Original Article

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, Multicenter, Multicountry, Clinical Trial

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Background. Influenza attack rates are high in 6- to 35-month-old children; vaccines containing both lineages of influenza B (Yamagata and Victoria), in addition to the H3N2 and H1N1 antigens, may improve protection rates.
Methods. In a randomized double-blind controlled trial, the immunogenicity and reactogenicity of an inactivated quadrivalent influenza vaccine (QIV) and a trivalent control vaccine (TIV) were assessed.
Results. Six hundred one children (QIV, n = 299; TIV, n = 302) were enrolled at 8 sites in 3 countries. The primary immunogenicity objective was met: the lower limit (LL) of the 2-sided 95% confidence interval (CI) for the seroconversion rate in QIV recipients ranged from 66.6% to 81.3%, which was ≥40% against all 4 strains. The immunogenic superiority of the additional B/Victoria strain in the QIV compared to that in the TIV was confirmed: the LL of the 2-sided 95% CI of the geometric mean titer ratio (QIV/TIV) (6.28 [95% CI, 5.32–7.41]) was greater than 1.5, and the LL of the 2-sided 95% CI for the difference in the seroconversion rate (QIV – TIV) (64.19% [95% CI, 57.65%–69.95%]) was greater than 10%. Injection-site pain and irritability/fussiness were the most commonly reported solicited injection-site and general adverse events, respectively, from days 0 to 6 and were similar in frequency between the groups.
Conclusions. In children aged 6 to 35 months, a QIV has superior immunogenicity for the added B strain and acceptable immunogenicity for shared strains, with no notable difference in reactogenicity and safety when compared to a TIV.

Key words. children; immunogenicity; influenza vaccine.

Annual influenza attack rates are highest in young children, and their rates of complicated influenza that require medical care and hospitalization parallel those in persons older than 65 years [1]. In the United States from 1993 to 2008, the estimated rates of influenza-associated hospitalizations were 91.5 per 100 000 for children in the first 12 months of life and 21.9 per 100 000 for children 1 to 4 years of age [1]. Influenza is also associated with outpatient and emergency department visits, diagnoses of otitis media, and prescriptions for antibiotics.

Although influenza vaccines are accepted worldwide as the most effective method for preventing influenza [2], there is a paucity of data on the efficacy of inactivated influenza vaccines in young children, especially those

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younger than 2 years. Indeed, recent systematic reviews of methodologically rigorous studies or randomized controlled trials [3-7] found few data demonstrating the immunogenicity or efficacy of the influenza vaccine in preventing laboratory-confirmed influenza in young children. More research on the influenza vaccine in this age group is needed [4].

The emergence of 2 lineages of influenza B virus since the 1980s, with a subsequent mismatch of circulating and vaccine strains, led to the development of quadrivalent influenza vaccines (QIVs) containing both the Yamagata and the Victoria lineages. QIVs, both live attenuated and inactivated, were approved in the United States beginning in the 2013–2014 season. QIVs containing both B virus lineages are estimated to reduce the numbers of illnesses, hospitalizations, and deaths caused by influenza [5]. Studies of the immunogenicity [6–8] and effectiveness [9] of various QIVs in children from 6 months to 17 years of age have met regulatory immunogenicity criteria for seasonal influenza vaccines; 1 of the approved QIVs showed 73% efficacy in preventing moderate-to-severe influenza in children aged 3 to 8 years [9].

In an open-label study in 6- to 35-month-olds conducted in the 2010–2011 season, a QIV was immunogenic and had a safety profile consistent with that of other inactivated influenza vaccines, and in a concurrent randomized controlled study in 3- to 17-year-olds, no interference with immune responses to the QIV occurred compared to a trivalent influenza vaccine (TIV) containing a B Victoria component [6].

In this Phase 3 randomized controlled study of children 6 through 35 months of age conducted in the 2012–2013 season, we assessed the immunogenicity, reactogenicity, and safety of a QIV containing influenza B strains from both lineages versus those of a TIV containing the same H1N1 and H3N2 and a B Yamagata component. In the 2012–2013 season, the recommended B Yamagata antigen had changed (to B/Hubei-Wujiagang/158/09) from that of the 2010–2011 season, providing an opportunity to assess the safety, reactogenicity, and immune responses to a vaccine containing influenza antigens from another combination of influenza strains.

METHODS

This study was a randomized (1:1) controlled double-blind comparison of QIV and TIV in 6- to 35-month-old children to determine safety and immunogenicity (Figure 1). The study was initiated on November 1, 2012, and the day 180 visit concluded on June 19, 2013.

The study (registered under ClinicalTrials.gov identifier NCT01711736) was undertaken in compliance with Good Clinical Practice guidelines, the Declaration of Helsinki, and national regulatory requirements and was approved by a local, regional, or national institutional review board at each study site.

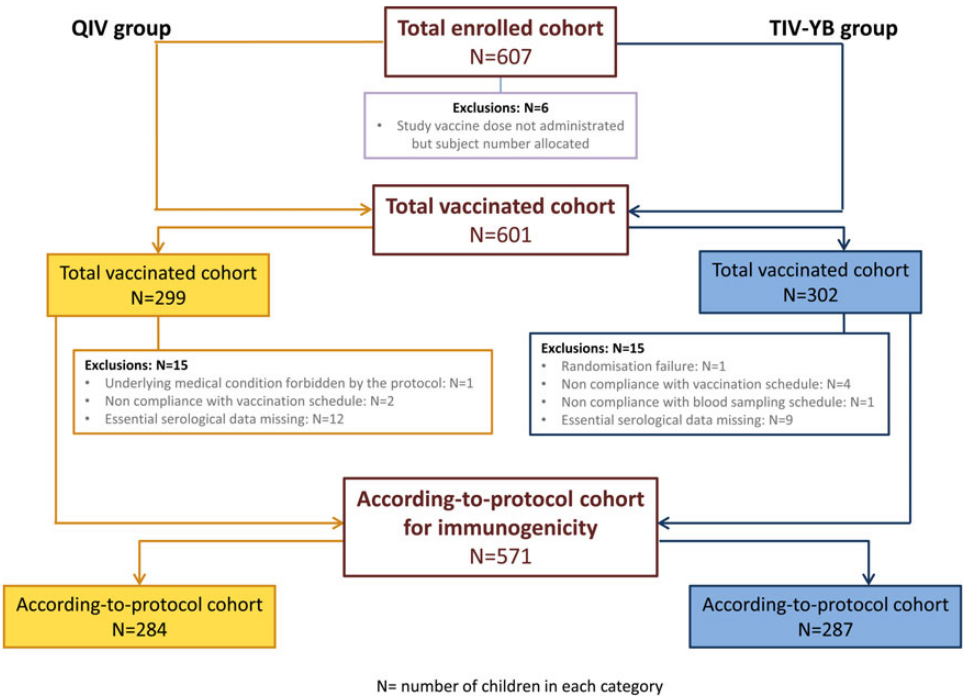

Figure 1. Participant flow. Abbreviations: QIV, quadrivalent influenza vaccine; TIV-YB, trivalent influenza vaccine containing Yamagata lineage of the B strain.

Participants

Eligible children were in stable health and between 6 and 35 months of age at the time of the first vaccination. Children were excluded if they were febrile (temperature, ≥38.0°C) or acutely ill at the time of enrollment, known to be immunocompromised, known to be allergic to any of the vaccine components, had a history of Guillain-Barré syndrome within 6 weeks of receipt of a previous influenza vaccine, had a coagulation disorder, had received influenza vaccine in the previous 6 months, had received immunoglobulins or blood products within 3 months, or had received an investigational product within 30 days before the first study vaccine. A parent/guardian provided written informed consent for each participant. The study was conducted at 8 sites in 3 countries (Canada, Dominican Republic, and Honduras).

Vaccines

All vaccines were provided in a single-dose thimerosal-free formulation and administered by the intramuscular route. The QIV contained 15 μg of hemagglutinin (HA) from 4 strains recommended for the 2012–2013 season [10]: A/California/7/2009(H1N1)pdm09, A/Victoria/361/2011(H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Hubei-Wujiagang/158/2009 (Yamagata lineage). The TIV contained the same H1N1, H3N2, and B Yamagata components but no B Victoria lineage component.

The vaccines were manufactured by GlaxoSmithKline Vaccines and provided in prefilled 0.5-mL syringes. The QIV was produced in Sainte-Foy, Quebec, Canada, according to the process used to manufacture *FluLaval*™, and the licensed TIV was produced in Dresden, Germany, according to the process used to manufacture *Fluarix*™. The vaccines, which were both opalescent off-white to grayish suspensions, were labeled with the treatment number but no identifying information.

Study Procedures

After the consent process, the study staff determined treatment allocation using an internet-based central randomization system. The randomization sequence was generated by using MATEX, a software program developed for use in SAS (SAS Institute, Cary, NC) that uses a minimization algorithm to balance treatment allocation between age groups (6–17 months and 18–35 months), between pre-study influenza vaccine priming statuses (primed vs unprimed), and among study centers.

Before the first vaccination, a brief history-directed physical examination was performed, and blood was drawn. Children who had previously received ≥2 doses of an influenza vaccine at least 1 month apart or who had received at least 1 dose before the previous season were considered vaccine primed. Vaccine-primed children received one 0.5-mL dose of study vaccine on day 0 and had blood collected on day 28 (visit 2). Vaccine-unprimed participants received a second 0.5-mL dose of vaccine on day 28 and had blood collected on day 56. Receipt of previous influenza vaccines was determined by parental/guardian history. Vaccines were administered intramuscularly in the anterolateral region of the left thigh (for children aged 6–11 months) or the deltoid region of the nondominant arm (for those aged ≥12 months) using a 25-mm (1-inch) 22- to 25-gauge needle, as determined by the vaccinator on the basis of participant age and muscle mass. The children were observed at the study site for 30 minutes after administration of the study vaccine. Routine childhood vaccines were permitted to be given concurrently.

Parents/guardians were instructed on the use of a diary card for recording any solicited injection-site or general adverse events (AEs) for 7 days and any unsolicited AEs for 28 days and to bring this diary with them at the next study visit. Parents were instructed to contact the investigator immediately if the child displayed any symptoms they perceived as serious. Antipyretics taken between 6 hours before and 12 hours after vaccination were recorded. On day 180, parents/guardians were asked if changes in the child's health had occurred since the last vaccination to identify unsolicited AEs.

Participant flow is seen in Figure 1.

Outcomes

Immunogenicity. Antibody titers against the vaccine strains were measured in serum samples by hemagglutination-inhibition (HI) assays performed at the GlaxoSmithKline Vaccines laboratory in Dresden, Germany, using standardized procedures [11].

The primary objective was to assess the immunogenicity of the QIV 28 days after the completion of dosing (day 28 for vaccine-primed children and day 56 for vaccine-unprimed children) on the basis of the Center for Biologics Evaluation and Research's (CBER's) seroconversion rate (SCR) criterion (lower limit [LL] of the 95% confidence interval [CI] for each of the 4 strains) [12].

A secondary objective was to determine the immunogenic superiority of the B/Victoria strain in the QIV, compared to that in the TIV, 28 days after the final vaccination in terms of the geometric mean titer (GMT) ratio (QIV/TIV) (success criterion, LL of the 95% CI, >1.5), adjusted by the baseline antibody titer (analysis of covariance model) and the SCR difference (QIV – TIV) (success criterion, LL of the 95% CI, >10%). Immunogenicity was also described for each group, age stratum, and priming status, with 95% CIs for the following parameters: the GMT of HI and

seroprotection rate (SPR) on day 0 and 28 days after the final vaccination and the SCR and mean geometric increase (MGI) 28 days after the final vaccination with 95% CIs.

Reactogenicity. The frequencies of solicited injection-site AEs (pain, redness, and swelling) and solicited general AEs (drowsiness, fever, irritability/fussiness, loss of appetite) were described (primary safety objective). Fever was defined as a temperature of ≥38.0°C as measured by any method. Intensity scales were used for the description of each symptom (Supplementary Table 1). The relative risk of fever in the 4 days after vaccination (QIV group/TIV group) was explored.

Unsolicited AEs were recorded for 28 days after each dose. Safety was assessed further by consideration of serious AEs (SAEs), medically attended AEs (MAEs), and potentially immune-mediated diseases (pIMDs) during the entire study period (up to day 180). MAEs were defined as events for which a child was hospitalized, visited the emergency department, or had a visit with a physician for any complaint.

All injection-site reactions were considered vaccine-related events. The causality of all other AEs was assessed by the investigators. All AEs were classified according to the *Medical Dictionary for Regulatory Activities* (MedRA).

Statistical Analysis

We planned for a sample size of 255 evaluable participants in each group to obtain an overall power of 99.99% to demonstrate the primary objective of meeting the CBER SCR criterion simultaneously for all 4 strains. A target of 600 children (300 per group) was set to account for an attrition rate of 15%.

The according-to-protocol (ATP) cohort for analysis of immunogenicity was defined as children who did not meet elimination or exclusion criteria during the study, those for whom we had assay results for at least 1 study vaccine antigen after vaccination, and those who complied with the time requirements of the study.

Reactogenicity and safety were assessed in the total vaccinated cohort (TVC), which included vaccinated participants for whom data were available. AEs, SAEs, pIMDs, and MAEs were analyzed descriptively by tabulating the percentage of subjects with at least 1 AE in each category and any AE after each vaccine dose and overall, with a 95% CI.

RESULTS

Participants

Six hundred one children (QIV, n = 299; TIV, n = 302) were enrolled and randomly assigned (Figure 1). The ATP cohort for immunogenicity consisted of 571 children (95%). The mean ages of the participants at the first vaccination visit were 18.2 months (standard deviation [SD], 8.17 months) in the TVC and 18.1 months (SD, 8.34 months) in the QIV and TIV groups (Table 1). Overall, 53% (317 of 601) of the children were 6 to 17 months of age, and 47% (284 of 601) were 18 to 35 months of age. Girls comprised 50.1% of the participants (301 of 601).

Of 601 children, only 39 (6.5%) had previously received an influenza vaccine in any previous season (QIV, n = 16; TIV, n = 23); thus, 573 (95%) of the 601 children were considered vaccine unprimed.

Immunogenicity

The primary immunogenicity objective was met: the LL of the 2-sided 95% CI for the SCR in QIV recipients ranged from 66.6% to 81.3%, which was ≥ 40% against all 4 strains, and the SCR point estimates for each of the 4 strains ranged from 72.2% to 85.9% (Figure 2 and Table 2) .

The immunogenic superiority of the B/Victoria strain present in the QIV (in terms of GMT and SCR) over that in the TIV was concluded, because the LL of the 2-sided

Table 1. Demographic Characteristics at Enrolment: Total Vaccinated Cohort

Characteristic	QIV (n = 299)	TIV (n = 302)	Total (n = 601)
Mean age in months (SD; median; range)	18.2 (8.17; 17.0; 6–35)	18.1 (8.34; 16.5; 6–35)	18.1 (8.25; 17.0; 6–35)
Male, n (%)	144 (48.2)	156 (51.7)	300 (49.9)
Female, n (%)	155 (51.8)	146 (48.3)	301 (50.1)
Hispanic/Latino ethnicity	231 (77.3)	233 (77.2)	464 (77.2)
Not Hispanic/Latino ethnicity	68 (22.7)	69 (22.8)	137 (22.8)
Heritage/race			
European heritage/Caucasian	47 (15.7)	52 (17.2)	99 (16.5)
Asian	16 (5.4)	16 (5.3)	32 (5.3)
African heritage/African American	4 (1.3)	2 (0.7)	6 (1.0)
American Indian or Native Alaskan	0	0	0
Pacific Islander/Native Hawaiian	0	0	0
Other	232 (77.6)	232 (76.8)	464 (77.2)
Median BMI (kg/m^2), SD	17.0 (1.94)	17.1 (2.42)	17.0 (2.2)

QIV, quadrivalent influenza vaccine; SD, standard deviation; TIV, trivalent influenza vaccine; BMI, body mass index, weight/ height.

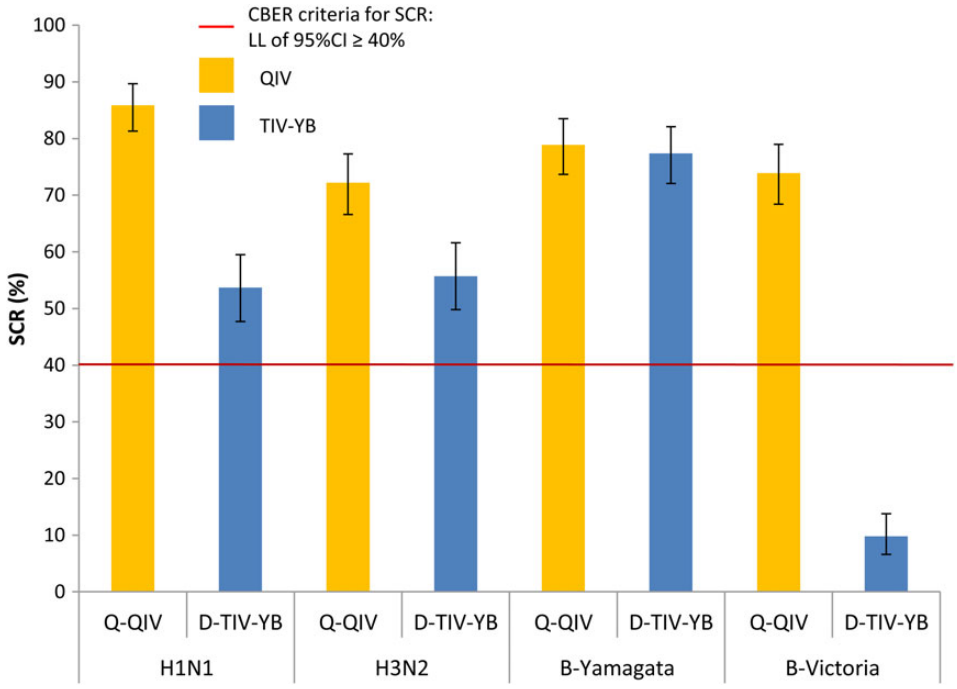

Figure 2. SCRs for HI antibodies 28 days after the last vaccine dose (28 days after dose 1 [day 28] for primed subjects; 28 days after dose 2 [day 56] for unprimed subjects) in the ATP cohort for immunogenicity. The SCR was defined as an antibody titer of ≥40 1/DIL after vaccination for initially seronegative subjects and an antibody titer after vaccination of ≥4-fold the prevaccination antibody titer for initially seropositive subjects. % indicates the percentage of seroconverted subjects; error bars indicate 95% CIs. Abbreviations: Q-QIV, quadrivalent influenza vaccine; Q, manufactured in Quebec; TIV-YB, trivalent influenza vaccine containing Yamagata lineage of B strain; D, manufactured in Dresden.

95% CI of the adjusted GMT ratio (QIV/TIV) (6.28 [95% CI, 5.32–7.41]) was greater than 1.5, and the LL of the 2-sided 95% CI for the difference in SCRs (QIV – TIV, 64.19% [95% CI, 57.65%–69.95%]) was greater than 10% (Figure 3). The adjusted GMT ratio (adjusted for HI antibody titers at baseline) was 6.28 (95% CI, 5.32–7.41).

The GMTs, SCRs, SPRs, and MGIs for the QIV and TIV recipients before and after vaccination (day 28 after the last dose) and according to age stratum (6–17 months or 18–35 months of age) are shown in Table 2. Immune responses were lower in the younger age stratum (6–17 months) than in the 18- to 35-month-olds. The SPR point estimates for the 3 antigens contained in both vaccines ranged from 76.2% to 82.8% for the 6- to 17-month-old QIV recipients and from 87.2% to 97.0% for the 18- to 35-month-old QIV recipients. In contrast, SPRs in the TIV recipients ranged from 39.6% to 71.8% and 79.7% to 88.4% in the younger and older age strata, respectively. SCRs for the 3 antigens contained in both study vaccines ranged from 72.2% to 80.8% in the 6- to 17-month-old QIV recipients and– from 72.2% to 91.7% in 18- to 35-month-old QIV recipients,. In TIV recipients, the SCRs ranged from 38.3% to 68.5% and 70.3% to 87.0% in the younger and older age strata, respectively.

Reactogenicity

The frequencies of injection-site and general solicited AEs overall during the 7 days after vaccination are shown in Figures 4 and 5. Injection-site pain was the most frequently reported solicited injection-site AE (QIV, 32.6%; TIV, 30.6%), with grade 3 pain in 2.4% and 1.0% of participants, respectively. Irritability/fussiness was the most frequently reported solicited general AE (40.7% and 41.6% of subjects in the QIV and TIV groups, respectively). Grade 3 irritability/fussiness was reported in 5.2% and 4.7% of the QIV and TIV recipients, respectively.

A temperature of ≥38°C in the 7-day postvaccine period was reported for 14.5% (95% CI, 10.7%–19.1%) (QIV) and 14.5% (95% CI, 10.7%–19.1%) (TIV) of the recipients after the first dose and in 10.3% (95% CI, 7.0%–14.5%) (QIV) and 9.1% (95% CI, 5.9%–13.1%) (TIV) of the children after the second dose. A temperature of ≥39.0°C in the same time period was reported in 3.8% (95% CI, 1.9%–6.7%) and 3.0% (95% CI, 1.4%–5.7%) of the QIV and TIV recipients, respectively.

In an exploratory analysis, the relative risks (RRs) (QIV/TIV) of a temperature of ≥38.5°C were 1.40 (95% CI, 0.66–2.94; $P = .43$) after the first vaccine dose and 1.62 (95% CI, 0.76–3.46; $P = .23$) after the second

Table 2. Immunogenicity of QIV and TIV in Children Aged 6 to 35 months: ATP Immunogenicity Cohort

Vaccine	Age (mo)	Timing	n	GMT Value (95% CI)	SCR* (% [95% CI])	SPR* (% [95% CI])	MGI Value (% [95% CI])
A/California/7/2009 (H1N1)							
QIV	6–35	Pre	284	9.6 (8.1–11.3)		16.2 (12.1–21.0)	
	6–35	Post	284	157.1 (132.8–185.9)	85.9 (81.3–89.7)	89.4 (85.3–92.8)	16.4 (14.3–18.7)
	6–17	Pre	151	7.0 (5.9–8.3)		7.3 (3.7–12.7)	
	6–17	Post	151	103.2 (82.2–129.5)	80.8 (73.6–86.7)	82.8 (75.8–88.4)	14.8 (12.1–18.1)
	18–35	Pre	133	13.7 (10.3–18.3)		26.3 (19.1–34.7)	
	18–35	Post	133	253.2 (201.7–317.7)	91.7 (85.7–95.8)	97.0 (92.5–99.2)	18.4 (15.6–21.8)
TIV	6–35	Pre	287	9.8 (8.3–11.6)		16.4 (12.3–21.2)	
	6–35	Post	287	61.2 (49.2–76.2)	53.7 (47.7–59.5)	58.9 (53.0–64.6)	6.2 (5.3–7.3)
	6–17	Pre	149	6.9 (5.8–8.2)		8.1 (4.2–13.6)	
	6–17	Post	149	28.6 (21.7–37.7)	38.3 (30.4–46.6)	39.6 (31.7–47.9)	4.1 (3.4–5.1)
	17–35	Pre	138	14.4 (10.8–19.1)		25.4 (18.3–33.5)	
	17–35	Post	138	139.3 (104.1–186.4)	70.3 (61.9–77.8)	79.7 (72.0–86.1)	9.7 (7.8–12.0)
A/Victoria/361/2011 (H3N2)							
QIV	6–35	Pre	284	17.4 (14.1–21.5)		32.7 (27.3–38.5)	
	6–35	Post	284	159.4 (129.4–196.3)	72.2 (66.6–77.3)	81.3 (76.3–85.7)	9.1 (8.0–10.5)
	6–17	Pre	151	12.4 (9.5–16.1)		24.5 (17.9–32.2)	
	6–17	Post	151	108.3 (81.0–144.8)	72.2 (64.3–79.2)	76.2 (68.6–82.7)	8.7 (7.3–10.5)
	18–35	Pre	133	25.6 (18.4–35.7)		42.1 (33.6–51.0)	
	18–35	Post	133	247.1 (185.9–328.6)	72.2 (63.7–79.6)	87.2 (80.3–92.4)	9.6 (7.9–11.8)
TIV	6–35	Pre	287	13.8 (11.4–16.8)		25.8 (20.8–31.3)	
	6–35	Post	287	103.0 (83.7–126.7)	55.7 (49.8–61.6)	66.6 (60.8–72.0)	7.5 (6.4–8.7)
	6–17	Pre	149	9.2 (7.3–11.6)		14.8 (9.5–21.5)	
	6–17	Post	149	53.5 (40.9–70.1)	42.3 (34.2–50.6)	50.3 (42.0–58.6)	5.8 (4.8–7.1)
	18–35	Pre	138	21.4 (15.9–29.0)		37.7 (29.6–46.3)	
	18–35	Post	138	208.7 (158.4–274.9)	70.3 (61.9–77.8)	84.1 (76.9–89.7)	9.7 (7.7–12.2)
B/Brisbane/60/2008 (Victoria)							
QIV	6–35	Pre	284	10.6 (9.1–12.4)		19.7 (15.3–24.8)	
	6–35	Post	284	111.4 (91.9–135.2)	73.9 (68.4–79.0)	76.1 (70.7–80.9)	10.5 (9.2–11.9)
	6–17	Pre	151	6.9 (6.0–7.9)		6.6 (3.2–11.8)	
	6–17	Post	151	66.6 (53.0–83.6)	66.9 (58.8–74.3)	68.2 (60.1–75.5)	9.7 (7.9–11.8)
	18–35	Pre	133	17.4 (13.4–22.7)		34.6 (26.6–43.3)	
	18–35	Post	133	200.1 (149.2–268.3)	82.0 (74.4–88.1)	85.0 (77.7–90.6)	11.5 (9.8–13.4)
TIV	6–35	Pre	287	9.3 (8.0–10.7)		15.7 (11.7–20.4)	
	6–35	Post	287	15.6 (13.3–18.5)	9.8 (6.6–13.8)	25.8 (20.8–31.3)	1.7 (1.5–1.9)
	6–17	Pre	149	5.6 (5.2–6.0)		1.3 (0.2–4.8)	
	6–17	Post	149	8.6 (7.4–9.9)	7.4 (3.7–12.8)	8.7 (4.7–14.5)	1.5 (1.3–1.8)
	18–35	Pre	138	16.1 (12.4–21.0)		31.2 (23.6–39.6)	
	18–35	Post	138	29.9 (22.8–39.2)	12.3 (7.3–19.0)	44.2 (35.8–52.9)	1.9 (1.6–2.2)
B/Hubei–Wujiagang/158/2009 (Yamagata)							
QIV	6–35	Pre	284	7.7 (6.9–8.7)		9.2 (6.1–13.1)	
	6–35	Post	284	114.2 (100.0–130.5)	78.9 (73.7–83.5)	85.2 (80.5–89.1)	14.8 (12.8–17.1)
	6–17	Pre	151	7.5 (6.4–8.8)		8.6 (4.7–14.3)	
	6–17	Post	151	93.3 (78.2–111.3)	74.2 (66.4–80.9)	81.5 (74.3–87.3)	12.5 (10.2–15.3)
	18–35	Pre	133	8.0 (6.8–9.4)		9.8 (5.3–16.1)	
	18–35	Post	133	143.8 (118.2–174.8)	84.2 (76.9–90.0)	89.5 (83.0–94.1)	18.0 (14.6–22.1)
TIV	6–35	Pre	287	7.2 (6.5–8.0)		8.4 (5.4–12.2)	
	6–35	Post	287	107.2 (92.2–124.6)	77.4 (72.1–82.1)	79.8 (74.7–84.3)	14.8 (12.8–17.2)
	6–17	Pre	149	6.9 (6.0–7.9)		6.7 (3.3–12.0)	
	6–17	Post	149	71.8 (59.0–87.5)	68.5 (60.3–75.8)	71.8 (63.9–78.9)	10.5 (8.6–12.7)
	18–35	Pre	138	7.7 (6.5–9.0)		10.1 (5.7–16.4)	
	18–35	Post	138	165.3 (134.1–203.7)	87.0 (80.2–92.1)	88.4 (81.9–93.2)	21.5 (17.4–26.6)

Abbreviations: Pre, prevaccination; Post, postvaccination.
*Each of the four strains contained in the QIV met CBER licensure criteria for immunogenicity (LL of the 95% CI for SCR of at least 40% and a postvaccination SPR of at least 70%).

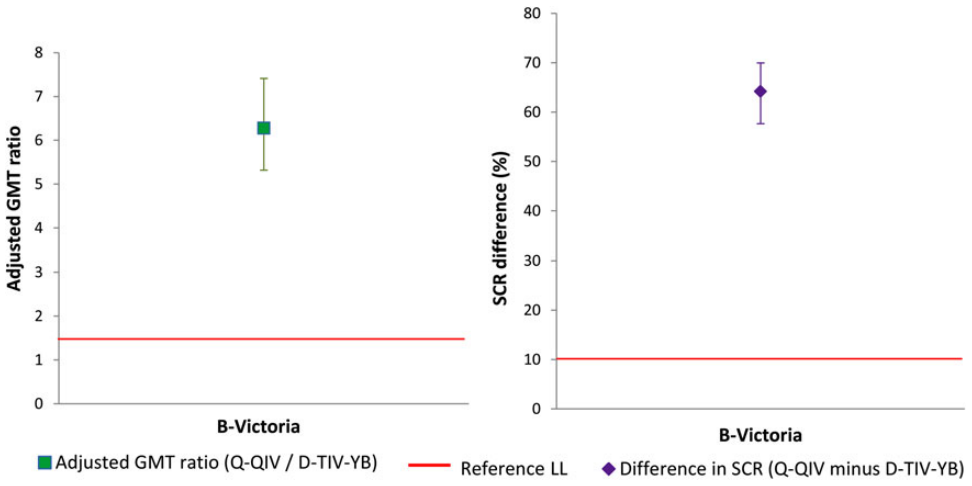

Figure 3. Immunogenic superiority of QIV versus TIV-YB for the B Victoria strain in the ATP cohort for immunogenicity. Adjusted GMT indicates GMT adjusted for baseline titer; SCRs, an antibody titer of ≥40 1/DIL after vaccination for initially seronegative subjects and an antibody titer after vaccination of ≥4-fold the prevaccination antibody titer for initially seropositive subjects. Criteria for superiority were that the LL of the 2-sided 95% CI of the GMT ratio (Q-QIV /D-TIV-YB) was >1.5 and the LL of the 2-sided 95% CI on the SCR difference (Q-QIV – D-TIV-YB) was >10%. Error bars indicate 95% CIs.

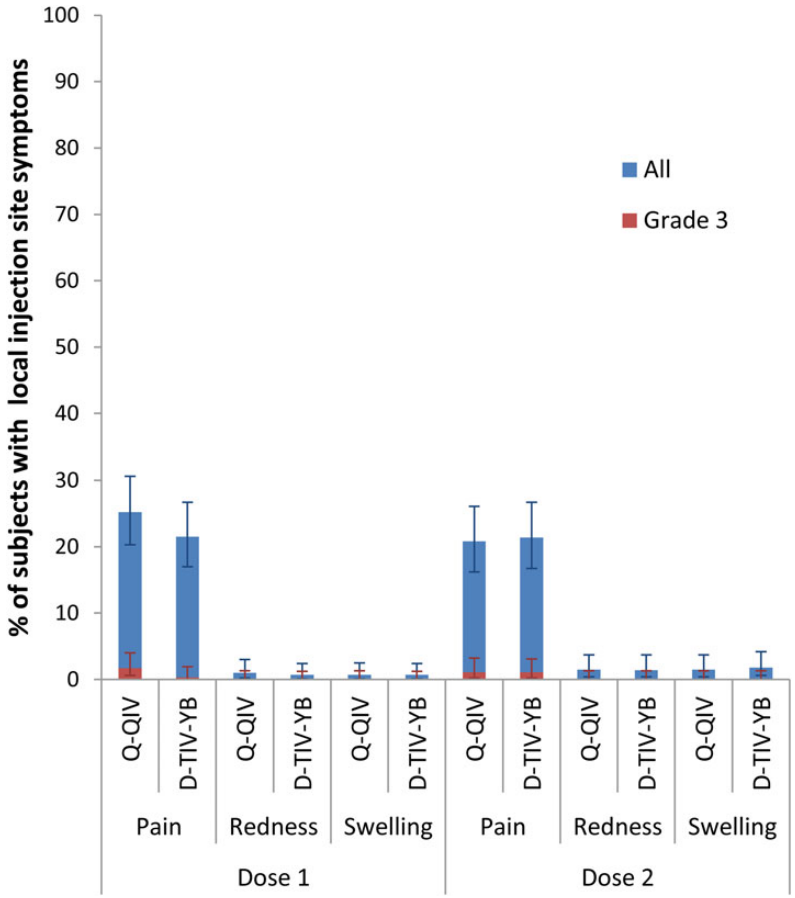

Figure 4. Incidence and nature of local injection-site adverse events on days 0 to 6 after each vaccine dose in the TVC. Grade 3 pain was defined as a child crying when his or her limb was moved and/or spontaneously painful; grade 3, redness/swelling of >100 mm. Error bars indicate 95% CIs.

vaccine dose. The RRs of a temperature of >39.0°C (102.2°F, grade 3) during the 4-day follow-up period were 1.54 (95% CI, 0.47–5.03; $P = .54$) after dose 1 and 2.54 (95% CI, 0.85–7.58; $P = .11$) after dose 2. The frequencies of fever were similar across the age strata (data not shown). An antipyretic agent was taken by

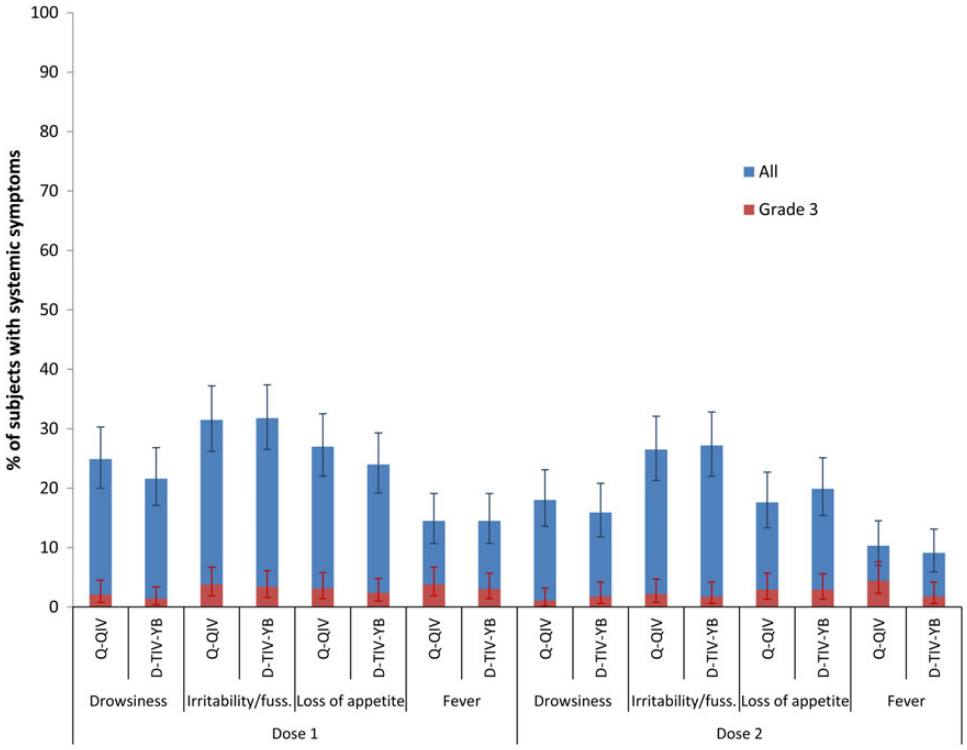

Figure 5. Incidence and nature of systemic adverse events on days 0 to 6 after each vaccine dose in the TVC. Grade 3 drowsiness was defined as drowsiness that prevented normal activity; grade 3 irritability/fussiness (irritability/fuss), crying that could not be comforted and/or prevented normal activity; grade 3 loss of appetite, child did not eat at all; grade 3 fever (or higher), temperature of ≥39.0°C (grade 4 fever also included here). Error bars indicate 95% CIs.

31.8% of QIV recipients and 32.8% of TIV recipients after the vaccine. Although the administration of routine childhood vaccines was permitted, only 2 of 601 subjects in the TVC received pneumococcal conjugate vaccine concomitantly.

During the 28-day postvaccination period, at least 1 unsolicited AE was reported for 47.5% and 54.6% of participants in the QIV and TIV groups, respectively. Nasopharyngitis (26.1% and 29.8% of participants) and diarrhea (12.7% and 12.6% of participants) were the only 2 unsolicited AEs reported for more than 5% of the children in the QIV and TIV groups, respectively. At least 1 unsolicited grade 3 AE was reported for 3.0% and 1.7% of participants in the QIV and TIV groups, respectively.

During the 180-day follow-up period, 25 nonfatal SAEs were reported for 17 participants (QIV, n = 9 [3%]; TIV, n = 8 [2.6%]). Only 1 SAE was considered by the investigators to be related to the study vaccine: a simple partial seizure associated with fever in an 18-month-old 6 hours after the first dose of QIV. The child recovered without sequelae, and the second dose of vaccine was given with no fever reported. All SAEs were resolved by the end of the study. At least 1 MAE was reported for 52.2% (n = 156) and 51.7% (n = 156) of the QIV and TIV recipients, respectively. The most common MAEs were the unsolicited AEs: nasopharyngitis, diarrhea, and pharyngitis. Two pIMDs were reported in the TIV group (MedRA codes "alopecia areata" and "colitis ulcerative"), and they resolved by day 180. No pIMDs were reported in the QIV group. No AEs led to participant discontinuation.

DISCUSSION

In this randomized double-blind controlled trial of 6- to 35-month-old children, the QIV met regulatory criteria for immunogenicity and was superior to the TIV for the B/Victoria strain present in the QIV. QIVs are expected to improve protection against influenza B when 2 B lineages are cocirculating or there is mismatch between the B lineage in the TIV and the circulating strain. Although immunogenicity is an indirect measure of clinical protection against influenza-associated illness, it is notable that in a recent phase 3 efficacy trial in 3- to 8-year-old children, the efficacy of the QIV was confirmed. The vaccine effectiveness rate in this older age group against reverse-transcriptase polymerase chain reaction–confirmed influenza was 55.4% (95% CI, 39.2%–67.3%) in the TVC and against moderate-to severe-influenza was 73.1% (95% CI, 47.1%–86.3%) [9]. An efficacy trial in children

aged 6 to 35 months (ClinicalTrials.gov identifier NCT01439360) is underway.

Attaining robust immune responses to influenza vaccine in infants in the first years of life has been an ongoing challenge. In this study, immune responses in the younger age stratum (6–17 months) were lower than those of the 18- to 35-month-olds, and the SPRs for H3N2 and the B virus (Victoria lineage) did not meet CBER immunogenicity criteria. We note that in a predominately vaccine-unprimed population, such as in this study, SCRs may be a better measure of vaccine response. Strategies for overcoming the reduced immunogenicity of vaccine have included using 2 doses in the first year a child is vaccinated, using a 0.5-ml "adult dose" [13–15], and adjuvantation with oil-in-water emulsions [16, 17] or virosomes [18].

The frequencies of both local and systemic AEs in the week after immunization were comparable in the QIV and TIV recipients, after dose 1 or 2, and across the age strata. Adverse events were somewhat less common than those observed in a previous open-label (unblinded) trial of this QIV product in the same age group except for fever, which was slightly higher [6]. Grade 3 events were also similar across groups and uncommon. There are few published data on the reactogenicity of inactivated nonadjuvanted influenza vaccines in children younger than 3 years to which our results can be compared. Most systematic reviews of randomized controlled trials of influenza vaccines in children have focused instead on immunogenicity and/or effectiveness. Two narrative reviews found few primary studies of inactivated split-virus vaccines that documented AEs in young children [19, 20].

Fever is probably the most concerning AE after influenza immunization in children under 5 years of age because of its association with febrile seizures. In the 2010–2011 and 2011–2012 seasons, the Centers for Disease Control and Prevention noted an increase in febrile seizures in the 48 hours after TIV receipt in 6- to 48-month-old children, at a frequency of less than 1 in 1000 children vaccinated [10]. No increase was seen in the subsequent season. In this study of 601 children from 6 to 35 months of age, an exploratory analysis of the RR of fever in QIV recipients compared to TIV recipients during a 4-day follow-up period did not detect any significant findings, but the CIs were wide. Similar to previous QIV study results, no excess reactogenicity seems to occur with the higher dose of influenza antigen (60 μg).

A limitation of this study is that it was conducted in children in stable health, and the results may not be generalizable to children in this age group with chronic conditions, particularly immunodeficiency. Also, the study end points, antibody responses, are surrogate outcomes for clinical protection. Although efficacy studies of this QIV in children aged 3 to 8 years have shown clinical protection [9], clinical efficacy for this QIV was shown in 3- to 8-year-olds but not in 6- to 35-month-olds. Other limitations are that most children in this study had not previously received influenza vaccine; children in this age group with previous influenza vaccine exposure (vaccine-primed) may respond differently to a subsequent inactivated influenza vaccine. Although routine childhood immunizations were permitted in this study, the number of children who concurrently received vaccines was insufficient to evaluate immune responses and reactogenicity with concurrent administration.

In summary, in this randomized controlled trial in children aged 6 to 35 months, an inactivated QIV had an immunogenicity that was superior to that of a TIV for the added B strain and acceptable immunogenicity for the shared strains, with no notable difference in reactogenicity and safety compared to the TIV.

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All authors participated in the implementation of the study, including providing substantial contributions to conception and design, gathering of the data, or analysis and interpretation of the data. The corresponding author drafted the manuscript; all authors were involved in revising the manuscript critically for important intellectual content and in its final approval.

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Supplementary Data

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

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