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

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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.

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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.

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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-

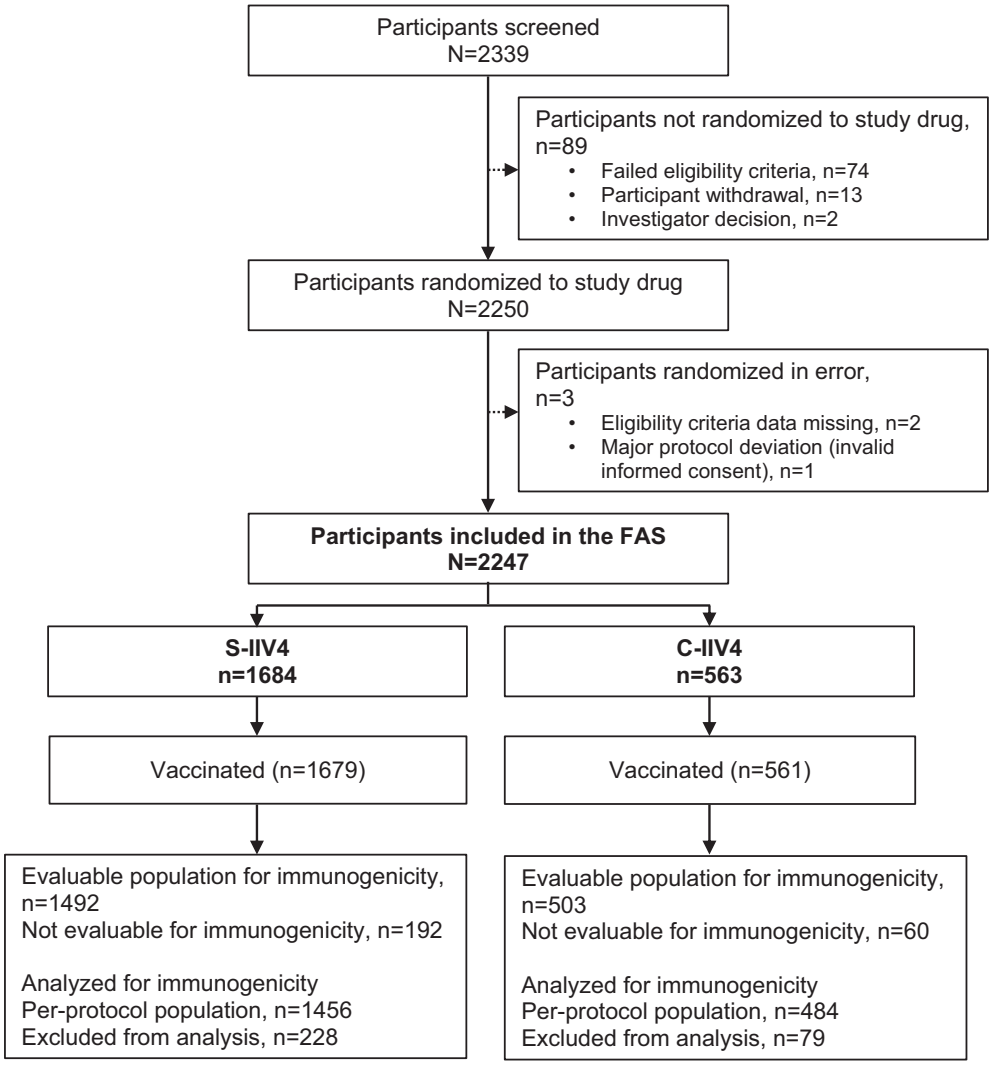

Fig. 1. Participant disposition. Abbreviations: C-IIV4 = comparator quadrivalent inactivated influenza vaccine; FAS = full analysis set; S-IIV4 = Seqirus quadrivalent inactivated influenza vaccine.

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

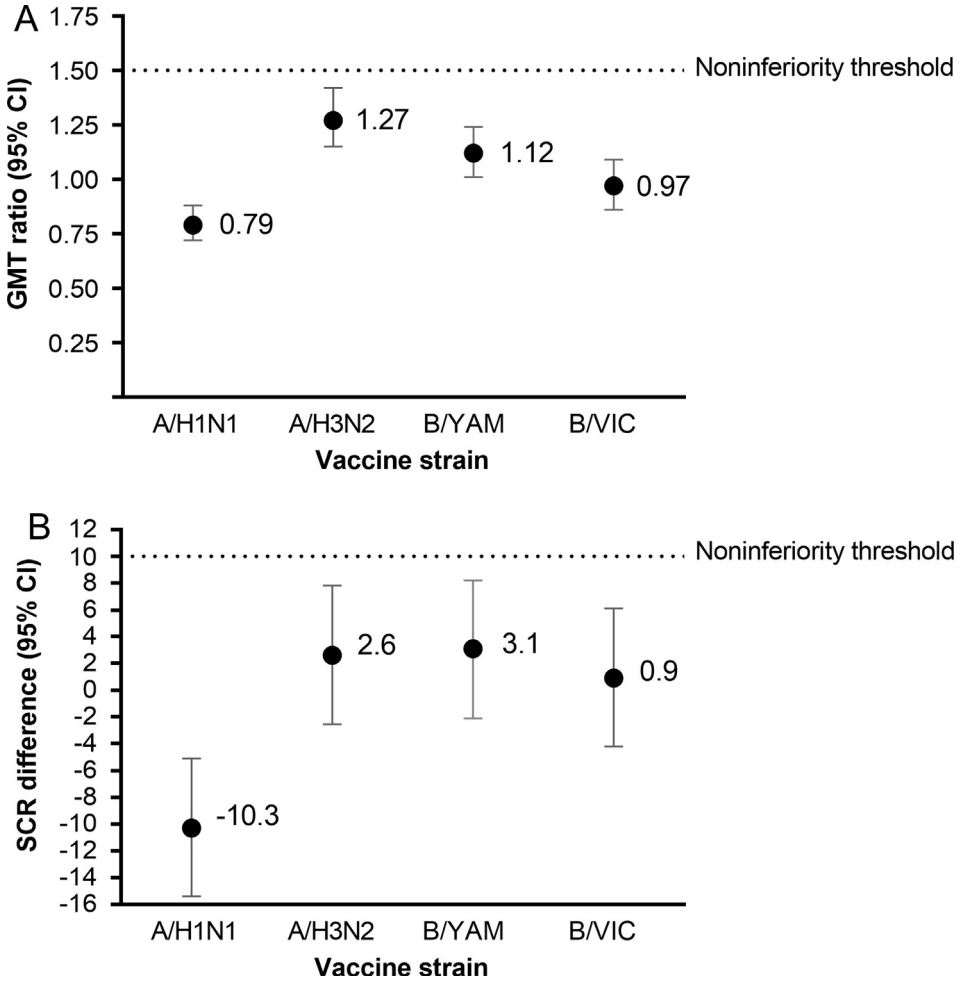

Fig. 2. Noninferiority analysis of S-IIV4 versus C-IIV4 in participants 6–59 months of age (per-protocol population for immunogenicity). Panel A: adjusted geometric mean titer ratio. Panel B: difference in seroconversion rates. Abbreviations: C-IIV4 = comparator quadrivalent inactivated vaccine; CI = 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.

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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.

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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.