

Safety and Immunogenicity of a Novel Influenza Subunit Vaccine Produced in Mammalian Cell Culture

Agnieszka Szymczakiewicz-Multanowska,¹ Nicola Groth,² Roberto Bugarini,^{2a} Maria Lattanzi,² Daniela Casula,² Anne Hilbert,³ Theodore Tsai,⁴ and Audino Podda²

¹Center for Clinical Pharmacology Research, Monipol, Krakow, Poland; ²Global Clinical Research and Development, Novartis Vaccines and Diagnostics, Siena, Italy; ³Clinical Serology Department, Novartis Vaccines and Diagnostics, Marburg, Germany; ⁴Scientific Affairs, Novartis Vaccines and Diagnostics, Cambridge, Massachusetts

(See the article by Reisinger et al and the editorial commentary by Neuzil and Bright, on pages 849–57 and 835–7, respectively.)

Background. Immunization remains the best prevention strategy for influenza, but production constraints for egg-based influenza vaccines have prompted the development of innovative cell culture manufacturing processes. Here, we describe a novel cell culture–derived influenza vaccine (CCIV) produced in Madin-Darby canine kidney cells.

Methods. This phase 3, observer-blind, randomized, multicenter study in Poland compared the immunogenicity of a CCIV and a conventional egg-based vaccine. Participants, stratified by age (adults 18–60 years, $n = 1300$; elderly persons ≥ 61 years, $n = 1354$), received a single intramuscular vaccination. Immunogenicity was assessed 21 days later by hemagglutination inhibition assay. Reactogenicity was assessed using self-completed diary cards.

Results. The immunogenicity of CCIV was noninferior to that of the conventional vaccine for all 3 vaccine strains in both age groups, regardless of underlying health status. Both vaccines fulfilled European Union registration criteria and were well tolerated, with similar incidences of solicited local and systemic reactions in both age groups; the only significant difference was an increased frequency of mild or moderate pain with CCIV than the conventional vaccine among adult (22% vs 17%; $P < .05$) and elderly (9% vs 5%; $P < .001$) vaccinees.

Conclusions. CCIV was well tolerated and highly immunogenic in adults 18 years of age or older. Cell culture may offer greater flexibility of supply during periods of high demand for both seasonal and pandemic vaccines.

Trial registration. ClinicalTrials.gov identifier: NCT00492063.

Influenza results in considerable morbidity and mortality, especially in elderly persons and other at-risk populations [1, 2], making it a serious health concern worldwide for which vaccination remains the cornerstone of disease control. The imminent threat of pandemic influenza has led to the development of prepandemic influenza vaccines and “mock-ups” of pandemic vaccines, based on the conventional technologies used for current seasonal vaccines. However, increasing demand for seasonal vaccines and potentially for pan-

demic and prepandemic vaccines is threatening to render the supply generated by the current methods of vaccine production insufficient.

These conventional methods rely on propagation of the influenza viruses in embryonated chicken eggs, a methodology that has inherent limitations in capacity.

Potential conflicts of interest: N.G., M.L., D.C., A.H., T.T., and A.P. are employees of Novartis Vaccines and Diagnostics. R.B. was an employee of Novartis Vaccines and Diagnostics at the time of the study. A.S.-M. was the coordinating investigator of this study.

Presented in part: Options for the Control of Influenza VI, Toronto, 17–23 June 2007 (poster P716 and presentation at a symposium entitled “New Approaches to Influenza Vaccine Production: Meeting the Challenge”); Second International Conference on Influenza Vaccines for the World, Vienna, 18–20 October 2006 (poster 47 and presentation at a symposium entitled “A New Paradigm for Influenza Vaccines”); Tenth Annual Conference on Vaccine Research, Baltimore, 30 April–2 May 2007 (poster P56); 13th International Conferences on Infectious Diseases, Kuala Lumpur, 19–22 June 2008 (poster 19.038).

Financial support: Novartis Vaccines and Diagnostics (study sponsor).

^a Present affiliation: Genomic Health, Inc, Redwood City, California.

Received 18 December 2008; accepted 1 April 2009; electronically published 12 August 2009.

Reprints or correspondence: Dr Nicola Groth, Via Fiorentina 1, 53100 Siena, Italy (nicola.groth@novartis.com).

The Journal of Infectious Diseases 2009;200:841–8

© 2009 by the Infectious Diseases Society of America. All rights reserved.

0022-1899/2009/20006-0003\$15.00

DOI: 10.1093/infdis/jin105

Each egg yields only 1 dose of vaccine antigen; production and purification procedures are laborious and require large numbers of fertilized eggs that must be ordered and prepared in advance; and virus strains must be adapted to grow in eggs, a time-consuming process that can result in alterations in antigens that lead to suboptimal immunogenicity [3–5]. Furthermore, the majority of currently circulating human A/H3N2 strains cannot be grown in eggs [6], which can restrict strain selection.

Recent developments in vaccine-production technology have used cell culture for virus propagation rather than embryonated chicken eggs. Cell culture technology offers a reliable and flexible production process because of the ready availability of raw material [7] and can be performed using closed aseptic techniques [8]. In addition, growth of a broad range of authentic virus strains is possible in cell culture without adaptation [5, 9]. Additional advantages are the suitability of cell culture–derived vaccines for persons with egg allergies and their greater acceptability for use in infants, given that they can be produced without using preservatives, antibiotics, or stabilizers [10].

One of the cell substrates investigated for influenza vaccine production is Madin-Darby canine kidney (MDCK) cells [11–13]. MDCK cells have been used for the isolation of a wide variety of human influenza viruses [14], resulting in high-yield virus propagation with good replication accuracy [15]. In a phase 1–2 trial, a novel MDCK cell–derived subunit influenza vaccine was shown to have an immune response and safety profile similar to that of a conventional egg-based vaccine [16]. Here, we report the results of a large phase 3 trial designed to evaluate the safety and noninferior immunogenicity of the cell culture–derived influenza vaccine (CCIV) compared with an egg-based vaccine in adult and elderly populations.

METHODS

Study population. This was a phase 3, randomized, observer-blind study conducted at 5 centers in Poland during the 2004–2005 Northern Hemisphere influenza season. Adult (18–60 years of age) and elderly (≥ 61 years of age) volunteers, in good health for their age, were enrolled. Those with a history of hypersensitivity to study vaccine components, impaired or altered immune function, laboratory-confirmed influenza, or vaccination against influenza during the 6 months before enrollment were excluded. Written informed consent was obtained from all participants, and the study was conducted under a protocol reviewed and approved by an institutional review board.

Study vaccines. The 2 subunit influenza vaccines used in the study were produced by Novartis Vaccines and Diagnostics: the test CCIV was produced in MDCK cells, and the licensed influenza control vaccine (Agrimppal) was conventionally produced in eggs. Seed viruses were egg adapted by the UK National Institute for Biological Standards and Control. Each 0.5-

mL dose of vaccine contained 15 μ g of viral hemagglutinin (HA) for each of the 3 recommended virus strains for the 2004–2005 Northern Hemisphere influenza season: A/New Caledonia/20/99(H1N1)-like, A/Fujian/411/2002(H3N2)-like, and B/Shanghai/361/2002(B)-like [17].

By means of randomization lists provided by designated study personnel, participants were randomly assigned (1:1) to receive 1 dose of either CCIV or control vaccine by intramuscular injection. The study was conducted in a blinded fashion, with neither the participant nor the investigation site personnel aware of the identity of the administered vaccine. Blood samples (~ 10 mL) for immunogenicity measurements were collected immediately before vaccination (day 1) and after 3 weeks (day 22). Safety assessment was performed up to 6 months after vaccination, as described below.

Safety assessment. Each participant maintained a daily diary card for 22 days after vaccination. For the first 7 days, participants were asked to record occurrences of solicited local and systemic reactions, other indicators of reactogenicity (ie, axillary temperature, the use of analgesic or antipyretic medication, the impact of vaccination on daily activities), and any other adverse events (AEs). All AEs were recorded up to 3 weeks after vaccination, and all serious AEs or those AEs resulting in premature withdrawal from the study were recorded up to 6 months after vaccination.

Immunogenicity assessment. Serum levels of antibodies against the egg-derived A/H1N1, A/H3N2, and B influenza virus strains contained in the vaccines were measured using the hemagglutination inhibition (HI) assay, as described elsewhere [18]. Immunogenicity was evaluated using the European Union Committee for Medicinal Products for Human Use (CHMP) criteria: for seroprotection, an HI titer ≥ 40 in $>70\%$ of adult and $>60\%$ of elderly participants; for geometric mean ratio (GMR), a postvaccination increase of >2.5 in adult and >2.0 in elderly participants; and for seroconversion, a change in HI titer from <10 to ≥ 40 or a ≥ 4 -fold increase in HI titer in $>40\%$ of adult and $>30\%$ of elderly participants.

Statistical analysis. The primary objective of the study was to evaluate the immunogenicity of the CCIV compared with that of the control vaccine according to the CHMP criteria. The secondary objective was to demonstrate the noninferiority of CCIV compared with control vaccine for correlates of protection. Analyses were performed using SAS software (version 8.2; SAS Institute), on the basis of a predefined analysis plan developed by Novartis Vaccines and Diagnostics.

To test the null hypothesis with 80% power, a total of 2650 participants were required, with at least 583 adult and 605 elderly participants in each vaccine group. Criteria for determining the noninferiority of the CCIV compared with the control vaccine were as follows for all 3 antigens: (1) the lower limit of the 95% confidence interval (CI) for the difference

Table 1. Immunogenicity of the Cell Culture–Derived Influenza Vaccine (CCIV) and the Conventional Egg-Based Vaccine (Control) among Adult and Elderly Participants

Strain	Adult participants		Elderly participants	
	CCIV (n = 650)	Control (n = 644)	CCIV (n = 672)	Control (n = 674)
H3N2				
Before vaccination	46 (42–51)	43 (39–48)	47 (42–52)	41 (37–45)
After vaccination	278 (258–300)	307 (285–332)	337 (309–368)	341 (313–373)
GMR	6.0 (5.4–6.7)	7.1 (6.3–7.9)	7.3 (6.5–8.1)	8.4 (7.5–9.4)
H1N1				
Before vaccination	16 (15–18)	16 (16–19)	16 (15–18)	16 (15–18)
After vaccination	185 (167–205)	186 (167–206)	95 (86–104)	95 (87–105)
GMR	11 (10–13)	11 (9.3–12)	5.7 (5.2–6.4)	6.0 (5.45–6.7)
B				
Before vaccination	10 (9.6–11)	11 (10–12)	13 (12–14)	13 (12–14)
After vaccination	139 (127–152)	131 (119–143)	159 (145–174)	120 (109–131)
GMR	13 (12–15)	12 (11–13)	12 (11–13)	9.3 (8.4–10)

NOTE. Data are geometric mean titers of antibodies to the 3 vaccine antigens before and 3 weeks after vaccination, along with geometric mean ratios of the responses (after to before). Values in parentheses are 95% confidence intervals. Adult participants were 18–60 years of age, and elderly participants were ≥61 years of age.

between the CCIV and control groups in the percentage of subjects achieving seroprotection was more than –10%; (2) the lower limit of the 95% CI for the difference between the CCIV and control groups in the percentage of subjects achieving seroconversion was more than –10%; and (3) the lower limit of the 95% CI for the day 22 GMR (CCIV to control vaccine) was >0.5.

Demographic information, safety, and tolerability were analyzed using descriptive statistics. For safety, significant differences ($P < .05$) between vaccine groups were determined using the Pearson χ^2 test. Demographic information was analyzed using all randomized subjects; safety and tolerability were evaluated for all subjects with at least 1 vaccination and some postbaseline safety data. Immunogenicity was analyzed for subjects who received the vaccination correctly, provided evaluable data before and after vaccination, and had no major protocol violations (per-protocol population).

Role of the funding source. As the study sponsor, Novartis Vaccines and Diagnostics designed the study in collaboration with the investigators and wrote the study protocol but had no role in on-site data collection.

RESULTS

Participant characteristics. A total of 2654 participants were enrolled, and >99% completed the study and were included in the per-protocol population analysis—1294 (99.5%) of the 1300 enrolled adult participants and 1346 (99.4%) of the 1354 enrolled elderly participants. Withdrawals were evenly balanced between the 2 vaccine groups, and none were for safety reasons. Medical histories and patient demographics were similar in the 2 vaccine groups, including age (mean $\pm$ SD, 38.7 ± 12.7 and

38.7 ± 12.7 years among adult participants and 69.1 ± 5.7 and 68.8 ± 5.6 years among elderly participants in the CCIV and control groups, respectively), the proportion of male participants (42% and 43% among adult participants and 42% and 43% among elderly participants in the CCIV and control groups, respectively), and the proportion previously vaccinated against influenza (38% and 42% among adult participants in the CCIV and control groups, respectively, and 59% among elderly participants in both the CCIV and the control group).

Immunogenicity. Serologic parameters before vaccination were comparable in the CCIV and control groups. The percentages of subjects who did not have seroprotective levels of antibody before vaccination were similar between vaccine groups among adult participants (29% and 33% for A/H1N1, 65% and 63% for A/H3N2, and 16% and 18% for B in the CCIV and control groups, respectively) and elderly participants (30% and 31% for A/H1N1, 66% and 59% for A/H3N2, and 23% and 20% for B in the CCIV and control groups, respectively). Strong immune responses were obtained in all 4 groups and were similar for both vaccines for each strain in each age range (table 1). Responses were also similar across the age groups, except for a lower response to H1N1 among elderly participants compared with adults 18–60 years of age (figure 1).

The immunological noninferiority of the CCIV to the control vaccine was demonstrated in both age groups for all 3 CHMP immunogenicity criteria for all 3 virus strains (figure 2). In both age cohorts, both vaccines elicited seroprotection rates that surpassed CHMP criteria for all 3 influenza strains (among adult participants, 90%–99% for CCIV and 91%–99% for control vaccine; among elderly participants, 85%–97% for CCIV and 85%–98% for control vaccine) (figure 1A). Similarly, GMRs

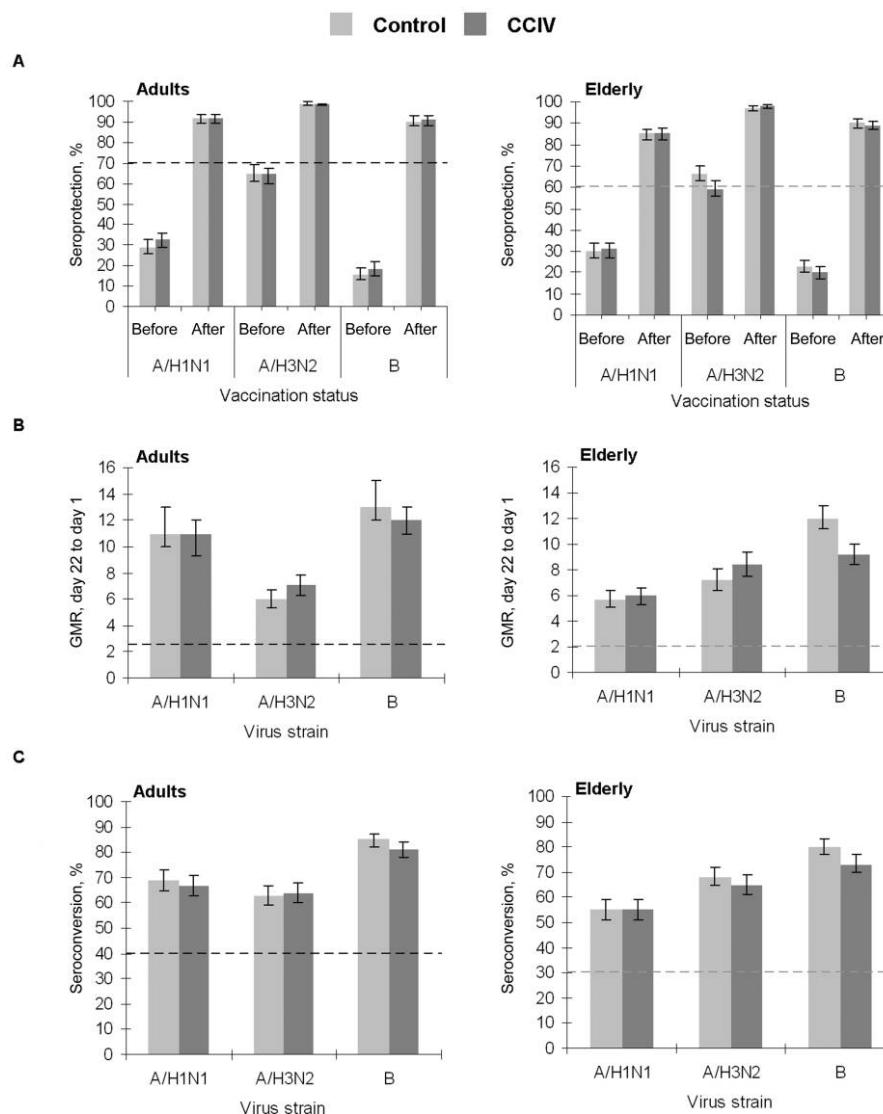


Figure 1. Assessment of seroprotection rates (A), geometric mean ratios (GMRs) of the hemagglutination inhibition (HI) titer on day 22 after vaccination to that before vaccination (B), and seroconversion rates (C) for cell culture-derived influenza vaccine (CCIV) and conventional egg-based vaccine (control) against egg-derived viral antigens in adult (18–60 years old; $n = 1294$) and elderly (≥ 61 years old, $n = 1346$) participants in the per-protocol population. Error bars represent 95% confidence intervals. Dashed lines indicate the European Union Committee for Medicinal Products for Human Use criteria for nonelderly adults (black) and elderly individuals (gray). Seroprotection was defined as an HI titer ≥ 40 , and seroconversion was defined as an HI titer < 10 before vaccination and ≥ 40 after vaccination or an HI titer ≥ 10 before vaccination and a ≥ 4 -fold increase after vaccination.

and seroconversion rates among both adult and elderly participants exceeded CHMP requirements for all 3 strains (figure 1B and 1C). Among the subset of subjects who were initially not seroprotected, all CHMP requirements were met for all 3 strains for both vaccines in both age groups.

A large proportion of the elderly participants and a smaller proportion of the adult participants had histories of chronic medical conditions. Therefore, an additional analysis was performed in a subset of this population (for elderly participants, $n = 341$ for CCIV and $n = 317$ for control vaccine; for adult participants, $n = 54$ for CCIV and $n = 67$ for control vaccine)

who had a history of at least 2 diseases from the following categories: circulatory system, endocrine and immune system, nutrition and metabolism, respiratory system, digestive system, genitourinary system, infection, and parasites. All 3 CHMP criteria were met for all 3 strains in both vaccine groups for these participants with an unfavorable medical background who would be considered a priority group for immunization by current recommendations.

Safety and tolerability. Both vaccines were generally safe and well tolerated, with similar reactogenicity profiles. Solicited local or systemic reactions were experienced by 40% of adult

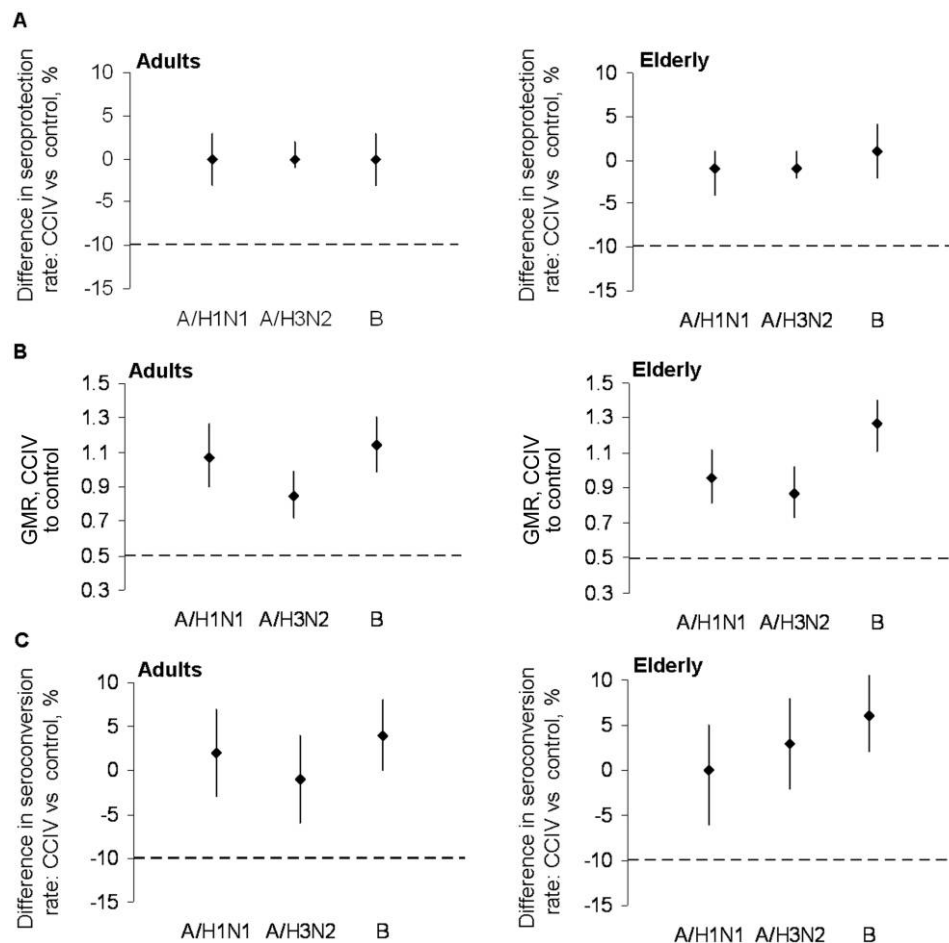


Figure 2. Assessment of the noninferiority of cell culture–derived influenza vaccine (CCIV) compared with conventional egg-based influenza vaccine in adult (18–60 years old; $n = 1294$) and elderly (≥ 61 years old; $n = 1346$) participants, on the basis of seroprotection rates (A), geometric mean ratios (GMRs) of the hemagglutination inhibition (HI) titer on day 22 in the CCIV group to that in the control group (B), and seroconversion rates (C). Dashed lines indicate the values above which noninferiority (lower confidence interval, more than -10%) was concluded. Seroprotection was defined as an HI titer ≥ 40 , and seroconversion was defined as an HI titer < 10 before vaccination and ≥ 40 after vaccination or an HI titer ≥ 10 before vaccination and a ≥ 4 -fold increase after vaccination. Vertical lines represent 95% confidence intervals.

participants and 33% of elderly participants. Among adult participants, local reactions (32% for CCIV vs 31% for control vaccine) were more frequent than systemic reactions (22% for CCIV vs 23% for control vaccine), whereas among elderly participants the incidences of local and systemic reactions were similar.

Incidences of individual solicited local and systemic reactions, together with other indicators of reactogenicity, are shown in table 2. The only significant difference between the CCIV and control groups was a higher frequency of injection site pain reported by adult (22% and 17%, respectively; $P < .05$) and elderly (9% and 5%, respectively; $P < .001$) CCIV recipients. However, $>99\%$ of pain reports were graded as mild or moderate and the pain usually resolved within 48 h of vaccination, so this difference was not considered to be clinically meaningful. The most frequent systemic reactions were head-

ache, fatigue, and malaise, which occurred in 10% to 12% of all participants. Fewer than 1% of vaccinees reported fever, all of which were mild to moderate ($\geq 38^\circ\text{C}$), with no reports of severe fever ($\geq 40^\circ\text{C}$). Analgesic or antipyretic use was low and similar in both vaccine groups, and there were no differences in the numbers who reported disruption of their usual activity (staying at home) because of vaccination.

During the study period, reported unsolicited AEs were mostly mild or moderate in severity, with no difference reported between vaccine groups (13%–15% among all groups). AEs considered to be possibly or probably related to the vaccine were reported in 2% of the CCIV group and in 4% of the control group among adult participants and in 2% of the CCIV and control groups among elderly participants. These AEs were generally mild influenza-like symptoms and were known adverse effects of influenza vaccination. None of the serious AEs,

Table 2. Reactogenicity of the Cell Culture–Derived Influenza Vaccine (CCIV) and the Conventional Egg-Based Vaccine (Control) among Adult and Elderly Participants

Type of reaction	Adults participants			Elderly participants		
	CCIV (n = 652)	Control (n = 648)	P ^a	CCIV (n = 678)	Control (n = 676)	P ^a
Local reactions						
Ecchymosis	18 (3)	22 (3)	.51	26 (4)	25 (4)	.89
Erythema	92 (14)	106 (16)	.26	72 (11)	72 (11)	.99
Induration	38 (6)	42 (6)	.62	37 (5)	29 (4)	.32
Swelling	25 (4)	27 (4)	.76	23 (3)	17 (3)	.34
Pain	141 (22)	111 (17)	.04 ^b	64 (9)	32 (5)	.001 ^c
Systemic reactions						
Chills	25 (4)	29 (4)	.56	23 (3)	26 (4)	.65
Malaise	74 (11)	74 (11)	.97	70 (10)	75 (11)	.65
Myalgia	45 (7)	49 (8)	.65	46 (7)	57 (8)	.25
Arthralgia	31 (5)	27 (4)	.61	41 (6)	44 (7)	.73
Headache	81 (12)	79 (12)	.90	69 (10)	70 (10)	.91
Sweating	28 (4)	27 (4)	.91	44 (6)	48 (7)	.66
Fatigue	73 (11)	73 (11)	.97	73 (11)	84 (12)	.34
Fever	2 (<1)	5 (1)	.29	5 (1)	5 (1)	1.00
Other reactions						
Stayed home because of reaction	14 (2)	16 (2)	.69	19 (3)	14 (2)	.38
Analgesic/antipyretic used	44 (7)	40 (6)	.67	34 (5)	29 (4)	.53

NOTE. Data are no. (%) of participants. Adult participants were 18–60 years of age, and elderly participants were ≥61 years of age.

^a Pearson χ^2 test for vaccine group differences.

^b $P < .05$.

^c $P < .001$.

which occurred in 1% of adult and 3% of elderly participants, were judged to be related to the vaccines. Three deaths occurred, all in elderly subjects (1 in the CCIV group and 2 in the control group); however, these were unrelated to the study vaccines and were most likely due to underlying comorbidities.

DISCUSSION

Constraints on vaccine-manufacturing capacity is a major concern against the background of an impending influenza pandemic and increased demand for seasonal influenza vaccines. The established technology using embryonated chicken eggs is slow, laborious, and limited in volume. Indeed, it has inherent drawbacks in strain selection, because not all viruses can be cultivated in this way, as was illustrated in 2003 when a poorly matching A/Panama/2007/99(H3N2) vaccine had to be used because the predominant circulating strain, A/Fujian/41/2002(H3N2), could not be isolated for growth in eggs until the year after [19]. One approach to overcoming these shortcomings and avoiding the reliance on eggs is to cultivate vaccine viruses in cell culture, which can then be scaled up in times of high demand; however, this technology must first be confirmed. In the present phase 3 study, the largest clinical trial undertaken with a CCIV to date, we compared a novel CCIV with an established egg-based control vaccine.

The statistical objective of the study was achieved, as we

demonstrated that both vaccines surpassed the regulatory requirements for immunogenicity of seasonal influenza vaccines; the actual immune responses (assessed as geometric mean titers) and the statistical comparison of the CHMP criteria demonstrated that the CCIV was noninferior to the conventional egg-based control vaccine. This was true not only in healthy adult and elderly populations but also among those with no baseline seroprotection or with defined comorbidities. Accepting the limitation of sample size within these subsets, there was no evidence that the immune response to either of the vaccines differed from that in the overall population, and inclusion of these subjects did not affect attainment of CHMP criteria or demonstration of noninferiority.

There were no clinically significant differences in the tolerability or safety profiles of the 2 vaccines—the only statistically significant difference being more frequent reports of transient, mild, or moderate injection site pain with the novel vaccine. However, the overall frequency was low—affecting ~15% and ~11% of all CCIV and control vaccine recipients, respectively—and the pain did not interfere with normal day-to-day activities of the recipients. The overall rates were comparable to the prevailing frequency in a number of studies with other influenza vaccines [20–23].

Our study is the first conducted in a large population to confirm the safety and immunogenicity of CCIV in both nonel-

derly and elderly adults. Smaller preliminary studies have demonstrated the similar immunogenicity of CCIVs and conventional egg-based vaccines in small populations typical of phase 1 and 2 trials [13, 16]. The CCIV evaluated in the present study was well tolerated and immunogenic in a recent phase 2 trial conducted in healthy adults <50 years of age [24] and in a previous phase 1–2 trial conducted in adults and elderly persons [16]. Cell lines other than MDCK, such as African monkey kidney (Vero) cells [25], have been investigated as cell substrates for influenza vaccine production. In a small trial, an experimental trivalent influenza virus HA vaccine produced using Vero cells was well tolerated and immunogenic in healthy adults ($n = 154$) [26]. In addition to vaccines produced using mammalian cell culture, a novel vaccine was recently produced in insect cells using recombinant baculoviruses [27].

MDCK cells are more permissive than chicken eggs for the propagation of human influenza viruses. Human influenza viruses bind to Sia- α 2-6gal receptors, which predominate on nonciliated respiratory epithelial cells, in contrast to the viral receptor on the egg allantoic membrane, which is a Sia- α 2-3gal disaccharide [28]. Inoculation of clinical samples into eggs selects for viral variants that bind to Sia- α 2-3gal receptors and, because the receptor binding site of the viral HA contains important antigenic domains, attendant changes in receptor-binding specificity can also result in significant antigenic changes in the HA [29]. MDCK cells, however, express both Sia- α 2-6gal and Sia- α 2-3gal receptors, minimizing the host cell selection of variants. In fact, inoculation of clinical isolates into MDCK cells results in viruses with HA that is structurally identical to that of the original isolate [5, 9, 30]. Therefore, the MDCK cell line could be used not only to produce influenza vaccines but also to isolate and select the seed strain to be used for vaccine production, providing a closer antigenic match to the circulating wild-type strains than egg-derived seed strains. CCIV derived from MDCK seed strains may therefore be expected to have a more authentic match to circulating wild-type viruses and, in theory, better efficacy than conventional egg-based vaccines. To date, this has been demonstrated only in animal models [5, 9].

The present phase 3 study, which involved >2650 adult and elderly participants, has demonstrated that the immune response to a cell culture–derived subunit influenza vaccine is noninferior to the immune response to a conventional egg-based influenza vaccine. Both vaccines elicited immune responses that surpassed the European requirements for the licensure of influenza vaccine without compromising safety or tolerability. Thus, it may be feasible to supplement current egg-based influenza vaccines with a CCIV produced by contemporary biotechnological processes. Indeed, in some patient groups the new vaccines may be preferable, to prevent reactions to the components of egg-based vaccines. Because CCIVs are

not dependent on eggs, they have the potential to propagate a wider selection of virus strains and offer benefits in terms of viral authenticity, supply reliability, and flexibility.

Acknowledgments

Editorial assistance was provided by Dr Pamela Obuchowski (Health Interactions, Atlanta, Georgia).

References

1. World Health Organization (WHO). Influenza vaccines. *Wkly Epidemiol Rec* **2005**; 80:277–88.
2. Fiore AE, Shay DK, Broder K. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. *MMWR Recomm Rep* **2008**; 57:1–60.
3. Roberston JS. Clinical influenza virus and the embryonated hen's egg. *Rev Med Virol* **1993**; 3:97–106.
4. Widjaja L, Ilyushina N, Webster RG, Webby RJ. Molecular changes associated with adaptation of human influenza A virus in embryonated chicken eggs. *Virology* **2006**; 350:137–45.
5. Harmon MW, Rota PA, Walls HH, Kendal AP. Antibody response in humans to influenza virus type B host-cell-derived variants after vaccination with standard (egg-derived) vaccine or natural infection. *J Clin Microbiol* **1988**; 26:333–7.
6. Lu B, Zhou H, Chan W, Kemble G, Jin H. Single amino acid substitutions in the hemagglutinin of influenza A/Singapore/21/04 (H3N2) increase virus growth in embryonated chicken eggs. *Vaccine* **2006**; 24: 6691–3.
7. Audsley JM, Tannock GA. The role of cell culture vaccines in the control of the next influenza pandemic. *Expert Opin Biol Ther* **2004**; 4:709–17.
8. Mabrouk T, Ellis RW. Influenza vaccine technologies and the use of the cell-culture process (cell-culture influenza vaccine). *Dev Biol (Basel)* **2002**; 110:125–34.
9. Ito T, Suzuki Y, Takada A, et al. Differences in sialic acid-galactose linkages in the chicken egg amnion and allantois influence human influenza virus receptor specificity and variant selection. *J Virol* **1997**; 71: 3357–62.
10. Çinkaya I, Gregersen JP, Hilbert AK. Novel process for a MDCK suspension cell culture derived influenza subunit vaccine [poster]. Presented at the 11th International Congress on Infectious Diseases (Cancun, Mexico) **2004**.
11. 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**; 98:115–25; discussion 133–4.
12. Tree JA, Richardson C, Fooks AR, Clegg JC, Looby D. Comparison of large-scale mammalian cell culture systems with egg culture for the production of influenza virus A vaccine strains. *Vaccine* **2001**; 19:3444–50.
13. 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**; 20:1240–7.
14. Tobita K. Permanent canine kidney (MDCK) cells for isolation and plaque assay of influenza B viruses. *Med Microbiol Immunol* **1975**; 162:23–7.
15. Trusheim H, Roth B, Wilms R. The MDCK 33016-PF cell-line is not only suitable for the production of cell-based influenza vaccine but is also an ideal substrate for influenza virus isolation [poster]. Presented at Options for the Control of Influenza VI (Toronto). **2007**.
16. 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**; 27:786–91.

17. Recommended composition of influenza virus vaccines for use in the 2004–2005 influenza season. *Wkly Epidemiol Rec* **2004**; 79:88–92.
18. Stephenson I, Wood JM, Nicholson KG, Charlett A, Zambon MC. Detection of anti-H5 responses in human sera by HI using horse erythrocytes following MF59-adjuvanted influenza A/Duck/Singapore/97 vaccine. *Virus Res* **2004**; 103:91–5.
19. Centers for Disease Control and Prevention. Assessment of the effectiveness of the 2003–04 influenza vaccine among children and adults—Colorado, 2003. *MMWR Morb Mortal Wkly Rep* **2004**; 53:707–10.
20. De Donato S, Granoff D, Minutello M, et al. Safety and immunogenicity of MF59-adjuvanted influenza vaccine in the elderly. *Vaccine* **1999**; 17:3094–101.
21. Frey S, Poland G, Percell S, Podda A. Comparison of the safety, tolerability, and immunogenicity of a MF59-adjuvanted influenza vaccine and a non-adjuvanted influenza vaccine in non-elderly adults. *Vaccine* **2003**; 21:4234–7.
22. Cooper CL, Davis HL, Morris ML, et al. Safety and immunogenicity of CPG 7909 injection as an adjuvant to Fluarix influenza vaccine. *Vaccine* **2004**; 22:3136–43.
23. Delore V, Salamand C, Marsh G, Arnoux S, Pepin S, Saliou P. Long-term clinical trial safety experience with the inactivated split influenza vaccine, Vaxigrip. *Vaccine* **2006**; 24:1586–92.
24. Reisinger KS, Block SL, Izu A, Groth N, Holmes S. Subunit influenza vaccines produced from cell culture or in embryonated chicken eggs: comparison of safety, reactogenicity, and immunogenicity. *J Infect Dis* **2009**; 200:849–57 (in this issue).
25. Kistner O, Barrett PN, Mundt W, Reiter M, Schober-Bendixen S, Dörner F. Development of a mammalian cell (Vero) derived candidate influenza virus vaccine. *Vaccine* **1998**; 16:960–8.
26. Govorkova EA, Kodihalli S, Alymova IV, Fanget B, Webster RG. Growth and immunogenicity of influenza viruses cultivated in Vero or MDCK cells and in embryonated chicken eggs. *Dev Biol Stand* **1999**; 98:39–51; discussion 73–4.
27. Treanor JJ, Schiff GM, Hayden FG, et al. Safety and immunogenicity of a baculovirus-expressed hemagglutinin influenza vaccine: a randomized controlled trial. *JAMA* **2007**; 297:1577–82.
28. Zeiger RS. Current issues with influenza vaccination in egg allergy. *J Allergy Clin Immunol* **2002**; 110:834–40.
29. Robertson JS, Cook P, Attwell AM, Williams SP. Replicative advantage in tissue culture of egg-adapted influenza virus over tissue-culture derived virus: implications for vaccine manufacture. *Vaccine* **1995**; 13: 1583–8.
30. Katz JM, Wang M, Webster RG. Direct sequencing of the HA gene of influenza (H3N2) virus in original clinical samples reveals sequence identity with mammalian cell-grown virus. *J Virol* **1990**; 64:1808–11.