

Safety, immunogenicity, and lot-to-lot consistency of a quadrivalent inactivated influenza vaccine in children, adolescents, and adults: A randomized, controlled, phase III trial



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ABSTRACT

Background: Inactivated quadrivalent influenza vaccine (IIV4) containing two influenza A strains and one strain from each B lineage (Yamagata and Victoria) may offer broader protection against seasonal influenza than inactivated trivalent influenza vaccine (IIV3), containing a single B strain. This study examined the safety, immunogenicity, and lot consistency of an IIV4 candidate.

Methods: This phase III, randomized, controlled, multicenter trial in children/adolescents (9 through 17 years) and adults (18 through 60 years) was conducted in Australia and in the Philippines in 2012. The study was double-blind for IIV4 lots and open-label for IIV4 vs IIV3. Children/adolescents were randomized 2:2:2:1 and adults 10:10:10:1 to receive one of three lots of IIV4 or licensed IIV3. Safety data were collected for up to 6 months post-vaccination. Hemagglutination inhibition and seroneutralization antibody titers were assessed pre-vaccination and 21 days post-vaccination.

Results: 1648 adults and 329 children/adolescents received IIV4, and 56 adults and 55 children/adolescents received IIV3. Solicited reactions, unsolicited adverse events, and serious adverse events were similar for IIV3 and IIV4 recipients in both age groups. Injection-site pain, headache, malaise, and myalgia were the most frequently reported solicited reactions, most of which were mild and resolved within 3 days. No vaccine-related serious adverse events or deaths were reported. Post-vaccination antibody responses, seroconversion rates, and seroprotection rates for the 3 strains common to both vaccines were comparable for IIV3 and IIV4 in both age groups. Antibody responses to IIV4 were equivalent among vaccine lots and comparable between age groups for each of the 4 strains. IIV4 met all European Medicines Agency immunogenicity criteria for adults for all 4 strains.

Conclusions: In both age groups, IIV4 was well tolerated and caused no safety concerns, induced robust antibody responses to all 4 influenza strains, and met all EMA immunogenicity criteria for adults.

Clinical trial registry number: NCT01481454.

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1. Introduction

Inactivated quadrivalent influenza vaccines (IIV4s) contain inactivated split virions from two influenza A virus strains (H1N1 and H3N2) and from the two B lineage strains (Yamagata and Victoria) [1–3]. Each year, the exact strains to be included in the quadrivalent and trivalent seasonal influenza vaccines are determined by the World Health Organization based on influenza disease surveillance data from the previous year [4]. Because the two influenza B lineages have co-circulated globally for over a decade, persons vaccinated with trivalent inactivated influenza vaccine (IIV3), which contains only one B strain, have been left unprotected against the B lineage not included in the vaccine [1,2]. IIV4 offers improved coverage over IIV3 and is expected to substantially reduce illness and hospitalizations due to influenza B [5,6]. Several quadrivalent influenza vaccines have been recently licensed for use in the US and are either approved or awaiting approval in Europe [3,4]. These vaccines have been consistently shown to be as safe and as immunogenic as their trivalent counterparts [7–12].

A recent phase III trial conducted in France during the 2011/2012 influenza season demonstrated the safety and immunogenicity of an IIV4 candidate in healthy adults [12]. Here, we report the results of a phase III trial investigating the safety, immunogenicity, and lot-to-lot consistency of this IIV4 candidate in children, adolescents, and adults, and the first results for this vaccine in children and adolescents.

2. Materials and methods

2.1. Study design

This was a phase III, randomized, controlled, multicenter trial in children/adolescents (9 through 17 years of age) and adults (18 through 60 years of age) performed at six centers in Australia and four centers in the Philippines in 2012 (ClinicalTrials.gov identifier: NCT01481454). The study was double-blind for IIV4 lots and open-label for IIV4 vs IIV3. The primary objective was to describe the safety profiles (injection-site reactions and systemic events) of IIV4 and IIV3 during the 21 days following vaccination and the serious adverse events (SAEs) for 6 months in all adult and child/adolescent participants. Secondary objectives were to demonstrate compliance of IIV4 with European Medicines Agency (EMA) immunogenicity criteria in adults, demonstrate the immunogenic consistency of three lots of IIV4, and describe post-vaccination seroneutralization titers in children/adolescents. The study was approved by the independent ethics committee and/or institutional review board responsible for each study site and was carried out in accordance with International Conference on Harmonization Guidelines for Good Clinical Practice, the Declaration of Helsinki, and with all national and local ethical requirements. Written informed consent was obtained from all adult participants and from all parents or legal representatives of 9–17-year-old participants included in the trial. Some 9–17-year-old participants also provided written informed assent where required by local regulations.

2.2. Study population

Individuals 9–60 years old who had not been previously vaccinated against influenza with a 2012 Southern Hemisphere formulation or a 2011–2012 Northern Hemisphere formulation in the previous 6 months were considered for study inclusion. They were excluded if they had received another vaccination within 4 weeks before inclusion; were allergic to or had a history of a serious adverse reaction (AR) to any influenza vaccine; had a known or

suspected congenital or acquired immunodeficiency; had moderate or severe acute illness/infection or a temperature $\geq 38.0^{\circ}\text{C}$; or had recently received immunosuppressive or corticosteroid treatment, immune globulins, blood, or blood-derived products. Women were excluded if they were pregnant, lactating, or of childbearing potential and not using adequate birth control. Enrollment was stratified by age at each site into children/adolescents 9 through 17 years old and adults 18 through 60 years old.

2.3. Randomization

Adult participants were randomized 10:10:10:1 and children/adolescents 2:2:2:1 to be immunized with one of three lots of IIV4 or with licensed IIV3. Participants were randomized using the permuted block method with stratification by site and age group and were assigned to the treatment groups via an interactive voice or web response system. All vaccines were administered by intramuscular injection with a 16-mm, 25-gauge needle. The study was open-label for receipt of either IIV4 or licensed IIV3 and was double-blind for IIV4 lots so that neither the investigator nor the subject or subject's legal representative knew which lot was administered. Blood samples were collected before vaccination (day 0) and 21 days after vaccination.

2.4. Vaccines

All vaccines were inactivated, split-virion preparations containing 15 μg hemagglutinin per strain in a total volume of 0.5 mL. IIV4 lot 1 (batch S4361), lot 2 (batch S4362), and lot 3 (batch S4363) contained the A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (Victoria lineage) strains (which were among those recommended for the 2011/2012 Northern Hemisphere and the 2012 Southern Hemisphere formulations), and the B/Florida/04/2006 strain (Yamagata lineage). The licensed IIV3 (batch H0290) was the 2011/2012 Northern Hemisphere formulation of Vaxigrip® (Sanofi Pasteur, Marcy-l'Étoile, France), which contained the A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (Victoria lineage) strains.

2.5. Hemagglutination inhibition (HAI) assay

The HAI assay was performed as previously described [11]. The highest serum dilution resulting in complete inhibition of hemagglutination was determined in duplicate for each sample. The titer for each strain was calculated as the geometric mean of the reciprocal of the duplicate values obtained for each sample. The lower limit of quantitation was a titer of 10, which is the reciprocal of the lowest dilution used in the assay. Samples with HAI antibody titers below 10 were assigned a titer of 5. The seroprotection rate for each group was the percentage of participants with a titer ≥ 40 . The seroconversion rate for each group was the percentage of participants with either a pre-vaccination titer < 10 and a post-vaccination titer ≥ 40 or a pre-vaccination titer ≥ 10 and a ≥ 4 -fold increase in titer at day 21.

2.6. Seroneutralization assay

The seroneutralization assay was a microneutralization assay based on methods previously described by the World Health Organization and the US Centers for Disease Control and Prevention [13,14]. Serially diluted, heat-inactivated serum samples from vaccinated child/adolescent participants were pre-incubated with a fixed amount of influenza A or B virus prior to adding the serum-virus mixture to Madin-Darby canine kidney cell cultures. After overnight incubation, viral nucleoprotein production in the infected cells was measured by enzyme-linked immunosorbent

assay using a monoclonal antibody specific to the nucleoprotein of either influenza A or B. Reduced or absent infectivity indicated the presence of influenza virus-specific neutralizing antibodies in human sera. The neutralization titer expressed by the reciprocal (1/dilution) was calculated by the intersection of the optical density curve of the test sample and the 50% neutralization point of the control optical density curve. The lower limit of quantitation was a titer of 10, which was the reciprocal of the lowest dilution used in the assay. Samples with neutralizing antibody titers below 10 were assigned a titer of 5.

2.7. Reactogenicity and safety

Solicited injection-site reactions (pain, erythema, swelling, induration, ecchymosis) and systemic reactions (fever, headache, malaise, myalgia, shivering) were recorded for 7 days after each vaccination. Unsolicited adverse events (AEs) and SAEs were collected according to the International Committee for Harmonization Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. Unsolicited AEs were collected for 21 days after vaccination. Immediate unsolicited adverse reactions (ARs) were those occurring within 30 min of vaccination and were considered to be related to the vaccination. SAEs and AEs of special interest were collected for 6 months after vaccination. AEs of special interest included anaphylaxis, Guillain-Barré syndrome, encephalitis, myelitis, neuritis, convulsions, and vasculitis. AEs and SAEs were classified by the center investigators as related or unrelated to the study vaccines.

2.8. Sample size determination

A total of 1980 participants (330 children/adolescents and 1705 adults) were planned to receive IIV4. This was estimated to allow AEs with a true incidence of 0.15% overall or 0.90% in children/adolescents to be detected with a probability of 95%. Based on simulations, 660 participants in each IIV4 lot group would provide 90% power to demonstrate lot-to-lot consistency for immunogenicity with an alpha of 5%.

2.9. Statistical analysis

Statistical analysis was performed using SAS version 9.2 (SAS Institute, Cary, NC). Safety was assessed in all participants who received a study vaccine according to the vaccine received. Lot-to-lot consistency was assessed in all participants who completed the study according to protocol. Lot-to-lot equivalence for each strain was demonstrated if the age group-stratified two-sided 95% confidence interval (CI) of the post-vaccination HAI geometric mean antibody titer (GMT) ratio for that strain in the two lots being compared was between 0.67 and 1.5. Age group-stratified 95% CIs were calculated using an analysis of variance model (type II analysis) of log₁₀-transformed titers. The 95% CIs for GMTs and GMT ratios (HAI and seroneutralization) were calculated from the Student's *t* distribution of log₁₀-transformed titers.

3. Results

3.1. Study participants

Between March 19, 2012 and April 28, 2012, 1390 participants in the Philippines and 700 participants in Australia were enrolled and randomized (Fig. 1). The last follow-up visit was on October 31, 2012. Of the 2090 participants, 1977 were vaccinated with IIV4 (1648 adults and 329 children/adolescents) and 111 with IIV3 (56 adults and 55 children/adolescents). A total of 2071 participants (1962 IIV4, 109 IIV3) completed the study. Sex ratios were nearly

equivalent in the adult IIV3 group and in both child/adolescent groups, however the adult IIV4 group contained more women (61.4%) than men (Supplementary Table 1). Within each age group, mean ages and influenza vaccination histories were similar for the two vaccine groups. Most of the participants in each group were Asian.

3.2. Reactogenicity and safety

Solicited reaction frequencies were similar for IIV4 and IIV3 in adults (IIV4: 61.2%, 95% CI [58.8; 63.6]; IIV3: 57.1%, 95% CI [43.2; 70.3]) as well as in children/adolescents (IIV4: 66.6%, 95% CI [61.2; 71.6]; IIV3: 67.3%, 95% CI [53.3; 79.3]) (Fig. 2). Reactogenicity profiles were also similar for the three lots of IIV4 in both age groups (data not shown). In all groups, pain was the most common solicited injection-site reaction, whereas headache, malaise, and myalgia were the most common solicited systemic reactions. Almost all injection-site (99.1%) and systemic reactions (96.2%) were mild or moderate in severity and most (88.2%) resolved within 3 days. Although 19 (1.2%) adult IIV4 recipients reported grade 3 headache and 16 (1.0%) reported grade 3 malaise, all other types of grade 3 reactions were reported by less than 1% of the IIV4 recipients in either age group. Less than 2% the IIV3 recipients reported a grade 3 injection-site reaction and none reported a grade 3 systemic reaction.

Frequencies of unsolicited AEs were also similar for both vaccines in adults (IIV4: 18.0%, 95% CI [16.1; 19.9]; IIV3: 12.5%, 95% CI [5.2; 24.1]) and in children/adolescents (IIV4: 17.6%, 95% CI [13.7; 22.2]; IIV3: 29.1%, 95% CI [17.6; 42.9]). Unsolicited AEs related to vaccination in both age groups were reported for less than 4% of IIV4 recipients (59/1648 adults, 6/329 children/adolescents) and less than 10% of IIV3 recipients (7/56 adults, 5/55 children/adolescents). An unsolicited immediate AR (within 30 min) was reported by one adult in the IIV4 group (urticaria) and one adult in the IIV3 group (cough). Both resolved within 2 days and both participants completed the study. The most frequently reported unsolicited AE related to vaccination was injection-site pruritus, which occurred in 13 adults (0.8%) and one child/adolescent (0.3%) vaccinated with IIV4 and in one child/adolescent (1.8%) vaccinated with IIV3. Grade 3 vaccine-related ARs were reported for 4 adults in the IIV4 group (0.2%): 3 had tremors, dizziness, or cough that resolved in 2 days or less and 1 had fatigue that resolved within 4 days. A total of 13 SAEs were reported during the 6-month study period; all occurred in IIV4 recipients (12 adults and one child/adolescent) and none were considered to be vaccine-related. No participants discontinued the study due to an AE and no AEs of special interest or deaths were reported during the study.

3.3. Immunogenicity

Within each age group, prevaccination HAI antibody titers were similar for participants receiving IIV3 and IIV4, and both vaccines increased HAI antibody titers by post-vaccination day 21 (Fig. 3). For the three strains common to both vaccines (A/H1N1, A/H3N2, and B/Brisbane), within each age group, post-vaccination HAI antibody responses induced by IIV4 were comparable to the responses induced by IIV3. In adults, mean GMTs for these three strains increased 7.3- to 10.0-fold with IIV4 and 6.6- and 9.3-fold with IIV3. In children/adolescents, GMTs increased 7.5- to 23.4-fold with IIV4 and 6.4- to 16.6-fold with IIV3. As expected for the IIV4-specific B/Florida strain, GMTs increased to a greater extent with IIV4 than with IIV3, both in adults (8.4-fold for IIV4 vs 3.2-fold for IIV3) and in children/adolescents (19.4-fold for IIV4 vs 3.8-fold for IIV3). Overall, post-vaccination GMTs for all 4 strains were at least as high in children/adolescents as in adults.

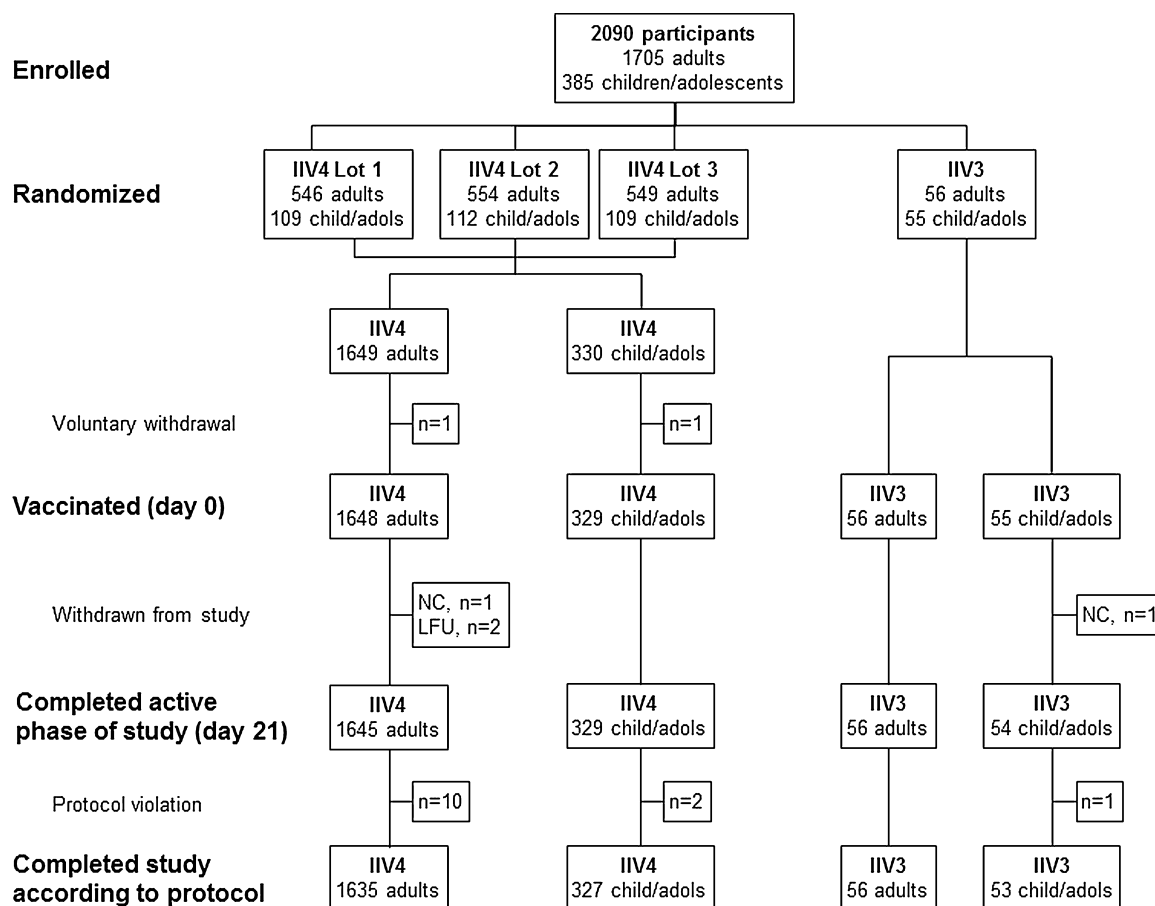


Fig. 1. Participant disposition. Adults (18–60 y) and children/adolescents (child/adols, 9–17 y) were enrolled and then randomized to receive one of three lots of IIV4 or IIV3. Two participants withdrew prior to vaccination. Following vaccination, 4 participants were withdrawn due to noncompliance (NC) or were lost to follow-up (LFU). Thirteen of the remaining participants had protocol violations and were removed from the Per Protocol Set. IIV3, trivalent inactivated influenza vaccine; IIV4, quadrivalent inactivated influenza vaccine.

IIV4 met all EMA criteria for adult vaccine recipients. In adults, post-vaccination seroprotection rates were $\geq 99\%$, seroconversion rates were $>59\%$, and post-vaccination/pre-vaccination GMT ratios were ≥ 7.3 for all four vaccine strains (Table 1). In addition, the lower limits of the 95% CIs for these values exceeded EMA criteria. Although there are no EMA criteria for children or adolescents, in these participants, post-vaccination seroprotection rates were $>98\%$, seroconversion rates were $>61\%$, and post-vaccination/pre-vaccination GMT ratios were ≥ 7.5 for all four strains (Table 1).

Lot-to-lot equivalence was demonstrated for all three lots of IIV4 for all four strains (Table 2, Supplementary Fig. 1). The ratios of the overall post-vaccination GMTs for each pair of lots for each strain were between 0.82 and 1.10, and the 95% CIs for these GMT ratios were all between 0.67 and 1.5.

Seroneutralization titers for the three strains common to IIV3 and IIV4 (A/H1N1, A/H3N2, and B/Brisbane) were similar for the two children/adolescent vaccine groups (Supplementary Table 2). Like the HAI titers, seroneutralization responses for the B/Florida strain were substantially higher in the IIV4 group (26.5-fold increase) than in the IIV3 group (3.7-fold increase).

4. Discussion

Difficulties in predicting the global circulation of influenza B viruses have resulted in missed opportunities for protection. Because quadrivalent influenza vaccines induce antibody responses against both influenza B lineages, they offer broader

protection than trivalent influenza vaccines. This study found comparable reactogenicity and safety profiles for the IIV4 candidate and IIV3 in both adults and in children/adolescents. The study also showed that the immunogenicity of three lots of IIV4 was equivalent for all four strains.

Both vaccines were well tolerated by both age groups. Most solicited injection-site and systemic reactions with either vaccine were mild to moderate and most resolved within a few days. Although no grade 3 systemic reactions were reported for IIV3 (small sample size), a few were reported for IIV4. The most frequent of these was headache in adult IIV4 recipients (1.2%). All other grade 3 systemic reactions (fever, malaise, myalgia, and shivering) were reported by $\leq 1\%$ of the IIV4 recipients in either age group. Similar findings in adults have been reported previously for IIV4 [7,11,12]. Frequencies of unsolicited AEs in the 21 days following vaccination were similar in adults and in children/adolescents vaccinated with IIV4 and few of these were considered to be treatment related.

Together with the previous phase III study [12], 328 children/adolescents, 2423 adults 18–60 years of age, and 784 older adults have now been vaccinated with the IIV4 candidate, for a total of 3525 recipients. This number of vaccinated participants should allow AEs with a true incidence of 0.085% overall to be observed with a probability of 95%. Among all IIV4 vaccinees, no participants have discontinued a study due to an AE and no vaccine-related SAEs, no AEs of special interest, and no safety issues or concerns have been reported. These two clinical trials demonstrate that the addition of a second B strain to IIV3 does not affect its safety,

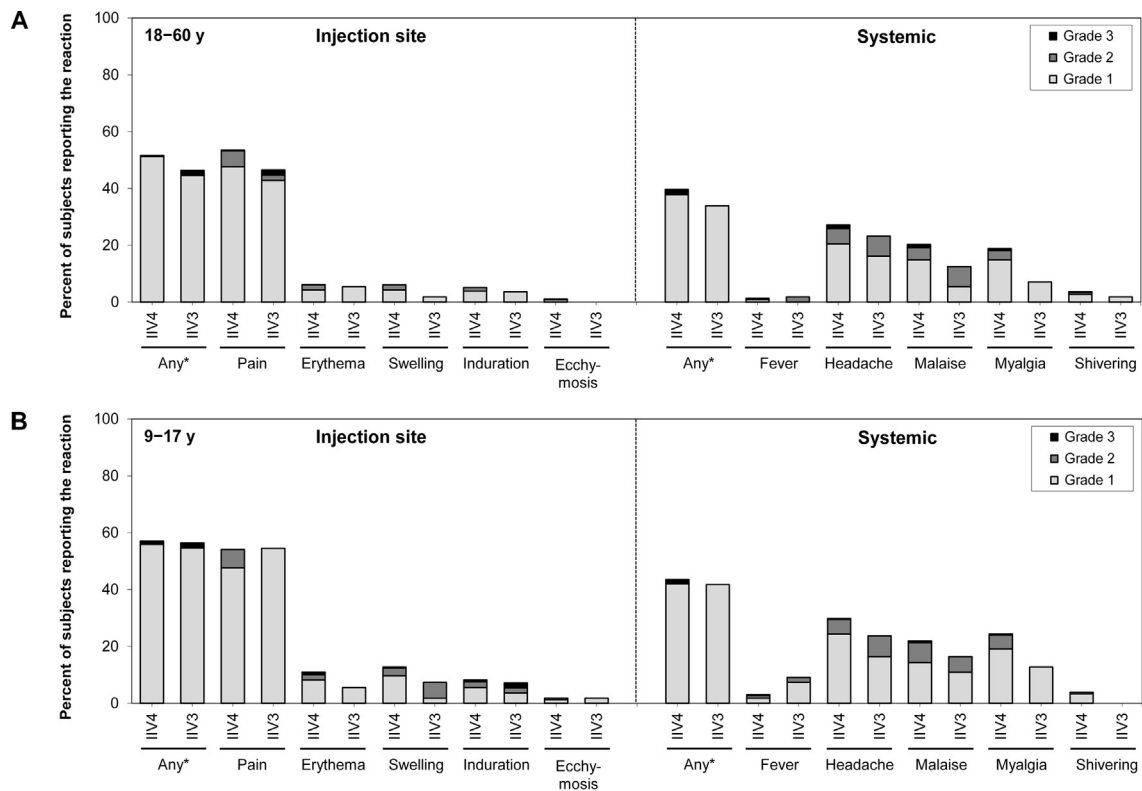


Fig. 2. Solicited injection-site and systemic reactions. The percentages of participants reporting any solicited reaction and specific solicited reactions are shown for adults (A) and for children/adolescents (B). Results are for the Safety Analysis Set that included all participants who received a study vaccine. Erythema, swelling, induration, and ecchymosis in children 9–11 years old were considered grade 1 if >0 to <25 mm, grade 2 if ≥ 25 to <50 mm, and grade 3 if ≥ 50 mm; in participants 12–60 years old, they were considered grade 1 if ≥ 25 to ≤ 50 mm, grade 2 if ≥ 51 to ≤ 100 mm, and grade 3 if >100 mm. Fever was considered grade 1 if ≥ 38.0 °C to ≤ 38.4 °C, grade 2 if ≥ 38.5 °C to ≤ 38.9 °C, and grade 3 if ≥ 39.0 °C. Pain in children 9–11 years old was considered grade 1 if easily tolerated, grade 2 if sufficiently discomforting to interfere with normal behavior or activities, and grade 3 if incapacitating and preventing performance of usual activities. Pain in subjects 12–60 years old and all other events in all age groups were considered grade 1 for no interference with activity, grade 2 for some interference with activity, and grade 3 for significant and preventing daily activities. IIV3, trivalent inactivated influenza vaccine; IIV4, quadrivalent inactivated influenza vaccine. *The “Any” category shows the overall percentage of participants reporting any solicited injection-site or systemic reaction and the percentage reporting any grade 3 reaction. Grade 1 and 2 reactions are not shown for this category.

which agrees with reports for other quadrivalent inactivated and live-attenuated vaccines [8–11].

IIV4 vaccination induced robust antibody responses for all four strains in both age groups. The antibody responses were similar

to those induced by IIV3 for the three strains in common and were higher with IIV4 for the B/Florida strain that was not in IIV3. In addition, IIV4 met all adult EMA immunogenicity criteria for seroprotection rates, GMT ratios, and seroconversion rates for

Table 1
Hemagglutination inhibition immunogenicity of IIV4.

Measure	EMA requirement	A/H1N1	A/H3N2	B/Brisbane	B/Florida
Adults (18–60 y)					
Post-vaccination (day 21) seroprotection ^a rate	$>70\%$	99.3 (98.7, 99.6)	99.0 (98.4, 99.4)	100.0 (99.8, 100.0)	100.0 (99.8, 100.0)
Post-vaccination (day 21) to pre-vaccination (day 0) GMT ratio ^b	>2.5	9.2 (8.5, 10.0)	7.3 (6.8, 7.9)	10.0 (9.2, 10.8)	8.4 (7.8, 9.0)
Rate of seroconversion ^c or significant increase ^d	$>40\%$	63.6 (61.2, 65.9)	59.3 (56.8, 61.7)	66.5 (64.2, 68.8)	65.9 (63.5, 68.1)
Children/adolescents (9–17 y)					
Post-vaccination (day 21) seroprotection ^a rate	–	98.8 (96.9; 99.7)	100.0 (98.9; 100.0)	99.4 (97.8; 99.9)	99.4 (97.8; 99.9)
Post-vaccination (day 21) to pre-vaccination (day 0) GMT ratio ^b	–	12.0 (10.2; 14.0)	7.5 (6.3; 8.9)	23.4 (19.6; 27.8)	19.4 (16.3; 23.2)
Rate of seroconversion ^c or significant increase ^d	–	77.7 (72.8; 82.1)	61.8 (56.3; 67.1)	83.8 (79.3; 87.6)	84.8 (80.4; 88.5)

Values are for the Other Analysis Set, which included randomized participants who received one dose of IIV4 and had valid pre- and post-vaccination HAI titers. IIV4, quadrivalent inactivated influenza vaccine.

^a Seroprotection is defined as a HAI titer ≥ 40 ; values are the percent of participants in each group (with 95% CIs in brackets) with a HAI titer ≥ 40 .

^b Values are the geometric means of the individual post-vaccination (day 21) HAI titer/pre-vaccination (day 0) HAI titer ratios (with 95% CIs in brackets) for the participants in each group.

^c Seroconversion is defined as a pre-vaccination (day 0) HAI titer <10 and post-vaccination (day 21) HAI titer ≥ 40 .

^d Significant increase is defined as a pre-vaccination (day 0) HAI titer ≥ 10 and post-vaccination (day 21) HAI titer/pre-vaccination (day 0) HAI titer ratio ≥ 4 . Values are the percent of participants in each group (with 95% CIs in brackets) who either seroconverted or had a significant increase in HAI titer.

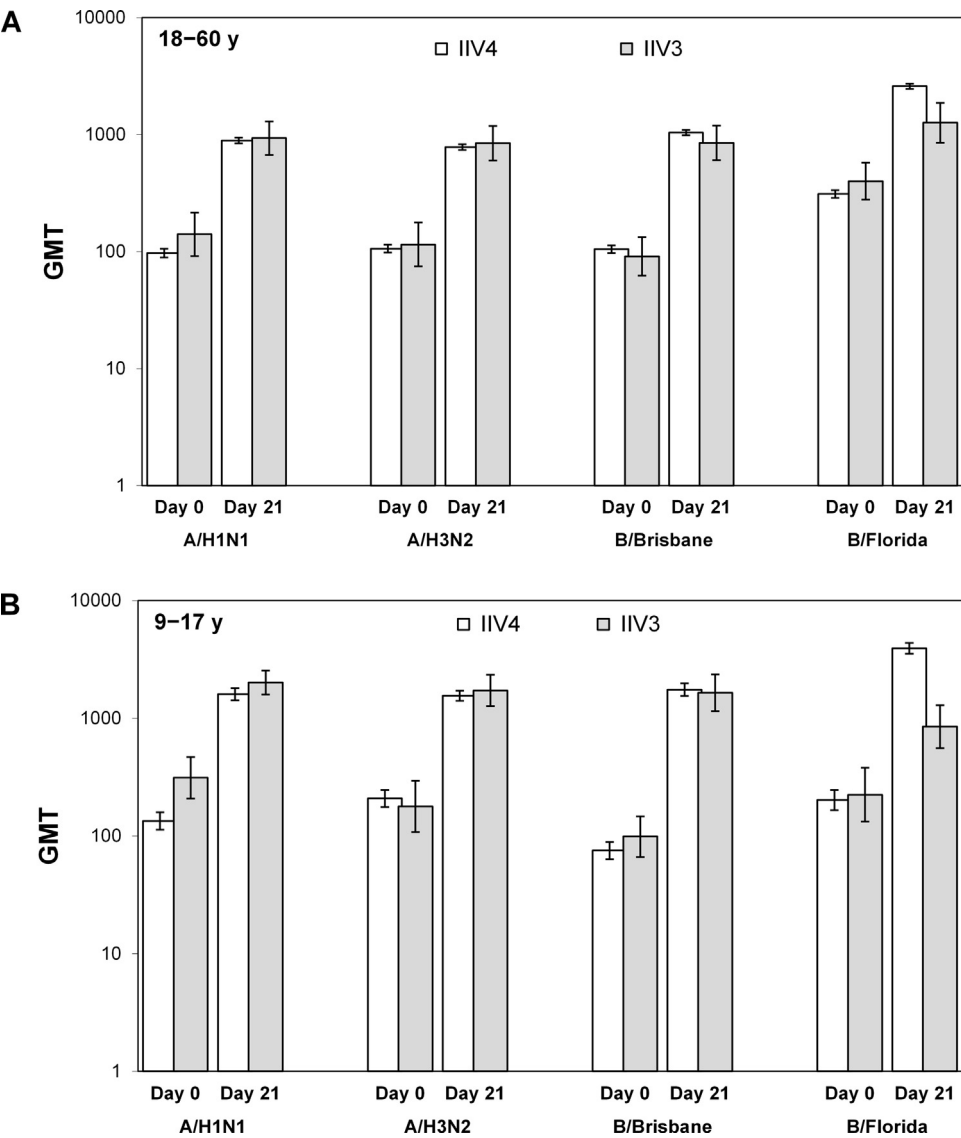


Fig. 3. Geometric mean HAI titers for IIV4 and IIV3. HAI titers were measured on day 0 (pre-vaccination) and 21 days post-vaccination. Bars indicate the geometric mean titers (GMTs) in adults (A) and adolescents/children (B), and whiskers indicate the 95% CIs. Results shown are for the Full Analysis Set, which included all participants who received a study vaccine and had a valid post-vaccination titer result. IIV4, quadrivalent inactivated influenza vaccine; IIV3, trivalent inactivated influenza vaccine.

Table 2
IIV4 lot-to-lot comparisons.

Strain	Comparison	GMT ratio (95% CI)	Equivalence
A/H1N1	Lot 1 vs Lot 2	1.10 (0.97; 1.24)	Yes
	Lot 1 vs Lot 3	0.89 (0.79; 1.01)	Yes
	Lot 2 vs Lot 3	0.82 (0.72; 0.92)	Yes
A/H3N2	Lot 1 vs Lot 2	1.01 (0.90; 1.14)	Yes
	Lot 1 vs Lot 3	0.91 (0.81; 1.03)	Yes
	Lot 2 vs Lot 3	0.90 (0.80; 1.02)	Yes
B/Brisbane	Lot 1 vs Lot 2	1.00 (0.89; 1.12)	Yes
	Lot 1 vs Lot 3	1.00 (0.89; 1.13)	Yes
	Lot 2 vs Lot 3	1.00 (0.89; 1.13)	Yes
B/Florida	Lot 1 vs Lot 2	0.98 (0.88; 1.10)	Yes
	Lot 1 vs Lot 3	0.95 (0.86; 1.06)	Yes
	Lot 2 vs Lot 3	0.97 (0.87; 1.08)	Yes

Values are for the Per Protocol Set, which included randomized participants who completed the study according to protocol. The age-stratified GMTs for each strain for each lot are shown in Supplementary Fig. 1. GMT, geometric mean HAI titer. Lot-to-lot equivalence for each strain was demonstrated if the two-sided 95% confidence interval (CI) of the post-vaccination ratio of the overall GMTs for that strain in the two lots being compared was between 0.67 and 1.5.

all four strains. Although there are currently no EMA criteria for children or adolescents, IIV4 responses tended to be higher in children/adolescents than in adults. These results suggest that a single dose of IIV4 provides sufficient immunity for adults and for children as young as 9 years old.

Although high HAI antibody titers generally correlate with protection for adults [15,16], the correlation is weaker in elderly adults [17,18] and in young children, the latter of which were shown to require HAI titers >110 to achieve 50% protection [19]. In addition, live-attenuated influenza vaccines are effective in young children but do not produce high HAI titers [20]. Hence, alternative serological assays are under evaluation to identify other protection correlates. Seroneutralization assays measure the capacity of all antibodies in the serum to neutralize live influenza viruses and have the potential to be more sensitive than HAI assays, which measure antibodies against only one viral protein [21–24]. Our microneutralization assay is based on enzyme immunoassay of viral nucleoprotein rather than on cytopathic effect. Although this assay was originally developed for use with pandemic influenza viruses (e.g. A/H5N1 and A/H1N1) [13,14], it is now frequently used

to measure responses to other human influenza A and B viruses [7,25,26]. The assay is strain-specific, highly sensitive, and requires less time than neutralization assays based on cytopathic effect and the results have been shown to correlate with HAI [25]. IIV4 and IIV3 both increased seroneutralization antibody titers substantially in children/adolescents with patterns similar to the HAI assay for each strain. This assay should be studied as an alternative measure of immunogenicity in future clinical trials.

The results of our study are in accordance with the previous phase III trial assessing this IIV4 candidate [12] and with several other clinical trials [7–11], which have shown that quadrivalent vaccines are as immunogenic as their trivalent counterparts for the matched influenza strains and that the two vaccine formulations have comparable safety profiles. Thus, the addition of a second B strain to a licensed trivalent vaccine offers additional protection against influenza B, does not interfere with the immunogenicity of the three shared strains, and has little, if any, impact on vaccine safety or reactogenicity.

This was a large study sufficiently powered to observe infrequent AEs and to confirm lot-to-lot equivalence. Because we focused on these objectives for the adult IIV4 group, the other groups in our study were too small to permit other statistically reliable comparisons. Thus, the study did not allow assessment of non-inferiority or superiority of the IIV4 responses to IIV3 nor comparisons between age groups.

In conclusion, this study demonstrated that IIV4 can be reproducibly manufactured to yield a well-tolerated, safe, and immunogenic vaccine in persons 9–60 years of age and that it met all EMA immunogenicity criteria in adults. Vaccination with this IIV4 candidate rather than IIV3 offers a broader immune response to influenza B and might help reduce influenza-related hospitalizations, costs, and deaths.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2015.03.065>.

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