Immunogenicity and Safety of an Inactivated Quadrivalent Influenza Vaccine Candidate: A Phase III Randomized Controlled Trial in Children

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(See the editorial commentary by Dolin on pages 539–40.)

Background. Mismatch between circulating influenza B viruses (Yamagata and Victoria lineages) and vaccine strains occurs frequently.

Methods. In a randomized controlled trial, immunogenicity and safety of an inactivated quadrivalent influenza vaccine candidate (QIV) versus trivalent inactivated influenza vaccine (TIV)-Victoria (Vic) and TIV-Yamagata (Yam) in children 3–17 years of age was evaluated. In an open-label study arm, QIV only was assessed in children 6–35 months of age.

Results. A total of 3094 children (932 QIV, 929 TIV-Vic, 932 TIV-Yam, and 301 QIV only) were vaccinated. QIV was noninferior to the TIVs for shared strains (A/H3N2 and A/H1N1) based on hemagglutination-inhibition (HI) antibodies 28 days after last vaccination, and superior for the unique B strains Victoria and Yamagata (geometric mean titer ratios 2.61, 3.78; seroconversion rate differences 33.96%, 44.63%). Among children in the randomized trial, adverse event rates were similar except for injection site pain (dose 1: 65.4% QIV, 54.6% TIV-Vic, 55.7% TIV-Yam).

Conclusion. QIV elicited superior HI responses to the added B strains compared to TIV controls, potentially improving its effectiveness against influenza B. HI responses were similar between QIV and TIV controls for the shared strains. QIV had an acceptable safety profile relative to TIVs.

Clinical Trials Registration. NCT01198756.

Keywords. influenza vaccine; immunogenicity; children.

The choice of influenza virus strains to be included in each year’s vaccine is a complex process in which worldwide surveillance data is used to predict the strains likely to circulate the next season, while taking into account ongoing antigenic drift [1]. Since 1978, there have been 3 strains in the annual vaccine [2]: 2 influenza A strains, A/H3N2 and H1N1; and 1 influenza B strain. However, the emergence of 2 lineages of B virus, Yamagata and Victoria [3], which may circulate simultaneously, has led to mismatch between the
versus 2 inactivated trivalent in vaccine (QIV) containing in
toxicity, and safety of an inactivated quadrivalent in
3 through 17 years of age, we assessed the immunogenicity, reac-
the same in
containing both B virus lineages are estimated to reduce illness,
mine safety and immunogenicity (Figure 1). In an open-label
parison of QIV and TIV in 3- to 17-year-old children to deter-
This was a randomized (1:1:1), controlled, double-blind com-
METHODS

In this Phase III randomized study of children and adolescents
3 through 17 years of age, we assessed the immunogenicity, reac-
togenicity, and safety of an inactivated quadrivalent influenza
vaccine (QIV) containing influenza B strains from both lineages
versus 2 inactivated trivalent influenza vaccines (TIV) containing
the same influenza A virus strains and a B strain from either the
B/Victoria lineage or the B/Yamagata lineage. In a concurrent
open-label study arm, the immunogenicity and safety of QIV
was assessed in children 6–35 months of age.

METHODS

This was a randomized (1:1:1), controlled, double-blind compa-
rison of QIV and TIV in 3- to 17-year-old children to deter-
mine safety and immunogenicity (Figure 1). In an open-label
study arm, the reactogenicity, safety, and immunogenicity of
QIV in children 6–35 months of age was described.

The study was undertaken in compliance with Good Clinical
Practice guidelines, the Declaration of Helsinki, and applicable
regulatory requirements, and was approved by local, regional,
or national Institutional Review Boards at each study site.

Participants

Eligible children were in stable health, between 6 months and
17 years of age, and not pregnant if female. Children were ex-
cluded if febrile (temperature ≥38.0°C), immunocompromised,
receiving aspirin, allergic to any vaccine component, known to
have a coagulation disorder, or had received influenza vaccine
in the prior 6 months, immunoglobulins or blood products
within 3 months, or an investigational product within 30 days.
A parent/guardian provided written informed consent for each
participant. The study was conducted in 32 centers in 5 coun-
tries (Canada, United States, Mexico, Spain, and Taiwan).

Vaccines

The TIVs contained either a Victoria lineage B strain (TIV-Vic),
or a Yamagata lineage B strain (TIV-Yam). Both TIVs contained
15 µg each of hemagglutinin antigen (HA) from each of A/H1N1
(A/California/7/2009) and A/H3N2 (A/Victoria/210/2009), TIV-Vic
contained 15 µg of B/Brisbane/60/2008, and TIV-Yam con-
tained 15 µg of B/Brisbane/3/2007. The QIV contained 60 µg of
HA: 15 µg of the same 2 influenza A strains as the TIVs, as well
as 15 µg of each of the B lineage strains.

All vaccines were manufactured by GlaxoSmithKline Vac-
cines using a thimerosal-free formulation and were opalescent
off-white to grayish suspensions provided in prefilled 0.5 mL
syringes. The TIVs were manufactured in Dresden, Germany,
and the QIV in Quebec City, Canada. Vaccines used in the 3-
to 17-year-old study were labeled with the treatment number
but no identifying information. Vaccines administered in the
QIV-only open-label study of 6- to 35-month-olds were labeled
as to contents.

Study Procedures

Following the consent process, study staff determined treat-
ment allocation using an Internet-based central randomization
system. The randomization sequence was generated using
MATEX, a software program developed for use in SAS (Cary,
NC) by GlaxoSmithKline (Rixensart, Belgium). Randomization
was stratified equally between groups by age (3–8 years and
9–17 years). A minimization procedure accounted for pre-
vious receipt of influenza vaccine (“priming” status), country,
and age subgroups (3–4 years and 5–8 years). Neither the
parents/guardians, participants, or study staff were aware of
treatment allocation for participants ≥3 years of age. In the
open-label study, parents/guardians and study staff were not
blinded.

Prior to the first vaccination, a brief history-directed physical
examination was performed and blood drawn. Children who
were ≥9 years of age or had prior receipt of ≥2 doses of an in-
fluenza vaccine at least 1 month apart were considered
“primed.” Primed children received one 0.5 mL dose of study
vaccine on day 0 and had blood collected on day 28 (visit 2).
Unprimed participants received a second 0.5 mL dose of
vaccine on day 28, and had blood collected on day 56. Vaccines
were administered intramuscularly in the anterolateral region
of the left thigh (age 6–11 months) or deltoid region of the left
arm (age ≥12 months). Children were observed at the study
site for 30 minutes after administration of study vaccine.

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 the diary to study visits. Parents/guardians of
children <5 years of age were asked to contact study staff
immediately if a seizure or fever ≥39°C occurred within 2
days of vaccination. On day 180, parents/guardians were
asked if changes in health or AEs had occurred since the last
vaccination.

Participant flow is shown in Figure 1.
Outcomes

Immunogenicity

The primary confirmatory objective was to determine if QIV was noninferior to TIV-Vic and TIV-Yam for the shared vaccine strains in children 3–17 years of age, at 28 days after completion of dosing (day 28 for primed children and day 56 for unprimed children). Immunogenic noninferiority was assessed between shared viral strains in terms of the geometric mean titer (GMT) ratio and seroconversion rate (SCR) difference. A secondary confirmatory objective was to assess the immunogenic superiority of QIV versus TIV-Vic for the B/Victoria strain and TIV-Yam for the B/Yamagata strain (strains absent from the TIV) in children 3–17 years of age at 28 days after dosing. Exploratory objectives were to describe GMT, SCR, seroprotection rate (SPR), and seroconversion factor (SCF) for all strains in each vaccine in children 3–17 years and for QIV in children 6–35 months of age.

Antibody titers against the vaccine strains were measured in serum samples by hemagglutination-inhibition (HI) assay performed at GlaxoSmithKline Vaccines’ laboratory in Laval, Canada, using standardized procedures [12].

Reactogenicity

Reactogenicity analyses for participants ≤5 years of age were the frequency of solicited injection site reactions (pain, redness, swelling) and solicited general AEs (fever, irritability/fussiness, drowsiness, loss of appetite) in the 7 days following each vaccination. Fever was defined as a temperature ≥38.0°C measured by any method. For children >5 years, the same injection site reactions were collected, but the solicited general reactions were fever, headache, fatigue, and gastrointestinal symptoms; and joint pain, muscle aches, and shivering. Intensity scales were used for the description of each symptom (Supplementary Table 1). Unsolicited AEs were recorded for 28 days.

Safety was further assessed by consideration of serious adverse events (SAEs), medically attended adverse events (MAEs), and potentially immune-mediated diseases (pIMDs), which were recorded during the entire study period (to day

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Figure 1. Participant flow. Abbreviations: ATP, according-to-protocol; QIV, quadrivalent influenza vaccine; TIV-Vic, trivalent influenza vaccine Victoria lineage B strain; TIV-Yam, trivalent influenza vaccine Yamagata lineage B strain.
Sample Size and Statistical Analysis
The sample size for the randomized controlled trial (RCT) was 2175 evaluable children (725 per group) age 3–17 years, to obtain an overall power of 90% to demonstrate the primary objectives of noninferiority of QIV to TIV-Yam and TIV-Vic for the alternate-lineage B strain. A target of 2700 children (900 per group) was set to account for an attrition rate of 20%. In the open-label QIV-only study, the target sample size was set at 300 infants age 6–35 months, and was not based on a power calculation.

The immunogenicity analyses were performed using the According to Protocol (ATP) cohort for immunogenicity, defined as children who did not meet elimination or exclusion criteria during the study, who had assay results for at least 1 study vaccine antigen, and who complied with study time requirements. In the randomized study of 3- to 17-year-olds, immunogenic noninferiority was concluded if the upper limit of the 2-sided 95% confidence interval (CI) of the GMT ratio (TIV/QIV) after the final vaccine did not exceed 1.5 for the 3 strains (A/H3N2, A/H1N1, and shared B strains), and if the upper limit of the 2-sided 95% CI for the difference in SCR (TIV minus QIV) did not exceed 10% for the 3 strains contained in TIV-Yam and TIV-Vic. Immunogenic superiority was confirmed if the lower limit of the 2-sided 95% CI of the GMT ratio (QIV/TIV) was greater than 1.5 and the difference in SCR (QIV minus TIV) was greater than 10%.

GMT, SCR, SPR, and SCF were tabulated with 95% CIs, and SCR and SPR were evaluated according to Center for Biologics Evaluation and Research (CBER) licensure criteria for immunogenicity of influenza vaccines in adults <65 years of age: the criteria were fulfilled if the lower limit of the 2-sided 95% CI on the SCR was ≥40%, and the lower limit of the 2-sided 95% CI on the SPR was ≥70% [13].

Reactogenicity and safety were assessed in the Total Vaccinated Cohort (TVC). The frequency of AEs after each vaccine dose and overall were tabulated. SAEs and MAEs were described by tabulating the percentage of subjects with at least 1 event in each of these categories, with a 95% CI.

RESULTS
The study was conducted from 1 October 2010 until 6 July 2011. A total of 3109 children were enrolled. The TVC comprised 3094 children (Figure 1); 15 children were not vaccinated. The ATP immunogenicity cohort had a total of 2886 children enrolled. The 6-month safety follow-up was completed by 2960 participants (2685/2793; 96.1%) in the randomized study and 275/301 (91.4%) children in the open study. The median age in the RCT was 8.9 years and in the open study 1.2 years; other demographic characteristics at enrollment are given in Table 1. Age, sex, and ethnicity were similar across groups. Stable health was required for enrollment. Overall, 12.4% (370/2960) of participants had stable asthma (QIV 121/932; TIV-Vic 130/929; QIV-Yam 119/932).
TIV-Yam 119/932), 2% had another chronic respiratory disease, and 1.5% had cardiovascular disease.

**Immunogenicity**

GMT, SCR, SPR, and SCF immunogenicity outcomes for each strain and vaccine in the ATP cohort are given in Table 2. In children 3–17 years of age, the QIV group was shown to be noninferior to both TIVs in terms of GMT ratios and SCR differences to all vaccine strains (Table 3). The upper limit of the 2-sided 95% CI for the adjusted GMT ratio of HI antibody titers did not exceed 1.5 for any comparison between TIV and QIV for shared vaccine strains. The upper limit of the 2-sided

<table>
<thead>
<tr>
<th>Group/Lineage</th>
<th>Timing</th>
<th>N</th>
<th>GMT (95% CI)</th>
<th>SCR (95% CI)</th>
<th>SPR (95% CI)</th>
<th>SCF (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/California/7/2009 (H1N1)</td>
<td>Pre</td>
<td>876</td>
<td>29.4 (26.8, 32.2)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>QIV</td>
<td>Post</td>
<td>878</td>
<td>362.7 (335.3, 392.3)</td>
<td>84.4 (81.8, 86.7)</td>
<td>96.8 (95.4, 97.9)</td>
<td>12.3 (11.3, 13.4)</td>
</tr>
<tr>
<td>TIV-Vic</td>
<td>Pre</td>
<td>870</td>
<td>2.9 (2.4, 3.5)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>871</td>
<td>429.1 (396.5, 464.3)</td>
<td>86.8 (84.3, 89.0)</td>
<td>97.4 (96.1, 98.3)</td>
<td>13.3 (12.3, 14.4)</td>
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</tr>
<tr>
<td>TIV-Yam</td>
<td>Pre</td>
<td>877</td>
<td>2.9 (2.6, 3.1)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>878</td>
<td>420.2 (388.8, 454.0)</td>
<td>85.5 (83.0, 87.8)</td>
<td>96.6 (95.2, 97.7)</td>
<td>14.4 (13.3, 15.7)</td>
<td></td>
</tr>
<tr>
<td>QIV-only (6–35 mo)</td>
<td>Pre</td>
<td>259</td>
<td>16.8 (13.9, 20.3)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>259</td>
<td>200.9 (166.6, 242.2)</td>
<td>84.9 (80.0, 89.1)</td>
<td>89.6 (85.2, 93.0)</td>
<td>12.0 (10.5, 13.6)</td>
<td></td>
</tr>
<tr>
<td>A/Victoria/210/2009 (H3N2)</td>
<td>Pre</td>
<td>876</td>
<td>18.1 (16.7, 19.7)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>QIV</td>
<td>Post</td>
<td>878</td>
<td>143.7 (134.2, 153.9)</td>
<td>70.1 (66.9, 73.1)</td>
<td>92.9 (91.0, 94.5)</td>
<td>7.9 (7.3, 8.6)</td>
</tr>
<tr>
<td>TIV-Vic</td>
<td>Pre</td>
<td>870</td>
<td>19.0 (17.4, 20.6)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>871</td>
<td>139.6 (120.5, 149.3)</td>
<td>67.8 (64.6, 70.9)</td>
<td>92.8 (90.8, 94.4)</td>
<td>7.4 (6.8, 8.0)</td>
<td></td>
</tr>
<tr>
<td>TIV-Yam</td>
<td>Pre</td>
<td>876</td>
<td>19.4 (17.8, 21.1)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>878</td>
<td>151.0 (141.0, 161.6)</td>
<td>69.6 (66.5, 72.7)</td>
<td>93.1 (91.4, 94.8)</td>
<td>7.8 (7.2, 8.5)</td>
<td></td>
</tr>
<tr>
<td>QIV-only</td>
<td>Pre</td>
<td>259</td>
<td>5.6 (5.3, 6.0)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>259</td>
<td>61.4 (53.8, 70.0)</td>
<td>73.0 (67.1, 78.3)</td>
<td>74.5 (68.8, 79.7)</td>
<td>10.9 (8.6, 12.4)</td>
<td></td>
</tr>
<tr>
<td>B/Brisbane/60/2008 (Victoria)</td>
<td>Pre</td>
<td>876</td>
<td>24.8 (22.5, 27.3)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>QIV</td>
<td>Post</td>
<td>878</td>
<td>250.5 (230.8, 272.0)</td>
<td>74.5 (71.5, 77.4)</td>
<td>95.4 (93.8, 96.7)</td>
<td>10.1 (8.2, 11.1)</td>
</tr>
<tr>
<td>TIV-Vic</td>
<td>Pre</td>
<td>870</td>
<td>25.8 (23.5, 28.4)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>871</td>
<td>245.9 (226.9, 265.4)</td>
<td>71.5 (68.4, 74.5)</td>
<td>96.3 (94.9, 97.5)</td>
<td>9.5 (8.6, 10.5)</td>
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</tr>
<tr>
<td>TIV-Yam</td>
<td>Pre</td>
<td>877</td>
<td>25.8 (23.5, 28.4)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>878</td>
<td>68.1 (61.9, 74.9)</td>
<td>29.9 (26.9, 33.1)</td>
<td>73.3 (70.3, 76.2)</td>
<td>2.6 (2.5, 2.8)</td>
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<tr>
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<td>Pre</td>
<td>259</td>
<td>8.7 (7.5, 10.0)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>259</td>
<td>127.3 (109.4, 148.1)</td>
<td>84.6 (79.6, 88.7)</td>
<td>88.0 (83.4, 91.7)</td>
<td>14.6 (12.8, 16.6)</td>
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</tr>
<tr>
<td>B/Florida/4/2006 (Yamagata)</td>
<td>Pre</td>
<td>876</td>
<td>57.9 (52.0, 64.4)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>QIV</td>
<td>Post</td>
<td>878</td>
<td>512.5 (477.6, 549.9)</td>
<td>75.2 (72.2, 78.1)</td>
<td>99.0 (91.1, 99.5)</td>
<td>8.9 (8.1, 9.7)</td>
</tr>
<tr>
<td>TIV-Vic</td>
<td>Pre</td>
<td>870</td>
<td>58.4 (52.6, 64.9)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>871</td>
<td>197.0 (180.7, 214.8)</td>
<td>41.3 (38.0, 44.6)</td>
<td>92.4 (90.5, 94.1)</td>
<td>3.4 (3.1, 3.6)</td>
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<td>TIV-Yam</td>
<td>Pre</td>
<td>877</td>
<td>65.9 (59.3, 73.2)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>878</td>
<td>579.0 (541.2, 619.3)</td>
<td>73.4 (70.4, 76.3)</td>
<td>99.4 (98.7, 99.8)</td>
<td>8.8 (8.1, 9.6)</td>
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<tr>
<td>QIV-only</td>
<td>Pre</td>
<td>259</td>
<td>7.7 (7.0, 8.6)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Post</td>
<td>259</td>
<td>192.7 (172.1, 215.7)</td>
<td>93.8 (90.2, 96.4)</td>
<td>96.5 (93.5, 98.4)</td>
<td>24.9 (22.0, 28.3)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ATP, according to protocol; CI, confidence interval; GMT, geometric mean titer; Post, postvaccination; Pre, prevaccination; QIV, quadrivalent influenza vaccine; SCF, seroconversion factor (defined as the geometric mean of the within-subjects ratios of the postvaccination reciprocal HI titer to the day 0 reciprocal HI titer); SCR, seroconversion rate (defined as the proportion of vaccinees with either a prevaccination titer <1:10 and a postvaccination titer ≥1:40, or a prevaccination titer ≥1:10 and at least a 4-fold increase in postvaccination titer); SPR, seroprotection rate (defined as the percentage of subjects who had a serum anti-HI antibody titer ≥1:40); TIV-Yam, trivalent influenza vaccine Yamagata lineage B strain; TIV-Vic, trivalent influenza vaccine Victoria lineage B strain.

* Each of the 4 strains contained in the QIV met Center for Biologics Evaluation and Research licensure criteria for immunogenicity (lower limit of 95% CI for SCR of at least 40% and a postvaccination SPR of at least 70%).
95% CI for the SCR difference did not exceed 10% for any comparisons between TIV and QIV for shared strains. Immunogenicity of QIV was superior versus TIV-Vic for the B/Yamagata strain, and versus TIV-Yam for the B/Victoria strain based on the GMT ratio (QIV/TIV) and the SCR difference (QIV minus TIV) (Table 4).

The QIV-fulfilled CBER criteria for immunogenicity. Seroconversion rates in the 3- to 17-year-old group, for strains

### Table 3. Immunogenic Noninferiority Comparison of QIV to TIV-Yam and TIV-Vic in Children 3–17 Years of Age: ATP Immunogenicity Cohort

<table>
<thead>
<tr>
<th>Cohort</th>
<th>TIV-Vic + TIV-Yam</th>
<th>QIV</th>
<th>GMT ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (adjusted GMT)</td>
<td>N (adjusted GMT)</td>
<td></td>
</tr>
<tr>
<td>A/California/7/2009 (H1N1)</td>
<td>1747 (421.4)</td>
<td>876 (366.3)</td>
<td>1.15 (1.06, 1.25)</td>
</tr>
<tr>
<td>A/Victoria/210/2009 (H3N3)</td>
<td>1746 (144.3)</td>
<td>876 (145.8)</td>
<td>0.99 (0.92, 1.07)</td>
</tr>
<tr>
<td>B/Brisbane/60/2008 (Victoria)</td>
<td>870 (243.4)</td>
<td>876 (252.5)</td>
<td>0.96 (0.87, 1.07)</td>
</tr>
<tr>
<td>B/Florida/4/2006 (Yamagata)</td>
<td>877 (564.6)</td>
<td>876 (525.2)</td>
<td>1.08 (0.99, 1.16)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SCR difference</th>
<th>% difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/California/7/2009 (H1N1)</td>
<td>1505/1747 (86.1%)</td>
</tr>
<tr>
<td>A/Victoria/210/2009 (H3N3)</td>
<td>1200/1746 (68.7%)</td>
</tr>
<tr>
<td>B/Brisbane/60/2008 (Victoria)</td>
<td>622/870 (71.5%)</td>
</tr>
<tr>
<td>B/Florida/4/2006 (Yamagata)</td>
<td>644/877 (73.4%)</td>
</tr>
</tbody>
</table>

Abbreviations: ATP, according to protocol; CI, confidence interval; GMT, geometric mean titer; N, number; QIV, quadrivalent influenza vaccine; SCR, seroconversion rate; TIV-Vic, trivalent influenza vaccine Victoria lineage B strain; TIV-Yam, trivalent influenza vaccine Yamagata lineage B strain.

a Immunogenic noninferiority demonstrated if the upper limit of the 2-sided 95% CI of the adjusted GMT was \( \leq 1.5 \) and the upper limit of the 2-sided 95% CI of the difference between SCR was \( \leq 10\% \) for all vaccine strains.

b (TIV-Vic + TIV-Yam) divided by QIV.

c TIV-Vic divided by QIV.

d TIV-Yam divided by QIV.

e SCR was defined as the proportion of vaccinees with either a prevaccination titer <1:10 and a postvaccination titer \( \geq 1:40 \), or a prevaccination titer \( \geq 1:10 \) and at least a 4-fold increase in postvaccination titer.

f (TIV-Vic + TIV-Yam) minus QIV.

g QIV minus QIV.

h TIV-Yam minus QIV.

### Table 4. Immunogenic Superiority Comparison of QIV to TIV-Yam and TIV-Vic in Children 3–17 Years of Age: ATP Immunogenicity Cohort

<table>
<thead>
<tr>
<th>Cohort</th>
<th>TIV-Vic</th>
<th>TIV-Yam</th>
<th>QIV</th>
<th>GMT ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (adjusted GMT)</td>
<td>N (adjusted GMT)</td>
<td>N (adjusted GMT)</td>
<td></td>
</tr>
<tr>
<td>B/Florida/4/2006 (Yamagata)</td>
<td>870 (196.5)</td>
<td>. . .</td>
<td>876 (513.8)</td>
<td>2.61 (2.41, 2.84)</td>
</tr>
<tr>
<td>B/Brisbane/60/2008 (Victoria)</td>
<td>. . .</td>
<td>876 (67.2)</td>
<td>876 (253.7)</td>
<td>3.78 (3.43, 4.16)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SCR difference</th>
<th>% difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/California/7/2009 (H1N1)</td>
<td>359/870 (41.3%)</td>
</tr>
<tr>
<td>A/Victoria/210/2009 (H3N3)</td>
<td>262/876 (29.9%)</td>
</tr>
</tbody>
</table>

Abbreviations: ATP, according to protocol; CI, confidence interval; GMT, geometric mean titer; N, number; QIV, quadrivalent influenza vaccine; SCR, seroconversion rate; TIV-Vic, trivalent influenza vaccine Victoria lineage B strain; TIV-Yam, trivalent influenza vaccine Yamagata lineage B strain.

a Immunogenic superiority demonstrated if the lower limit of the 2-sided 95% CI of the adjusted GMT was \( >1.5 \) and the lower limit of the 2-sided 95% CI of the difference between SCRs was \( >10\% \) with respect to each B strain in the QIV compared with corresponding TIV lacking the same B strain.

b QIV divided by TIV-Vic.

c QIV divided by TIV-Yam.

d SCR was defined as the proportion of vaccinees with either a prevaccination titer <1:10 and a postvaccination titer \( \geq 1:40 \), or a prevaccination titer \( \geq 1:10 \) and at least a 4-fold increase in postvaccination titer.

e QIV minus TIV-Vic.

f QIV minus TIV-Yam.
contained in the vaccine, ranged from 92% to 99%. The TIV controls did induce HI antibodies directed to the alternate B lineage strain not in the vaccine, but with reduced seroconversion rates (29.9%–41.3%) relative to the response of the B strain contained in the vaccines (71.5%–75.2%).

GMTs for each of the 4 strains prior to vaccination and 28 days after the final dose in primed compared to unprimed children 3–8 years of age are seen in Supplementary Figure 1. In general, vaccination in unprimed children (who received 2 doses) resulted in as high or higher GMTs than in primed children (who received 1 dose). GMTs to the B/Yamagata strain in recipients of QIV and TIV-Yam were twice as high compared with the B/Victoria in recipients of QIV or TIV-Vic. In the QIV-only 6- to 35-month-old group, HI antibody titers fulfilled CBER criteria for immunogenicity for the strains contained in the QIV with the exception of SPR for the A/Victoria/210/2009 (H3N2) strain (74.5, 95% CI, 68.8, 79.9). Postvaccination SPRs in this age group ranged from 73% to 96%.

**Reactivity**

The frequency of injection site and general solicited AEs overall during 7 days after vaccination in children ≤5 years of age is shown in Figure 2. Pain was the most common injection site AE in children 3–17 years of age and in children 6–35 months of age receiving QIV. Grade 3 pain was reported by <4% of participants. The most common solicited general AEs in children 3–5 years of age were drowsiness, irritability, and loss of appetite; in children 6–35 months of age, the most common solicited general AE was irritability (Figure 2). In children >5 year of age, muscle ache, fatigue, and headache were the most frequent general solicited AEs (Supplementary Table 2). Any fever >38°C occurred in less than 10% of children, and grade 3 solicited AEs (including fever ≥39°C [≥102.2°F]) occurred in <3.2% of 3- to 5-year-olds, and less than 1.8% of 5- to 17-year-olds.

At least 1 unsolicited AE during the 28-day postvaccination period was reported by 283/932 (30.4%), 291/929 (31.3%), and 275/932 (29.5%) of children in the QIV, TIV-Vic, and
In this large multicenter, randomized, controlled trial in children age 3–17 years, respectively, and reported by 160/301 (53.2%) of the 6–35 months QIV group. In all children, cough was the most frequently reported unsolicited AE in this period (4.1%–11.3%).

At least 1 MAE was reported during the 6-month follow-up by 346/932 (37.1%), 335/929 (36.1%), and 350/932 (37.6%) of children in the randomized study in the QIV, TIV-Vic, and TIV-Yam groups, respectively, and reported by 147/301 (48.8%) of children in the QIV-only arm. Among all participants, upper-respiratory-tract infection was the most common MAE. Thirty-five SAEs were reported in 21 children overall during the 6-month follow-up (Supplementary Table 3); there were neither deaths nor withdrawals due to a SAE. Among children age 3–17 years, 3 (0.3%) children from the QIV group, 6 (0.6%) children from the TIV-Vic group, and 5 (0.5%) children from the TIV-Yam group reported 4, 12, and 9 SAEs, respectively, over the 6-month follow-up. Among children age 6–35 months in the QIV-only arm, 7 children (2.3%) reported a total of 10 SAEs. Four SAEs in 3 children were considered by the investigator to be related to the study vaccines: 2 SAEs (angioedema and acute conjunctivitis), which resolved within 7 days, were reported for a 12-year-old boy given TIV-Yam. A 1-year-old girl had a generalized seizure on the day after QIV receipt, and a 2-year-old boy had a febrile seizure 18 days after QIV receipt; both recovered within 1 day.

**DISCUSSION**

In this large multicenter, randomized, controlled trial in children 3–17 years of age, the immunogenicity of a candidate QIV was noninferior to TIV for shared vaccine strains, and was superior to TIV with respect to the added B strains. Inclusion of a fourth strain in QIV did not interfere with immune responses to the other 3 strains, which met all CBER criteria. Immune responses to all strains contained in the 3 vaccines were robust, with the highest responses to the B Yamagata and A/California/7/2009 (H1N1) strains. In the superiority analysis, the ratio of the GMT of QIV to TIV for the B strains showed that QIV elicited an immune response 2.5- to 3.7-fold higher than that of TIV. Similarly, the seroconversion rates of QIV were 32 to 40 percentage points higher than TIV. In brief, the immune responses to each B component of the quadrivalent vaccine exceed the cross-reactive immune responses to the B component not contained in each trivalent vaccine. The superior QIV immunogenicity is expected to correspond with superior protection against influenza B relative to TIV in a season when there is lineage mismatch or cocirculation of 2 influenza B lineages.

These immunogenicity results suggest that the candidate QIV can improve protection against influenza B without compromising protection against influenza A. Because influenza B exacts a special toll on the young and particularly those with comorbidities (increasing their risk for complicated influenza), and mismatch of vaccine and circulating B strain has been frequent with TIVs, the option of a QIV for children is particularly relevant.

An open-label study arm of children 6–35 months of age was included in this study. Vaccines for this age group were given at the same dosage as older children, 0.5 mL, which is considered the “full” or “adult” dose. Practice regarding dose for young children varies around the world, with some jurisdictions routinely using a “half dose” for children up to 35 months of age and a 0.5 mL dose for older children. Public health in Canada first recommended the 0.5 mL dose for the 2011–2012 season [14]; recent evidence suggests that this dose results in moderate improvement in antibody response without increase in reactogenicity [15–16]. HI antibody responses in 6- to 35-month-olds were lower than in older children, but fulfilled CBER criteria [13] except for the SPR for the A/H3N2 strain. Because no TIV comparator arm was included for the youngest age group, no conclusions about relative immunogenicity or reactogenicity differences can be made. Based on the results of this open-label study, a randomized controlled study of this population is underway (NCT01711736). We speculate that copresentation of antigens from 2 lineages of influenza B virus to young children might result in improved responses to both B lineage strains. Priming with 1 influenza B lineage has been shown to affect immunogenicity to subsequent doses of the other lineage [17–18], without affecting humoral responses to influenza A antigens. In the open-label QIV-only arm, the candidate QIV elicited notably high SCRs of 94.6% and 93.8% for B/Victoria and B/Yamagata strains, respectively, in children 6–35 months old. Because there was no comparator arm for this component of the study, no conclusions can be made, but this possibility warrants further investigation.

Quadrivalent influenza vaccine contains 60 µg of HA antigen, and it is therefore plausible that reactogenicity could be higher than with a vaccine containing 45 µg of HA antigen. Injection site pain was modestly more common with QIV than TIV controls in 3- to 17-year-old recipients after the first dose (65.4% in QIV recipients, 54.6% in TIV-Vic recipients, and 55.7% in the TIV-Yam group), although redness and swelling were not. Pain was also the most common injection site symptom in 6- to 35-month-olds, but less frequent than in older participants. The overall percentage of participants presenting with at least 1 AE during the 7 days following their vaccination ranged from 69.0% to 77.3% in the 3–17 year age group, and was 74.8% in the 6–35 month age group. Other than an increase in injection site pain, the AE profile of the candidate QIV was similar to that of the TIV controls.

There are several limitations to this study. The intermediate outcome of immunogenicity was assessed; therefore, clinical protection cannot be predicted with accuracy. As well, the 6- to 35-month-old group had no TIV comparator, and their outcomes cannot be compared to older children. Finally, we do not know the duration of antibody responses to the 4 strains, as immunogenicity was only assessed to 28 days after final vaccination.
In summary, the evolution of influenza B viruses has led to 2 cocirculating, antigenically distinct B lineages, and immune responses to one confers limited or no cross-lineage protection. A live attenuated QIV has received regulatory approval for use in persons 2–49 years of age in the United States [19] as has an injected QIV [20], and the World Health Organization made its first recommendations for constituents of QIVs for the southern hemisphere for the coming season [21]. The inactivated QIV assessed in this study can potentially provide protection against both B lineages, as well as to the 2 influenza A strains, and thus reduce influenza morbidity in children.

Supplementary Data

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

Notes

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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


