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Safety and immunogenicity of a quadrivalent influenza vaccine in adults 65 y of age and older

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ABSTRACT

Frequent mismatches between the predominant circulating B strain lineage and the B strain lineage in trivalent influenza vaccines have resulted in missed opportunities to prevent influenza illness. Quadrivalent influenza vaccines containing B strains from each of the 2 lineages have been developed for improved prevention of influenza B infections. Here, we describe the results of a phase III, randomized, double-blind, active-controlled, multicenter trial examining the safety and immunogenicity of a split-virion inactivated quadrivalent influenza vaccine (IIV4) in 675 adults ≥ 65 y of age (NCT01218646). Participants were randomly assigned 1:1:1 to receive a single intramuscular injection with the investigational IIV4, or one of 2 split-virion trivalent inactivated influenza vaccines (IIV3s): a licensed IIV3 containing a B Victoria-lineage strain or an investigational IIV3 containing a B Yamagata-lineage strain. Post-vaccination (day 21) hemagglutinin inhibition titers to all strains induced by IIV4 were statistically non-inferior to those induced by the 2 IIV3s. In addition, for each B strain, rates of seroconversion in the IIV4 group were superior to those induced by the comparator IIV3 not containing that B strain. For all vaccines, the most common solicited reaction was injection-site pain, and most reactions were mild to moderate in intensity and transient. Overall safety profiles were similar between IIV4 and the IIV3s, and no vaccine-related serious adverse events were reported. These results confirm that in adults ≥ 65 y of age, IIV4 was well tolerated and immunogenic against the additional B lineage strain without compromising the immunogenicity of the other 3 vaccine strains.

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Introduction

Since 2001, 2 antigenically distinct lineages of influenza B have co-circulated globally, leading to frequent mismatches between the predominant circulating B strain and the single B strain in trivalent influenza vaccines.^{1,2} The result has been a missed opportunity to reduce morbidity and mortality related to seasonal influenza, a disease that contributes to an estimated 3000 to 49,000 deaths and 55,000 to 431,000 hospitalizations each year in the US.^{1,2} Quadrivalent influenza vaccines containing B strains from both lineages have been developed to provide improved protection against influenza and have been available in the US since the 2013–2014 influenza season.³ Economic modeling suggests that if quadrivalent vaccines had been used instead of trivalent vaccines in the US during the 1999–2000 to 2008–2009 influenza seasons, an additional 2.7 million influenza cases, 21,440 influenza-related hospitalizations, and 1371 influenza-related deaths could have been prevented,⁴ with substantial savings to society and third-party payers.⁵

A split-virion quadrivalent influenza vaccine (IIV4; Fluzone[®] Quadrivalent, Sanofi Pasteur) was approved in the US in 2013 for individuals ≥ 6 months of age.⁶ A phase II trial in healthy adults demonstrated the safety and immunogenicity of IIV4.⁷ In that study, IIV4 was compared with 2 split-virion trivalent influenza vaccines (IIV3s), each lacking one of the 2 B strains included in

IIV4. Hemagglutination inhibition (HAI) antibody titers induced by IIV4 were statistically non-inferior to those induced by the comparator IIV3 for the 2 A strains and the B strain present in each of the comparators. Also, IIV4 induced higher HAI antibody titers to each B strain than the control IIV3 lacking the same B strain. The study found no differences in the incidence or severity of solicited reactions or unsolicited adverse events (AEs) between the quadrivalent and trivalent formulations.

Because the phase II study included a limited number of older adults (≥ 65 y of age), a phase III study was conducted to better assess safety and immunogenicity of IIV4 in this age group, which accounts for approximately 63% of influenza-related hospitalizations and 90% of influenza-related deaths.^{8,9} The study also included a small open-label cohort to confirm the safety and immunogenicity of the same year's formulation of IIV3 in adults 18–64 y of age.


Results

Randomized cohort

Participants

The study included 675 adults ≥ 65 y of age randomized (n = 225 per group) to receive IIV4, the licensed IIV3

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 Supplemental data for this article can be accessed on the [publisher's website](#).

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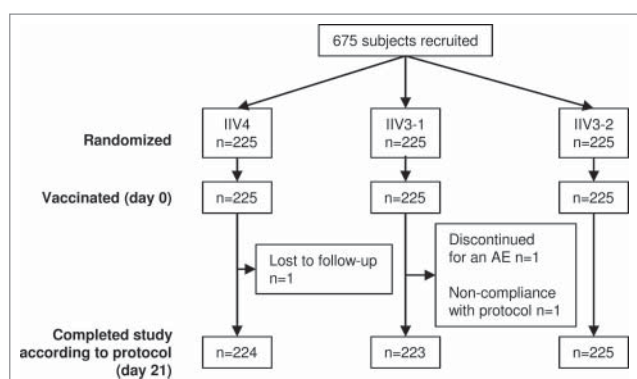


Figure 1. Participant disposition and study flow in the randomized cohort (adults ≥ 65 y of age). A total of 675 adults ≥ 65 y of age were randomly assigned 1:1:1 to receive the quadrivalent split-virion influenza vaccine (IIV4), the licensed trivalent split-virion influenza vaccine containing the B Victoria-lineage strain (IIV3-1), or an investigational split-virion IIV3 containing the B Yamagata-lineage strain (IIV3-2). AE, adverse event; n, number of participants in the group.

containing a B strain of the Victoria lineage (IIV3-1), or an investigational IIV3 containing a B strain of the Yamagata lineage (IIV3-2) (Fig. 1). All but 3 randomized participants completed the study. Baseline characteristics were generally similar in the 3 vaccine groups (Table 1).

Immunogenicity

This study met its primary objective, which was to demonstrate non-inferiority of post-vaccination HAI geometric mean titers (GMTs) for IIV4 vs. the pooled IIV3s for all 4 strains (Table 2). Also, for the B Yamagata-lineage strain (but not the B Victoria-lineage strain), the HAI GMT in participants vaccinated with IIV4 was superior to that in participants vaccinated with the IIV3 lacking the homologous B strain. In addition, for the A/H3N2, B Yamagata-lineage, and B Victoria-lineage strains, post-vaccination seroconversion rates for IIV4 were non-

inferior to those for the pooled IIV3s, and for each B lineage strain, seroconversion rates were superior to those for the IIV3 lacking the homologous B strain.

HAI titers at baseline were similar in all study groups (Table 3). Immunization with all 3 vaccines increased HAI GMTs by at least 5-fold against the 2 A strains and by 1.8 to 2.6-fold against the homologous B strains. Post-vaccination seroprotection rates were $\geq 91\%$ against the A strains and 67% to 78% against the B strains, and seroconversion occurred in $> 55\%$ of participants for the A strains and in 19% to 33% for the B strains in each vaccine. Interestingly, 9.1% (95% confidence interval [CI], 5.7 to 13.8) of participants vaccinated with IIV3-1, which contained a Victoria-lineage B strain, seroconverted against the B Yamagata-lineage strain, while 8.6% (95% CI, 5.3 to 13.1) of participants vaccinated with IIV3-2 seroconverted against the B Victoria-lineage strain.

Solicited reactions and AEs

Pain was the most common injection-site reaction and myalgia the most common systemic reaction for all vaccines (Table 4). Most solicited reactions were grade 1 or 2. Grade 3 solicited reactions included injection-site pain, fever, headache, malaise, and myalgia, each reported by no more than 2 participants ($< 2\%$) per group. All solicited reactions resolved within 8 d (data not shown). Proportions reporting solicited injection-site and systemic reactions were similar in participants vaccinated with IIV4 or IIV3.

No vaccine-related serious adverse events (SAEs), no AEs of special interest, and no immediate (< 20 min) unsolicited AEs were reported (Table 5). Proportions of participants reporting unsolicited AEs considered to be vaccine-related were similar for the 3 vaccines. The most common AEs considered to be vaccine-related were at the injection site ($< 2\%$ per group) and included hematoma, induration, pain, and pruritus. One participant vaccinated with IIV3-1 discontinued for events considered to be vaccine-related (diarrhea and injection-site pruritus).

Table 1. Participant characteristics in the randomized cohort (≥ 65 years).

Characteristic	IIV4 N = 225	IIV3-1 N = 225	IIV3-2 N = 225
Sex, n (%)			
Male	96 (42.7)	99 (44.0)	104 (46.2)
Female	129 (57.3)	126 (56.0)	121 (53.8)
Age (y)			
Mean \pm standard deviation	72.4 \pm 5.7	72.8 \pm 5.3	72.8 \pm 5.6
Range	65.1–92.2	65.0–94.6	65.1–92.3
Race/Ethnicity, n (%)			
Caucasian	197 (87.6)	202 (89.8)	205 (91.1)
Black	9 (4.0)	4 (1.8)	2 (0.9)
Hispanic	19 (8.4)	17 (7.6)	14 (6.2)
Other	0 (0.0)	2 (0.9)	4 (1.8)
Received 2009–2010 seasonal influenza vaccine, n (%)			
Yes	183 (81.3)	180 (80.0)	167 (74.2)
No	41 (18.2)	42 (18.7)	52 (23.1)
Unknown	1 (0.4)	3 (1.3)	6 (2.7)
Received 2009 H1N1 monovalent influenza vaccine, n (%)			
Yes	72 (32.0)	69 (30.7)	52 (23.1)
No	141 (62.7)	135 (60.0)	152 (67.6)
Unknown	12 (5.3)	21 (9.3)	21 (9.3)

Values are for all participants vaccinated. Abbreviations: IIV3-1, licensed split-virion trivalent influenza vaccine containing the B Victoria-lineage strain; IIV3-2, investigational split-virion trivalent influenza vaccine containing the B Yamagata-lineage strain; IIV4, quadrivalent split-virion inactivated influenza vaccine.

Open-label cohort

In addition to the 3 randomized cohorts, the study included an open-label cohort in which 64 healthy adults 18–64 y of age were vaccinated with the licensed IIV3 to document its immunogenicity and safety in this age group as part of the manufacturer's annual vaccine assessment. Results from this cohort are presented in the supplemental online material.

Discussion

This study, performed during the 2010–2011 influenza season in the US, showed that in adults ≥ 65 y of age, IIV4 induced non-inferior antibody titers compared with control IIV3s for all 4 vaccine strains. This finding is in line with the results of a phase II trial using similar IIV4 and IIV3s performed during the 2009–2010 influenza season in adults ≥ 18 y of age.⁷ The current study further showed that, in adults ≥ 65 y of age, IIV4 induced superior HAI antibody titers for the B Yamagata-lineage strain when compared with the IIV3 lacking the homologous strain and that IIV4 was as well tolerated as the IIV3s.

Table 2. Immunogenicity comparisons in the randomized cohort (≥ 65 years).

Measure/comparison	Strain/lineage	Comparator	Ratio of GMT and difference in seroconversion rate (95% CI)	Criteria met?
			–Ratio of GMTs–	
HAI GMT	A/H1N1	Pooled IIV3s ^a	0.85 (0.67, 1.09)	Yes
Non-inferiority ^b	A/H3N2	Pooled IIV3s ^a	1.55 (1.25, 1.92)	Yes
	B Victoria	IIV3–1	1.11 (0.90, 1.37)	Yes
Superiority ^c	B Yamagata	IIV3–2	1.27 (1.05, 1.55)	Yes
	B Victoria	IIV3–2	1.75 (1.43, 2.14)	No
	B Yamagata	IIV3–1	2.14 (1.74, 2.65)	Yes
			–Seroconversion difference–	
Seroconversion rate (%)	A/H1N1	Pooled IIV3s	–3.86 (–11.50, 3.56)	No
Non-inferiority ^d	A/H3N2	Pooled IIV3s	9.77 (1.96, 17.20)	Yes
	B Victoria	IIV3–1	1.96 (–6.73, 10.60)	Yes
Superiority ^e	B Yamagata	IIV3–2	9.91 (1.96, 17.70)	Yes
	B Victoria	IIV3–2	20.04 (12.90, 27.00)	Yes
	B Yamagata	IIV3–1	24.05 (16.60, 31.20)	Yes

Values are for all participants who completed the study according to protocol and had valid serology results. Abbreviations: CI, confidence interval; GMT, geometric mean titer; HAI, hemagglutination inhibition titer; IIV3–1, the licensed split-virion trivalent inactivated influenza vaccine containing the B Victoria-lineage strain; IIV3–2, an investigational split-virion trivalent inactivated influenza vaccine containing the B Yamagata-lineage strain; IIV4, split-virion quadrivalent inactivated influenza vaccine

^aIncludes participants vaccinated with either IIV3–1 or IIV3–2

^bNon-inferiority for HAI GMT was defined as a lower limit of the 2-sided 95% CI > 0.66 for the ratio of GMT for IIV4 vs. the GMT for IIV3

^cSuperiority for HAI GMT was defined as a lower limit of the 2-sided 95% CI > 1.5 for the ratio of the GMT for IIV4 vs. the GMT for IIV3

^dNon-inferiority for seroconversion rate was defined as a lower limit of the 2-sided 95% CI $> -10\%$ for the difference in seroconversion rate for IIV4 vs. IIV3

^eSuperiority for seroconversion was defined as a the lower limit of the 2-sided 95% CI $> 10\%$ for the difference in seroconversion rate for IIV4 vs. IIV3

Although HAI GMTs were superior for the B Yamagata-lineage strain, they were not for the B Victoria-lineage strain. This lack of superiority for the B Victoria-lineage strain might have been due to interference by existing antibody, cross-reactivity between the B strains, or other unknown factors. Low-level B-strain cross-reactivity has

been documented in adults,^{7,10} and was also observed in this study along with high baseline seroprotection rates against the B strains. The ability to detect superiority might have also been limited by the well-documented weaker immune responses in older adults as a result of immunosenescence.¹¹ Irrespective of the reason, the failure to meet

Table 3. Immunogenicity measures in the randomized cohort (≥ 65 years).

Vaccine	Measure	A/H1N1	A/H3N2	B Victoria	B Yamagata
IIV4 (N = 220)	HAI GMT (95% CI)				
	Day 0	21.7 (17.9, 26.3)	52.3 (42.1, 65.0)	27.1 (23.3, 31.5)	20.2 (17.5, 23.3)
	Day 21	231 (188, 283)	501 (422, 593)	73.8 (63.9, 85.3)	61.1 (52.5, 71.2)
	Day 21/day 0 GMTR (95% CI)	8.81 (7.06, 11.0)	8.72 (7.13, 10.7)	2.46 (2.16, 2.80)	2.65 (2.33, 3.03)
	Seroprotection, % (95% CI)				
	Day 0	31.7 (25.6, 38.2)	53.8 (47.0, 60.6)	48.9 (42.1, 55.7)	30.3 (24.3, 36.8)
IIV3–1 (N = 219)	Day 21	91.4 (56.8, 94.7)	100.0 (98.3, 100.0)	77.7 (71.6, 83.0)	73.2 (66.8, 78.9)
	Seroconversion, % (95% CI)	65.9 (59.2, 72.1)	69.1 (62.5, 75.1)	28.6 (22.8, 35.1)	33.2 (27.0, 39.8)
	HAI GMT (95% CI)				
	Day 0	24.8 (20.4, 30.1)	48.3 (40.0, 58.4)	29.0 (25.0, 33.7)	18.7 (16.4, 21.3)
	Day 21	269 (221, 328)	291 (243, 347)	57.9 (50.6, 66.4)	28.5 (24.6, 33.0)
	Day 21/day 0 GMTR (95% CI)	9.18 (7.33, 11.5)	5.65 (4.65, 6.86)	1.83 (1.62, 2.07)	1.40 (1.27, 1.54)
IIV3–2 (N = 221)	Seroprotection, % (95% CI)				
	Day 0	45.8 (32.7, 59.2)	45.8 (32.7, 59.2)	35.6 (23.6, 49.1)	30.5 (19.2, 43.9)
	Day 21	91.3 (86.8, 94.7)	95.4 (91.8, 97.8)	71.7 (65.2, 77.6)	46.1 (39.4, 53.0)
	Seroconversion, % (95% CI)	66.7 (60.0, 72.9)	55.7 (48.9, 62.4)	18.7 (13.8, 24.5)	9.1 (5.7, 13.8)
	HAI GMT (95% CI)				
	Day 0	21.1 (17.5, 25.5)	42.3 (34.9, 51.4)	28.5 (24.2, 33.6)	19.7 (17.2, 22.6)
Pooled IIV3s ^a (N = 440)	Day 21	271 (221, 331)	360 (302, 429)	42.2 (36.5, 48.7)	54.8 (47.5, 63.3)
	Day 21/day 0 GMTR (95% CI)	10.6 (8.60, 13.0)	7.73 (6.38, 9.36)	1.34 (1.25, 1.45)	2.47 (2.18, 2.80)
	Seroprotection, % (95% CI)				
	Day 0	45.8 (32.7, 59.2)	45.8 (32.7, 59.2)	35.6 (23.6, 49.1)	30.5 (19.2, 43.9)
	Day 21	91.9 (87.4, 95.1)	95.9 (92.4, 98.1)	60.2 (53.4, 67.0)	67.4 (60.8, 73.6)
	Seroconversion, % (95% CI)	72.9 (66.5, 78.6)	62.9 (56.2, 69.3)	8.6 (5.3, 13.1)	31.2 (25.2, 37.8)
	Day 21 HAI GMT (95% CI)	270 (234, 311)	324 (285, 267)	—	—
	Day 21 Seroconversion, % (95% CI)	69.8 (62.3, 74.0)	59.3 (54.6, 64.0)	—	—

Hemagglutinin inhibition (HAI) geometric mean titers (GMTs) were calculated at baseline (day 0) and 21 d post-vaccination. The geometric mean titer ratio (GMTR) was calculated as the geometric mean of the individual post-vaccination/pre-vaccination titer ratios. Seroprotection was defined as a HAI titer ≥ 40 . Seroconversion was defined as a pre-vaccination titer < 10 and post-vaccination titer ≥ 40 or a pre-vaccination titer ≥ 10 and a ≥ 4 -fold increase in post-vaccination titer. Values are for all participants vaccinated with valid results. Abbreviations: CI, confidence interval; IIV3–1, the licensed split-virion trivalent inactivated influenza vaccine containing the B Victoria-lineage strain; IIV3–2, an investigational split-virion trivalent inactivated influenza vaccine containing the B Yamagata-lineage strain; IIV4, split-virion quadrivalent inactivated influenza vaccine.

^aIncludes participants vaccinated with either IIV3–1 or IIV3–2

Table 4. Proportions reporting solicited reactions in the randomized cohort (≥ 65 years).

Event	Maximum intensity	IIV4	IIV3-1	IIV3-2
		(N = 225) % (95% CI)	(N = 225) % (95% CI)	(N = 225) % (95% CI)
Any solicited reaction	Any	43.8 (37.2; 50.5)	38.4 (32.0; 45.1)	33.3 (27.2; 39.9)
	grade 3	0.9 (0.1; 3.2)	0.0 (0.0; 1.6)	1.3 (0.3; 3.8)
Solicited injection site reaction	Any	33.5 (27.3; 40.1)	29.5 (23.6; 35.9)	24.0 (18.6; 30.1)
	grade 3	0.9 (0.1; 3.2)	0.0 (0.0; 1.6)	0.0 (0.0; 1.6)
Solicited systemic reaction	Any	24.6 (19.1; 30.7)	24.1 (18.7; 30.3)	20.9 (15.8; 26.8)
	grade 3	0.4 (0.0; 2.5)	0.0 (0.0; 1.6)	1.3 (0.3; 3.8)
Injection-site pain	Any	32.6 (26.5; 39.2)	28.6 (22.8; 35.0)	23.1 (17.8; 29.2)
	grade 3	0.9 (0.1; 3.2)	0.0 (0.0; 1.6)	0.0 (0.0; 1.6)
Injection-site erythema	Any	2.7 (1.0; 5.7)	1.3 (0.3; 3.9)	1.3 (0.3; 3.9)
	grade 3	0.0 (0.0; 1.6)	0.0 (0.0; 1.6)	0.0 (0.0; 1.6)
Injection-site swelling	Any	1.8 (0.5; 4.5)	1.3 (0.3; 3.9)	0.0 (0.0; 1.6)
	grade 3	0.0 (0.0; 1.6)	0.0 (0.0; 1.6)	0.0 (0.0; 1.6)
Fever	Any	1.3 (0.3; 3.9)	0.0 (0.0; 1.6)	0.9 (0.1; 3.2)
	grade 3	0.4 (0.0; 2.5)	0.0 (0.0; 1.6)	0.4 (0.0; 2.5)
Headache	Any	13.4 (9.2; 18.6)	11.6 (7.7; 16.5)	11.6 (7.7; 16.5)
	grade 3	0.4 (0.0; 2.5)	0.0 (0.0; 1.6)	0.4 (0.0; 2.5)
Malaise	Any	10.7 (7.0; 15.5)	6.3 (3.5; 10.3)	11.6 (7.7; 16.5)
	grade 3	0.4 (0.0; 2.5)	0.0 (0.0; 1.6)	0.9 (0.1; 3.2)
Myalgia	Any	18.3 (13.5; 24.0)	18.3 (13.5; 24.0)	14.2 (9.9; 19.5)
	grade 3	0.4 (0.0; 2.5)	0.0 (0.0; 1.6)	0.4 (0.0; 2.5)

Swelling and erythema were graded based on the diameter of the reaction as any for ≥ 25 mm and grade 3 for > 100 mm. Fever was graded as any for $\geq 38.0^\circ\text{C}$ and grade 3 for $\geq 39.0^\circ\text{C}$. All other solicited reactions were graded as any for no interference or at least some interference with daily activity and grade 3 for significant interference preventing daily activity. Abbreviations: IIV3-1, the licensed split-virion trivalent inactivated influenza vaccine containing a B strain of the Victoria lineage; IIV3-2, an investigational split-virion trivalent inactivated influenza vaccine containing a B strain of the Yamagata lineage; IIV4, split-virion quadrivalent inactivated influenza vaccine.

the superiority criterion for the B Victoria-lineage strain is not expected to adversely affect clinical protection because IIV4 was non-inferior to the IIV3s for post-vaccination HAI GMTs. The study also showed that seroconversion rates were non-inferior for IIV4 vs. IIV3 for all vaccine strains except for A/H1N1. This failure to reach non-inferiority for the A/H1N1 strain is not expected to affect protection provided by IIV4 because the seroprotection rate for this strain was 91%.

In this study, IIV4 induced post-vaccination seroprotection rates in the older adult participants that were at least 91% for the A strains and at least 73% for the B strains. For this study, we used the widely accepted HAI titer of at least 1:40 to define seroprotection, but whether this definition and serum antibody titers in general are valid correlates of protection continues to be debated.¹²⁻¹⁶ According to current US guidelines, however, the HI antibody response remains an acceptable surrogate marker that is likely to predict clinical benefit.¹⁷

Table 5. Proportions reporting unsolicited adverse events in the randomized cohort (≥ 65 years).

Event	IIV4	IIV3-1	IIV3-2
	N = 224 n (%)	N = 224 n (%)	N = 225 n (%)
Immediate unsolicited AE (< 20 min)	0 (0.0)	0 (0.0)	0 (0.0)
Unsolicited AE	28 (12.4)	24 (10.7)	23 (10.2)
Vaccine-related	6 (2.7)	6 (2.7)	4 (1.8)
SAE	0 (0)	2 (0.9)	1 (0.4)
Vaccine-related	0 (0.0)	0 (0.0)	0 (0.0)
Death	0 (0.0)	0 (0.0)	0 (0.0)

Values are for all participants randomized and for whom safety data were available. Abbreviations: AE, adverse event; IIV3-1, the licensed split-virion trivalent inactivated influenza vaccine containing a B strain of the Victoria lineage; IIV3-2, an investigational split-virion trivalent inactivated influenza vaccine containing a B strain of the Yamagata lineage; IIV4, split-virion quadrivalent inactivated influenza vaccine; SAE, serious adverse event.

Although this study met its primary objective of showing non-inferior antibody titers in older adults for IIV4 vs. IIV3, immune responses in this population were substantially lower than those observed in the younger open-label cohort that received licensed IIV3-1. Reduced immunogenicity of influenza vaccines in older adults is well documented¹¹ and is why a high-dose split-virion inactivated influenza vaccine (Fluzone High-Dose, Sanofi Pasteur) containing 60 μg hemagglutinin per strain¹⁸ and a subunit vaccine containing a squalene-based oil-in-water emulsion as an adjuvant (Fluad, Sequirus)¹⁹ have been developed specifically for this age group. These vaccines are currently trivalent, but quadrivalent formulations of both are under clinical development.

In addition to confirming non-inferior antibody titers for IIV4 vs. IIV3, this study demonstrated that the IIV4 had a safety profile similar to that of IIV3. No important safety issues or substantial differences in the occurrence or severity of solicited reactions or AEs were detected.

One limitation of this study is that the trial population consisted of medically stable, community-dwelling older adults. Accordingly, safety and immunogenicity findings from this trial may not be generalizable to other older adults, such as those who are frail or institutionalized. However, because the immune responses (for homologous strains) and safety profile between IIV4 and the comparator IIV3s were similar in the current study, it is reasonable to expect that the performance of IIV4 relative to IIV3 would also be similar within specific sub-populations of older adults. Another limitation is that the size of the study population was too small to detect adverse reactions that could occur with relatively low frequency post-vaccination. Even so, given the extensive safety experience of IIV3 and similar reactogenicity being observed for IIV4 and IIV3, it is unlikely that the safety profile for IIV4 would be materially

different from IIV3 with respect to any rare adverse reactions that might occur after influenza vaccination.

To conclude, this study showed that the addition of a second B strain in IIV4 did not affect tolerability or compromise the immunogenicity of the other vaccine strains in older adults.

Patients and methods

Study design

This was a phase III trial conducted at 12 sites in the US between October and December, 2010 (NCT01218646). The study included a double-blind, randomized, controlled cohort of older adults (≥ 65 y of age) and an open-label cohort of younger adults (18–64 y of age). The primary objective for the randomized cohorts was to demonstrate that IIV4 induced non-inferior antibody responses compared with those induced by comparator IIV3s containing matching A and B strains in adults ≥ 65 y of age. The objective of the open-label cohort study was to document the immunogenicity and safety of the 2010–2011 formulation of the licensed IIV3 in healthy adults.

Ethics

The study was approved by all institutional review boards and performed in accordance with International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Participants

Healthy older adults (≥ 65 y of age) were recruited for the randomized, double-blind cohort, and healthy younger adults (18–64 y of age) were recruited for the open-label cohort. Participants were excluded if they had an allergy to egg proteins, latex, or any vaccine component; a history of serious adverse reactions to any influenza vaccine; received any vaccine in the 4 weeks preceding study vaccination or an influenza vaccine after August 1, 2010; a history of Guillain-Barré syndrome; a known or suspected immunodeficiency; received immunosuppressive therapy within the preceding 6 months or long-term systemic corticosteroid therapy for more than 2 consecutive weeks within the past 3 months; a developmental delay, neurologic disorder, or seizure disorder; or received blood or blood-derived products in the past 3 months.

Vaccines

IIV4 contained A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage) strains. IIV3–1 was the licensed 2010–2011 formulation of Fluzone (Sanofi Pasteur) containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008. IIV3–2 was an investigational trivalent vaccine containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Florida/04/2006. All were split-virion inactivated vaccines provided in prefilled 0.5-mL single-dose syringes or in single-dose vials containing 15 μ g hemagglutinin per strain.

Study conduct

Participants in the randomized cohort (adults aged 65 y and older) were randomly assigned using a pre-programmed interactive voice response system 1:1:1 to be vaccinated with a single dose of IIV4, IIV3–1, or IIV3–2. Neither participants nor investigators knew which vaccine was administered. Participants in the open-label cohort (adults aged 18–64 years) were all vaccinated with IIV3–1. All vaccines were administered by intramuscular injection into the deltoid muscle using 25 gauge, 1-inch (25 mm)-long needle.

HAI assay

HAI titers were measured at baseline (day 0) and 21 (window, 21–28) days after vaccination and were recorded as the reciprocal of the dilution as described previously.²⁰ The lower limit of quantitation was set at the reciprocal of the lowest dilution (10) and the upper limit of quantitation as the highest dilution (10,240) used in the assay. Seroprotection was defined as an HAI titer ≥ 40 . Seroconversion rate was defined as a pre-vaccination titer < 10 and post-vaccination titer ≥ 40 or a pre-vaccination titer ≥ 10 and a ≥ 4 -fold increase in post-vaccination titer.

Safety

Unsolicited AEs and SAEs were collected by investigators according to International Committee for Harmonisation Guideline (E2A) for Clinical Safety Data Management. Unsolicited AEs were collected up to day 21 post-vaccination. Immediate events were those occurring within 20 min after vaccination. SAEs and AEs of special interest were collected for 6 months after vaccination. AEs of special interest included Guillain-Barré syndrome, Bell's palsy, encephalitis/myelitis, optic neuritis, Stevens-Johnson syndrome, and toxic epidermal necrolysis.

Solicited injection-site and systemic reactions were recorded by participants for 7 d after vaccination using a diary card. Solicited injection-site reactions included pain, erythema, and swelling. Solicited systemic reactions included fever, headache, malaise, and myalgia. Swelling and erythema were assigned grade 1 for a diameter ≥ 25 to ≤ 50 mm, grade 2 for a diameter ≥ 51 to ≤ 100 mm, and grade 3 for a diameter > 100 mm. Fever was assigned grade 1 for $\geq 38.0^\circ\text{C}$ to $\leq 38.4^\circ\text{C}$, grade 2 for $\geq 38.5^\circ\text{C}$ to $\leq 38.9^\circ\text{C}$, and grade 3 for $\geq 39.0^\circ\text{C}$. All other AEs and solicited reactions were assigned grade 1 for no interference with daily activity, grade 2 for some interference with daily activity, and grade 3 for significant interference preventing daily activity.

Sample size calculation

A sample size calculation was performed only for the randomized, double-blind cohort. For each group, 225 participants were planned to be enrolled. Assuming an attrition rate no greater than 5%, this provided 90.3% power to demonstrate non-inferiority in GMTs between IIV4 and the IIV3s.

Statistical analysis

All analyses were performed using SAS[®] version 9.1 or higher (SAS Institute). Missing and incomplete data were not replaced and no search for outliers was performed. Safety was assessed in all participants who received a study or control vaccine. Immunogenicity was assessed in all participants who received the study or a control vaccine, had a valid post-vaccination serology result, and completed the study according to protocol.

Non-inferiority and superiority of IIV4 vs. comparator IIV3s was assessed for the randomized cohort. Non-inferiority of the HAI GMTs and seroconversion rates was assessed for all 4 viral strains in IIV4 compared with the control IIV3s containing the homologous B strains. For comparison of A/H1N1 and A/H3N2 responses, data were pooled for the 2 IIV3s. For non-inferiority comparisons of B-strain responses, IIV4 was compared with the respective IIV3 containing the same B-lineage strain. For each strain, the HAI GMT for IIV4 was considered non-inferior to that for the pooled IIV3s if the lower limit of the 2-sided 95% CI of the GMT ratio (IIV4 divided by IIV3) was > 0.66 . Similarly, for each strain, the seroconversion rate for IIV4 was considered non-inferior to that for the pooled IIV3s if the lower limit of the 2-sided 95% CI of the difference in rates (IIV4 minus IIV3) was $> -10\%$. Superiority of HAI GMTs and seroconversion rates was assessed for each B-lineage strain in IIV4 compared with each respective IIV3 lacking the matched B-lineage strain. For each B strain, the HAI GMT for IIV4 was considered superior to that for the IIV3 lacking the homologous B strain if the lower limit of the 2-sided 95% CI of the GMT ratio (IIV4 divided by IIV3) was > 1.5 , and the seroconversion rate for IIV4 was considered superior to that for the IIV3 lacking the homologous B strain if the lower limit of the 2-sided 95% CI of the difference in rates (IIV4 minus IIV3) was $> 10\%$.

Abbreviations

AE	adverse event
CI	confidence interval
GMT	geometric mean titer
HAI	hemagglutination inhibition
IIV3	trivalent split-virion inactivated influenza vaccine
IIV3-1	licensed IIV3 containing a B strain of the Victoria lineage
IIV3-2	investigational IIV3 containing a B strain of the Yamagata lineage
IIV4	quadrivalent split-virion inactivated influenza vaccine

Disclosure of potential conflicts of interest

D.P.G., C.A.R., and M.D.D. were employees of Sanofi Pasteur during the period of this study. H.K.T. has received research support from Sanofi Pasteur, Gilead, and MedImmune and serves as an advisor for Seqirus and VaxInnate.

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