (88.5–98.0) | 110 | 93.6 (87.3–97.4) | 110 | 100 (96.7–100) | 110 | 85.5 (77.5–91.5) |
| SCR, % (95% CI) | | | | | | | | |
| Double-dose | 98 | 91.8 (84.5–96.4) | 98 | 89.8 (82.0–95.0) | 98 | 99.0 (94.4–100) | 98 | 93.9 (87.1–97.7) |
| Standard-dose | 108 | 87.0 (79.2–92.7) | 108 | 85.2 (77.1–91.3) | 108 | 94.4 (88.3–97.9) | 108 | 84.3 (76.0–90.6) |
| MGI (95% CI) | | | | | | | | |
| Double-dose | 98 | 13.9 (11.6–16.8) | 98 | 18.0 (14.1–23.1) | 98 | 32.5 (26.3–40.1) | 98 | 18.0 (15.3–21.2) |
| Standard-dose | 108 | 12.4 (10.2–15.1) | 108 | 20.7 (15.6–27.4) | 108 | 22.8 (18.4–28.3) | 108 | 14.2 (11.9–16.8) |

Abbreviations: CI, confidence interval; DIL, dilution; GMT, geometric mean titer; MGI, mean geometric increase; N, number of participants included in analysis; SCR, seroconversion rate; SPR, seroprotection rate.

Corresponding values in all unprimed children were 1.85 (95% CI, 1.59–2.13) and 2.04 (95% CI, 1.79–2.33). Superior immunogenicity of the double-dose was also observed for the same groups in terms of SCR difference (Figure 4 and Supplementary Table 1).

#### Safety and Reactogenicity

Pain was the most common solicited injection site symptom, occurring in approximately 40% of children in both vaccine groups; severe (grade 3) pain occurred in 2.9% (95% CI, 2.0–4.1) and 1.7% (95% CI, 1.0–2.6) of children with the double-dose and standard-dose, respectively (Table 4). Fever (≥38.0°C) was reported in approximately 8% of children up to 7 days postvaccination; fever >39.0°C occurred in approximately 2% of children (Table 4). During the 2-day postvaccination period (days 0–1), the incidence of fever (≥38.0°C) was similar in both groups (Table 4), and the relative risk (double-dose/standard-dose) was 0.97 (95% CI, 0.62–1.52; $P$ = .9777). Twenty-two SAEs occurred in the double-dose group and 21 in the standard-dose group (Table 4), none considered related to vaccination. Febrile seizure was reported in 5 children in the double-dose group and in 4 children in the standard-dose group (Table 4).

There was a modest increase in reactogenicity with regard to general symptoms in children 6–17 months of age compared with those aged 18–35 months with both the double-dose and standard-dose vaccines. With the double-dose vaccine, the fold-difference between the younger and older age groups ranged from 1.3 for loss of appetite to 2.7 for fever ≥38.0°C. With the standard-dose, the fold-difference ranged from 1.2 for loss of appetite to 1.6 for drowsiness and fever ≥38.0°C. The difference between age groups was unlikely to be due to chance because, in general, 95% CIs did not overlap. However, there were overlapping 95% CIs and thus no apparent age group differences with the standard-dose vaccine for fever ≥38.0°C and loss of appetite.

## DISCUSSION

The introduction of IIV4 provides an opportunity to review long-accepted practices in administration of influenza vaccines. Since the 1970s, the standard-dose of IIVs in children less than 3 years of age has been 7.5 μg per antigen, half the dose given to older children and adults. The lower dose was intended to reduce reactogenicity and febrile convulsions observed with the whole virus vaccines that were in use at the time [7–11]. However, young children mount a variable immune response to this lower dose, especially against vaccine B strains [12–14]. In particular, vaccine-naive children less than 3 years of age mount a lower immune response compared with older or

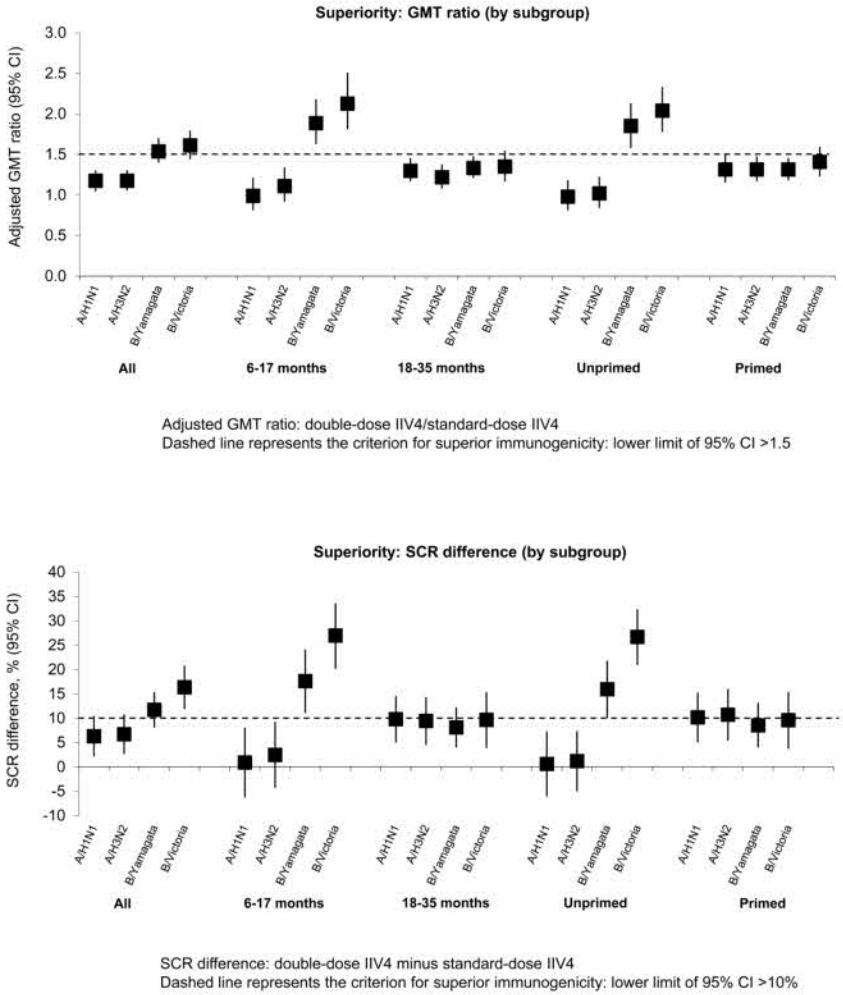



**Figure 4.** Comparison of immunogenicity of the double-dose versus the standard-dose in all children 6–35 months of age regardless of priming status and in each subgroup: geometric mean titer (GMT) ratio and difference in seroconversion rate (SCR) at 28 days after completion of vaccination series (per-protocol cohort). CI, confidence interval; IIV4, inactivated quadrivalent influenza vaccine.

vaccine-primed children [14, 21–23]. The immune response in this vulnerable group could be improved by a change in practice to administer the double-dose, ie, same dose as used for children 3 years of age and above, and for adults. Increasing the immunogenicity of IIVs for young children is expected to improve their effectiveness, because the postvaccination HI antibody titer is inversely related to the risk of illness [24, 25]. However, there is controversy regarding the HI antibody titer necessary to offer high-level effectiveness [24, 25].

In the present study, both the double-dose and the standard-dose IIV4s were immunogenic against all vaccine strains in primed and unprimed children 6–35 months of age. The primary objective of the study—to fulfill US licensure criteria by demonstrating immunogenic noninferiority of the investigational IIV4 to a licensed IIV4 and acceptable safety of the investigational IIV4—was achieved. Most children receiving the double-dose IIV4 seroconverted (SCRs, 64.9%–85.5%), and most children achieved seroprotection (SPRs, 66.0%–97.0%). Similar immune responses have been achieved with the double-dose IIV4 in children of the same age in small studies conducted in 3 prior seasons [26–28].

Greater antibody responses were observed with the double-dose IIV4 compared with the standard-dose, prompting us to perform a post hoc analysis to evaluate whether the double-dose elicited a superior immune response in terms of the CBER criteria usually applied to establish lot-to-lot consistency of influenza vaccines. In this analysis, the double-dose IIV4 did not reach superiority to the standard-dose in the overall population, the older age group (18–35 months), or previously primed children. However, in the younger age group (6–17 months) and in all unprimed children, the double-dose IIV4 met the applied superiority immune response criteria compared with the standard-dose against the B strains. It should be noted that the unprimed group was predominantly 6–17 months of age.

Several previous studies have compared the HI antibody response elicited by a double-dose versus a standard-dose IIV. The results of the present large phase III study contrast with

**Table 4. Safety Outcomes Reported Throughout the Study (Intent-to-Treat Cohort)**

| Adverse event | Double-Dose IIV4 N = 1207[a] | | Standard-Dose IIV4 N = 1217[a] | |
|---|---|---|---|---|
| | No. Patients With Symptom | % (95% CI) | No. Patients With Symptom | % (95% CI) |
| **Solicited[b] injection site symptoms during 7-day postvaccination period** | | | | |
| Pain | 509 | 44.0 (41.1–46.9) | 462 | 40.1 (37.3–43.0) |
| Grade 3[c] | 34 | 2.9 (2.0–4.1) | 19 | 1.7 (1.0–2.6) |
| Redness | 16 | 1.4 (0.8–2.2) | 16 | 1.4 (0.8–2.2) |
| Grade 3[c] | 0 | - | 0 | - |
| Swelling | 11 | 1.0 (0.5–1.7) | 5 | 0.4 (0.1–1.0) |
| Grade 3[c] | 0 | - | 0 | - |
| **Solicited general symptoms during 7-day postvaccination period** | | | | |
| Drowsiness | 471 | 40.6 (37.8–43.5) | 471 | 40.9 (38.0–43.8) |
| Grade 3[c] | 36 | 3.1 (2.2–4.3) | 34 | 3.0 (2.1–4.1) |
| Fever (≥38.0°C) | 91 | 7.9 (6.4–9.6) | 86 | 7.5 (6.0–9.1) |
| >39.0°C | 25 | 2.2 (1.4–3.2) | 17 | 1.5 (0.9–2.4) |
| Irritability/fussiness | 630 | 54.4 (51.4–57.3) | 582 | 50.5 (47.6–53.4) |
| Grade 3[c] | 61 | 5.3 (4.0–6.7) | 45 | 3.9 (2.9–5.2) |
| Loss of appetite | 391 | 33.7 (31.0–36.5) | 385 | 33.4 (30.7–36.2) |
| Grade 3[c] | 26 | 2.2 (1.5–3.3) | 19 | 1.6 (1.0–2.6) |
| **Unsolicited (spontaneously reported) symptoms during 28-day postvaccination period** | | | | |
| All | 549 | 45.5 (42.6–48.3) | 537 | 44.1 (41.3–47.0) |
| Grade 3[c] | 70 | 5.8 (4.5–7.3) | 75 | 6.2 (4.9–7.7) |
| Related to vaccine | 71 | 5.9 (4.6–7.4) | 71 | 5.8 (4.6–7.3) |
| **Fever reported during 2-day postvaccination period** | | | | |
| All (≥38.0°C) | 42 | 3.6 (2.6–4.9) | 43 | 3.7 (2.7–5.0) |
| **Febrile seizure[d] during entire study period** | | | | |
| All | 5 | 0.4 (0.1–1.0) | 4 | 0.3 (0.1–0.8) |
| **Medically attended event[e] during entire study period** | | | | |
| All | 727 | 60.2 (57.4–63.0) | 719 | 59.1 (56.3–61.9) |
| **Potential immune-mediated disease during entire study period[f]** | | | | |
| All | 1[g] | 0.1 (0.0–0.5) | 1[g] | 0.1 (0.0–0.5) |
| **Serious adverse event during entire study period[h]** | | | | |
| All | 22 | 1.8 (1.1–2.7) | 21 | 1.7 (1.1–2.6) |

Abbreviations: CI, confidence interval; IIV4, inactivated quadrivalent influenza vaccine; N, number of participants included in analysis.

[a]For solicited injection site and general symptoms, only children for whom diary cards were returned are included (injection site symptoms: N = 1156 for double-dose IIV4 and N = 1151 for standard-dose IIV4; general symptoms: N = 1159 for double-dose IIV4 and N = 1152 for standard-dose IIV4).

[b]All solicited injection-site symptoms were considered related to vaccination.

[c]Grade 3 events were defined as follows: pain: child cried when the limb was moved or the limb was spontaneously painful; redness and swelling: >100 mm surface diameter; drowsiness and irritability/fussiness: prevented normal activity; loss of appetite: did not eat at all; spontaneously reported symptom: prevented normal activity.

[d]In the double-dose group, seizures occurred 5, 50, 88, 106, and 168 days after the first vaccine dose. In the standard-dose group, 1 seizure occurred 178 days after the first vaccine dose and the others 39, 74, and 80 days after the second vaccine dose. All children recovered, and none of the seizures was considered by the investigator to be related to vaccination.

[e]Hospitalization, emergency room visit, medical practitioner visit.

[f]Autoimmune diseases and other inflammatory and/or neurologic disorders that may or may not have an autoimmune etiology, according to a protocol-specified list or investigators' judgment.

[g]Kawasaki's disease in the double-dose group and erythema multiforme in the standard-dose group, neither related to vaccination.

[h]Serious adverse events were defined as any untoward medical occurrence that results in death, is life-threatening, requires hospitalization or prolongs hospitalization, or results in disability or incapacity.

those of a phase II study comparing GSK's double-dose IIV4 with the United States-approved standard-dose inactivated trivalent influenza vaccine (IIV3) (the corresponding IIV3 to the licensed IIV4 comparator used in the present study) [26]. In the phase II study, the immune response with the double-dose and the standard-dose was similar against the strains common to both vaccines, but the sample size was too small to reliably detect differential immunogenicity against the vaccine B strains, especially in children 6–17 months of age [26]. Two other small studies compared the immunogenicity of the United States-approved IIV3 administered as a standard or double-dose to young children in different years, with contrasting results [21, 29]. A 2008–09 trial found that a double-dose IIV3 elicited a higher immune response than a standard-dose in vaccine-unprimed children 6–23 months of age, reaching statistical significance in children 6–11 months of age for 2 of 3 vaccine strains [21]. However, a 2010–12 trial found no difference in immunogenicity between standard-dose versus double-dose IIV3s in unprimed children [29]. This prior experience highlights the necessity for trials of adequate size to reliably establish treatment benefit, and it suggests that observations made in 1 year may not be repeated in other years, because the baseline

immunity of young children may vary. Furthermore, the dose effect on immunogenicity among IIVs may differ according to their manufacturing process [23, 26].

In the present study, the double-dose and standard-dose IIV4s had a similar reactogenicity profile despite the higher antigen content and volume of the double-dose. Injection site symptoms, including pain, occurred at a similar rate in both groups. There was no difference in the rate of fever over the 2-day postvaccination period between the 2 groups. Febrile seizures occurred at a similar rate in both groups, none were reported within 2 days of vaccination, and none were considered related to the vaccine. The finding that the higher antigen dose and volume in this study did not adversely affect tolerability in children confirms previous findings from studies comparing reactogenicity and safety of double-dose versus standard-dose IIVs [21–23, 26, 29], and IIV4s versus IIV3s [26, 27, 30–32].

## CONCLUSIONS

In conclusion, a double-dose IIV4 may afford greater protection in young children against influenza B. Increased protection against influenza B, a potentially serious and life-threatening illness particularly in young children [33], would be a beneficial clinical outcome. Use of the same vaccine dose for all eligible ages would also simplify the annual influenza vaccine campaign and reduce cost [34] and logistic complexity. This study provides evidence to support a change in clinical practice to use a double-dose IIV4 (15 μg per antigen) in all children 6 months of age and older, once that dosing for a vaccine product has been approved.

### Supplementary Data

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online.

### Notes

***Acknowledgments.*** We are indebted to the participating study volunteers and their parents, clinicians, nurses, and laboratory technicians at the study sites. In particular, we thank Christopher Chambers, Larkin Wadsworth, Wendy Daly, William Johnston, Michael Levin, William Douglas, Agnes Schultz, Edward Zissman, Terry Poling, Paul Bernhardson, Brad Brabec, Stephen Russel, and Mary Tipton who provided support and cared for study participants. We thank the teams of GSK Vaccines, in particular, Silvija Jarnjak, Arshad Amanullah, Els Praet, and Rafik Bekkat-Berkani. Finally, we thank Mary L. Greenacre (An Sgriobhadair, UK, on behalf of GSK Vaccines) for providing medical writing services and Bruno Dumont and Véronique Gochet (Business and Decision Life Sciences, on behalf of GSK Vaccines) for editorial assistance and manuscript coordination.

***Author contributions.*** All authors participated in the design or implementation or analysis of the study, interpretation of the study, and the development of this manuscript. All authors had full access to the data and gave final approval before submission.

***Financial support.*** This work was supported by GlaxoSmithKline Biologicals SA. GlaxoSmithKline Biologicals SA paid for all costs associated with the development of this manuscript.

***Potential conflicts of interest.*** V. K. J., L. W., and P. L. were employed by the GSK group of companies at the time of the study. V. K. J. is currently employed by the Bill and Melinda Gates Foundation. L. W. is currently employed by Merck Research Laboratory. P. L. is currently employed by Pfizer. O. O.-A., J. S., V. C., and B. L. I. are currently employed by the GSK group of companies. V. K. J., L. W., O. O.-A., P. L., and B. L. I. currently hold shares in the GSK group of companies. J. B. D. reports payments from the GSK group of companies, during the conduct of the study, and reports grants and others from the GSK group of companies, Pfizer, Sanofi Pasteur, and MedImmune for consultancy services, outside the submitted work. M. L. L. reports payments from the GSK group of companies, during the conduct of the study. N. P. K. reports payments from the GSK group of companies, during the conduct of the study, and grants from Sanofi Pasteur, Novartis, Pfizer, Protein Science, MedImmune, Merck & Co, and Nuron Biotech, outside the submitted work. B. L. H. reports payments and nonfinancial support from the GSK group of companies, during the conduct of the study. J. S. H. reports payments from the GSK group of companies, during the conduct of the study. A. C. M. reports payments from the GSK group of companies, during the conduct of the study.

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.

### References

1. O'Brien MA, Uyeki TM, Shay DK, et al. Incidence of outpatient visits and hospitalizations related to influenza in infants and young children. Pediatrics **2004**; 113:585–93.
2. Izurieta HS, Thompson WW, Kramarz P, et al. Influenza and the rates of hospitalization for respiratory disease among infants and young children. N Engl J Med **2000**; 342:232–9.
3. Molinari NA, Ortega-Sanchez IR, Messonnier ML, et al. The annual impact of seasonal influenza in the US: measuring disease burden and costs. Vaccine **2007**; 25:5086–96.
4. Bourgeois FT, Valim C, Wei JC, et al. Influenza and other respiratory virus-related emergency department visits among young children. Pediatrics **2006**; 118:e1–8.
5. Centers for Disease Control and Prevention (CDC). Influenza-associated pediatric deaths–United States, September 2010-August 2011. MMWR Morb Mortal Wkly Rep **2011**; 60:1233–8.
6. Grohskopf LA, Sokolow LZ, Olsen SJ, et al. Prevention and control of influenza with vaccines: Recommendations of the Advisory Committee on Immunization Practices, United States, 2015–16 influenza season. MMWR Morb Mortal Wkly Rep **2015**; 64:818–25.
7. Wright PF, Dolin R, La Montagne JR. From the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, the Center for Disease Control, and the Bureau of Biologics of the Food and Drug Administration. Summary of clinical trials of influenza vaccines–II. J Infect Dis **1976**; 134:633–8.
8. Wright PF, Thompson J, Vaughn WK, et al. Trials of influenza A/New Jersey/76 virus vaccine in normal children: an overview of age-related antigenicity and reactogenicity. J Infect Dis **1977**; 136(Suppl):S731–41.
9. Wright PF, Vaughn WK, Thompson J, et al. Inactivated influenza A/New Jersey/76 vaccines in children: results of a multi-center trial. Dev Biol Stand **1977**; 39:309–13.
10. Gross PA, Ennis FA, Gaerlan PF, et al. A controlled double-blind comparison of reactogenicity, immunogenicity, and protective efficacy of whole-virus and split-product influenza vaccines in children. J Infect Dis **1977**; 136:623–32.
11. Gross PA. Reactogenicity and immunogenicity of bivalent influenza vaccine in one- and two-dose trials in children: a summary. J Infect Dis **1977**; 136(Suppl):S616–25.
12. Englund JA, Walter EB, Gbadebo A, et al. Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. Pediatrics **2006**; 118:e579–85.
13. Walter EB, Neuzil KM, Zhu Y, et al. Influenza vaccine immunogenicity in 6- to 23-month-old children: are identical antigens necessary for priming? Pediatrics **2006**; 118:e570–8.
14. Walter EB, Rajagopal S, Zhu Y, et al. Trivalent inactivated influenza vaccine (TIV) immunogenicity in children 6 through 23 months of age: do children of all ages respond equally? Vaccine **2010**; 28:4376–83.
15. Bernstein DI, Zahradnik JM, DeAngelis CJ, Cherry JD. Clinical reactions and serologic responses after vaccination with whole-virus or split-virus influenza vaccines in children aged 6 to 36 months. Pediatrics **1982**; 69:404–8.
16. Bernstein DI, Zahradnik JM, DeAngelis CJ, Cherry JD. Influenza immunization in children and young adults: clinical reactions and total and IgM antibody responses after immunization with whole-virus or split-product influenza vaccines. Am J Dis Child **1982**; 136:513–7.

17. Baxter R, Jeanfreau R, Block SL, et al. A Phase III evaluation of immunogenicity and safety of two trivalent inactivated seasonal influenza vaccines in US children. Pediatr Infect Dis J **2010**; 29:924–30.
18. Nolan T, Bravo L, Ceballos A, et al. Enhanced and persistent antibody response against homologous and heterologous strains elicited by a MF59-adjuvanted influenza vaccine in infants and young children. Vaccine **2014**; 32:6146–56.
19. Li-Kim-Moy J, Booy R. The manufacturing process should remain the focus for severe febrile reactions in children administered an Australian inactivated influenza vaccine during 2010. Influenza Other Respir Viruses **2016**; 10:9–13.
20. Brady RC, Hu W, Houchin VG, et al. Randomized trial to compare the safety and immunogenicity of CSL Limited's 2009 trivalent inactivated influenza vaccine to an established vaccine in United States children. Vaccine **2014**; 32:7141–7.
21. Skowronski DM, Hottes TS, Chong M, et al. Randomized controlled trial of dose response to influenza vaccine in children aged 6 to 23 months. Pediatrics **2011**; 128:e276–89.
22. Langley JM, Vanderkooi OG, Garfield HA, et al. Immunogenicity and safety of 2 dose levels of a thimerosal-free trivalent seasonal influenza vaccine in children aged 6–35 months: a randomized, controlled trial. J Pediatric Infect Dis Soc **2012**; 1:55–63.
23. Pavia-Ruz N, Angel Rodriguez Weber M, Lau YL, et al. A randomized controlled study to evaluate the immunogenicity of a trivalent inactivated seasonal influenza vaccine at two dosages in children 6 to 35 months of age. Hum Vaccin Immunother **2013**; 9:1978–88.
24. Black S, Nicolay U, Vesikari T, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. Pediatr Infect Dis J **2011**; 30:1081–5.
25. Ng S, Fang VJ, Ip DK, et al. Estimation of the association between antibody titers and protection against confirmed influenza virus infection in children. J Infect Dis **2013**; 208:1320–4.
26. Wang L, Chandrasekaran V, Domachowske JB, et al. Immunogenicity and safety of an inactivated quadrivalent influenza vaccine in US children 6–35 months of age during 2013–2014: results from a phase II randomized trial. J Pediatric Infect Dis Soc **2016**; 5:170–9.
27. Langley JM, Wang L, Aggarwal N, et al. Immunogenicity and reactogenicity of an inactivated quadrivalent influenza vaccine administered intramuscularly to children 6 to 35 months of age in 2012–2013: a randomized, double blind, controlled, multi-centre, multi-country, clinical trial. J Pediatric Infect Dis Soc **2014**; 4:242–51.
28. Langley JM, Carmona Martinez A, Chatterjee A, et al. Immunogenicity and safety of an inactivated quadrivalent influenza vaccine candidate: a phase III randomized controlled trial in children. J Infect Dis **2013**; 208:544–53.
29. Halasa NB, Gerber MA, Berry AA, et al. Safety and immunogenicity of full-dose trivalent inactivated influenza vaccine (TIV) compared with half-dose TIV administered to children 6 through 35 months of age. J Pediatric Infect Dis Soc **2015**; 4:214–24.
30. Greenberg DP, Robertson CA, Landolfi VA, et al. Safety and immunogenicity of an inactivated quadrivalent influenza vaccine in children 6 months through 8 years of age. Pediatr Infect Dis J **2014**; 33:630–6.
31. Greenberg DP, Robertson CA, Noss MJ, et al. Safety and immunogenicity of a quadrivalent inactivated influenza vaccine compared to licensed trivalent inactivated influenza vaccines in adults. Vaccine **2013**; 31:770–6.
32. Domachowske JB, Pankow-Culot H, Bautista M, et al. A randomized trial of candidate inactivated quadrivalent influenza vaccine versus trivalent influenza vaccines in children aged 3–17 years. J Infect Dis **2013**; 207:1878–87.
33. Paddock CD, Liu L, Denison AM, et al. Myocardial injury and bacterial pneumonia contribute to the pathogenesis of fatal influenza B virus infection. J Infect Dis **2012**; 205:895–905.
34. Centers for Disease Control and Prevention. Vaccine for Children Program (VFC). CDC Vaccine Price List. Available at: www.cdc.gov/vaccines/programs/vfc/awardees/vaccine-management/price-list/. Accessed 8 February 2016.

Vaccine 37 (2019) 343–351

Contents lists available at ScienceDirect

Vaccine

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

# Immunogenicity and safety of a quadrivalent inactivated influenza vaccine in children 6–59 months of age: A phase 3, randomized, noninferiority study

Victoria A. Statler [a], Frank R. Albano [b,*], Jolanta Airey [b], Daphne C. Sawlwin [c], Alison Graves Jones [c], Vince Matassa [b], Esther Heijnen [d], Jonathan Edelman [e], Gary S. Marshall [a]

[a] Division of Pediatric Infectious Diseases, University of Louisville School of Medicine, Louisville, KY, USA
[b] Clinical Development, Seqirus Pty Ltd, Parkville, Victoria, Australia
[c] Global Pharmacovigilance and Risk Management, Seqirus Pty Ltd, Parkville, Victoria, Australia
[d] Clinical Development, Seqirus Netherlands B.V., Amsterdam, The Netherlands
[e] Clinical Development, Seqirus USA Inc., Cambridge, MA, USA

ARTICLE INFO

*Article history:*
Received 8 May 2018
Received in revised form 11 July 2018
Accepted 15 July 2018
Available online 26 July 2018

*Keywords:*
Immunogenicity
Inactivated influenza vaccine
Paediatrics
Quadrivalent influenza vaccine
Safety

ABSTRACT

*Background:* In the Southern Hemisphere 2010 influenza season, Seqirus' split-virion, trivalent inactivated influenza vaccine was associated with increased reports of fevers and febrile reactions in young children. A staged clinical development program of a quadrivalent vaccine (Seqirus IIV4 [S-IIV4]; Afluria® Quadrivalent/Afluria Quad™/Afluria Tetra™), wherein each vaccine strain is split using a higher detergent concentration to reduce lipid content (considered the cause of the increased fevers and febrile reactions), is now complete.

*Methods:* Children aged 6–59 months were randomized 3:1 and stratified by age (6–35 months/36–59 months) to receive S-IIV4 (n = 1684) or a United States (US)-licensed comparator IIV4 (C-IIV4; Fluzone® Quadrivalent; n = 563) during the Northern Hemisphere 2016–2017 influenza season. The primary objective was to demonstrate noninferior immunogenicity of S-IIV4 versus C-IIV4. Immunogenicity was assessed by hemagglutination inhibition (baseline, 28 days postvaccination). Solicited, unsolicited, and serious adverse events were assessed for 7, 28, and 180 days postvaccination, respectively.

*Results:* S-IIV4 met the immunogenicity criteria for noninferiority. Adjusted geometric mean titer ratios (C-IIV4/S-IIV4) for the A/H1N1, A/H3N2, B/Yamagata, and B/Victoria strains were 0.79 (95% CI: 0.72, 0.88), 1.27 (1.15, 1.42), 1.12 (1.01, 1.24), and 0.97 (0.86, 1.09), respectively. Corresponding values for differences in seroconversion rates (C-IIV4 minus S-IIV4) were −10.3 (−15.4, −5.1), 2.6 (−2.5, 7.8), 3.1 (−2.1, 8.2), and 0.9 (−4.2, 6.1). Solicited, unsolicited, and serious adverse events were similar between vaccines in both age cohorts, apart from fever. Fever rates were lower with S-IIV4 (5.8%) than C-IIV4 (8.4%), with no febrile convulsions reported with either vaccine during the 7 days postvaccination.

*Conclusion:* S-IIV4, manufactured with a higher detergent concentration, demonstrated noninferior immunogenicity to the US-licensed C-IIV4, with similar postvaccination safety and tolerability, in children aged 6–59 months. This completes the program demonstrating the immunogenicity and safety of S-IIV4 in participants aged 6 months and older.

*Funding:* Seqirus Pty Ltd; **ClinicalTrials.gov identifier:** NCT02914275.



## 1. Introduction

Seasonal influenza vaccination is recommended for all persons in the United States (US), including children as young as 6 months of age [1]. Estimates suggest that in the 2014–2015 influenza season, vaccination prevented 1.9 million influenza illnesses and 67,000 influenza-associated hospitalizations in the US; the estimated 40 million cases of influenza that season underscores the continual need for a robust supply of effective vaccines [2]. This

*Abbreviations:* AE, adverse event; C-IIV4, comparator quadrivalent inactivated influenza vaccine; CBER, Center for Biologics Evaluation and Research; CI, confidence interval; DSMB, Data and Safety Monitoring Board; eDiary, electronic diary; FAS, full analysis set; FDA, Food and Drug Administration; GMFI, geometric mean fold increase; GMT, geometric mean titer; HA, hemagglutinin; HI, hemagglutination inhibition; S-IIV3, Seqirus trivalent inactivated influenza vaccine; S-IIV4, Seqirus quadrivalent inactivated influenza vaccine; SAE, serious adverse event; SCR, seroconversion rate; SD, standard deviation; US, United States; VIC, Victoria; YAM, Yamagata.

* Corresponding author at: Clinical Development, Seqirus Pty Ltd, 63 Poplar Rd, Parkville, Victoria 3052, Australia.
*E-mail address:* frank.albano@seqirus.com (F.R. Albano).

https://doi.org/10.1016/j.vaccine.2018.07.036
0264-410X/

is especially true for young children, as only a few products are licensed in this age group.

The Seqirus trivalent inactivated influenza vaccine (S-IIV3), used in the Southern Hemisphere during the 2010 influenza season, was associated with increased reports of fevers and febrile seizures in children, especially those <5 years of age; as a result, S-IIV3 was not recommended for continued use in this age group [3,4]. Investigations by Seqirus identified residual lipid under the previous splitting conditions as a likely cause of the fevers [5]. *In vitro* studies showed that increasing the concentration of the detergent used to split the virus reduced the lipid content and the pyrogenicity of the vaccine [5]. Accordingly, the concentration of splitting agent used in the manufacturing process for the Seqirus quadrivalent inactivated influenza vaccine (S-IIV4) was increased. The immunogenicity and safety of S-IIV4 were evaluated in two previous phase 3 randomized studies involving adults aged ≥18 years [6] and children aged 5–17 years [7]; S-IIV4 showed similar immunogenicity, safety, and fever rates to US-licensed comparator vaccines. The objective of the current study was to assess the safety and immunogenicity of S-IIV4 compared with a US-licensed IIV4 in children 6–59 months of age.

## 2. Materials and methods

### 2.1. Study design

This phase 3, randomized, observer-blind, controlled, multicenter study (ClinicalTrials.gov identifier: NCT02914275) evaluated the immunogenicity and safety of S-IIV4 compared with a US-licensed comparator IIV4 (C-IIV4), both containing the four influenza strains recommended for the Northern Hemisphere 2016–2017 influenza season [2]. The study was conducted at 39 US sites between September 2016 and August 2017. The protocol was approved by the relevant Institutional Review Board at each study site, and the study was conducted in accordance with the Declaration of Helsinki [8], International Conference of Harmonisation – Good Clinical Practice [9], and all applicable laws and regulations. Written informed consent was obtained from parents/guardians before any study-related procedures were performed.

### 2.2. Study population

Healthy children 6–59 months of age were enrolled. Children were excluded if they were febrile (axillary temperature ≥ 99.5 °F [≥37.5 °C]), acutely ill, immunocompromised, or allergic to egg proteins or any study vaccine component. Children were also excluded if they had a history of serious adverse reactions to any influenza vaccine; a known coagulation disorder; a history of seizures (with the exception of a single febrile seizure); or had received any influenza vaccine within the last 6 months, any immunoglobulin or blood product within the last 3 months, an investigational product within the last 28 days, or any licensed vaccine within the last 21 days.

### 2.3. Randomization

Participants were randomized 3:1 (interactive response technology system) to receive either S-IIV4 or C-IIV4. Randomization was stratified by age (6–35 month cohort and 36–59 month cohort), with no more than 60% of the total sample size represented in either age cohort. Enrollment was staged by age cohort; approximately one third of participants in the 36–59 month cohort were to have received their first vaccination and provided ≥7 days of postvaccination safety data such that an interim safety analysis could be conducted before enrollment of the 6–35 month cohort.

### 2.4. Vaccines and vaccination schedule

Participants in the 6–35 month cohort received 0.25 mL of vaccine and those in the 36–59 month cohort received 0.5 mL. For S-IIV4 (Afluria® Quadrivalent/Afluria Quad™/Afluria Tetra™, Seqirus Pty Ltd), the respective lot numbers were 090403501 and 090403502. During manufacturing, each vaccine strain was split using 1.5% w/v sodium taurodeoxycholate. For C-IIV4 (Fluzone® Quadrivalent, Sanofi Pasteur), the lot numbers were UT5583UA, UT5583MA, and UT5663UA (0.25 mL dose) and UI683AA and UI693AA (0.5 mL dose). Each 0.25 mL dose of either vaccine contained 7.5 mcg of hemagglutinin (HA) from each influenza virus strain, and each 0.5 mL dose contained 15 mcg of HA from each influenza virus strain (A/California/7/2009 [H1N1] pdm09-like virus; A/Hong Kong/4801/2014 [H3N2]-like virus; B/Phuket/3073/2013-like virus [Yamagata lineage]; B/Brisbane/60/2008-like virus [Victoria lineage]). Participants received one dose (Day 1, vaccination-experienced participants) or two doses (Day 1 and Day 29; vaccination-naïve participants) [10]. Vaccines were administered intramuscularly in the deltoid area or anterolateral aspect of the thigh, and participants were observed for 30 min postvaccination.

## 3. Immunogenicity

### 3.1. Primary endpoints

The primary immunogenicity objective was to demonstrate that vaccination with S-IIV4 elicits an immune response that is noninferior to C-IIV4 28 days after the last vaccination in participants 6–59 months of age. The eight co-primary immunogenicity endpoints were hemagglutination inhibition (HI) geometric mean titer (GMT) ratio and difference in seroconversion rate (SCR) for each of the four viral strains. The HI GMT ratio was defined as the geometric mean of the postvaccination (28 days after last vaccination) HI titer for C-IIV4 divided by the geometric mean of the postvaccination HI titer for S-IIV4. The SCR was defined as the percentage of participants with either a prevaccination HI titer < 1:10 and a postvaccination HI titer ≥1:40, or a prevaccination HI titer ≥1:10 and a ≥4-fold increase in postvaccination HI titer [11]. The difference in SCR was the C-IIV4 SCR minus the S-IIV4 SCR.

Blood samples were collected for HI assay before the first study vaccination (Day 1) and ≥28 days after the last study vaccination (at or after Day 29 for participants receiving a single dose; at or after Day 57 for participants receiving two doses).

### 3.2. Secondary endpoints

Immunogenicity was assessed in the overall study population and separately in the two age cohorts. HI antibody titers for each viral strain were used to calculate GMTs, SCRs, percentage of participants with an HI titer ≥1:40, and geometric mean fold increase (GMFI) in antibody titer (the geometric mean of the fold increase of postvaccination HI antibody titer divided by the prevaccination HI antibody titer).

## 4. Safety

Safety and tolerability were assessed in the overall study population and two age cohorts. Using an electronic diary (eDiary), parents/guardians recorded participants' daily axillary temperature and the occurrence and intensity grade of any solicited local (pain, redness, or swelling at the vaccination site) and systemic (overall study population: fever, nausea and/or vomiting, diarrhea; 6–35 month cohort: loss of appetite, irritability; 36–59 month cohort:

malaise and fatigue, headache, myalgia) adverse events (AEs) for 7 days postvaccination. Fever and severe fever were defined as axillary temperature ≥99.5 °F (≥37.5 °C) and ≥101.3 °F (≥38.5 °C), respectively. Unsolicited AEs, cellulitis-like reactions, and concomitant medication occurring up to 28 days postvaccination were also recorded. Serious AEs (SAEs) and AEs of special interest (including febrile events) were collected for 180 days after the last vaccination. AEs were coded using Medical Dictionary for Regulatory Activities, Version 19.0. Participants who continued to experience an SAE at study completion were followed up until the event had resolved or stabilized. An independent Data and Safety Monitoring Board (DSMB) provided study safety oversight.

## 5. Statistical analysis

Accounting for a 10% dropout rate and a 3:1 randomization schedule, a sample size of 2222 participants (S-IIV4, n = 1667; C-IIV4, n = 555) was estimated to provide at least 80% power to demonstrate noninferiority for all eight co-primary endpoints using a one-sided alpha of 0.025 for each comparison. No adjustment for multiple endpoints was made.

Per the US Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) criteria [11], noninferiority was confirmed if: (1) the upper limit of the two-sided 95% confidence interval (CI) of the GMT ratio (C-IIV4/S-IIV4) for all four vaccine strains did not exceed 1.5; and (2) if the upper limit of the two-sided 95% CI for the difference in SCRs (C-IIV4 minus S-IIV4) for all four vaccine strains did not exceed 10%. For the GMT ratio (adjusted analysis), a general linear model was fitted on log-transformed postvaccination HI titer as the outcome variable, with vaccine, age cohort, sex, vaccination history, log-transformed prevaccination HI titer, study site, number of vaccine doses, and age-by-vaccine interaction as covariates.

The frequency and intensity of solicited and unsolicited AEs were summarized for each age cohort and by vaccine group. All solicited local adverse reactions were considered related to study vaccine; causality assessments were performed by the investigator for all other AEs. Two interim safety analyses were conducted by the DSMB after approximately one third of participants in each age cohort had received vaccination and provided ≥7 days of postvaccination safety data.

The full analysis set (FAS) was used to analyze participant characteristics and comprised all participants whose parent(s)/guardian(s) had provided informed consent and who were randomized to treatment. The per-protocol population was used for immunogenicity analyses and was defined as all participants who were vaccinated at Day 1, had prevaccination and postvaccination HI titers available, and did not have any laboratory-confirmed influenza illness, prohibited medications, or protocol deviations assessed as potentially affecting immuno-

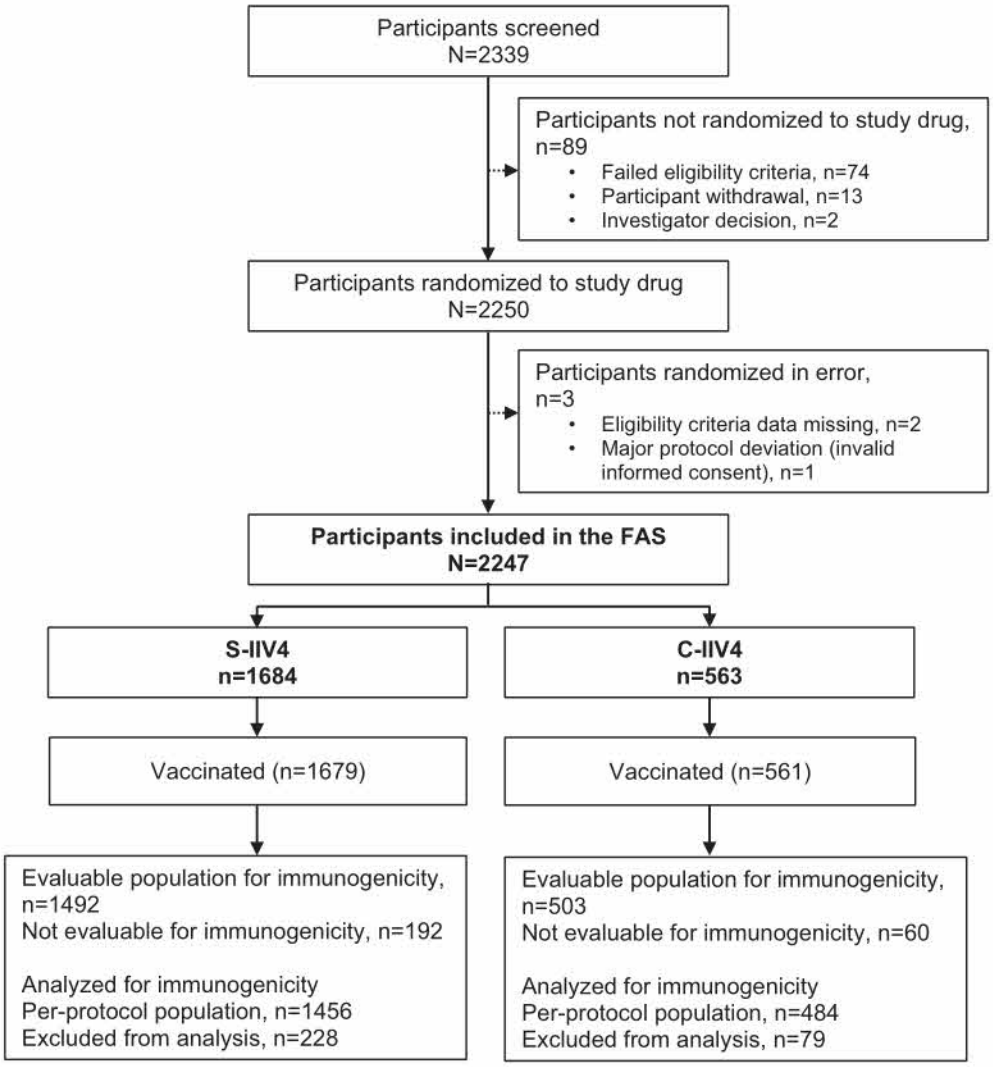



**Fig. 1.** Participant disposition. Abbreviations: C-IIV4 = comparator quadrivalent inactivated influenza vaccine; FAS = full analysis set; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine.

genicity results. The overall safety population comprised all FAS participants who received at least one dose/partial dose of study vaccine and had evaluable follow-up safety data. The solicited safety population included all FAS participants who received at least one dose/partial dose of study vaccine and had evaluable data on solicited events. Analyses were conducted with SAS Version 9.3 (SAS Institute, Inc., Cary, NC).

## 6. Results

### 6.1. Disposition and baseline characteristics

Of the 2250 participants randomized, 2247 were included in the FAS (S-IIV4, n = 1684; C-IIV4, n = 563; Fig. 1). A total of 160 participants were discontinued from the study; the main reason was

**Table 1**
Demographics and baseline clinical characteristics (full analysis set).[a]

| Characteristic | S-IIV4 | | | C-IIV4 | | | Overall |
|---|---|---|---|---|---|---|---|
| | 6–35 month cohort (n = 700) | 36–59 month cohort (n = 984) | Total (n = 1684) | 6–35 month cohort (n = 235) | 36–59 month cohort (n = 328) | Total (n = 563) | N = 2247 |
| Age, mean (SD) months | 21.8 (8.55) | 47.2 (7.02) | 36.6 (14.70) | 21.7 (8.73) | 47.1 (6.71) | 36.5 (14.68) | 36.6 (14.69) |
| Sex, n (%) | | | | | | | |
| Male | 358 (51.1) | 506 (51.4) | 864 (51.3) | 133 (56.6) | 162 (49.4) | 295 (52.4) | 1159 (51.6) |
| Female | 342 (48.9) | 478 (48.6) | 820 (48.7) | 102 (43.4) | 166 (50.6) | 268 (47.6) | 1088 (48.4) |
| Ethnicity, n (%) | | | | | | | |
| Hispanic or Latino | 190 (27.1) | 244 (24.8) | 434 (25.8) | 70 (29.8) | 90 (27.4) | 160 (28.4) | 594 (26.4) |
| Not Hispanic or Latino | 509 (72.7) | 734 (74.6) | 1243 (73.8) | 164 (69.8) | 236 (72.0) | 400 (71.0) | 1643 (73.1) |
| Not reported | 1 (0.1) | 5 (0.5) | 6 (0.4) | 1 (0.4) | 2 (0.6) | 3 (0.5) | 9 (0.4) |
| Unknown | 0 | 1 (0.1) | 1 (0.1) | 0 | 0 | 0 | 1 (<0.1) |
| Race, n (%) | | | | | | | |
| American Indian/Alaska Native | 2 (0.3) | 3 (0.3) | 5 (0.3) | 0 | 2 (0.6) | 2 (0.4) | 7 (0.3) |
| Asian | 6 (0.9) | 9 (0.9) | 15 (0.9) | 4 (1.7) | 6 (1.8) | 10 (1.8) | 25 (1.1) |
| Black or African American | 146 (20.9) | 215 (21.8) | 361 (21.4) | 44 (18.7) | 79 (24.1) | 123 (21.8) | 484 (21.5) |
| Native Hawaiian or Other Pacific Islander | 4 (0.6) | 9 (0.9) | 13 (0.8) | 0 | 3 (0.9) | 3 (0.5) | 16 (0.7) |
| White | 512 (73.1) | 693 (70.4) | 1205 (71.6) | 174 (74.0) | 217 (66.2) | 391 (69.4) | 1596 (71.0) |
| Other | 30 (4.3) | 55 (5.6) | 85 (5.0) | 13 (5.5) | 21 (6.4) | 34 (6.0) | 119 (5.3) |
| Previous vaccination | 393 (56.1) | 841 (85.5) | 1234 (73.3) | 137 (58.3) | 291 (88.7) | 428 (76.0) | 1662 (74.0) |
| In the preceding season | 349 (49.9) | 496 (50.4) | 845 (50.2) | 122 (51.9) | 172 (52.4) | 294 (52.2) | 1139 (50.7) |
| Allocated to two doses | 428 (61.1) | 249 (25.3) | 677 (40.2) | 144 (61.3) | 74 (22.6) | 218 (38.7) | 895 (39.8) |
| Weight, kg, mean (SD) | 12.21 (2.69) | 17.60 (3.65) | 15.36 (4.22) | 12.48 (3.42) | 17.49 (3.27) | 15.40 (4.15) | 15.37 (4.21) |
| Prevaccination axillary temperature, mean (SD) °F | 97.19 (0.907) | 97.15 (0.956) | 97.17 (0.936) | 97.29 (0.940) | 97.22 (0.932) | 97.25 (0.935) | 97.19 (0.936) |
| Mean °C[b] | 36.2 | 36.2 | 36.2 | 36.3 | 36.2 | 36.3 | 36.2 |

Abbreviations: C-IIV4 = comparator quadrivalent inactivated influenza vaccine; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine; SD = standard deviation.
[a] Included all participants who provided informed consent and who were randomized to treatment.
[b] Converted from Fahrenheit.

**Table 2**
Postvaccination HI antibody GMTs, SCRs, and analyses of noninferiority of S-IIV4 relative to C-IIV4 for each strain 28 days after last vaccination (per-protocol population).

| Virus strain | Postvaccination GMT (adjusted) | | Adjusted GMT Ratio[a] (95% CI) | SCR, % (95% CI)[b] | | SCR difference[c] (95% CI) | Met both predefined noninferiority criteria[d] |
|---|---|---|---|---|---|---|---|
| | S-IIV4 (n = 1456[e]) | C-IIV4 (n = 484) | | S-IIV4 (n = 1456) | C-IIV4 (n = 484) | | |
| A/H1N1 | 353.5 (n = 1455[e]) | 281.0 | 0.79 (0.72, **0.88**) | 79.1 (76.9, 81.1) | 68.8 (64.5, 72.9) | −10.3 (−15.4, **−5.1**) | Yes |
| A/H3N2 | 393.0 (n = 1454[e,f]) | 500.5 | 1.27 (1.15, **1.42**) | 82.3 (80.2, 84.2) (n = 1455[f]) | 84.9 (81.4, 88.0) | 2.6 (−2.5, **7.8**) | Yes |
| B/Yamagata | 23.7 (n = 1455[e]) | 26.5 | 1.12 (1.01, **1.24**) | 38.9 (36.4, 41.4) | 41.9 (37.5, 46.5) | 3.1 (−2.1, **8.2**) | Yes |
| B/Victoria | 54.6 (n = 1455[e]) | 52.9 (n = 483 [g]) | 0.97 (0.86, **1.09**) | 60.2 (57.6, 62.7) | 61.1 (56.6, 65.4) (n = 483 [g]) | 0.9 (−4.2, **6.1**) | Yes |

Abbreviations: C-IIV4 = comparator quadrivalent inactivated influenza vaccine; CI = confidence interval; GMT = geometric mean titer; HI = hemagglutination inhibition; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine; SCR = seroconversion rate.
[a] Adjusted GMT Ratio = C-IIV4/S-IIV4. Adjusted analysis model: Log-transformed Postvaccination HI Titer = Vaccine + Age Strata [6–35 months, 36–59 months] + Sex + Vaccination History [y/n] + Log-transformed Prevaccination HI Titer + Site + Number of Doses (one vs two) + Age * Strata Vaccine. (The Age Strata by Vaccine interaction term was excluded from the model fit for the strains B/Yamagata and B/Victoria as the interaction result was non-significant [p > 0.05].) Least square means were back transformed.
[b] SCR was defined as the percentage of participants with either a prevaccination HI titer < 1:10 and a postvaccination HI titer ≥ 1:40 or a prevaccination HI titer ≥ 1:10 and a 4-fold increase in postvaccination HI titer.
[c] SCR difference = C-IIV4 SCR percentage minus S-IIV4 SCR percentage.
[d] Noninferiority criterion for the GMT ratio: upper bound of two-sided 95% CI on the GMT ratio of C-IIV4/S-IIV4. GMT should not exceed 1.5. Noninferiority criterion for the SCR difference: upper bound of two-sided 95% CI on the difference between SCR C-IIV4 minus S-IIV4 should not exceed 10%.
[e] Results from three participants were excluded from the primary analysis due to a lack of information (n = 1; unknown prevaccination history),
[f] missing A/H3N2 postvaccination titer (n = 1),
[g] or missing B/Victoria prevaccination titer (n = 1).

loss-to-follow-up (n = 113). No participants discontinued due to AEs. Baseline characteristics were generally well matched between vaccine groups and within age cohorts (Table 1).

### 6.2. Immunogenicity

S-IIV4 was noninferior to C-IIV4 in participants 6–59 months of age (Table 2). For all strains, the upper limit of the two-sided 95% CI did not exceed the prespecified noninferiority margin of 1.5 for the GMT ratios (adjusted analysis; Fig. 2A) or 10% for the difference in SCR between vaccines (Fig. 2B). Both study vaccines elicited strong immune responses against the respective vaccine strains in children 6–59 months of age (Table 2). Postvaccination HI GMTs for both vaccines were higher for A strains than B strains, and significantly higher for S-IIV4 relative to C-IIV4 for the A/H1N1 strain. In contrast, postvaccination HI GMTs were significantly higher for C-IIV4 relative to S-IIV4 for the A/H3N2 and B/Yamagata strains. Postvaccination HI GMTs were similar between vaccines for the B/Victoria strain. Postvaccination SCRs were similar between S-IIV4 and C-IIV4, and were higher for A strains than B strains for both vaccines.

Immune responses were similar across age cohorts and vaccine groups (Table 3) and were higher for A strains than B strains. Postvaccination immune responses to B strains were higher in the older age cohort than the younger age cohort for both vaccines.

## 7. Safety and tolerability

### 7.1. Overall safety events

Both vaccines were well tolerated. In the overall safety population (n = 2232), 65.2% of participants reported at least one AE (combined solicited and unsolicited AEs), with most participants experiencing AEs of mild (35.1%) or moderate (23.1%) intensity.

### 7.2. Solicited adverse events

In the solicited safety population (n = 2163), solicited AEs (combined local and systemic solicited AEs) were reported by 58.1% and 57.2% of participants in the S-IIV4 and C-IIV4 groups, respectively.

Similar proportions of participants experienced solicited local adverse reactions in the two vaccine groups (S-IIV4, 39.9%; C-IIV4, 38.2%). The most common solicited local adverse reaction in both vaccine groups in the overall study population was vaccination-site pain (S-IIV4, 24.9%; C-IIV4: 24.0%). Vaccination-site pain was also the most common solicited local adverse reaction in both vaccine groups in the two age cohorts (along with redness in the 6–35 month cohort) (Table 4). Severe local adverse reactions were more common in the C-IIV4 group

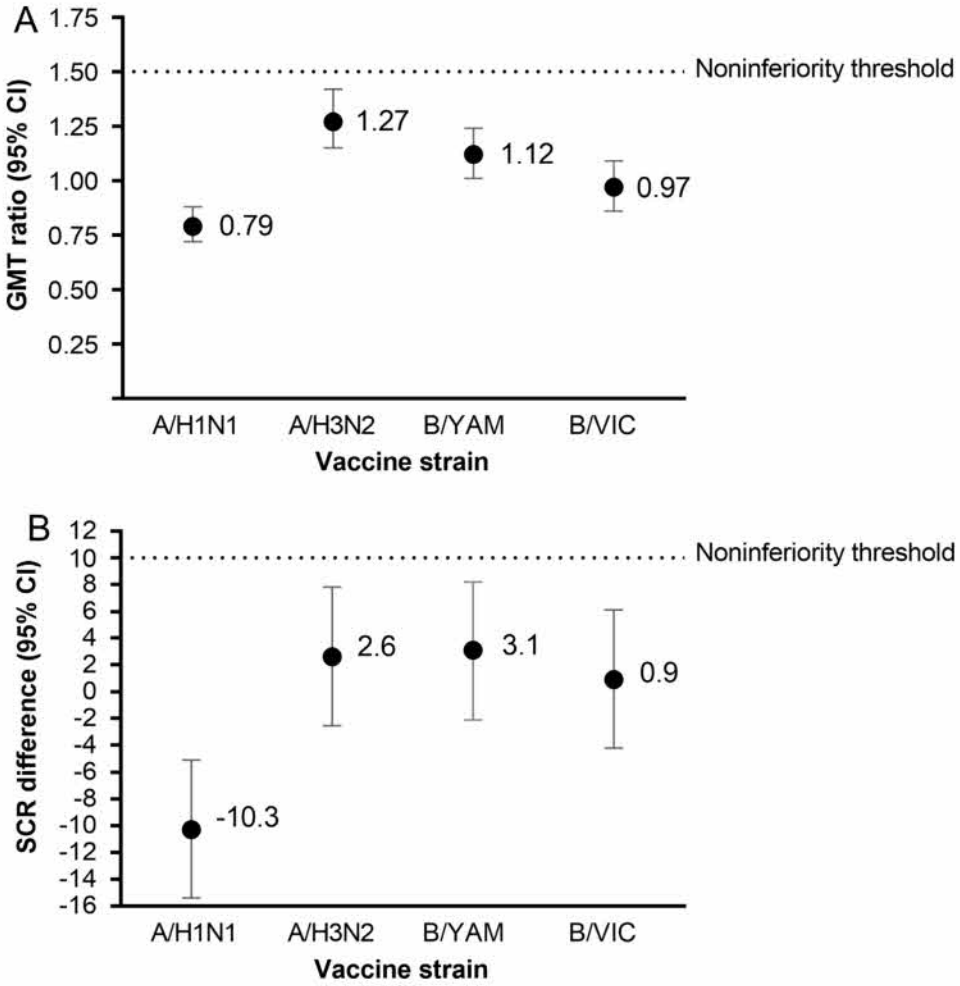



**Fig. 2.** Noninferiority analysis of S-IIV4 versus C-IIV4 in participants 6–59 months of age (per-protocol population for immunogenicity). Panel A: adjusted geometric mean titer ratio. Panel B: difference in seroconversion rates. Abbreviations: C-IIV4 = comparator quadrivalent inactivated vaccine; CI = confidence interval; GMT = geometric mean titer; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine; SCR = seroconversion rate; VIC = Victoria; YAM = Yamagata. Note: The error bars indicate the two-sided 95% CIs. The dashed line represents the margin of noninferiority. Noninferiority criterion for the GMT ratio: upper bound of two-sided 95% CI on the ratio of C-IIV4/S-IIV4 for all four vaccine strains should not exceed 1.5. Noninferiority criterion for the SCR difference: upper bound of two-sided 95% CI on the difference between SCR C-IIV4 minus S-IIV4 for all four vaccine strains should not exceed 10%.

**Table 3**
Immune responses against each vaccine strain overall and according to age cohorts (per-protocol population).[a]

| Virus strain | 6–35 Month Cohort | | 36–59 Month Cohort | | Overall | |
|---|---|---|---|---|---|---|
| | S-IIV4 (n = 586) | C-IIV4 (n = 193) | S-IIV4 (n = 870) | C-IIV4 (n = 291) | S-IIV4 (n = 1456) | C-IIV4 (n = 484) |
| **A/H1N1** | | | | | | |
| GMT (95% CI) | 184.9 (165.15, 207.05) | 168.3 (137.69, 205.62) | 590.2 (548.62, 634.93) | 469.2 (413.72, 532.05) | 370.0 (345.13, 396.55) | 311.7 (276.88, 350.90) |
| GMFI[b] (95% CI) | 13.4 (11.98, 14.90) | 11.3 (9.40, 13.49) | 9.7 (8.90, 10.64) | 6.8 (5.83, 7.99) | 11.1 (10.31, 11.85) | 8.3 (7.39, 9.40) |
| Percentage of participants with an HI titer ≥ 1:40, % (95% CI) | 90.1 (87.4, 92.4) | 88.6 (83.3, 92.7) | 99.1 (98.2, 99.6) | 98.3 (96.0, 99.4) | 95.5 (94.3, 96.5) | 94.4 (92.0, 96.3) |
| Seroconversion[c], % (95% CI) | 81.9 (78.6, 84.9) | 80.3 (74.0, 85.7) | 77.1 (74.2, 79.9) | 61.2 (55.3, 66.8) | 79.1 (76.9, 81.1) | 68.8 (64.5, 72.9) |
| **A/H3N2** | | | | | | |
| GMT (95% CI) | 184.9 (164.57, 207.65) (n = 585) | 247.5 (202.14, 302.95) | 778.6 (710.83, 852.82) | 1047 (911.69, 1202.48) | 436.8 (403.04, 473.28) (n = 1455) | 589.1 (516.42, 671.91) |
| GMFI[b] (95% CI) | 13.0 (11.62, 14.50) | 15.1 (12.60, 18.08) | 12.5 (11.42, 13.62) | 16.0 (13.62, 18.74) | 12.7 (11.83, 13.58) (n = 1455) | 15.6 (13.86, 17.60) |
| Percentage of participants with an HI titer ≥1:40, % (95% CI) | 92.5 (90.0, 94.5) (n = 585) | 95.3 (91.3, 97.8) | 98.4 (97.3, 99.1) | 98.6 (96.5, 99.6) | 96.0 (94.8, 97.0) (n = 1455) | 97.3 (95.5, 98.6) |
| Seroconversion[c], % (95% CI) | 82.4 (79.1, 85.4) (n = 585) | 85.0 (79.1, 89.7) | 82.2 (79.5, 84.7) | 84.9 (80.2, 88.8) | 82.3 (80.2, 84.2) (n = 1455) | 84.9 (81.4, 88.0) |
| **B/Yamagata** | | | | | | |
| GMT (95% CI) | 15.6 (14.33, 17.00) | 16.3 (14.03, 18.95) | 35.4 (32.73, 38.26) | 44.1 (38.30, 50.87) | 25.5 (23.93, 27.06) | 29.7 (26.51, 33.21) |
| GMFI[b] (95% CI) | 2.6 (2.45, 2.86) | 2.8 (2.45, 3.19) | 4.5 (4.17, 4.77) | 5.3 (4.67, 6.00) | 3.6 (3.43, 3.81) | 4.1 (3.73, 4.52) |
| Percentage of participants with an HI titer ≥1:40, % (95% CI) | 24.7 (21.3, 28.4) | 29.0 (22.7, 36.0) | 57.1 (53.8, 60.4) | 61.5 (55.7, 67.1) | 44.1 (41.5, 46.7) | 48.6 (44.0, 53.1) |
| Seroconversion[c], % (95% CI) | 22.5 (19.2, 26.1) | 26.9 (20.8, 33.8) | 49.9 (46.5, 53.3) | 51.9 (46.0, 57.8) | 38.9 (36.4, 41.4) | 41.9 (37.5, 46.5) |
| **B/Victoria** | | | | | | |
| GMT (95% CI) | 39.8 (36.02, 44.04) | 31.9 (26.88, 37.81) | 72.1 (65.62, 79.25) | 85.9 (73.16, 100.96) | 56.8 (52.90, 60.96) | 57.9 (51.04, 65.62) |
| GMFI[b] (95% CI) | 5.6 (5.11, 6.09) | 4.6 (3.97, 5.42) | 7.5 (6.93, 8.15) | 8.2 (7.18, 9.42) (n = 290) | 6.7 (6.27, 7.08) | 6.5 (5.89, 7.27) (n = 483) |
| Percentage of participants with an HI titer ≥1:40, % (95% CI) | 55.6 (51.5, 59.7) | 52.8 (45.6, 60.1) | 71.0 (67.9, 74.0) | 75.3 (69.9, 80.1) | 64.8 (62.3, 67.2) | 66.3 (61.9, 70.5) |
| Seroconversion[c], % (95% CI) | 52.9 (48.8, 57.0) | 49.7 (42.5, 57.0) | 65.1 (61.8, 68.2) | 68.6 (62.9, 73.9) (n = 290) | 60.2 (57.6, 62.7) | 61.1 (56.6, 65.4) (n = 483) |

Abbreviations: C-IIV4 = comparator inactivated influenza vaccine; CI = confidence interval; GMFI = geometric mean fold increase; HI = hemagglutination inhibition; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine.

[a] Defined as all participants who received one dose of the study vaccine and had prevaccination and postvaccination titers available. These participants did not have any protocol deviations that were medically assessed as potentially impacting on immunogenicity results (n = 1940).

[b] GMFI was defined as the geometric mean of the fold increase of postvaccination HI antibody titer over the prevaccination HI antibody titer.

[c] Seroconversion rates were defined as percentage of participants with either a prevaccination HI titer <1:10 and a postvaccination HI titer ≥1:40 or a prevaccination titer ≥1:10 and a ≥4-fold increase in postvaccination titer.

**Table 4**
Adverse events (all grades) experienced after vaccination according to age cohorts (solicited safety population).

| **6–35 Month cohort** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | S-IIV4 (n = 669) | | | | C-IIV4 (n = 227) | | | | Relative risk (95% CI)[a] |
| | Mild | Moderate | Severe | All grades | Mild | Moderate | Severe | All grades | |
| **Solicited Local Adverse Reactions[b] After Any Vaccination, %[c]** | | | | | | | | | |
| Any | 23.7 | 8.4 | 0.7 | 32.9 | 23.5 | 8.0 | 2.7 | 34.4 | 0.96 (0.78, 1.18) |
| Pain | 15.2 | 5.4 | 0.1 | 20.8 | 19.8 | 5.3 | 0.4 | 25.6 | 0.81 (0.62, 1.06) |
| Swelling | 4.5 | 1.2 | 0.4 | 6.1 | 4.4 | 0.9 | 0.9 | 6.2 | 0.99 (0.55, 1.79) |
| Redness | 16.6 | 3.4 | 0.6 | 20.8 | 13.7 | 1.8 | 1.8 | 17.6 | 1.18 (0.86, 1.62) |
| **Solicited Systemic AEs[d] After Any Vaccination, %[c]** | | | | | | | | | |
| Any | 28.3 | 17.5 | 3.1 | 48.9 | 32.6 | 13.2 | 4.0 | 49.8 | 0.98 (0.84, 1.14) |
| Irritability | 19.0 | 13.2 | 0.7 | 32.9 | 16.7 | 11.0 | 0.4 | 28.2 | 1.17 (0.92, 1.47) |
| Loss of appetite | 15.8 | 3.9 | 0.3 | 20.0 | 16.3 | 2.6 | 0.4 | 19.4 | 1.03 (0.76, 1.40) |
| Nausea and/or vomiting | 4.6 | 4.0 | 0.7 | 9.4 | 9.3 | 1.8 | 0 | 11.0 | 0.86 (0.55, 1.33) |
| Diarrhea | 19.4 | 4.6 | 0.1 | 24.2 | 22.5 | 2.6 | 0.4 | 25.6 | 0.95 (0.73, 1.23) |
| Fever | 3.1 | 1.5 | 2.5 | 7.2 | 6.6 | 2.6 | 2.6 | 11.9 | 0.60 (0.39, 0.94) |
| **36–59 Month Cohort** | | | | | | | | | |
| | S-IIV4 (n = 949) | | | | C-IIV4 (n = 318) | | | | Relative Risk (95% CI)[a] |
| | Mild | Moderate | Severe | All grades | Mild | Moderate | Severe | All grades | |
| **Solicited Local Adverse Reactions[b] After Any Vaccination, %[c]** | | | | | | | | | |
| Any | 35.0 | 7.0 | 2.7 | 44.8 | 26.2 | 8.8 | 5.7 | 40.9 | 1.10 (0.94, 1.27) |
| Pain | 31.7 | 3.8 | 0 | 35.5 | 27.0 | 3.8 | 0.6 | 31.4 | 1.13 (0.94, 1.36) |
| Swelling | 5.8 | 2.6 | 1.7 | 10.1 | 4.4 | 5.7 | 2.5 | 12.9 | 0.78 (0.56, 1.11) |
| Redness | 16.5 | 3.5 | 2.3 | 22.4 | 10.7 | 4.7 | 5.3 | 20.8 | 1.08 (0.85, 1.38) |
| **Solicited Systemic AEs[d] After Any Vaccination, %[c]** | | | | | | | | | |
| Any | 21.8 | 8.4 | 2.0 | 32.2 | 24.8 | 5.7 | 1.6 | 32.1 | 1.01 (0.84, 1.21) |
| Headache | 4.4 | 1.4 | 0.4 | 6.2 | 4.7 | 0.3 | 0 | 5.0 | 1.24 (0.72, 2.12) |
| Myalgia | 7.9 | 1.9 | 0.1 | 9.9 | 8.2 | 1.3 | 0 | 9.4 | 1.05 (0.71, 1.55) |
| Malaise and fatigue | 8.3 | 5.5 | 0.5 | 14.3 | 8.5 | 4.4 | 0.3 | 13.2 | 1.09 (0.79, 1.50) |
| Nausea and/or vomiting | 5.3 | 3.5 | 0.4 | 9.2 | 4.1 | 2.2 | 0.3 | 6.6 | 1.39 (0.88, 2.20) |
| Diarrhea | 10.3 | 1.7 | 0.1 | 12.1 | 7.5 | 0.6 | 0.6 | 8.8 | 1.38 (0.93, 2.04) |
| Fever | 2.6 | 1.1 | 1.2 | 4.8 | 3.8 | 1.3 | 0.9 | 6.0 | 0.81 (0.48, 1.36) |

Abbreviations: AE = adverse event; C-IIV4 = comparator quadrivalent inactivated influenza vaccine; CI = confidence interval; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine.

[a] Relative risk for S-IIV4 compared to C-IIV4 = proportion of participants with a given symptom in the IIV4 group/proportion of participants with a given symptom in the C-IIV4 group. If the value 1 is not in the range of the CI, it can be concluded that the proportions are significantly different between the two groups, and that there is an increased risk in one group compared to the other.

[b] Solicited local adverse reactions: pain at the vaccination site was graded as none (Grade 0), mild (Grade 1; does not interfere with daily activities), moderate (Grade 2; interferes with daily activities), and severe (Grade 3; prevents daily activity). Swelling and redness was graded by size as absent (Grade 0), mild (Grade 1; <10 mm), moderate (Grade 2; ≥10 mm to ≤30 mm), and severe (Grade 3; >30 mm).

[c] Proportion of subjects based on the number of participants in the respective group.

[d] Solicited systemic adverse events: nausea, vomiting, diarrhea, headache, malaise and fatigue, and myalgia were graded as none (Grade 0), mild (Grade 1; does not interfere with daily activities), moderate (Grade 2; interferes with daily activities), and severe (Grade 3; prevents daily activities). Fever (axillary) was graded as absent (Grade 0; <99.5 °F [<37.5 °C]), mild (Grade 1; ≥99.5 °F to <100.4 °F [≥37.5 to <38.0 °C]), moderate (Grade 2; ≥100.4 °F to 101.3 °F [≥38.0 to <38.5 °C]), and severe (Grade 3; ≥101.3 °F [≥38.5 °C]).

than the S-IIV4 group in the 6–35 month cohort (S-IIV4, 0.7%; C-IIV4, 2.7%) and the 36–59 month cohort (S-IIV4, 2.7%; C-IIV4, 5.7%).

Similar proportions of participants experienced solicited systemic AEs in the two vaccine groups (S-IIV4, 39.1%; C-IIV4, 39.4%). Irritability was the most common solicited systemic AE in both vaccine groups in the overall study population (S-IIV4, 32.9%; C-IIV4, 28.2%), as well as in the 6–35 month cohort. Malaise and fatigue was the most common solicited systemic AE in the 36–59 month cohort (Table 4). The rate of severe systemic AEs was similar in the two vaccine groups in the 6–35 month cohort (S-IIV4, 3.1%; C-IIV4, 4.0%) and in the 36–59 month cohort (S-IIV4, 2.0%; C-IIV4, 1.6%).

For both S-IIV4 and C-IIV4, systemic AEs were more common than local adverse reactions in the 6–35 month cohort, whereas local adverse reactions were more common than systemic AEs in the 36–59 month cohort (Table 4).

### 7.3. Fever

Fever of any grade was less likely to occur with S-IIV4 (5.8%) than with C-IIV4 (8.4%) in the overall study population (relative risk: 0.69; 95% CI; 0.49, 0.97). Fever of any grade was also less likely to occur with S-IIV4 than C-IIV4 in the 6–35 month cohort; the rates of fever of any grade were similar between the two vaccines in the 36–59 month cohort (Table 4).

The rates of severe fever were similar between the S-IIV4 and C-IIV4 groups (1.7% in each group) in the overall study population. The rates of severe fever were also similar between S-IIV4 and C-IIV4 in the two age cohorts (Table 4).

No febrile convulsions were observed with either vaccine within 7 days following vaccination (considered the risk window for febrile convulsions related to influenza vaccine). Two febrile convulsions occurred in the 6–35 month cohort in the S-IIV4 group >7 days after the vaccination (Days 43 and 104); both were assessed as unrelated to the study vaccine.

### 7.4. Unsolicited adverse events

Unsolicited AEs were reported by 32.0% and 30.6% of participants in the S-IIV4 and C-IIV4 groups, respectively. The most common unsolicited AEs (≥1% overall) reported were cough (S-IIV4, 8.8%; C-IIV4, 7.2%) and rhinorrhea (S-IIV4, 7.5%; C-IIV4, 9.3%).

### 7.5. Serious adverse events

Overall, 15 SAEs were reported in 14 participants; all were assessed as unrelated to the study vaccine. The nature of the SAEs reported was consistent with illnesses (such as respiratory tract infections) and injuries commonly occurring in this age group.

## 8. Discussion

In this study of children 6–59 months of age, similar immune responses were demonstrated for S-IIV4 and C-IIV4 for all four strains, as assessed by GMTs, SCRs, percentage of participants with an HI titer $\geq 40$, and GMFIs. The FDA CBER criteria for noninferiority of immunogenicity were met for all eight co-primary immunogenicity endpoints for all four strains. S-IIV4 was well tolerated and had a similar safety profile to C-IIV4, except for any grade fever, which occurred less frequently with S-IIV4. These findings are consistent with results of the other S-IIV4 phase 3 studies in adults [6] and children aged 5–17 years [7].

The rates of any grade and severe fever observed with S-IIV4 were similar to or lower than those observed with C-IIV4 in the 6–35 month and 36–59 month cohorts. In the phase 3 study conducted in children aged 5–17 years, the rates of any grade and severe fever observed with S-IIV4 were numerically higher than, but not statistically different from, those observed with a different comparator IIV4 [7]. Taken together, these results indicate that the modified manufacturing process for S-IIV4 attenuated the febrile reactogenicity in young children that was associated with the S-IIV3 used in the 2010 influenza season in the Southern Hemisphere.

Strengths of the study include its prospective, randomized, multicenter design and robust recruitment; the sample size within each group was sufficient to allow for meaningful comparisons of fever, and participants without prior vaccination exposure were well represented. This study has some limitations. First, approximately 14% of participants were excluded from the per-protocol population. However, this rate of per-protocol population exclusion is not unexpected given the age of the population and is consistent with rates observed in similar studies [12–14]. Second, immunogenicity is a surrogate marker of protection and may not represent the true clinical efficacy of the vaccine (a limitation of other similar studies). Thirdly, participants with moderate or severe acute illnesses were excluded from the study; therefore, extrapolation of study results to the real-world situation, where moderately ill persons might be vaccinated, should be made with caution.

In conclusion, S-IIV4 demonstrated noninferior immunogenicity and a similar safety profile relative to a US-licensed comparator IIV4 in children 6–59 months of age. The favorable immunogenicity and safety profiles of S-IIV4 observed in this study support its potential for use in this population.

## Funding support

This study was sponsored by Seqirus Pty Ltd, Australia, manufacturer/licensee of Afluria® Quadrivalent/Afluria Quad™/Afluria Tetra™ (trademarks of Seqirus UK Limited or its affiliates). Medical writing assistance was provided by Jordana Campbell, BSc and Justine Southby, PhD, CMPP of ProScribe – Envision Pharma Group, and was funded by Seqirus Pty Ltd, Australia. ProScribe's services complied with international guidelines for Good Publication Practice (GPP3).

## Role of the sponsor

Seqirus Pty Ltd was involved in the study design, in the collection, analysis, and interpretation of the data, in the writing of the report, and in the decision to submit the article for publication.

## Role of contributors

All authors participated in the study design, interpretation of study results, and in the drafting, critical revision, and approval of the final version of the manuscript. VM supervised the statistical analysis. All authors attest they meet the ICMJE criteria for authorship.

## Conflicts of interest

VS has been an investigator for Seqirus, Merck, and Novartis. GSM has been an investigator and consultant for Seqirus, GlaxoSmithKline, Merck, Novartis, Pfizer, and Sanofi Pasteur, and has also received honoraria for educational lectures from some of these companies. AGJ is an employee of Seqirus Pty Ltd. FRA, JA, DCS, VM, EH, and JE are employees of Seqirus Pty Ltd and own shares in CSL Pty Ltd. Seqirus Pty Ltd is a subsidiary of the CSL group.

## Other contributors/acknowledgments

The authors would like to thank all investigators, site staff, and study participants.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2018.07.036.

## References

[1] Grohskopf LA, Sokolow LZ, Broder KR, Walter EB, Bresee JS, Fry AM, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the advisory committee on immunization practices – United States, 2017–18 influenza season. MMWR Recomm Rep 2017;66:1–20.

[2] Centers for Disease Control and Prevention. Estimated influenza illnesses and hospitalizations averted by vaccination — United States, 2014–15 influenza season; 2015 <https://www.cdc.gov/flu/about/disease/2014-15.htm> [accessed 30 January 2018].

[3] Therapeutic Goods Administration. Investigation into febrile reactions in young children following 2010 seasonal trivalent influenza vaccination. Status Report as at 2 July 2010. Updated 24 September 2010 <https://www.tga.gov.au/sites/default/files/alerts-medicine-seasonal-flu-100702.pdf> [accessed 24 February 2018].

[4] Therapeutic Goods Administration. Overview of vaccine regulation and safety monitoring and investigation into adverse events following 2010 seasonal influenza vaccination in young children. 8 October 2010 <https://www.tga.gov.au/sites/default/files/alerts-medicine-seasonal-flu-101008.pdf> [accessed 24 February 2018].

[5] Rockman S, Becher D, Dyson A, Koernig S, Morelli AB, Barnden M, et al. Role of viral RNA and lipid in the adverse events associated with the 2010 Southern Hemisphere trivalent influenza vaccine. Vaccine 2014;32:3869–76.

[6] Treanor JT, Albano FR, Sawlwin DC, Graves Jones A, Airey J, Formica N, et al. Immunogenicity and safety of a quadrivalent inactivated influenza vaccine compared with two trivalent inactivated influenza vaccines containing alternate B strains in adults: a phase 3, randomized noninferiority study. Vaccine 2017;35:1856–64.

[7] Airey J, Albano FR, Sawlwin DC, Jones AG, Formica N, Matassa V, et al. Immunogenicity and safety of a quadrivalent inactivated influenza virus vaccine compared with a comparator quadrivalent inactivated influenza vaccine in a pediatric population: a phase 3, randomized noninferiority study. Vaccine 2017;35:2745–52.

[8] World Medical Association Declaration of Helsinki – Ethical principles for medical research involving human subjects; 2013 <https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/> [accessed 30 January 2018].

[9] U.S. Food and Drug Administration. Guidance for industry. E6 good clinical practice: consolidated guidance; 1996 <https://clinicalcenter.nih.gov/ccc/clinicalresearch/guidance.pdf> [accessed 30 January 2018].

[10] Grohskopf LA, Sokolow LZ, Broder KR, Olsen SJ, Karron RA, Jernigan DB, et al. Prevention and control of seasonal influenza with vaccines. MMWR Recomm Rep 2016;65:1–54.

[11] U.S. Food and Drug Administration. Guidance for industry. Clinical data needed to support the licensure of pandemic influenza vaccines; 2007 <https://www.

fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091985.pdf> [accessed 30 January 2018].

[12] Domachowske JB, Pankow-Culot H, Bautista M, Feng Y, Claeys C, Peeters M, et al. A randomized trial of candidate inactivated quadrivalent influenza vaccine versus trivalent influenza vaccines in children aged 3–17 years. J Infect Dis 2013;207:1878–87.

[13] Brady RC, Hu W, Houchin VG, Eder FS, Jackson KC, Hartel GF, et al. Randomized trial to compare the safety and immunogenicity of CSL Limited's 2009 trivalent inactivated influenza vaccine to an established vaccine in United States children. Vaccine 2014;32:7141–7.

[14] Greenberg DP, Robertson CA, Landolfi VA, Bhaumik A, Senders SD, Decker MD. Safety and immunogenicity of an inactivated quadrivalent influenza vaccine in children 6 months through 8 years of age. Pediatr Infect Dis J 2014;33:630–6.