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Immunogenicity and safety of a cell culture-based quadrivalent influenza vaccine in adults: A Phase III, double-blind, multicenter, randomized, non-inferiority study

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ABSTRACT

Quadrivalent influenza vaccines (QIVs), which include both B lineage strains, are expected to provide broader protection than trivalent influenza vaccines (TIVs). The non-inferiority, immunogenicity, and safety of a cell culture-based investigational QIVc and 2 TIVs (TIV1c, TIV2c), in adults (≥18 y), were evaluated in this Phase III, double-blind, multicenter study. A total of 2680 age-stratified subjects were randomized (2:1:1) to receive 1 dose of QIVc (n = 1335), TIV1c (n = 676), or TIV2c (n = 669). TIV1c (B/Yamagata) and TIV2c (B/Victoria) differed only in B strain lineage. The primary objective was to demonstrate non-inferiority of the hemagglutinin-inhibition antibody responses of QIVc against TIVc, 22 d post-vaccination. Secondary objectives included the evaluation of immunogenicity of QIVc and TIVc in younger (≥18 – <65 y) and older (≥65 y) adults. Hemagglutinin inhibition assays were performed at days 1 and 22. Solicited local and systemic adverse events (AEs) were monitored for 7 d post-vaccination, and unsolicited AEs and serious AEs until day 181. QIVc met the non-inferiority criteria for all 4 vaccine strains and demonstrated superiority for both influenza B strains over the unmatched B strain included in the TIV1c and TIV2c, when geometric mean titers and seroconversion rates with TIVc were compared at day 22. Between 48%–52% of subjects experienced ≥1 solicited AE, the most common being injection-site pain and headache. Serious AEs were reported by ≤1% of subjects, none were vaccine-related. The results indicate that QIVc is immunogenic and well tolerated in both younger and older adults. The immunogenicity and safety profiles of QIVc and TIVc were comparable at all ages evaluated.

Introduction

Influenza viruses undergo frequent genetic mutations (antigenic drifts) that result in accumulating changes in the viral hemagglutin (HA) surface protein, giving rise to antigenically new strains which enable the virus to cause repetitive outbreaks.[1] Consequently, seasonal influenza vaccines have to be reformulated annually to replace current strains with those that are most likely to circulate in the coming influenza season.[1] Trivalent influenza vaccines (TIV) contain strains of 2 subtypes of influenza A: A/H1N1 and A/H3N2, and a single type B influenza strain.

There are 2 distinct phylogenetic lineages of influenza B virus, B/Yamagata and B/Victoria, whose strains cause human infection.[2] There have been several instances, particularly in the past decade, where a lineage-level mismatch between the circulating and the recommended vaccine B strains occurred, thereby reducing the effectiveness of TIV.[3-7] Increasingly, strains of both B lineages have been co-dominantly circulating in the same season.[3,6,7] In a Finnish study spanning 12 influenza seasons (1999–2012), approximately 42% of the influenza B infections were found to have been caused by the virus strain of opposite lineage than the virus included in the vaccine.[8]

Quadrivalent influenza vaccines (QIV), which incorporate strains from both influenza B lineages, are developed to overcome the risk of selecting the incorrect B lineage for the vaccine composition and to improve the immunity of individuals against both lineages. In 2012, the first seasonal QIV was licensed for use in the US.[9]

Cell-culture technology is now available for the production of influenza vaccines. The first mammalian cell culture-derived trivalent inactivated influenza vaccine (TIVc) approved for use in adults (≥18 y) was Optaflu® (Novartis Vaccines and Diagnostics, GmBH, Marburg, Germany). This vaccine, produced using Madin-Darby Canine Kidney (MDCK) suspension cell lines, has been licensed in Europe since 2007 and in the US since 2012 (under the trade name Flucelvax®).[9,10] The safety and immunogenicity of this TIVc have been evaluated in clinical trials in individuals aged ≥6 mo.[11-13]

Using the same MDCK manufacturing platform, Novartis Vaccines and Diagnostics has developed an investigational, cell culture-based, inactivated, quadrivalent influenza vaccine (QIVc). This study assessed the non-inferiority, immunogenicity, and safety of this QIVc compared with TIVc in adults.

Results

Out of the 2680 subjects who were vaccinated on day 1, 2585 (96.5%) subjects completed the study. The reasons for premature withdrawal are provided in Figure 1. A total of 98.2% (n = 2632) of enrolled subjects were included in the full analysis set and approximately 94% (n = 2523) of enrolled subjects were included in the per-protocol set. A total of 99.2% (n = 2662) of subjects were included in the overall safety set.

The baseline characteristics were balanced across the vaccine groups (Table 1). The mean age was 57 y, the majority of subjects were Caucasians, and approximately 25% of subjects in each group had received influenza vaccination within 6–12 mo prior to study participation. At baseline, 2.5%–14% of all subjects had hemagglutinin inhibition (HI) titers <1:10, and 84%–96% of ≥1:10 across all 4 vaccine strains (Table 1).

Immunogenicity analyses

Non-inferiority
At 3 wks post-vaccination (day 22), the immune responses to QIVc were non-inferior to TIV1c and TIV2c, for A/H1N1 and A/H3N2 strains, and for B/Yamagata and B/Victoria strains, respectively, in the overall population (Fig. 2A and 2B).
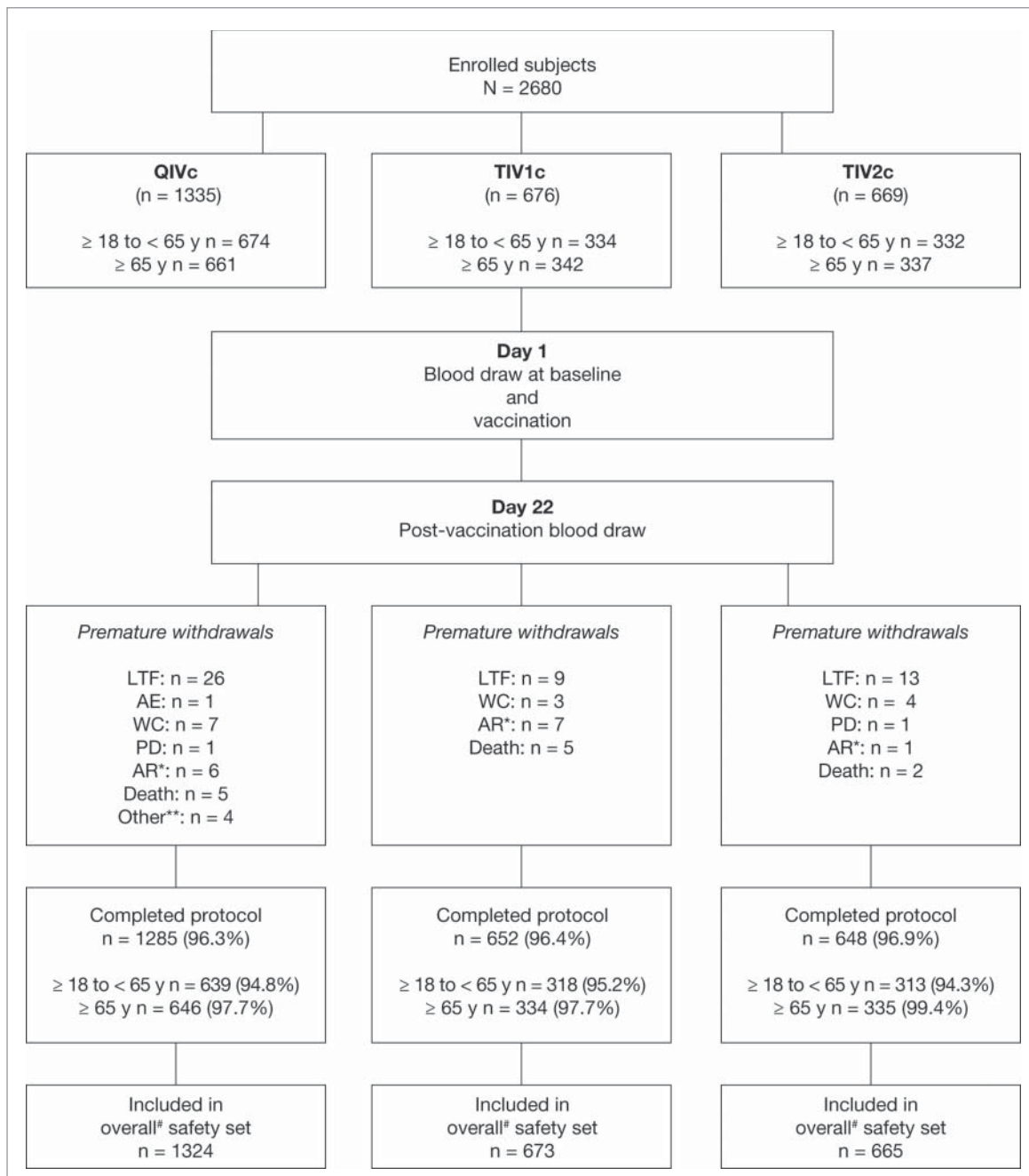

Figure 1. Subject disposition flowchart. * Administrative reasons: insurance issues or relocation. **Other reasons were: 1 subject was enrolled at 2 different study sites; 1 subject went abroad for studies; 2 were withdrawn based on investigators decision (1 subject was unable to come to study site for assessments and another subject admitted to substance abuse). #Overall safety set included subjects who provided solicited and unsolicited AEs data for period day 1 through day 181. AR = administrative reason; PD = protocol deviation; LTF = lost to follow-up; WC = withdrawal of consent.

Table 1. Demographics and baseline characteristics of all enrolled populations.

	QIVc n = 1335	TIV1c n = 676	TIV2c n = 669
Age (y ± SD)	57.4 ± 17.8	57.2 ± 18.0	57.1 ± 18.1
Male, n (%)	603 (45.2)	284 (42.0)	277 (41.4)
Race/ethnicity, n (%)			
Asian	4 (0.3)	3 (0.4)	3 (0.4)
American Indian	10 (0.7)	7 (1.0)	2 (0.3)
African-American	179 (13.4)	80 (11.8)	81 (12.1)
Native Hawaiian	2 (0.1)	2 (0.3)	0 (0.0)
Hispanic	122 (9.1)	59 (8.7)	53 (7.9)
Caucasian	1009 (75.6)	519 (76.8)	525 (78.5)
Other	9 (0.7)	6 (0.9)	5 (0.7)
Height (cm ± SD)	169.4 ± 9.9	168.8 ± 10.3	168.6 ± 10.1
Weight (kg ± SD)	86.9 ± 22.7	86.2 ± 21.3	85.8 ± 22.1
Body mass index (kg/m^2 ± SD)	30.2 ± 7.3	30.2 ± 6.7	30.2 ± 7.4
Received influenza vaccine within 6–12 months prior to study vaccination	326 (24.4%)	172 (25.4%)	168 (25.1%)
Percentage of Patients with baseline HI titer of <1:10 / ≥1:10			
Total			
A/H1N1	14.0 / 84.2	14.3 / 83.9	13.8 / 84.5
A/H3N2	6.1 / 92.1	6.2 / 92.0	4.2 / 94.2
B1	5.6 / 92.6	4.4 / 93.8	5.5 / 92.7
B2	2.8 / 95.4	2.5 / 95.7	3.6 / 94.8
Age group 18–64			
A/H1N1	10.9 / 38.7	11.8 / 36.7	9.4 / 39.3
A/H3N2	4.0 / 45.5	4.7 / 43.8	3.1 / 45.6
B1	3.6 / 45.9	3.8 / 45.4	3.4 / 45.3
B2	1.9 / 47.6	1.8 / 46.7	2.2 / 46.5
Age group ≥65			
A/H1N1	3.1 / 45.5	2.5 / 47.2	4.3 / 45.1
A/H3N2	2.1 / 46.6	1.5 / 48.2	1.0 / 48.6
B1	2.0 / 46.7	1.3 / 48.4	2.1 / 47.4
B2	0.9 / 47.8	0.7 / 49.0	1.3 / 48.3

SD = standard deviation.

Antibody responses

Overall, HI antibody responses were similar in the QIVc and TIV1c/TIV2c groups. In the ≥18 to <65 y age cohort, QIVc and TIV1c/TIV2c met both Center for Biologics Evaluation and Research (CBER) immunogenicity criteria. At day 22, the percentages of subjects with a HI titer ≥1:40 ranged between 96%–99% for all 4 vaccine strains in the QIVc and in the TIV1c/TIV2c groups (Fig. 3A). At 3 wks post-vaccination, 52%–63% of subjects in the QIVc and 47%–60% of subjects in the TIV1c/TIV2c groups achieved seroconversion.

At day 22, in the ≥65 y age cohort, 92%–98% of subjects in the QIVc and 88%–98% of subjects in the TIV1c/TIV2c groups demonstrated an HI titer ≥1:40 and thereby met the CBER immunogenicity criterion (HI titer ≥1:40) for all vaccine strains (Fig. 3A). The seroconversion rates (SCRs) of subjects aged ≥65 y were lower compared with the younger age group: 21%–35% in the QIVc group and 19%–37% in the TIV1c/TIV2c groups, for all 4 vaccine strains (49%–63% and 47%–60%, respectively, for subjects aged ≥18–≤65 y). The CBER criterion for seroconversion was only met for the A/H1N1 strain in the QIVc and TIV1c/TIV2c groups.

In subjects aged ≥18 to ≤60 y, all the Committee for Medicinal Products for Human Use (CHMP) immunogenicity criteria were met in the QIVc and TIV1c/TIV2c groups (Fig. 4). In older adults (≥61 y), the QIVc and TIV1c/TIV2c groups met all CHMP criteria (seroconversion, HI titer ≥1:40, and geometric mean ratio [GMR]) for the A/H1N1 strain and 2 CHMP criteria (GMR and HI titer ≥1:40) for the A/H3N2 and B/Victoria strains. For the B/Yamagata strain, the QIVc group met the HI titer ≥1:40 and GMR criteria, and the TIV1c/TIV2c group met the CHMP criteria for HI titer ≥1:40 (Fig. 4).

In subset analyses across all age groups at day 22, subjects who were seronegative at baseline (HI < 1:10) demonstrated substantially higher SCRs (70%–86% QIVc; 80%–88% TIVc) and GMRs for the 4 influenza strains (13.4–40.7-fold increase QIVc; 18–35.1-fold increase TIVc) than subjects who were seropositive at baseline (baseline HI ≥ 1:10; SCR: 35%–43%; QIVc: 32%–42% TIVc; GMR: 2.7–3.6-fold increase QIVc; 2.4–3.6-fold increase TIVc) (Fig. 3B and Table 2). There were no significant differences in the antibody responses between QIVc and TIV1c/TIV2c recipients when analyzed according to sex and race/ethnicity.

Superiority

At day 22, the geometric mean titers (GMTs) and the percentage of subjects with seroconversion for the unmatched B strains were higher in the QIVc group than in the TIV1c and TIV2c groups. Superiority of the antibody responses in the QIVc group over the TIV1c group against the B/Victoria strain and over the TIV2c group for the B/Yamagata strain was demonstrated (Table 3).
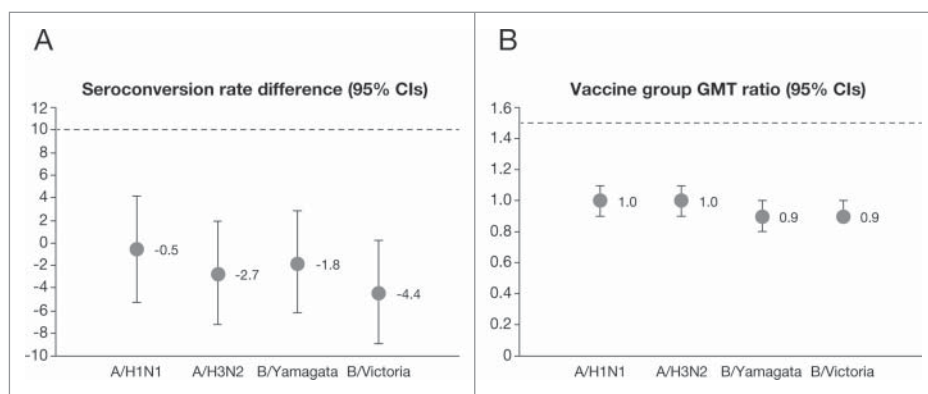

Figure 2. The non-inferiority of HI antibody responses of QIVc to TIV1c (matched strains including B/Yamagata) and TIV2c (matched strains including B/Victoria strain) at 3 wks after vaccination in terms of the differences in percentages of subjects achieving seroconversion (A) and the between group GMT ratios (B). The horizontal dashed line indicates CBER non-inferiority threshold, for each of the 4 strains: 1) the upper limit of the 2-sided 95% confidence interval (CI) on the difference between the SCRs (TIV1c/TIV2c−QIV) must be < 10%; 2) the upper limit of the 2-sided 95% CI for the ratio of GMTs (GMT TIVc/GMTQIVc) for HI antibody should be <1.5.
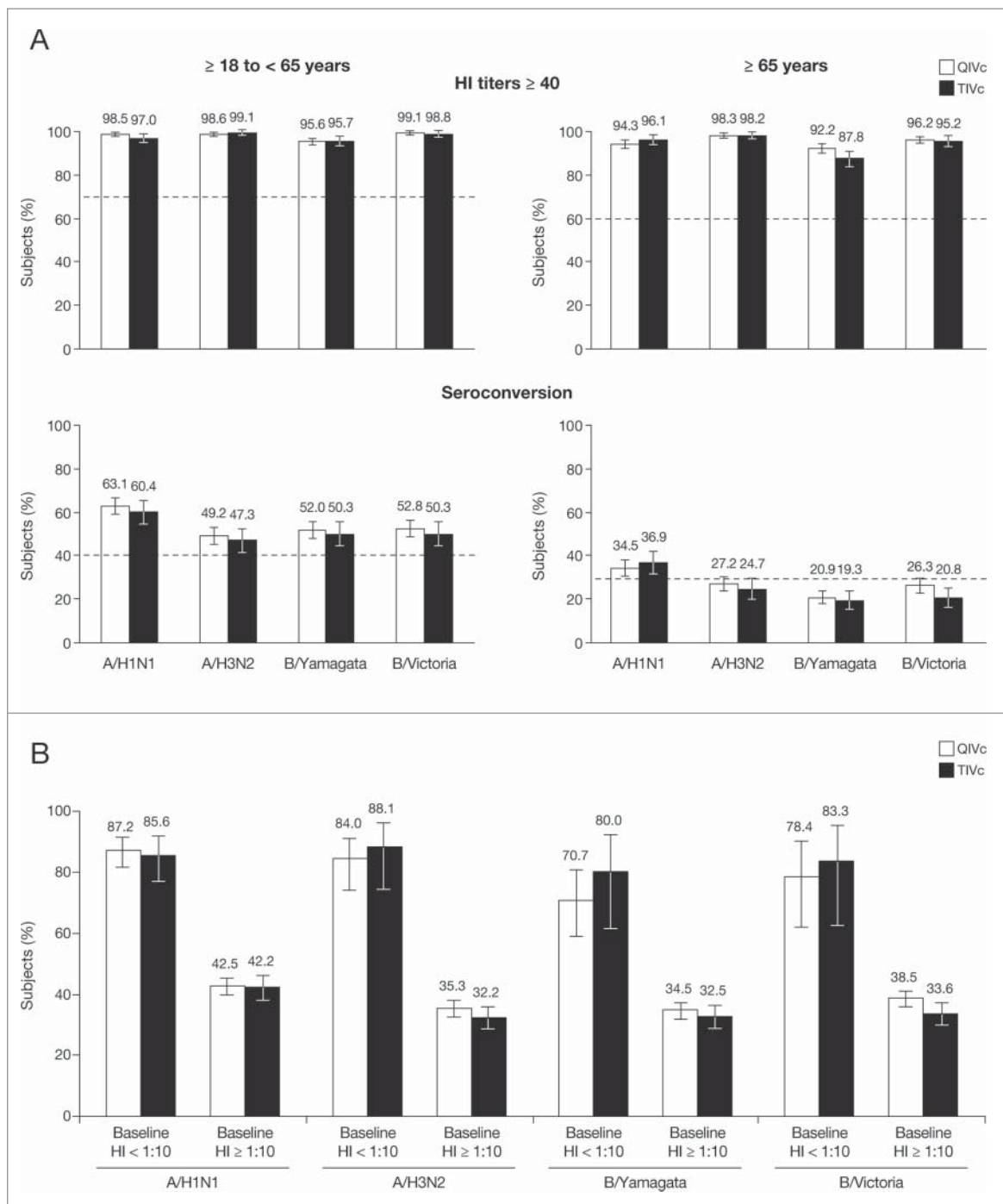
Figure 3. (A) The percentage of subjects with HI titers ≥40 (±95% CI) and percentage of subjects showing seroconversion or 4-fold increase (±95% CI) for all 4 vaccine strains at day 22 post-vaccination in subjects ≥18 to<65 y and ≥65 y of age. Error bars represent 95% CIs. Lines represent the relevant CBER* criterion for each measure. Data presented are for the full analysis set. *For subjects ≥18 to <65 y: the LL of the 2-sided 95% CIs for the percentage of subjects achieving an HI titer ≥1:40 is ≥70% and the LL of the 95% CIs for the percentage of subjects with seroconversion or significant increase in HI antibody is ≥40% for all 4 strains. For ≥65 y; the LL of the 95% CIs for the percentage of subjects achieving an HI titer ≥1:40 is ≥60%; the LL of the 95% CIs for the percentage of subjects with seroconversion or significant increase in HI antibody is ≥30% for all 4 strains. QIVc compared with TIV1c for A/H1N1, A/H3N2, and B/Yamagata strain and TIV2c for B/Victoria strain. LL = lower limit. (B) The percentage of subjects showing seroconversion or 4-fold increase in HI titers from baseline for all 4 vaccine strains* at day 22 post-vaccination in subjects stratified by baseline serostatus (HI < 1:10 and HI ≥ 1:10). Error bars represent 95% CIs. Data presented are for the full analysis set. *QIVc compared with TIV1c for A/H1N1, A/H3N2, and B/Yamagata strain and TIV2c for B/Victoria strain.

Safety

Compliance to study protocol was similar across all 3 cohorts; In total, 94.7 % of all subjects (2537 subjects) were assessed at the first return visit and 95.5% of subjects (2545 subjects) were assessed at the second return visit.

Solicited AEs

A similar percentage of subjects reported ≥1 solicited adverse event (AE) across all 3 vaccine groups. The most commonly reported solicited AE was injection-site pain, the overall incidence of which was slightly higher in the QIVc group (33.6%) compared with the TIV1c (27.8%) and TIV2c (29.4%) groups.
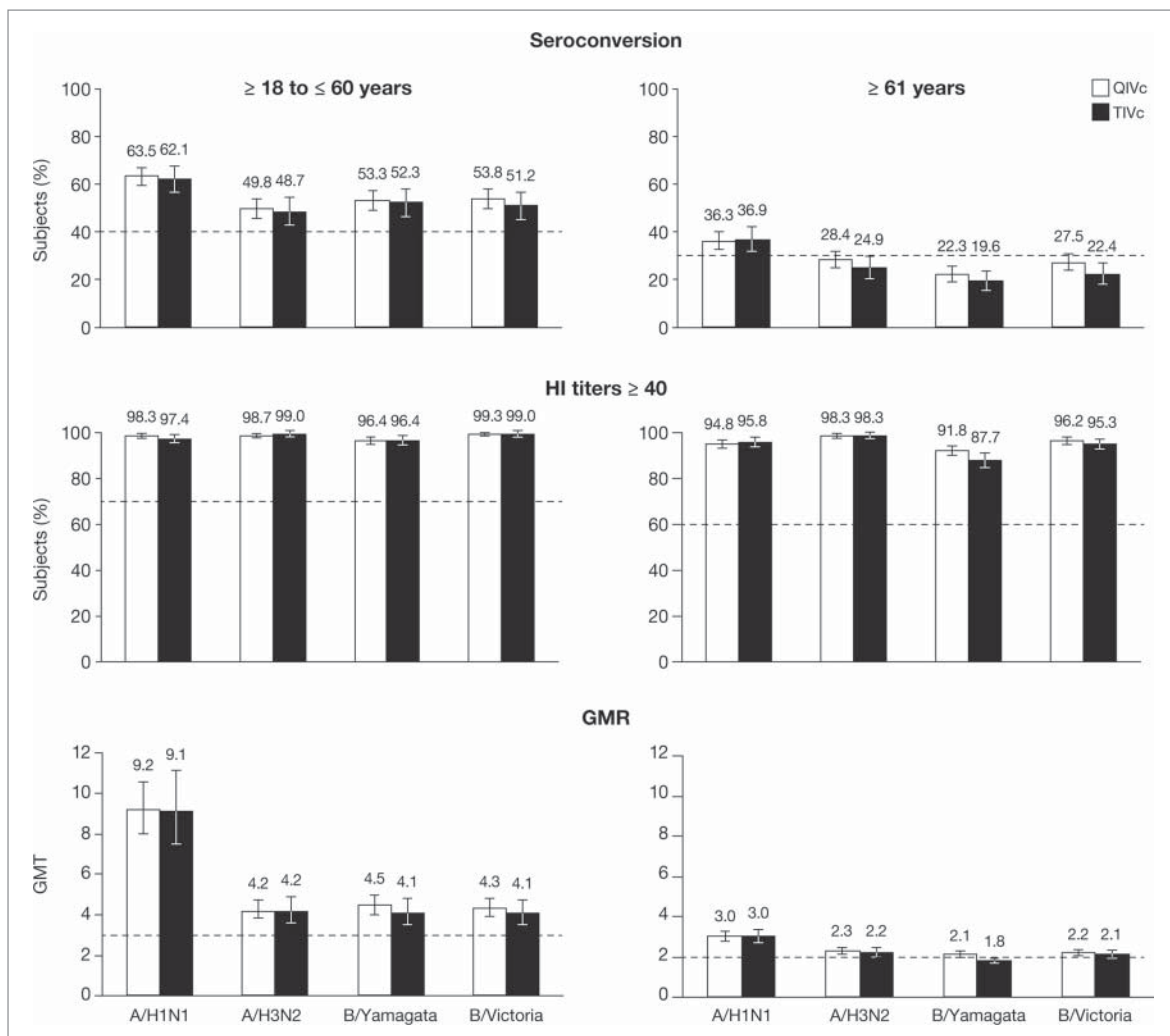
Figure 4. The percentage of subjects HI titers ≥40, percentage of subjects showing seroconversion and GMR, for all 4 vaccine strains at day 22 post-vaccination in subjects ≥18 to ≤60 y and ≥61 y of age. Lines represent the relevant CHMP* criterion for each measure. Error bars represent 95% CIs. Data presented are for the full analysis set. *For subjects ≥18 to<60 y; the percentage of subjects achieving an HI titer ≥1:40 is >70%; the percentage of subjects with seroconversion or significant increase in HI antibody is >40%; the GMR is >2.5. For ≥61 y; the percentage of subjects achieving an HI titer ≥1:40 is >60%; the percentage of subjects with seroconversion or significant increase in HI antibody is >30%.the GMR is >2.0. QIVc compared with TIV1c for A/H1N1, A/H3N2, and B/Yamagata strain and TIV2c for B/Victoria strain.

Table 2. The percentages of subjects with HI titers ≥40 (±95% CI), GMTs (±95% CI), and GMR (±95% CI) for all 4 vaccine strains at day 22 post-vaccination, in subjects stratified by baseline serostatus (HI < 1:10 and HI ≥ 1:10) (FAS).

Baseline serostatus		HI <1:10		HI ≥1:10	
		QIVc	TIV1c/TIV2c	QIVc	TIV1c/TIV2c
A/H1N1		n = 187	n = 97	n = 1124	n = 567
	GMTs	208.6 (163.2–266.5)	178.1 (125.0–253.7)	319.3 (297.6–342.7)	327.5 (297.1–361.0)
	GMR	40.7 (31.8–52.0)	35.1 (24.6–50.1)	3.5 (3.3–3.8)	3.6 (3.3–4.0)
	HI titers >1:40	87.2 (81.5–91.6)	85.6 (77.0–91.9)	98.0 (96.9–98.7)	98.4 (97.0–99.3)
A/H3N2		n = 81	n = 42	n = 1172	n = 595
	GMTs	98.2 (72.1–133.6)	113.1 (74.0–173.0)	407.7 (384.0–433.0)	412.8 (378.2–450.0)
	GMR	19.6 (14.4–26.8)	22.6 (14.8–34.6)	2.7 (2.5–2.9)	2.6 (2.4–2.8)
	HI titers >1:40	84.0 (74.1–91.2)	88.1 (74.4–96.0)	99.4 (98.8–99.8)	99.4 (98.4–99.8)
B/Yamagata		n = 75	n = 30	n = 1236	n = 634
	GMTs	68.4 (48.3–96.6)	87.7 (52.1–147.7)	141.1 (133.1-149.7)	118.3 (109.0-128.3)
	GMR	13.7 (9.7–19.4)	17.5 (10.4–29.5)	2.7 (2.5–2.9)	2.4 (2.2–2.6)
	HI titers >1:40	70.7 (59.0–80.6)	80.0 (61.4–92.3)	95.3 (94.0–96.4)	92.3 (89.9–94.2)
B/Victoria		n = 37	n = 24	n = 1215	n = 615
	GMTs	78.5 (45.4–135.9)	89.8 (48.9–165.0)	181.4 (172.0–191.3)	168.1 (155.4–181.7)
	GMR	15.7 (9.1–27.2)	18.0 (9.8–33.0)	2.8 (2.7–3.0)	2.6 (2.4–2.8)
	HI titers >1:40	78.4 (61.8–90.2)	83.3 (62.6–95.3)	98.2 (97.3–98.9)	97.5 (95.9–98.6)

FAS = full analysis set; GMR, geometric mean ratio (day 22/day 1).

Table 3. The superiority of HI antibody responses of QIVc to TIV1c and TIV2c over the unmatched B strain, at 3 wks (day 22) after vaccination in terms of the differences in percentages of subjects achieving seroconversion and the between group GMT ratios (FAS).

	QIVc n = 1311	TIV1c n = 664	TIV2c n = 657	
		GMTs (95% CI)		Vaccine Group ratio
B/Yamagata strain	177.1 (167.8–187.1)	–	76.3 (70.4–82.7)	0.5 (0.5–**0.5**)
B/Victoria strain	135.4 (127.6–143.7)	91.7 (85.7–98.2)	–	0.6 (0.6–**0.7**)
		% seroconversion (95% CI)		Vaccine Group difference
B/Yamagata strain	39.7 % (37.0–42.4)	–	18% (15.1–21.1)	−21.7% (−25.5, −**17.7%**)
B/Victoria strain	36.6% (34.0–39.3)	17.2% (14.4–20.3)	–	−19.4% (−23.2%, −**15.5**)

Bold = superiority criteria met.
Superiority margin: the upper limit of the 2-sided 95% CIs for the ratio of GMTs (GMT TIV1c or TIV2c/GMT QIVc) for HI antibody should be <1 and the upper limit of the 2-sided 95% CIs for the difference between SCRs (% seroconversion TIV1c or TIV2c − % seroconversion QIVc) for HI antibody should be <0.
FAS = full analysis set.

Severe pain was reported by 0.2% (n = 3/1319) and 0.1% (n = 1/670) of subjects in the QIVc and TIV1c groups, respectively. Rates of other solicited local AEs were similar among the vaccine groups. There was 1 case of severe ecchymosis and 1 case of severe induration in the TIV1c group.

The most commonly solicited systemic AEs reported were fatigue (13.5% in QIVc, 16.3% in TIV1c, and 12.2% in TIV2c) and headache (14.0%, 13.4%, and 13.4%). Severe systemic AEs were reported by <1% of subjects. Fever was reported by 15 subjects (7 [0.5%] in QIVc, 5 [0.7%] in TIV1c, 3 [0.5%] in TIV2c) and there were no reports of body temperature ≥40°C. The majority of reported solicited local and systemic AEs were mild to moderate in intensity. The reported solicited local and systemic reactions by overall study cohort are shown in Figure 5.

When analyzed by age cohorts, the rates of any solicited AEs were higher in the ≥18 to <65 y age cohort (61.8% in QIVc, 56.7% in TIV1c, 59.6% in TIV2c) than in the ≥65 y age cohort (41.3% in QIVc, 39.1% in TIV1c, 43.2% in TIV2c). Based on sex, across all 3 vaccine groups, reported incidences of any solicited AE were higher among female (57.9% in QIVc, 54.1% in TIV1c, 54.2% in TIV2c) than among male subjects (43.9% in QIVc, 38.9% in TIV1c, 47.1% in TIV2c). The rates of any solicited AEs did not differ among subjects from different ethnicities (data not shown).

Unsolicited AEs

Across the whole study group (≥18 y), the percentages of subjects reporting unsolicited AEs were similar between the QIVc (16.1%), TIV1c (14.7%), and TIV2c (16.5%) groups. The most commonly reported possibly or probably related unsolicited AEs by the Medical Dictionary for Regulatory Activities (MedDRA) preferred Term were injection-site hemorrhage (0.8% in QIVc, 0.4% in TIV1c, 0.6% in TIV2c), fatigue (0.5% in QIVc, 0.4% in TIV1c, 0.6% in TIV2c), and myalgia (0.5% in QIVc, 0.1% in TIV1c, 0.5% in TIV2c).

Medically attended AEs were reported by 26.0%, 25.6%, and 25.0% of subjects in the QIVc, TIV1c, and TIV2c groups, respectively. The most frequently recorded AEs by MedDRA preferred term were sinusitis (1.8% in QIVc, 2.5% in TIV1c, 2.4% in TIV2c) and bronchitis (2.2% in QIVc, 1.5% in TIV1c, 0.9% in

TIV2c). Overall, new onset of chronic diseases (NOCDs) were reported in 4.4% (116/2662) of subjects. The most commonly reported NOCDs were metabolism and nutritional disorders (0.8% in QIVc, 0.7% in TIV1c, 0.5% in TIV2c), cardiac disorders (0.8% in QIVc, 0.6% in TIV1c, 0.3% in TIV2c), and musculoskeletal and connective tissue disorders (0.8% in QIVc, 0.4% in TIV1c, 0.3% in TIV2c). No significant differences were observed between vaccine groups or age groups in the proportion of subjects with NOCDs. There were no vaccine-related serious adverse events (SAEs) in the study. One subject, from the QIVc group (≥65 y cohort), withdrew from the study due to acute myeloid leukemia and worsening of diabetes. In total, 12 deaths were reported (Table 4). None of the AEs leading to premature withdrawal and none of the deaths were considered related to study vaccine.

Subgroup analyses of AE profiles of QIVc, TIV1c, and TIV2c, by age cohorts, sex, and race/ethnicity did not show any notable differences. Unsolicited AEs and medically attended AEs were reported by relatively higher percentages of subjects in the ≥65 y age cohort than in the ≥18 to <65 y age cohort, though rates of possibly vaccine-related AEs were similar among the 2 age cohorts (Table 4). The rates of unsolicited AEs and medically attended AEs were 32.7% and 22.3%, respectively, in male subjects and 40.5% and 28.5%, respectively, in female subjects. Among different ethnic groups, unsolicited AEs were 40.6% (n = 832/2047) in Caucasians, 31.6% (n = 6/19) in American Indians (n = 6/19), 20% (n = 2/10) in Asians, 26.5% (n = 88/332) in African-American, 22.5% in Hispanics (n = 52/231) and 42.1% (n = 8/19) in other populations.

Discussion

Influenza B is a significant contributor to influenza-related morbidity and mortality in all age groups, causing on average 24%–30% of all influenza cases per season.[6,7,14-16] With a reduced efficacy of 22%–52%, TIVs containing only 1 B strain provide low cross-protection against the opposite lineage.[4,5,11] QIVs are potentially more beneficial because they are not only likely to reduce influenza-related outcomes, but potentially also generate substantial cost savings.[2,17–19]

To elaborate, while taking into account potentially higher costs for QIVs, a Monte Carlo simulation model estimated
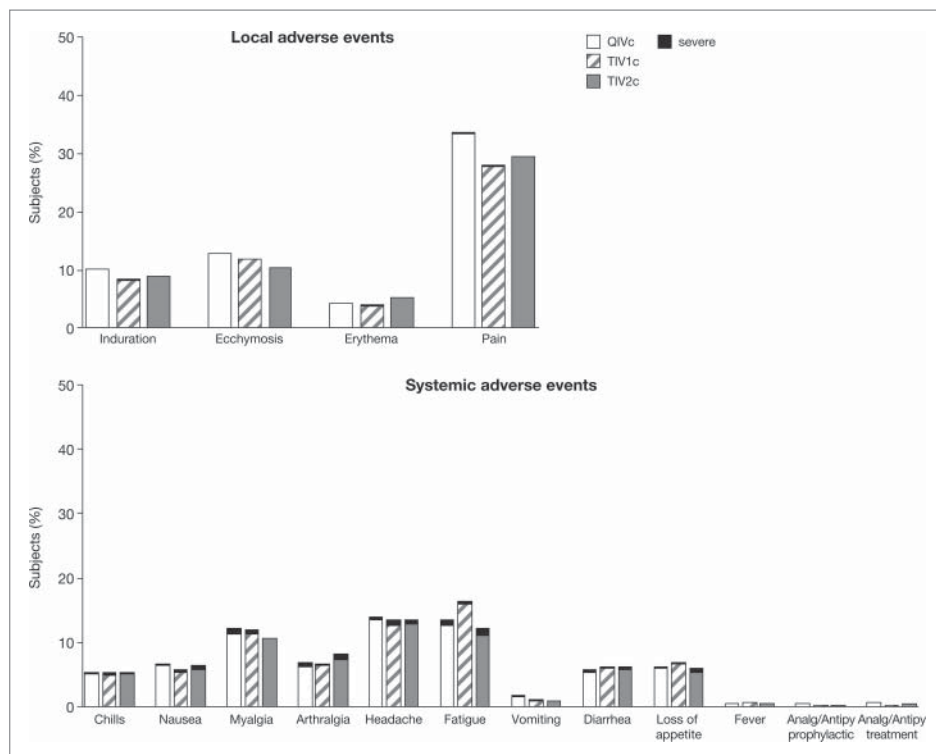
Figure 5. Percentage of subjects reporting solicited local and systemic AEs between day 1 through day 7 after vaccination with QIVc, TIV1c, and TIV2c in overall study population (≥18 y). Data are presented for the solicited data set. Analg/Antipy prophylactic = Prophylactic use of analgesics/antipyretics; Analg/Antipy treatment = Treatment with analgesics/antipyretics.

that the inclusion of both influenza B-strains in TIVs between 1999 and 2009 would have resulted in substantial cost savings for society (median of $3.1 billion) and third party payers (median $292 million) in the US.[17] In a more recent publication, a dynamic compartmental model accounting for the interactions between influenza B lineages predicted that routine vaccination with QIVs between 2000 and 2013 would have prevented, on average, 16% more B lineage cases than vaccination with TIVs in the US (assuming a cross protection of 70% of the vaccine efficacy).[19] This model highlighted that 2 groups in particular would benefit from QIV vaccination, the elderly (≥65 years) and the young seniors (50–65 y), in whom QIV has the potential to prevent 21% and 18% more B-lineage cases than TIVs, respectively.[19]

The current study is the first to assess the immunogenicity and safety of a cell culture-based QIV in adults and to assess non-inferiority as compared with a licensed comparator vaccine. The HI antibody responses of a single dose of QIVc were demonstrated to be non-inferior to 1 dose of TIVc for the A/H1N1, A/H3N2, B/Victoria, and B/Yamagata strains, as measured by the ratio of GMT and differences in SCRs at day 22. The primary objective of the study was therefore met.

Furthermore, QIVc demonstrated superior antibody responses to TIVc against the unmatched influenza B vaccine strain. Overall, for the strains common to the study vaccines, the immunogenicity of QIVc was comparable with the immunogenicity of TIVc formulations. Antibody responses were more robust in younger than in older adults. The lower

Table 4. Number (%) of subjects with unsolicited AEs following vaccination with QIVc, TIV1c, and TIV2c, throughout the study period (day 1 to day 181), by age cohorts (unsolicited safety set).

	≥18 to <65 y			≥65 y		
Unsolicited AEs	QIVc n = 665	TIV1c n = 330	TIV2c n = 328	QIVc n = 659	TIV1c n = 343	TIV2c n = 337
Any AE	212 (31.9)	88 (26.7)	107 (32.6)	282 (42.8)	155 (45.2)	144 (42.7)
Possibly or probably related AE	28 (4.2)	9 (2.7)	15 (4.6)	29 (4.4)	13 (3.8)	15 (4.5)
Any SAE	11 (1.7)	6 (1.8)	5 (1.5)	41 (6.2)	16 (4.7)	16 (4.7)
Possibly or probably related SAE	0	0	0	0	0	0
AEs leading to premature withdrawal*	0	0	1 (0.3)	2 (0.3)	1 (0.3)	0
Medically attended AE	141 (21.2)	58 (17.6)	67 (20.4)	203 (30.8)	114 (33.3)	99 (29.4)
NOCDs	24 (3.6)	10 (3.0)	12 (3.7)	38 (5.8)	15 (4.4)	17 (5.0)
Death	0	0	1 (0.3)	5 (0.8)	5 (1.5)	1 (0.3)

Unsolicited AEs were collected from day 1 through day 22. Unsolicited AEs that were SAEs, medically attended AEs, AEs leading to withdrawal from the study, and new onset of chronic diseases (NOCDs) were collected from day 1 through day 181. *One subject from the QIVc group withdrew from the study due to AEs (acute myeloid leukemia and worsening of diabetes). For 2 subjects (1 each in TIV1c [≥65 y] and TIV2c [≥18–65 y] groups), the reason for premature withdrawal was death. One subject had a SAE amyotrophic lateral sclerosis (the onset date of this AE was before the vaccination date but the final diagnosis was made after vaccination; however, the reason for premature withdrawal was captured as withdrawal of consent in the case report form).

antibody response to influenza vaccines in older adults has also been observed in other QIV studies[20,21] and is attributed largely to age-related immunosenescence.[22] The high pre-vaccination antibody titers and the prior influenza vaccination history could also partially account for the low SCRs, although the older and younger adult cohorts in our study were balanced with respect to both of these factors. The impact of baseline serostatus on vaccine responses was evident from the relatively higher SCRs observed in seronegative subjects when compared with seropositive subjects, in both QIVc and TIVc groups.

All currently licensed QIVs are produced using embryonated eggs, a conventional method that has several limitations in terms of flexibility and capability to scale-up production.[9] In comparison, advantages of the cell-culture technology are reduced risk of contamination, absence of trace egg components, a more efficient downstream process, no addition of antibiotics, a shorter lead time, higher yield, and faster production cycles.[10,23] The MDCK suspension cell lines are the most permissive for growth of both influenza A and B strains.[24] Studies have also shown that any residual cell substrate in the vaccine produced with MDCK technology do not have either tumerogenic or oncogenic potential.[10,23,25] To date, the safety of TIVc/Optaflu® has been assessed in >12,500 subjects in several trials, across age groups with no novel safety signals identified relative to the safety signals for egg-derived vaccines.[11-13,26-28]

Although the CDC recommends routine influenza vaccinations, especially for at-risk populations,[29] vaccination hesitancy among the general public is increasing and strongly influenced by the media. Incidents, such as occurred in Italy in 2014,[30] where negative media coverage following the wrongful precautionary suspension of an adjuvanted influenza vaccine resulted in panic and decreased vaccination rates, demonstrate the urgency of communicating the results of drug safety assessments, such as the current study's results that demonstrate QIVc and TIVc are well tolerated, through adequate health communication. Overall, solicited AEs were reported in a lower percentage of older adults than in younger adults. Vaccine-related unsolicited AEs were few and medically attended AEs were reported by a similar proportion of study subjects. The reactogenicity and safety profiles of QIVc were consistent with those of TIVc, which has previously been established to have a similar safety profile to other licensed influenza vaccines.[11-13,18,21,31-33]

While the study participants were representative of the general population, including individuals with different underlying medical conditions, the study is limited by the fact that it was conducted only in 1 country and did not include individuals with impaired immunity and/or specific immunocompromising conditions. Another limitation, related to the safety analyses, is that no formal statistical comparisons between the 2 vaccine groups were performed. The study was neither powered to capture nor compare rare AEs following vaccination. The randomized, double-blind, multicenter design of the study aimed to control for confounding environmental factors, such as possible effects of local influenza outbreaks on the subject's antibody responses. Because the study enrolled its first participants in November 2013, it captured all local influenza outbreaks between December 2013 and January 2014. To further control for any confounding effects of circulating antigens on

HI titers, all confirmed influenza cases were excluded from the immunogenicity analysis.

In conclusion, 1 dose of the investigational cell culture-derived QIVc induced antibody responses against the 4 influenza strains (A/H1N1, A/H3N2, and both influenza B strain lineages) tested in adults similar to those responses produced by the trivalent influenza vaccine. The addition of the second influenza B strain in the vaccine did not interfere with the immune responses to the other 3 influenza strains. QIVc demonstrated an acceptable AE profile during the 6 mo follow-up. Overall, the results of this study established an acceptable immunogenicity and safety profile of the cell culture-based QIVs, making cell culture-based QIV a good alternative to TIVs with the added value of providing a broader coverage of influenza B-strains.

Patients and methods

Study design

This was a Phase III, double-blind, randomized study, undertaken in individuals ≥18 y of age, conducted across 40 centers in the US, from November 2013 to July 2014. The majority of study centers were operated under different site management organizations (SMOs). The SMO subject database was screened to identify potential subjects eligible for study participation. The study was performed in accordance with the principles of Good Clinical Practices, the Declaration of Helsinki, the US Code of Federal Regulations Title 21, and Novartis codes on protection of human rights.[34] The study was approved by Institutional Review Boards (central Institutional Review Boards or at individual study sites). Informed consent was obtained from all participants before enrollment. The study was registered with ClinicalTrials.gov (NCT01992094).

The primary objective of the study was to demonstrate the non-inferiority of the HI antibody responses of QIVc with a comparator TIVc, which was assessed for each of the 4 vaccine strains by between group GMT ratios and by the differences in SCR, at 3 wks post-vaccination. The key secondary objective was to evaluate the immunogenicity of QIVc and TIVc based on the CBER criteria in 2 age cohorts: ≥18 to <65 y and ≥65 y.[35] Other secondary immunogenicity objectives included assessing the immunogenicity of QIVc and TIVc according to the CHMP criteria for each of the 4 vaccine strains and to demonstrate superiority of QIVc against the unmatched B strain in TIVc.[36]

Eligible subjects were age stratified (1:1) into ≥18 to <65 y and ≥65 y and randomized (2:1:1) to receive a single dose of either QIVc, TIV1c (B/Yamagata lineage), or TIV2c (B/Victoria lineage) on day 1. Subjects were randomized using an interactive response technology system. Blood samples for immunogenicity analyses were drawn at baseline (day 1) immediately before vaccination and at 3 wks post-vaccination (day 22).

To encourage completion of the study protocol by subjects (Fig. 1); site staff were encouraged to contact the subjects by telephone and in writing (at least 3 documented attempts by telephone and at least 1 documented attempt in writing) before subjects were deemed lost to follow up. Safety follow-up was conducted for 6 months post-vaccination (day 23 through 181). The percentages of subjects adhering to study protocol

were recorded at the first (between days 20 and 29) and second return visit (between days 165 and 195).

Subjects

Subjects were included if they were ≥18 y of age, were willing/capable of providing informed consent, could comply with study procedures, and were available for follow-up.

Subjects were excluded if they had a body temperature measurement ≥38°C (≥ 100.4°F) within 3 d prior to vaccination; had received influenza vaccination or had documented influenza disease within the past 6 mo; had a chronic or acute illness that, in the opinion of the investigator, would interfere with the subject's safety during study participation and/or compliance with study-related procedures and/or with the evaluation of study vaccine; were potentially pregnant, pregnant, or breastfeeding; had a history of Guillain-Barré Syndrome; had current alcohol abuse or drug addiction; had any contraindication to vaccination, blood draw, or were allergic to latex; had participated in any other clinical trial within 30 d prior to first study visit; had known or suspected congenital or acquired immunodeficiency, or received immunosuppressive therapy within the previous 6 m or systemic corticosteroid therapy at any dose for ≥14 d within the past 3 mo; or had received blood, blood products, and/or plasma derivatives within the previous 12 wks.

Vaccines

Each 0.5 ml dose of the investigational QIVc contained approximately 15 μg of purified viral HA antigens for each of the 4 influenza strains recommended by the World Health Organization (WHO) for the 2013/14 influenza vaccine composition for the Northern Hemisphere season: A/Brisbane/10/2010 (H1N1), A/Texas/50/2012 (H3N2), B/Massachusetts/2/2012, and B/Brisbane/60/2008.

A 0.5 ml dose of the comparator vaccines TIV1c (Flucelvax®, approved by the Food and Drug Administration in the US and Optaflu® approved by the European Medicines Agency in the European Union) and TIV2c contained the same A influenza strains. TIV1c also contained HA antigens for B/Massachusetts/2/2012 (Yamagata lineage), as recommended by the WHO for inclusion in the trivalent vaccine composition for the 2013/2014 influenza season, while TIV2c contained HA antigens for B/Brisbane/60/2008 (Victoria lineage), not in the official TIV recommendation but recommended by the WHO for the composition of quadrivalent influenza vaccines. The vaccines were administered in the deltoid muscle, preferably of the non-dominant arm.

Immunogenicity assessment

Serological evaluations were conducted at the Novartis Vaccines Serology Laboratory, Marburg, Germany. Antibody responses against the influenza type A and B strains were measured by HI assays. The antibody responses of QIVc for influenza A/H1N1, A/H3N2, and B (Yamagata lineage) strains were compared with TIV1c, whereas the influenza B strain (Victoria lineage) was compared with TIV2c. HI titer was expressed as the reciprocal of the highest dilution at which hemagglutination was completely inhibited. Antibody responses were expressed in terms of GMTs, GMR, percentages of subjects with seroconversion, and percentages of subjects with HI titers ≥40. For seronegative subjects (i.e., HI titer <1:10), seroconversion was defined at baseline as a post-vaccination HI titer ≥1:40. For seropositive subjects (i.e., HI titer ≥1:10), seroconversion was defined at baselines as a minimum of a 4-fold increase in post-vaccination HI titer.

Safety assessment

Subjects were observed for a minimum of 30 min after vaccine administration to monitor for possible immediate reactions. Thereafter, subjects were provided with diary cards to record local, systemic, and other AEs occurring from day 1 through day 7 after vaccination. Solicited local AEs were injection-site pain, erythema, induration, swelling, and ecchymosis. Solicited systemic AEs were fever (≥38°C), shivering, malaise, generalized myalgia, generalized arthralgia, headache, nausea, fatigue, vomiting, diarrhea, and loss of appetite. Other measures of safety were the use of analgesics/antipyretics. The solicited AEs were graded as mild, moderate, or severe, if resulting in no limitation of, some limitation of, or an inability to perform normal daily activities, respectively. All unsolicited AEs were recorded for 3 wks after vaccination (day 1–22). All medically attended AEs, AEs leading to withdrawal from the study, new onset of chronic diseases (NOCDs), SAEs, and concomitant medications associated with these events were recorded throughout the study period (day 1–181).

Statistics

Non-inferiority

The co-primary endpoints of the study were the ratio of GMTs and the differences in seroconversion between vaccine groups, which were used to assess non-inferiority. QIVc was considered non-inferior to TIV1c and TIV2c if, for each matched vaccine strain, the upper limit (UL) of the 2-sided 95% confidence intervals (CI) of the vaccine group ratio of GMTs (TIV1c or TIV2c divided by QIVc) was <1.5, and the UL of the 2-sided 95% CI for the difference in SCR (TIV1c or TIV2c minus QIVc) was <10%. If both co-primary non-inferiority endpoints were achieved for all 4 vaccine strains, the study was to be considered a success.

Sample size

Assuming a dropout rate and exclusions of approximately 14% of subjects across the study, 1340 subjects in the QIVc arm and 670 in each of the TIV1c and TIV2c arms were considered to be sufficient to evaluate the co-primary objective in the pooled age cohort (≥18 y) with an overall power of 90% and a 1-sided α of 0.025%. No formal power assumptions were made for the secondary outcomes evaluated in the study.

Immunogenicity

The following endpoints were applicable according to CBER immunogenicity criteria: the lower limit of the 2-sided 95% CIs for the percentage of subjects achieving an HI antibody titer ≥1:40 should be ≥70% and ≥60% for subjects aged ≥18 to

<65 y and ≥65 y, respectively, and the lower limit of the 2-sided 95% CIs for the percentage of subjects achieving sero-conversion should be 40% and ≥30% for subjects aged ≥18 to <65 y and ≥65 y, respectively.

In order to reach the immunogenicity endpoints based on the CHMP criteria for HI antibody responses for subjects aged 18 to ≤60 y and ≥61 y of age, percentages of subjects achieving seroconversion should be >40% and >30%, respectively; GMRs should be >2.5 and >2.0, respectively; and the percentages of subjects achieving an HI titer ≥1:40 should be >70% and >60%, respectively.[15]

Superiority

The superiority margins were calculated for the influenza B strains included in QIVc. QIVc was considered superior to the unmatched B strain in TIV1c and TIV2c, if the UL of the 2-sided 95% CI for the ratio of GMTs (GMT TIV1c or TIV2c/GMT QIVc) was <1, and the UL of the 2-sided 95% CI for the difference between SCRs (% of seroconversion by TIV1c or TIV2c minus % of seroconversion by QIVc) was <0.

Safety

Safety data were evaluated descriptively and presented by vaccine and age groups (≥18–<65 y and ≥65 y).

Data sets

Non-inferiority was assessed using the per-protocol set population, i.e., all enrolled subjects who correctly received the study vaccine, provided blood samples at days 1 and 22, and who had no major protocol deviations or any other reasons leading to study exclusion. The full analysis set population, i.e., all vaccinated subjects providing evaluable serum samples on days 1 and 22, was used for all secondary immunogenicity measures and for superiority testing. The overall safety population consisted of all exposed subjects who provided post-vaccination solicited AE data (safety set solicited AEs) or post-vaccination unsolicited AE data (safety set unsolicited AEs).

Abbreviations

AE	adverse event
CBER	Center for Biologics Evaluation and Research
CHMP	Committee for Medicinal Products for Human Use
GMR	geometric mean ratio
GMT	geometric mean titer
HA	hemagglutinin
HI	hemagglutinin inhibition
MDCK	Madin-Darby Canine Kidney
NOCD	new onset of chronic diseases
QIVc	cell-based quadrivalent influenza vaccine
TIVc	cell-based trivalent influenza vaccine
SAE	serious adverse event
SCR	seroconversion rate
UL	upper limit
WHO	World Health Organization

Author contributions

Ahmed Abdul Mateen and Kelly Lindert participated in the conception and design of the trial. Stephan Bart, Kevin Cannon, Darrell Herrington, and Richard Mills managed study sites and enrolled participants. Ahmed Abdul Mateen, Kelly Lindert, and Eduardo Forleo-Neto performed study management for the study sponsor. All authors were involved in the interpretation of analyzed data, development of the manuscript and the decision to submit for publication.

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