Clinical Efficacy of Cell Culture–Derived and Egg-Derived Inactivated Subunit Influenza Vaccines in Healthy Adults

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(See the editorial commentary by Bernstein, on pages 1005–1006.)

Background. More efficient methods are needed to manufacture influenza vaccines. This trial compared the efficacy of cell culture–derived influenza vaccine (CCIV) and egg-derived trivalent inactivated vaccine (TIV) with placebo against laboratory-confirmed influenza illness in healthy adults in the United States, Finland, and Poland during the 2007–2008 influenza season.

Methods. A total of 11,404 study participants aged 18–49 years were randomized equally to receive CCIV (Optaflu; $n = 3828$), TIV (Agrippal; $n = 3676$), or placebo ($n = 3900$). Each participant was observed during a 6-month study surveillance period. Nasal and throat swabs for virus isolation and characterization were collected from all patients with influenza-like illness. Vaccine immunogenicity was evaluated in a subset of 1045 participants.

Results. Efficacy of CCIV and TIV against vaccine-like (83.8% [1-sided 97.5% confidence interval [CI] lower limit, 61.0%] and 78.4% [1-sided 97.5% CI lower limit, 52.1%], respectively) and all circulating influenza virus strains (69.5% [1-sided 97.5% CI lower limit, 55.0%] and 63.0% [1-sided 97.5% lower limit, 46.7%], respectively) exceeded the Center for Biologics Evaluation and Research efficacy criteria. Immunogenicity of both vaccines exceeded the Center for Biologics Evaluation and Research licensing criteria. Both vaccines were well tolerated, with similar safety profiles. Most solicited reactions were mild to moderate in severity and transient. No vaccination-related serious adverse events were reported; no withdrawals resulted from vaccine-related adverse events.

Conclusions. Both CCIV and TIV were effective in preventing influenza caused by vaccine-like and by all circulating influenza virus strains, were well tolerated, and had good safety profiles. Both vaccines can be considered for annual influenza vaccination campaigns.

Clinical trials registration. NCT00630331.

Influenza virus infections are a major cause of respiratory illness, morbidity, and mortality in elderly people, very young people, and those with intercurrent disease [1, 2]. Vaccination remains the principal control for seasonal infections and is a core strategy in pandemic influenza preparedness. Seasonal influenza vaccines are typically produced from viruses propagated in hen eggs. The supply of eggs is limited and obtaining them requires advance planning, potentially making rapid increase in production difficult in case of increased demand or a pandemic. The World Health Organization has recommended using established mammalian cell lines as alternative culture systems [3]. Cell culture propagation offers assured availability of substrate for virus growth, which could increase flexibility to meet shifts in demand [4, 5]. Three cell lines have shown promise for vaccine production, Madin Darby canine kidney (MDCK), Vero, and PER.C6. Vero cells are used to produce poliovirus, rabies, and rotavirus vaccines [6, 7]. A Vero cell–derived whole-cell H5N1 influenza vaccine has been evaluated [8], and the PER.C6 line has been shown to yield influenza viral titers sufficient for vaccine production [9]. In 2007,

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the European Medicines Agency approved an MDCK cell culture–derived influenza vaccine (CCIV) (Optaflu; Novartis Vaccines) [10, 11].

Approximately 4000 doses of this CCIV have been administered to healthy adult and elderly individuals in 5 trials that evaluated the safety and immunogenicity, compared with Novartis Vaccines' European (Agrippal) or American (Fluvirin) licensed trivalent inactivated vaccines (TIVs) [11–15]. CCIV was as immunogenic, safe, and well tolerated as the TIVs; all 3 vaccine virus strains met European (Committee for Medicinal Products for Human Use) and United States (Center for Biologics Evaluation and Research [CBER]) immunogenicity criteria for licensing [16, 17].

This trial (NCT00630331), conducted in healthy adults in the United States, Finland, and Poland during the 2007–2008 influenza season, evaluated the clinical efficacy of CCIV (Optaflu) and TIV (Agrippal), compared with placebo, to prevent laboratory-confirmed influenza. To our knowledge, this was the largest randomized, placebo-controlled trial yet conducted to evaluate the efficacy of inactivated influenza vaccines against virus-confirmed influenza and the first to evaluate the efficacy of a trivalent CCIV.

METHODS

Study design and objectives. This randomized, placebo-controlled, observer-blind trial evaluated the efficacy, safety, and immunogenicity of influenza vaccination in healthy adults in the United States, Finland, and Poland during the 2007–2008 influenza season. The ethics committee or institutional review board of each center approved the study. Healthy individuals were eligible if they could comply with study procedures. All gave written informed consent before enrollment. Major exclusion criteria included a health condition for which inactivated vaccine is recommended, employment prone to influenza transmission, influenza vaccination or laboratory-confirmed influenza within 6 months of enrollment, history of Guillain-Barré syndrome, a temperature of ⩾37.8°C and/or acute illness within 3 days of enrollment, and pregnancy or breast-feeding. Female individuals of childbearing potential agreed to continue using a reliable birth control method. A urine pregnancy test was performed on female participants before vaccination. Individuals aged 18–49 years were randomized equally, with use of an interactive voice response system, to receive a single dose of CCIV, TIV, or placebo. Influenza surveillance began 21 days after vaccination. Each study participant was observed during the 6-month study surveillance period or for 6 months after vaccination, whichever was longer. Study duration was ~9 months.

The primary objective was to demonstrate the efficacy, compared with placebo, of each study vaccine against confirmed influenza illness caused by virus strains antigenically similar to those of the vaccines. Secondary objectives included demonstration of vaccine efficacy against nonvaccine viruses and all circulating strains and the safety and tolerability of each vaccine. Influenza was defined as an episode of influenza-like illness (ie, fever [temperature, ⩾37.8°C] plus sore throat or cough), with subsequent laboratory confirmation of influenza virus. Seroprotection and seroconversion rates and antihemagglutinin geometric mean titers were determined on day 1 (baseline) and 3 weeks after vaccination in the first 1045 participants enrolled at United States sites, who were randomized 8:25:2 to CCIV ($n = 240$), TIV ($n = 746$), or placebo ($n = 59$) for immunogenicity analysis.

Vaccines. CCIV and TIV (Novartis Vaccines and Diagnostics) contained 15 μg of hemagglutinin per 0.5-mL dose of each virus strain recommended for the 2007–2008 Northern Hemisphere influenza season: A/Solomon Islands/3/2006 (H1N1)–like, A/Wisconsin/67/2005 (H3N2)–like, and B/Malaysia/2506/2004–like. Vaccines and placebo (0.5 mL of phosphate-buffered saline) were administered in the deltoid muscle of the nondominant arm. Only the vaccine administrator had access to the randomization code.

Reactogenicity and safety. Study participants were monitored for 30 min after vaccination for immediate reactions. Participants recorded the occurrence, duration, and severity of local injection site (ecchymosis, erythema, induration, swelling, and pain) and systemic (chills, malaise, myalgia, arthralgia, headache, sweating, fatigue, and fever [oral temperature, ⩾37.8°C]) reactions for 7 days after vaccination. Solicited reactions were graded as follows: mild, no limitation of normal daily activities; moderate, some limitation; or severe, unable to perform normal daily activities. Unsolicited reactions were recorded for 21 days after vaccination; serious adverse events were monitored for the entire study.

Influenza surveillance. Beginning 21 days after vaccination, participants reported influenza-like illness symptoms, defined as fever (temperature, ⩾37.8°C) plus sore throat or cough, as well as body aches, chills, headache, and runny or stuffy nose, to investigators. Active influenza-like illness surveillance was also conducted during weekly telephone calls from the call center. Participants reporting influenza-like illness symptoms underwent clinical evaluations; nasal and throat specimens were obtained for laboratory confirmation of influenza virus. Because peak shedding of virus may occur during the first 24–72 h of illness [18], specimens were targeted for collection within 24 h after symptom onset, with a window of 120 h.

Laboratory methods. Combined nasal and throat specimens were evaluated for influenza virus (Covance) in RhMK cell culture and by polymerase chain reaction testing (ResPlex III Plus, Qiagen). Confirmation and subtyping of infected cells was performed by fluorescence staining with fluorescein isothiocyanate–conjugated antibody against type-specific influenza an-

tigens [19]. Further characterization of polymerase chain reaction–positive specimens was not possible.

The hemagglutinin viral titers were performed on influenza-positive cultures using a standard erythrocyte assay [19]. The hemagglutinin titer was the highest dilution of virus to cause agglutination. All specimens with hemagglutinin titers ⩾1:4 were typed according to strain and evaluated for antigenic relatedness to vaccine strains at the Centers for Disease Control and Prevention. Isolates were considered to be vaccine matching if there was a ⩽4-fold difference in the titer of the participant's isolate and the vaccine strain against a reference antiserum. Nonvaccine strain isolates were characterized by an hemagglutination inhibition antibody titer ⩾1:8 against specific reference strain antisera.

Serum samples for immunogenicity evaluation were assessed by hemagglutination inhibition assays against egg-derived vaccine A/H1N1, A/H3N2, and B-strain antigens at the Novartis Vaccines Clinical Serology Laboratory. Serum samples were pretreated with receptor destroying enzyme to block nonspecific inhibitors [20] and tested at an initial dilution of 1:10. The hemagglutination inhibition titer was the highest dilution that showed complete inhibition of hemagglutination. Negative samples were assigned a titer of 1:5.

Statistical analysis. Vaccinated study participants who were evaluable 21 days after vaccination were included in the efficacy-modified intention-to-treat population. The efficacy per protocol population included those individuals who were evaluable during the individual 6-month surveillance period. The vaccine efficacy was estimated as (1− relative risk) × 100, where relative risk was the ratio of the percentages of vaccine recipients with influenza to placebo recipients with influenza ($P_{vaccine}/P_{placebo}$). Vaccine efficacy was assessed for significance versus placebo with use of simultaneous $100 \times (1 - \alpha)$ Sidak-corrected 1-sided confidence intervals (CIs) for the 2 relative risks, where $\alpha = .025$.

The study population size calculation of 11,700 individuals (3900 per group) was based on determining the vaccine efficacy for each vaccine compared with placebo separately. Comparison of the 2 vaccines was not planned. For a vaccine efficacy of 70%, a projected confirmed influenza attack rate of 3%, and a 1-sided α of .0125, the power to reject the null hypotheses that the vaccine efficacy was ⩽40% for each of the vaccines was 92%. To adjust for this double comparison, the Bonferroni method was used to estimate the power. The analyses were conducted using SAS software, version 9.1 (SAS Institute).

Seroprotection and seroconversion rates, geometric mean titers on days 1 and 22, and geometric mean ratios of geometric mean titers on days 1 and 22 were calculated. Seroprotection rates met the CBER immunogenicity criteria if the lower limit of the 2-sided 95% CI for the percentage of seroprotected individuals was ⩾70%. The seroconversion criterion was met if the lower limit of the 2-sided 95% CI for the percentage of seroconverted individuals was ⩾40%. The 95% CIs were calculated using the Clopper-Pearson method.

RESULTS

Study participants. A total of 11,404 participants were randomized, 11,382 were vaccinated, and 10,844 (95%) completed the study (Figure 1). Overall, 560 participants withdrew from the study: 206 in the CCIV group, 166 in the TIV group, and 188 in the placebo group. One participant in the CCIV group withdrew after an adverse event (head injuries), and 4 died, 2 in the CCIV and 1 each in the TIV and placebo groups. None of these events was judged as related to study treatment. The most frequent reason for withdrawal was loss to follow-up, which occurred at a rate of 5% in the CCIV and 4% in the TIV and placebo groups. Less than 1% of the participants in each group withdrew for other reasons (eg, withdrawal of consent, inappropriate enrollment, protocol violation, and inability to classify). A total of 11,301 study participants who were vaccinated (3791, 3648, and 3862 in the CCIV, TIV, and placebo groups, respectively) were evaluable 21 days after vaccination and were included in the efficacy-modified inten-

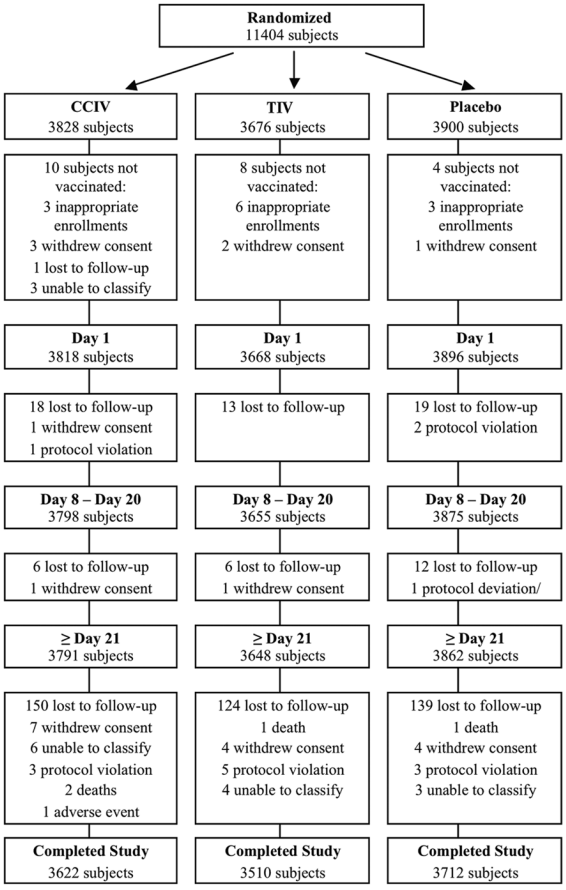

Figure 1. Disposition of the study participants. CCIV, cell culture–derived influenza vaccine; TIF, egg-derived trivalent inactivated vaccine.

Table 1. Cases of Influenza-Like Illness (ILI) Reported, Nasal and Pharyngeal Swabs Taken, and the Percentages of ILI Cases Confirmed as Influenza

Variable	CCIV (n = 3776)	TIV (n = 3638)	Placebo (n = 3843)	All (n = 11,257)
ILI cases	189 (5)	243 (7)	353 (9)	785 (7)
Swabs taken				
Total	174 (92)	226 (93)	326 (92)	726 (92)
Within 120 h	168/174 (97)	218/226 (96)	318/326 (98)	704/726 (97)
Interval from ILI to swab, mean h ± SD[a]	39.4 ± 26.6	38.2 ± 26.5	38.7 ± 27.2	38.7 ± 26.8
Confirmed influenza cases	42/168 (25)	49/218 (22)	140/318 (44)	231/704 (33)

NOTE. Data are no. or proportion (%) of participants, unless otherwise indicated. CCIV, cell culture–derived influenza vaccine; TIV, egg-derived trivalent inactivated vaccine; SD, standard deviation.

[a] For interval from ILI to swab, 168, 218, 318, and 704 participants were included for CCIV, TIV, placebo, and all groups, respectively.

tion-to-treat population. The efficacy per protocol population included 11,257 participants in the CCIV (n = 3776), TIV (n = 3638), and placebo (n = 3843) groups, respectively, who were evaluable during the individual 6-month surveillance period. The demographic and baseline characteristics of the 3 study groups were similar. The mean age was 32.7–33.0 years; 44%–45% were male. White, Hispanic, and black persons represented 84%–85%, 8%, and 7% of the study participants, respectively; 13%–15% of participants had previously received influenza vaccinations.

Efficacy. A total of 785 study participants in the CCIV (5%), TIV (7%), and placebo (9%) groups reported influenza-like illness symptoms during the surveillance period, with samples taken from 92% (Table 1). The mean interval from symptom onset to collection samples was ~39 h; 96%–98% were obtained within the specified 120-h window, and influenza vi-

Table 2. Vaccine Efficacy (VE) against Culture-Confirmed Influenza Caused by Vaccine-Like, Non–Vaccine-Like, and All Circulating Strains

Strain	Proportion (%) of participants with influenza			VE, % (97.5% CI lower limit)[a]		P[b]	
	CCIV	TIV	Placebo	CCIV vs placebo	TIV vs placebo	CCIV vs placebo	TIV vs placebo
All circulating strains[c]							
Overall	42/3776 (1.11)	49/3638 (1.35)	140/3843 (3.64)	69.5 (55.0)	63.0 (46.7)	<.001	.003
A/H1N1	6/3776 (0.16)	10/3638 (0.27)	57/3843 (1.48)	89.3 (73.0)	81.5 (60.9)	<.001	<.001
A/H3N2	6/3776 (0.16)	12/3638 (0.33)	25/3843 (0.65)	75.6 (35.1)	49.3 (−9.0)	.040	.53
B	30/3776 (0.79)	27/3638 (0.74)	61/3843 (1.59)	49.9 (18.2)	53.2 (22.2)	.37	.26
Vaccine-like strains							
Overall	7/3776 (0.19)	9/3638 (0.25)	44/3843 (0.011)	83.8 (61.0)	78.4 (52.1)	<.001	.004
A/H1N1	5/3776 (0.13)	8/3638 (0.22)	43/3843 (0.011)	88.2 (67.4)	80.3 (54.7)	<.001	.002
A/H3N2	2/3776 (0.05)	1/3638 (0.03)	0/3843 (0)	Not evaluable[d]	Not evaluable[a]	.999	.992
B	0/3776 (0)	0/3638 (0)	1/3843 (0.03)	100.0	100.0	.394	.400
Non–vaccine-like strains							
Overall	30/3776 (0.79)	29/3638 (0.80)	74/3843 (1.93)	58.7 (33.5)	58.6 (32.9)	.078	.085
A/H1N1	1/3776 (0.03)	0/3638 (0)	8/3843 (0.21)	87.3 (4.6)	100.0 (33.9)	.104	.033
A/H3N2	0/3776 (0)	2/3638 (0.05)	8/3843 (0.21)	100.0 (36.3)	73.6 (−30.1)	.030	.265
B	29/3776 (0.0077)	27/3638 (0.0074)	59/3843 (0.0154)	50.0 (17.5)	51.7 (19.4)	.376	.319

NOTE. CCIV, cell culture–derived influenza vaccine; CI, confidence interval; NE, not evaluable; TIV, egg-derived trivalent inactivated vaccine.

[a] VE = (1 − relative risk) × 100. Simultaneous 1-sided 97.5% CIs for the VE of each vaccine relative to placebo were based on Sidak-corrected score CIs for the 2 relative risks.

[b] Adjusted P values from the score statistic with Sidak correction testing the null hypothesis that the VE of each vaccine relative to placebo ⩽40% against the alternative hypothesis that the VE >40%. If the adjusted P < .025, then VE is significantly larger than 40%. This test procedure strongly controls the family-wise error rate at 2.5%.

[c] Five influenza cases in the CCIV group, 11 in the TIV group, and 22 in the placebo group were included in this category because influenza was confirmed by culture or polymerase chain reaction but the virus strain could not be characterized.

[d] The VE against A/H3N2 was <0% (ie, not evaluable); 2 cases of A/H3N2 influenza occurred in the CCIV group and 1 in the TIV group, but none were reported in the placebo group.

Table 3. Prevaccination and Postvaccination Seroprotection, Seroconversion, Geometric Mean Titer (GMT), and Geometric Mean Ratio (GMR) for the Cell Culture–Derived Influenza Vaccine (CCIV), Egg-Derived Trivalent Inactivated Vaccine (TIV), and Placebo Groups

	CCIV (n = 228)			TIV (n = 695)			Placebo (n = 55)		
Variable	A/H1N1	A/H3N2	B	A/H1N1	A/H3N2	B	A/H1N1	A/H3N2	B
Prevaccination (day 1)									
Seroprotection, % (95% CI)	48 (42–55)	63 (57–69)	25 (20–31)	53 (49–57)	58 (54–61)	23 (20–27)	60 (46–73)	71 (57–82)	22 (12–35)
GMT (95% CI)	34 (27–42)	48 (39–58)	14 (12–16)	35 (31–40)	41 (37–46)	13 (12–14)	37 (24–57)	56 (37–83)	12 (8.89–16)
Postvaccination (day 22)									
Seroprotection, % (95% CI)	99 (97–100)	99 (98–100)	78 (72–83)	98 (97–99)	99 (98–100)	92 (90–94)	60 (46–73)	65 (51–78)	22 (12–35)
Seroconversion, % (95% CI)	78 (72–83)	59 (53–66)	51 (45–58)	75 (71–78)	68 (64–71)	68 (65–72)	0 (0–6)	0 (0–6)	0 (0–6)
GMT (95% CI)	566 (483–663)	332 (289–383)	72 (63–84)	499 (455–546)	357 (330–387)	120 (111–131)	36 (26–50)	53 (40–71)	12 (8.81–16)
GMR (95% CI)	17 (13–21)	6.94 (5.68–8.46)	5.2 (4.31–6.28)	14 (12–16)	8.68 (7.74–9.73)	9.41 (8.45–10)	0.99 (0.62–1.56)	0.96 (0.64–1.44)	0.99 (0.68–1.46)

NOTE. CI, confidence interval. Center for Biologics Evaluation and Research criteria for seroprotection (the lower limit of the 2-sided 95% CI for the percentage of seroprotected individuals ⩾70%) and seroconversion (the lower limit of the 2-sided 95% CI for the percentage of seroconverted individuals ⩾40%) rates for each strain for adults aged 18– 64 years were used in assessing immunogenicity. Seroprotection was defined as an hemagglutination inhibition (HI) titer ⩾40. Seroconversion rate was defined as seroconversion (negative prevaccination serum [HI titer <10] and postvaccination HI titer ⩾40) or significant increase (⩾4-fold increase from nonnegative [⩾10] prevaccination HI titer).

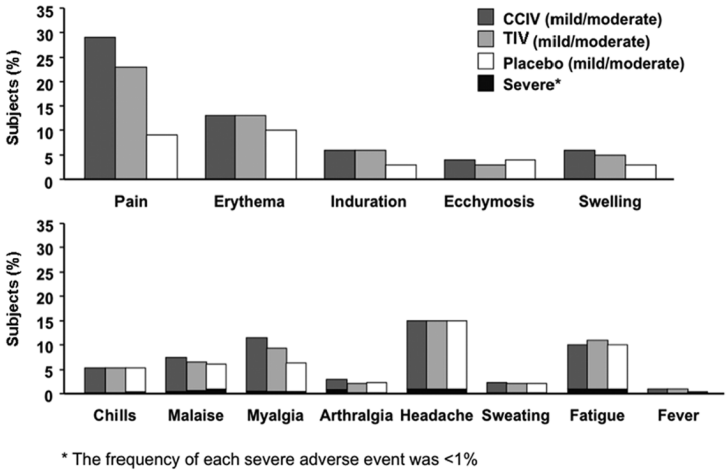

Figure 2. Solicited local and systemic reactions in the 7 days after vaccination. CCIV, cell culture–derived influenza vaccine; TIF, egg-derived trivalent inactivated vaccine.

rus was confirmed in 25% (CCIV), 22% (TIV), and 44% (placebo) of those influenza-like illness case samples.

A total of 231 influenza cases were caused by all circulating strains (ie, vaccine-like, non–vaccine-like, and untyped), including 140 of 3843 in the placebo group, 42 of 3776 in the CCIV group, and 49 of 3638 in the TIV group (vaccine efficacy of 69.5% [1-sided 97.5% CI lower limit, 55.0%] and 63.0% [1-sided 97.5% CI lower limit, 46.7%], respectively). The placebo attack rate was 3.64% (Table 2). For all circulating strains, the vaccine efficacies against the A/H1N1 strains were 89.3% (1-sided 97.5% CI lower limit, 73.0%) for the CCIV and 81.5% (1-sided 97.5% CI lower limit, 60.9%) for the TIV groups, with efficacies of 75.6% (1-sided 97.5% CI lower limit, 35.1%) and 49.3% (1-sided 97.5% CI lower limit, −9.0%) against A/H3N2 strains and 49.9% (1-sided 97.5% CI lower limit, 18.2%) and 53.2% (1-sided 97.5% CI lower limit, 22.2%) against B strains, respectively. B strains predominated among the nonvaccine strains, causing all but 1 of the 116 cases of confirmed B strain influenza.

Overall, 60 influenza cases were caused by vaccine-like strains, including 44 of 3843 in the placebo group, 7 of 3776 in the CCIV group, and 9 of 3638 in the TIV group (Table 2). The overall vaccine efficacy was 83.8% (1-sided 97.5% CI lower limit, 61.0%) for the CCIV and 78.4% (1-sided 97.5% CI lower limit, 52.1%) for the TIV group. An A/H1N1 virus was isolated from 56 of 60 cases, including 43 in the placebo group, 5 in the CCIV group (vaccine efficacy, 88.2%), and 8 in the TIV group (vaccine efficacy, 80.3%). Three cases were caused by vaccine-like H3N2 strains and only 1 by a vaccine-like B strain.

There were 134 culture-confirmed influenza cases caused by non–vaccine-like strains, including 74 of 3843 in the placebo group, 30 of 3776 in the CCIV group, and 29 of 3638 in the TIV group, giving vaccine efficacies of 58.7% (1-sided 97.5% CI lower limit, 33.5%) for the CCIV and 58.6% (1-sided 97.5% CI lower limit, 32.9%) for the TIV group (Table 2). Again, most of these (115 cases; 85.8%) were caused by B strain viruses.

The overall and anti-A/H1N1 vaccine efficacies of each vaccine were highly significant, compared with placebo, for all circulating and for vaccine-like strains, and the lower limits of the 1-sided 97.5% CIs for vaccine efficacy of the CCIV and TIV groups were well above the 40% predefined study objective and CBER efficacy criterion. Only 43 influenza cases were caused by A/H3N2, an attack rate of 0.65%, making reliable vaccine efficacy estimation difficult. The vaccine efficacy point estimate against influenza B was ~50% despite a vaccine mismatch, but the 97.5% CI lower limit was <40%.

Immunogenicity. Baseline seroprotection rates, seroconversion rates, and antihemagglutinin geometric mean titers did not differ among the study groups. Both vaccines were highly immunogenic, with postvaccination responses to type B slightly higher in the TIV group (Table 3). Seroprotection rates, seroconversion rates, and geometric mean ratios before to after vaccination for both vaccines and all 3 vaccine virus stains exceeded both European (Committee for Medicinal Products for Human Use) and United States (CBER) immunogenicity licensing criteria [16, 17].

Reactogenicity and safety. The percentage of study participants reporting solicited reactions was similar in each group. Most reactions occurred within 2 days after vaccination and were transient in duration. The most frequent local reaction was injection site pain, reported by 30%, 24%, and 10% of participants in the CCIV, TIV, and placebo groups, respectively. Erythema was reported by 13% of participants in each vaccine group and by 10% given placebo (Figure 2). The most common

solicited systemic reactions were headache (15% in all groups), fatigue (10% in the CCIV and placebo groups and 11% in the TIV group), myalgia (12%, 10%, and 7% in the CCIV, TIV, and placebo groups, respectively), and malaise (8%, 7%, and 6% in CCIV, TIV, and placebo groups, respectively) (Figure 2). Severe solicited reactions were reported in <1% of participants in any study group.

Possibly or probably related unsolicited adverse events were reported by 1%–2% of study participants on days 1–7 and by <1% of participants from days 8–23; none were reported on days 23–181. Three participants in the CCIV group and 1 each in the TIV and placebo groups withdrew after adverse events that were not considered to be related to vaccination. Four deaths occurred, 2 in the CCIV group and 1 each in the TIV and the placebo groups (all of which were judged unrelated to the study vaccines). The remaining 127 serious adverse events (42 participants in the CCIV group, 35 in the TIV group, and 38 in the placebo group) were determined by the investigator to be unrelated to the study vaccine. No individuals withdrew from the study because of a vaccine-related adverse event.

DISCUSSION

To our knowledge, this was the largest randomized, placebo-controlled trial yet conducted to evaluate the efficacy of inactivated influenza vaccines and the first to evaluate the efficacy of a vaccine produced using a continuous cell culture system. The study was designed to demonstrate the efficacy of CCIV and TIV separately against placebo, an approach taken to decrease the size of the required placebo group, thus reducing the number of participants potentially developing influenza. The total number of 785 (7%) of 11,257 participants who reported influenza-like illness symptoms was consistent with influenza surveillance data from Europe and the United States for the 2007–2008 influenza season [21, 22]. The vaccine efficacies of both CCIV and TIV against influenza caused by all circulating influenza strains and by vaccine-like strains were highly significant, compared with placebo, and both exceeded CBER vaccine efficacy criteria.

The vaccine efficacies of both study vaccines against vaccine-like influenza virus strains were consistent with results obtained in healthy adults with conventional TIVs, which have been the standard for many years. A recent meta-analysis of 48 randomized trials involving >66,000 healthy adults found that the overall vaccine efficacy was 80% (95% CI, 56%–91%) against influenza when the vaccine matched the circulating strain and circulation was high but decreased to 50% (95% CI, 27%–65%) when it did not match [23]. In this study, the vaccine-matched strains were nearly all A/H1N1. However, most influenza cases were caused by type B strains that were not vaccine-like, and the vaccine efficacy point estimate against these non-matching B strains was ~50%. This reduced the efficacy against all circulating strains. A large TIV trial conducted in Europe in the 2006–2007 influenza season using surveillance similar to ours found a comparable placebo attack rate of 3.2% and efficacy against culture-confirmed influenza of 66.9% (95% CI, 51.9%–77.4%), compared with placebo, against vaccine-like strains and 61.6% (95% CI, 46.0%–72.8%) against any circulating strain [24].

Both CCIV and TIV were well tolerated. Only mild-moderate injection site pain occurred more frequently in the CCIV group. The safety results were consistent with previous studies of this CCIV vaccine and with results observed in studies that compared another MDCK-propagated CCIV and TIV [11–15, 25–28].

Both CCIV and TIV met all CBER immunogenicity criteria for all 3 viral strains. The influenza B strain seroprotection rates, seroconversion rates, and geometric mean titers were lower for the CCIV than the TIV group, but that was not reflected by any differences in the B strain efficacy results. The immunogenicity results were in line with other trials of vaccines propagated in MDCK cells and egg-derived controls [11–15, 25–28].

Influenza virus propagation in cell culture offers potential benefits, compared with propagation in eggs. Most cell stocks, including MDCK cells, can be stored frozen, and the supply needed for vaccine production can be generated quickly [29]. Thus, the time between the choice of seasonal influenza virus strains and availability of vaccine might be shortened to allow a change in production and incorporate late emerging strains. Importantly, cell culture could assist in providing supplies of prepandemic and strain-specific pandemic vaccines more quickly than is possible with egg-based systems alone [30]. Cell culture avoids an egg allergy contraindication and, as a closed system, carries a reduced risk of microbial or chemical contamination. Finally, MDCK cells adapted to rapid growth in suspension media do not require microcarriers, enzyme treatment for subculture, or protein supplements to promote adhesion to a substrate [31].

This study has shown that CCIV (Optaflu) and TIV (Agippal), which have been in routine use in the European Union and other countries, both exceeded efficacy and immunogenicity criteria as defined by regulatory authorities. Both CCIV and TIV can be considered for use in annual influenza vaccination campaigns.

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References

1. World Health Organization. Avian influenza: assessing the pandemic threat. http://www.who.int/csr/disease/influenza/H5N1-9reduit.pdf. Accessed 9 November 2009.
2. Sprenger MJ, Mulder PG, Beyer WE, Van Strik R. Masurel N. Impact of influenza on mortality in relation to age and underlying disease, 1967–1989. Int J Epidemiol **1993**; 22:334–340.
3. World Health Organization. Cell culture as a substrate for the production of influenza vaccines: memorandum from a WHO meeting. Bull World Health Organ **1995**; 73:431–435.
4. Audsley JM, Tannock GA. The role of cell culture vaccines in the control of the next influenza pandemic. Expert Opin Biol Ther **2004**; 4:709–717.
5. Merten OW, Hannoun C, Manuguerra JC, Ventre F, Petres S. Production of influenza virus in cell cultures for vaccine production. Adv Exp Med Biol **1996**; 397:141–151.
6. Montagnon BJ, Vincent-Falquet JC. Experience with the Vero cell line. Dev Biol Stand **1998**; 93:119–123.
7. Barrett PN, Mundt W, Kistner O, Howard MK. Vero cell platform in vaccine production: moving towards cell culture-based viral vaccines. Expert Rev Vaccines **2009**; 8:607–618.
8. Keitel WA, Dekker CL, Mink C, et al. Safety and immunogenicity of inactivated, Vero cell culture-derived whole virus influenza A/H5N1 vaccine given alone or with aluminum hydroxide adjuvant in healthy adults. Vaccine **2009**; 27:6642–6648.
9. Koudstaal W, Hartgroves L, Havenga M, et al. Suitability of PER.C6 cells to generate epidemic and pandemic influenza vaccine strains by reverse genetics. Vaccine **2009**; 27:2588–2593.
10. EMEA. Optaflu European Public Assessment Report. 2007. http://www.emea.europa.eu/humandocs/PDFs/EPAR/optaflu/H-758-en6.pdf. Accessed 9 November 2009.
11. Doroshenko A, Halperin S. Trivalent MDCK cell culture-derived influenza vaccine Optaflu (Novartis Vaccines). Expert Rev Vaccines **2009**; 28: 679–688.
12. Groth N, Montomoli E, Gentile C, Manini I, Bugarini R, Podda A. Safety, tolerability and immunogenicity of a mammalian cell-culture-derived influenza vaccine: a sequential Phase I and Phase II clinical trial. Vaccine **2009**; 27:786–791.
13. Szymczakiewicz-Multanowska A, Groth N, Bugarini R, et al. Safety and immunogenicity of a novel influenza subunit vaccine produced in mammalian cell culture. J Infect Dis **2009**; 200:841–848.
14. Reisinger KS, Block SL, Izu A, Groth N, Holmes SJ. Subunit influenza vaccines produced from cell culture or in embryonated chicken eggs: comparison of safety, reactogenicity, and immunogenicity. J Infect Dis **2009**; 200:849–857.
15. Ambrozaitis A, Groth N, Bugarini R, Sparacio V, Podda A, Lattanzi M. A novel mammalian cell-culture technique for consistent production of a well-tolerated and immunogenic trivalent subunit influenza vaccine. Vaccine **2009**; 27:6022–6029.
16. European Agency for the Evaluation of Medicinal Products European Committee for Medicinal Products for Human Use: note for guidance on harmonization of requirements for influenza vaccines 1997 (CPMP/BWP/214/96). http://www.emea.europa.eu/pdfs/human/bwp/021496en.pdf. Accessed 6 October 2009.
17. US Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for industry: clinical data needed to support the licensure of seasonal inactivated influenza. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074794.htm. Accessed 21 November 2009.
18. Bell D, Nicoll A, Fukuda K, Horby P, Monto A. Nonpharmaceutical interventions for pandemic influenza, international measures. World Health Organization Writing Group. Emerg Infect Dis **2006**; 12:81–87.
19. Cox NJ, Ziegler T. Influenza viruses. In: Murray PR, Barron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds. Manual of clinical microbiology. 8th ed. Washington, DC: ASM Press, 2003:1360–1365.
20. Hierholzer JC, Suggs, MT, Hall EC. Standardized viral hemagglutination and hemagglutination inhibition tests, II: description and statistical evaluation. Appl Microbiol **1969**; 18:824–833.
21. Centers for Disease Control and Prevention. 2007–08 US Influenza Season Summary. http://www.cdc.gov/flu/weekly/weeklyarchives2007-2008/07-08summary.htm. Accessed 21 November 2009.
22. WHO/Europe influenza surveillance. EuroFlu Weekly Electronic Bulletin. Influenza season 2007–2008: http://www.euroflu.org/cgi-files/bulletin_v2.cgi?season = 2007&menu = y. Accessed 21 November 2009.
23. Demicheli V, Di Pietrantonj C, Jefferson T, Rivetti A, Rivetti D. Vaccines for preventing influenza in healthy adults. Cochrane Database Syst Rev **2007**; 2:CD001269.
24. Beran J, Vesikari T, Wertzova V, et al. Efficacy of inactivated split-virus influenza vaccine against culture-confirmed influenza in healthy adults: a prospective, randomized, placebo-controlled trial. J Infect Dis **2009**; 200:1861–1869.
25. Halperin SA, Smith B, Mabrouk T, et al. Safety and immunogenicity of a trivalent, inactivated, mammalian cell culture-derived influenza vaccine in healthy adults, seniors, and children. Vaccine **2002**; 20:1240–1247.
26. Palache AM, Brands R, Van Scharrenburg GJM. Immunogenicity and reactogenicity of influenza subunit vaccines produced in MDCK cells or fertilized chicken eggs. J Infect Dis **1997**; 176(Suppl 1):S20–S23.
27. Percheson PB, Trépanier P, Dugré R, Mabrouk T. A phase 1 randomized controlled clinical trial to study the reactogenicity and immunogenicity of a new split influenza vaccine derived from a non-tumorigenic cell line. Dev Biol Stand **1999**; 98:127–132.
28. Palache AM, Scheepers HS, de Regt V, et al. Safety, reactogenicity and immunogenicity of Madin Darby Canine Kidney cell-derived inactivated influenza subunit vaccine: a meta-analysis of clinical studies. Dev Biol Stand **1999**; 98:115–125.
29. World Health Organization. Initiative for Vaccine Research. Use of cell lines for the production of influenza virus vaccines: an appraisal of technical, manufacturing and regulatory considerations. Geneva, Switzerland, 10 April 2007. http://www.who.int/vaccine_research/diseases/influenza/WHO_flu_cell_substrate_version3.pdf. Accessed 20 November 2009.
30. Wright PF. Vaccine preparedness– are we ready for the next influenza pandemic? N Engl J Med **2008**; 358:2540–2543.
31. Doroshenko A, Halperin SA. Trivalent MDCK cell culture-derived influenza vaccine Optaflu (Novartis Vaccines). Expert Rev Vaccines **2009**; 8:679–688.