EXPERT REVIEWS

Flucelvax (Optaflu) for seasonal influenza

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Conventional egg-based manufacturing technology for seasonal influenza vaccines has several drawbacks, including its inflexibility, reliance on egg supplies, risk of contamination, absence of growth of some isolates and egg-adaptive viral mutations that threaten vaccine matching. To overcome these limitations, cell culture-derived vaccines have been designed, including the trivalent inactivated vaccine Flucelvax®/Optaflu® (brand names in the US/EU, respectively). Flucelvax/Optaflu has gained wide regulatory approval and is currently implemented in several countries. Non-clinical studies have assuaged hypothetical concerns regarding oncogenicity and use in persons allergic to dogs. Ample clinical data suggest the non-inferiority of Flucelvax/Optaflu to egg-based vaccines in terms of immunogenicity, safety and tolerability, and it has fulfilled American and European mandatory requirements. Although Flucelvax/Optaflu is currently indicated only for adults and the elderly, pediatric data indicate its good immunogenicity and safety. This paper provides an update on the clinical development of Flucelvax/Optaflu, its seasonal trials and available post-marketing surveillance data.

Keywords: Flucelvax • influenza vaccine • MDCK cell culture • Optaflu • vaccination

Seasonal influenza has a great impact on society, causing approximately half a million deaths each year worldwide [1]. In the USA, the annual burden of seasonal flu averages 610,660 life-years lost, 3.1 million days of hospitalization and more than 31 million outpatient visits [2]. The economic burden of the disease is also high; in Italy alone, the average cost of a seasonal epidemic exceeds €1 billion [3]. Influenza morbidity is highly age-dependent; in a typical year, the flu attack rate has been estimated to be 5–10% in adults and 20–30% in children [4]. A disproportionately high burden of seasonal influenza is placed on people at particularly high risk of developing severe disease and its complications (including young children under 5 years old, the elderly, and subjects with underlying medical conditions) and individuals at high risk of exposure to the virus, such as healthcare professionals [4].

It is well established that vaccination is the most effective single public health intervention able to dramatically reduce the impact of seasonal influenza [4,5]. Nevertheless, across the globe, policies on the implementation of seasonal influenza vaccination programs vary at national and supranational levels. In 2010, the US Advisory Committee on Immunization Practices recommended universal influenza vaccination for everyone over 6 months of age [6]. Conversely, in the EU, there are some differences among member states regarding the definition of individuals at high risk [7], and hence recommendations. In any case, the EU Council recommends that 75% vaccination coverage be reached as soon as possible in the elderly and high-risk groups with chronic conditions [8]. The latest WHO position paper [4] provides explicit recommendations on priority groups for influenza vaccination; these include children between 6 months and 5 years of age, the over-65s, persons with specific chronic conditions, pregnant women and healthcare workers.

Nowadays, the seasonal influenza vaccine market is more competitive than ever before, and several products are available in the US and the EU in the 2014–2015 influenza season [9,10]. Most of these preparations are inactivated and trivalent, that is, they contain three different viruses: two subtypes belonging to the A type (H1N1 and H3N2) and one belonging the B type. Trivalent-inactivated vaccines may be either split virus or subunit, unadjuvanted or adjuvanted. Most of the previously available inactivated whole-virus vaccines have been replaced by split or subunit vaccines as less reactogenic alternatives [4].

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More recently, inactivated quadrivalent vaccines containing both Victoria and Yamagata lineages of B virus have been marketed [11]. Another important public health tool is live attenuated influenza vaccines; first developed half a century ago, these were licensed in 2003 in the US and in 2011 the EU [12,13]. All the above-mentioned vaccine types have a common feature, that is, the egg-based technology used during their production. Each year, vaccine manufacturers order millions of high-quality fertilized hens' eggs well in advance of production (up to 12 months). The growth conditions of virus strains in eggs must then be adapted and optimized, a step that may delay delivery of the final product [14]. This may lead to vaccine shortages in the face of the increasing use of seasonal influenza vaccines worldwide [15]. Indeed, this increased demand puts pressure on vaccine supply [16]. The scant flexibility of egg-based manufacturing technology has prompted manufacturers to explore alternative production techniques. Several substrates for vaccine production have been investigated over the past few years [17]. Among the various seasonal vaccine candidates, the first to be widely used was the cell culture-derived influenza vaccine (CCIV) commercialized as Optaflu® in the EU [18] and Flucelvax® in the US [19]. Egg-independent technology for the production of influenza vaccines may be regarded as the first major innovation in recent decades. It is now appropriate to critically appraise issues surrounding the successful development and adoption of Optaflu/Flucelvax (henceforth referred to as CCIV). This paper reports the latest results of the clinical development program of CCIV and of the most relevant non-clinical studies.

Advantages of licensed cell culture-derived vaccines & an overview of their market

Among the most pressing needs of the influenza vaccine market are improved immunogenicity and more flexible and faster production processes [20,21]. The use of cell culture-based technology may, at least in part, meet these needs. Indeed, cell-based vaccine production offers several advantages over traditional egg-based production. First, it reduces reliance on egg supplies and thus increases flexibility, given that the raw material is readily available [16] and may be stored frozen [22]. Second, in the event of outbreaks of avian influenza in poultry, which frequently occur in various continents [23], the readily available supply of fertilized eggs may be insufficient. Third, greater control during the standardized manufacturing process and sterility of the cell culture medium and raw material reduce the risk of microbial contamination of the final product [24,25]. Fourth, cell cultures theoretically allow the growth of all influenza viruses, while recent data have suggested that most (over 90%) human isolates belonging to H3N2 are not recoverable in eggs [26,27]. Fifth, the process of serial passages in eggs may introduce important adaptive mutations, thereby altering matching and vaccine effectiveness [28]. By contrast, propagation of the virus in cell lines does not lead to major changes in the amino acid sequence of hemagglutinin (HA) [26]. Sixth, this approach overcomes the well-known contraindication (or at least precaution) of allergy to egg proteins. Indeed, conventional egg-based vaccines contain detectable amounts of some egg proteins; a risk of severe adverse allergic events following influenza immunization among egg-allergic vaccinees is well-documented [29]. Egg allergy is the commonest food allergy, especially in young children; a recent meta-analysis reported an overall lifetime prevalence of self-reported egg allergy of 2.5% (95% CI: 2.3–2.7%) [30].

The choice of a cell line for the production of inactivated influenza vaccines is based on certain criteria: *in primis*, it must be permissive for different virus isolates, allow virus growth at high titer and be safe [31]. Two continuous cell lines, namely Madin–Darby canine kidney (MDCK) and Vero, are most extensively studied and used in the development of inactivated vaccines [17,24]. Originally isolated from the kidney of a healthy female cocker spaniel in 1958, the MDCK cell line includes various derivatives, such as MDCK CCL34 (American Type Culture Collection), MDCK 841211903 (European Collection of Cell Cultures) and the MDCK 33016PF suspension cell line derived from CCL34 (Novartis) [24]. It has been shown that, in the primary isolation of both A and B subtypes, the MDCK suspension cell line is more sensitive than eggs by at least one order of magnitude, and thus yields high virus isolation efficiency. Indeed, all clinical isolates belonging to H1N1, H3N2 and both B lineages grew in this cell line after a blind passage following primary inoculation [26]. Three vaccines produced by using MDCK cells have been authorized so far. The subunit vaccine Influvac® TC (Solvay), the safety and tolerability profiles of which are equivalent to those of the conventional egg-based trivalent influenza vaccine (TIV) [32], was licensed in the Netherlands in 2001 but was never marketed, owing to manufacturing delays [33]. Later, Novartis Vaccines developed and commercialized a seasonal trivalent subunit CCIV (the subject of the review described in detail below) and a pandemic monovalent (H1N1) vaccine adjuvanted with MF59 (Celtura®); both vaccines were produced with the proprietary MDCK 33016PF suspension cell line [24,33]. The good safety, tolerability and immunogenicity profiles of Celtura have been documented in children, adolescents [34] and adults [35,36]. The second cell line, Vero, established from the kidney of a normal adult African green monkey, was for several years exploited by Baxter [24,33]. In 2002, a seasonal trivalent whole-virion vaccine, Inflúject®, was approved in the Netherlands, but then suspended owing to some safety concerns (a high rate of fever) [33]. The Vero cell-derived whole-virion monovalent pandemic vaccine Celvapan® was licensed and commercialized in the EU in October 2009 [24]. More recently, Baxter's second trivalent split seasonal flu vaccine, Preflucel®, was granted approval in Europe (as part of the mutual recognition after initial authorization in Austria in 2010); this vaccine has proved effective, displaying overall protective efficacy of 78.5% against vaccine-matched strains [37]. However, after an increase in reports of suspected side effects, such as severe allergic reactions, the EMA recalled Preflucel batches from the EU market [38]. Finally, FluBlok®, developed by Protein Sciences, is the first vaccine containing a recombinant trivalent HA; it is produced

in insect cell culture using the baculovirus expression system. FluBlok met the regulatory requirements of safety and immunogenicity and was approved by the US FDA in 2013 [39,40]. No flu vaccines derived from other cell lines, such as PER.C6®, have been approved for human use so far [24]. Notably, there are many other flu vaccine candidates that exploit fundamentally new approaches and targets (see, e.g., [17], and [24]); these, however, fall outside the primary aim of the present drug profile.

CCIV main characteristics

CCIV is an inactivated subunit trivalent flu vaccine for intramuscular (deltoid muscle) administration and is prepared from influenza virus propagated in qualified MDCK suspension cells (MDCK 33016PF). β-propiolactone is used for virus inactivation, which is followed by a detergent disruption process that uses cetyltrimethylammonium bromide and several steps of purification. A 0.5 ml dose of CCIV contains a total amount of 45 μg of HA (15 μg for each strain). This vaccine formulation is free from antibiotics, thimerosal, gelatin and formalin. It is currently indicated only for those ≥18 years old [24,41,42].

MDCK 33016PF is an approved certified proprietary cell line that grows efficiently in suspension in serum-free medium [43]. These cells yield a statistically higher isolation rate (89%) of H1N1pdm than allantoically inoculated eggs (66%), although HA titers of viruses isolated in MDCK 33016PF cells may be lower than those isolated in eggs after two passages [44]. Viruses isolated in MDCK 33016PF have shown almost no amino acid changes in HA sequence, unlike the majority of viruses isolated in eggs, which display 1–2 amino acid changes [44].

The application of MDCK-based technology has aroused some theoretical safety concerns regarding tumorigenicity, oncogenicity and the risk of viral contamination. Indeed, like other continuous cell lines (but not the originally established MDCK), the MDCK 33016PF derivative is tumorigenic in immunocompromised mice. In order to address this theoretical risk, the production process of CCIV includes several steps (e.g., centrifugation, filtration, chemical inactivation, membrane disruption) to ensure complete removal and inactivation of potentially oncogenic DNA. In their study based on a quantitative risk assessment, Onions *et al.* [31] showed that the probability of a residual cell in a vaccine dose is about 10^{-34}, residual MDCK-DNA per dose amounts to ≤10 ng; moreover, these authors found that β-propiolactone induced a reduction in detectable DNA to <200 base pairs, and that the vaccine was free from oncogenic viruses. In another risk assessment model [25], it was established that the maximum worst-case residual titers for over 20 different viruses ranged from 10^{-6} to 10^{-16} lg residual infectious units per vaccine dose, which is far below an infectious dose. Indeed, it has been confirmed that only few viruses can replicate in MDCK 33016PF cells (similar to those able to grow in eggs), while the growth of several avian viruses is blocked [25,45].

Another hypothetical concern regards CCIV administration to subjects with an allergy to dog-derived proteins, whose prevalence may be as high as 5–10% of the adult population [46]. *In vitro* studies [47,48] have not supported this hypothesis, as CCIV does not trigger rat basophilic leukemia cells sensitized with human anti-dog IgE in individuals allergic to dogs. More generally, across Phase I–III trials (see below) involving thousands of participants – and thus presumably hundreds of dog-allergic individuals – no acute adverse events of hypersensitivity have been reported [47].

CCIV clinical development

During the clinical development program, both in the US and EU, immunogenicity endpoints across the trials were formulated in accordance with the criteria of the EU Committee for Medicinal Products for Human Use (CHMP) [49] or the US Center for Biological Evaluation and Research (CBER) [50], or both. While both sets of criteria are similar in many respects, they also present important differences. Indeed, the US criteria are entirely based on the hemagglutination inhibition (HI) assay, while the European criteria allow seroprotection (SPR) and seroconversion (SCR) rates to be defined by means of the single radial hemolysis (SRH) assay. The EU immunogenicity criteria include post-vaccination geometric mean titer increase or geometric mean ratio (GMR), while the US criteria do not. Age-group definitions also vary. Moreover, the US criteria explicitly define a non-inferiority issue (Table 1). Finally, the CBER criteria indicate that a trial should have a statistical power to evaluate the lower limit of the 95% CI of vaccine effectiveness, which is anticipated to be significantly higher than 0, for example, 40–45%.

Phase I/II clinical trials

A description of Phase I and II clinical trials is given in Table 2. The first human trial was a combined Phase I/II single-center randomized controlled study [16] conducted in Germany in the fall of 2002. The Phase I part involved 40 healthy adults randomized on a 1:1 basis to receive CCIV or TIV. As no serious adverse events (SAEs) were registered, the Phase II part of the trial proceeded; this involved 200 subjects (82 adults aged 18–60 years and 118 elderly subjects). In the adults, the percentage of local and systemic reactions was similar between the two vaccine groups (local: 58 and 50%; systemic: 48 and 45% in CCIV and TIV groups, respectively). The most common local reactions among adults vaccinated with CCIV were injection-site pain (38%) and induration (23%), while systemic reactions were headache (32%) and fatigue (30%). Among the elderly, the frequency of local reactions was lower in CCIV than TIV vaccinees (47 and 62%, respectively), while that of systemic reactions was slightly higher in the CCIV group (40 vs 33%). Among the over-60s, unlike the non-elderly adults, the most frequently experienced local reaction was erythema (22%), while the most frequent systemic reactions were again fatigue (23%) and headache (17%). Of 13 severe reactions (5 local and 8 systemic) reported in Phase II of the trial, 6 (5 of these were in the adult group) were observed in CCIV vaccinees. These were: one case of erythema >50 mm, three

Table 1. EU Committee for Medicinal Products for Human Use or US Center for Biological Evaluation and Research criteria for influenza vaccines.

Endpoint	Criterion	Definition of criterion	Age-class (y)	Threshold
CHMP criteria				
Immunogenicity	Geometric mean ratio	Increase in GMT from pre- to post-vaccination	Adults (18–60)	>2.5
			Elderly (≥61)	>2.0
	Seroprotection	% of vaccinees with HI titer ≥40	Adults (18–60)	>70%
			Elderly (≥61)	>60%
	Seroconversion in HI assay	Post-vaccination increase in HI titer from <10 to ≥40 or at least fourfold increase	Adults (18–60)	>40%
			Elderly (≥61)	>30%
	Seroconversion in SRH assay	Post-vaccination increase in SRH titer from ≤4 mm^2 to ≥25 mm^2 or at least 50% area increase	Adults (18–60)	>40%
			Elderly (≥61)	>30%
CBER criteria				
Immunogenicity	Seroprotection	% of vaccinees with HI titer ≥40	Children (< 18)	>60%
			Adults (18–64)	>70%
			Elderly (≥65)	>60%
	Seroconversion in HI assay	Post-vaccination increase in HI titer from < 10 to ≥40 or at least fourfold increase	Adults (18–64)	>40%
			Elderly (≥65)	>30%
Non-inferiority	Geometric mean ratio	$GMT_{licenced\ vaccine}/GMT_{new\ vaccine}$	–	The lower limit of 2-sided 95% CI of GMR <1.5
	Seroconversion rate difference	$SCR_{licenced\ vaccine} - SCR_{new\ vaccine}$	–	The lower limit of 2-sided 95% CI of SCR difference <10%
Bioequivalence	Clinical lot consistency	Pairwise comparison of the 95% CI on the GMRs for each strain contained in the three vaccine lots	–	The two-sided 95% CI of GMR within 0.67–1.5

CBER: Center for biological evaluation and research; CHMP: Committee for medicinal products for human use; GMT: Geometric mean titer; HI: Hemagglutination inhibition; SRH: Single radial hemolysis; SCR: Seroconversion rate.

cases of severe headache and two cases of severe fatigue. The only SAE was judged to be unrelated to vaccination. With regard to immunogenicity endpoints, CCIV vaccinees of both age classes met all CHMP criteria for all three strains on using both cell- and egg-derived antigens in the SRH assay. SPRs determined by means of cell-derived antigens against H1N1-, H3N2- and B-like strains were higher in both age groups than those determined by means of egg-derived antigens.

The only trial conducted in the Southern Hemisphere (New Zealand, 2003) involved both adults and over-60-year-olds [42]. The frequency of solicited reactions was similar in CCIV and TIV vaccinees in both age groups, while unsolicited adverse events were rarer in the CCIV group. No SAEs were registered. Among both non-elderly and elderly adults immunized with CCIV, the CHMP criterion on GMR was met for H3N2 and B strains but not for the H1N1 strain (2.39 and 1.59 in adults and the elderly, respectively). The CHMP criterion on 3-week post-vaccination SPR was not met for the B strain (46 and 43% in adults and the elderly, respectively). SCRs were low, especially among adults (neither SCR exceeded 40%), probably owing to high baseline titers. In sum, the immunogenic profiles of CCIV and TIV were apparently similar in both age classes.

An American Phase II trial also failed to reveal any clinically significant difference in safety and tolerability profiles between CCIV and TIV [51]. Local reactions, which were mostly of mild and moderate severity, were reported by 54% of subjects randomized to the CCIV group compared with 61% in the TIV group; injection-site pain was the most frequent, accounting for approximately half of the cases. The only statistically significant between-group difference concerned the incidence of ecchymosis, which was less frequent among CCIV recipients (4 vs 9%). The two most common systemic reactions in both groups were headache (35 and 40% in CCIV and TIV groups, respectively) and malaise (25 and 24%, respectively). Unsolicited adverse events were more frequent in the TIV group (25 vs 16%, $p = 0.009$). None of the SAEs ($n = 8$) were vaccine-related. The study's immunogenicity endpoint highlighted the non-inferiority of CCIV, as the lower limit of the 95% CIs of the GMRs (CCIV vs TIV) for all vaccine strains was >0.5, thus fulfilling the CBER criterion. In the CCIV group, SPRs against H1N1-, H3N2- and

Table 2. Summary information on published Phase I and II clinical trials.

NCT ID	Phase	Study design	Study location (hemisphere, season)	Study population (age, years)	Vaccine strain-like composition	Comparator TIV vaccine	Immunogenicity endpoints	Schedule	Sample size on enrollment	Main results†	Ref.
n/a	I/II	Single-center sequential randomized (1:1) active-controlled observer-blind	Germany (northern) (2001–02)	Phase I part: adults (18–40) Phase II part: adults (18–60), elderly (≥61)	H1N1: A/New Caledonia/20/99 H3N2: A/ Moscow/10/99 B: B/Sichuan/ 379/99	Agrippal/ Agriflu	CHMP criteria	One dose of either vaccine	Phase I part: 40 (20 + 20)‡ Phase II part: 82 adults (40 + 42)‡, 118 elderly (60 + 58)‡ Total: 240	Safety: CCIV judged safe. No clinically significant difference between CCIV and TIV Immunogenicity: In both age-groups CCIV and TIV met all CHMP criteria for each strain in hemagglutination inhibition assay	[16]
n/a	II	Single-center randomized (1:1) active-controlled observer-blind	New Zealand (southern; 2003)	Adults (18–60), elderly (≥61)	H1N1: A/New Caledonia/20/99 H3N2: A/Panama/ 2007/99 B: B/Shangdong/ 120/2000	Agrippal/ Agriflu	CHMP criteria	One dose of either vaccine	CCIV: 110 (56 + 54)§ TIV: 113 (57 + 56)§ Total: 223 (113 + 110)§	Safety: CCIV judged safe. No clinically significant difference between CCIV and TIV Immunogenicity: both CCIV and TIV met at least one CHMP criterion in both adults and the elderly	[42]
00264576 [63]	II	Multicenter randomized (1:1) active-controlled observer-blind non-inferiority	US (northern; 2005–06)	Adults (18–49)	H1N1: A/New Caledonia/20/99 H3N2: A/California/ 7/2004 B: B/Shanghai/361/ 2002	Fluvirin	CBER criteria	One dose of either vaccine	CCIV group: 308 TIV group: 305 Total: 613	Safety: CCIV judged safe. No clinically significant difference between CCIV and TIV Immunogenicity: non-inferiority of CCIV to TIV for all strains	[51]

†See the main text for more detailed information.
‡Number of participants is reported as total (CCIV + TIV).
§Number of participants is reported as total (adults + elderly).
CBER: Center for biological evaluation and research; CCIV: Cell culture-derived inactivated vaccine; CHMP: Committee for medicinal products for human use; n/a: Not available; NCT: National clinical trial; TIV: Trivalent inactivated vaccine.

B-like strains were 96% (95% CI: 94–98%), 91% (95% CI: 87–94%) and 94% (95% CI: 91–96%), respectively, and were similar to those in the TIV group, except for SPR against the H3N2 strain, which was significantly higher in the TIV group (96 vs 91%). An analogous picture was seen with regard to SCRs 3 weeks post-immunization with CCIV (H1N1: 62% [95% CI: 57–68%]; H3N2: 85% [95% CI: 81–89%]; B: 77% [95% CI: 72–81%]). Again, the between-group difference in SCR against the H3N2-like strain proved to be statistically significant in favor of TIV when egg-derived antigens were used; when cell-derived antigens were used in the HI assay, however, the difference disappeared.

Phase III clinical trials

To date, the results of eight Phase III clinical trials are publically available [22,52–57]. These are summarized in Table 3 and described in detail below.

Children & adolescents

Immunogenicity

According to CBER criteria, CCIV is highly immunogenic and non-inferior to conventional egg-based vaccines in children and adolescents [52]. This was documented in a large trial involving 3604 healthy children and adolescents; in that study, the HI assay used both cell culture and egg-derived H1N1, H3N2 and B antigens. As in the trial by Groth *et al.* [16] the use of cell-derived antigens produced higher immunogenicity measures: CCIV was non-inferior to TIV according to the CBER criterion on the difference in SCRs for all three strains, and according to the criterion on geometric mean titer ratio CCIV versus TIV for H1N1 and B strains, but not for the H3N2 strain. Conversely, on using egg-derived antigens, CBER criteria were met for only two of six measures (SCR difference and geometric mean titer ratio for H1N1 strain). In children aged 3–8 years, the CHMP criterion on GMR (from before vaccination to 29th and 50th days post-vaccination) was met for all three antigens,

both cell- and egg-derived. SCR and SPR criteria were also fulfilled for all antigens except for the B strain. However, SCR measured against the B strain in HI using cell-derived antigen on day 50 did meet the SCR criterion (58% [95% CI: 54–63%]). In older children and adolescents aged 9–17 years, the immune response was stronger, in that all criteria were exceeded on using both cell- and egg-derived antigens [52].

Safety & tolerability

Overall, no significant differences between CCIV and egg-based TIV, in terms of the frequency and severity of both local and systemic reactions, were reported. Among children aged 3–8 years immunized with CCIV, local reactions were reported in 38 and 35% of cases after the first and second dose, respectively. This proportion was slightly higher (42%) among older children and adolescents. Injection-site pain and erythema were the most frequent reactions in both age groups. Systemic events were less frequent in both 3- to 8-year-olds (15 and 23% after first and second dose, respectively) and 9- to 17-year-olds (29%). Among these events, myalgia, headache, malaise and fatigue were relatively frequent. None of the serious adverse events (n = 28) registered during the study period was judged vaccine-related [52].

Adults & the elderly

Efficacy

A large (n = 11,404) cornerstone clinical trial of CCIV determined vaccine efficacy as its primary endpoint [22]. During the surveillance period of 6 months, 5.0% (189/3776), 6.7% (243/3638) and 9.2% (353/3843) of participants in the CCIV, TIV and placebo groups, respectively, reported ILI symptoms. Culture-confirmed influenza was detected in 1.11%, 1.35% and 3.64% in the CCIV, TIV and placebo groups, respectively, yielding an overall vaccine efficacy of CCIV versus placebo of 69.5%, which was higher than TIV versus placebo (63.0%). Most laboratory-confirmed cases were caused by non-vaccine-like strains, especially of B influenza type. As expected, vaccine efficacy against vaccine-like strains was much higher (83.8% [lower 97.5% CI limit: 61.0%]) than against non-vaccine-like ones (58.7% [lower 97.5% CI limit: 33.5%]).

The secondary endpoint of a lot-to-lot consistency trial by Ambrozaitis *et al.* [53] also aimed to evaluate the number of ILI cases developed by a randomized subset of vaccinees over a 6-month follow-up. Thirty-one of 494 vaccinees (6.3%) reported ILI symptoms and 7 (5 and 2 in CCIV and TIV groups, respectively) of these were confirmed influenza B, which was the predominant type in the 2005–06 influenza season in Europe. Vaccination failure in these seven subjects was very probably due to mismatching between the vaccine B strain (B/Malaysia/2506/2004-like) and the strain circulating in the northern hemisphere (B/Shanghai/361/2002-like).

Immunogenicity

In a large Polish study [54], CCIV was compared with TIV in terms of non-inferior immunogenicity, in both adults and the elderly. In both vaccine arms, elderly vaccinees showed similar immune responses to adults of 18–60 years against H3N2 and B strains, but significantly lower anti-H1N1 responses. The non-inferiority of CCIV to the egg-based vaccine was demonstrated for each of the three strains in both age classes. Moreover, in the CCIV arm, all CHMP criteria for all vaccine strains were achieved. Similarly, CCIV was non-inferior to TIV in a subset of vaccinees (n = 779) with at least two chronic conditions, exceeding all CHMP criteria. A study by Szymczakiewicz-Multanowska *et al.* [54] had two extensions [55]. The first one was of randomized observer-blind design and aimed to investigate issues of CCIV and TIV immunogenicity among subjects revaccinated with the same or alternate vaccine (with respect to [54]). The second extension involved patients who were revaccinated with TIV if they had been randomized to TIV in the parent study or the first extension, or CCIV otherwise; a subset of elderly subjects were also randomized to receive either TIV or CCIV, alone or concomitantly with a 23-valent polysaccharide pneumococcal vaccine (PCV23). In the first extension study, only elderly recipients of either vaccine met all three CHMP criteria for H1N1, H3N2 and B strains. By contrast, adults in both the CCIV and TIV groups satisfied SCR criterion for only the H3N2-like strain. In the CCIV group, the GMR criterion was met for H3N2 and B strains, while in the TIV group only for H3N2. As in the elderly, the SPR criterion was met for all strains in both vaccine groups. In the second extension study, as in the first, elderly subjects immunized with either vaccine met CHMP criteria for all three strains, except for SPR against the B strain in the TIV group and SCR against the B strain in both vaccine arms; this suggests that concomitant administration of PCV23 has no impact on the magnitude of the immune response in the elderly. Among adults, too, SPR was not met for the B strain, while GMR and SCR criteria in those vaccinated with CCIV were satisfied for all three strains, as against only one strain (H1N1) in the TIV group.

The lot-to-lot bioequivalence and antibody persistence after a dose of CCIV was later demonstrated in a Lithuanian trial [53]. Three lots of CCIV induced a similar immune response to all strains both 3 weeks and 6 months post-immunization, meeting all CHMP criteria; antibody levels in the CCIV arm were also similar to those in the TIV arm. Upper and lower 95% CIs of all pairwise lot comparisons ranged from 0.67 to 1.36, thus meeting the CBER clinical lot consistency criterion.

In an efficacy trial conducted by Frey *et al.* [22], immunogenicity criteria were also evaluated as secondary endpoints. Both CBER/CHMP criteria were met by both CCIV and TIV, although the 3-week post-vaccination immune response against the B strain was higher in the TIV group.

CHMP criteria in HI tests for the three strains were also met among both adults and elderly subjects aged ≥ 61years in an open-label uncontrolled study [56]. SPRs were particularly high – at least 98 and 85% in adults and the elderly, respectively. Adults showed much higher GMRs (5.6–13) than over-60s (3.5–5.8). In the SRH assay, criteria were almost in line

Table 3. Summary information on published Phase III clinical trials.

NCT ID	Study design	Study location (hemisphere, season)	Study population (age, years)	Vaccine strain-like composition	Comparator TIV vaccine	Immunogenicity and efficacy endpoints	Schedule	Sample size on enrollment	Main results[†]	Ref.
00492063 [64]	Multicenter randomized (1:1) active-controlled observer-blind non-inferiority	Poland (northern; 2004–05)	Adults (18–60), elderly (≥61)	H1N1: A/New Caledonia/20/99 H3N2: A/Fujian/411/2002 B: B/Shanghai/361/2002	Agrippal/ Agriflu	CHMP and CBER criteria	One dose of either vaccine	CCIV: 1330 (652 + 678)[‡] TIV: 1324 (648 + 676)[‡] Total: 2654 (1300 + 1354)[‡]	Immunogenicity: In both age groups CCIV and TIV met all CHMP criteria for each strain. Non-inferiority of CCIV to TIV for all strains. Safety: CCIV judged safe. No clinically significant difference between CCIV and TIV	[54]
00306527 [65]	Randomized observer-blind extension of NCT00492063	Poland (northern; 2005–06)	Adults (18–60), elderly (≥61)	H1N1: A/New Caledonia/20/99 H3N2: A/California/7/2004 B: B/Shanghai/361/2002	Agrippal/ Agriflu	CHMP criteria	Subjects immunized with CCIV in NCT00492063: 1 dose of CCIV (group 1), one dose of TIV (group 2). Subjects immunized with TIV in NCT00492063: 1 dose of CCIV (group 3), one dose of TIV (group 4)	Group 1: 562 (272 + 290)[‡] Group 2: 571 (274 + 297)[‡] Group 3: 542 (261 + 281)[‡] Group 4: 560 (260 + 300)[‡] Total: 2235 (1067 + 1168)[‡]	Immunogenicity: In adults, both CCIV and TIV met at least one CHMP criterion for each strain. In the elderly, both vaccines met all CHMP criteria for each strain. Safety: CCIV judged safe. No clinically significant difference between CCIV and TIV	[55]
00310804 [66]	Multicenter randomized (2:2:2:1) active-controlled observer-blind lot-to-lot consistency	Lithuania (northern; 2005–06)	Adults (18–60)	H1N1: A/New Caledonia/20/99 H3N2: A/California/7/2004 B: B/Shanghai/361/2002	Agrippal/ Agriflu	CHMP and CBER criteria; ILI surveillance for 6 months in a subset of subjects (n = 494)	One dose of CCIV from 3 lots (A, B, C) or TIV	Lot A: 342 Lot B: 344 Lot C: 343 TIV: 171 Total: 1200	Lot-to-lot consistency: demonstrated. Immunogenicity: both CCIV and TIV met all CHMP criteria. Safety: CCIV judged safe. No clinically significant difference between CCIV and TIV	[53]

[†]See the main text for more detailed information.
[‡]Number of participants is reported as total (adults + elderly).
[§]Combined II/III Phase.
[¶]Number of participants is reported as total (CCIV + TIV).
[#]Phosphate-buffered saline (0.5 ml).
[††]Registered as multicenter; the required sample size, however, was reached at one center.
CBER: Center for biological evaluation and research; CCIV: Cell culture-derived inactivated vaccine; CHMP: Committee for medicinal products for human use; GMR: Geometric mean ratio; ILI: Influenza-like illness; NCT: National clinical trial; SCR: Seroconversion rate; SPR: Seroprotection rate; TIV: Trivalent inactivated vaccine.

Table 3. Summary information on published Phase III clinical trials (cont.).

NCT ID	Study design	Study location (hemisphere, season)	Study population (age, years)	Vaccine strain-like composition	Comparator TIV vaccine	Immunogenicity and efficacy endpoints	Schedule	Sample size on enrollment	Main results[†]	Ref.
00579345 [67]	Non-randomized (3:1 allocation) single-blind extension of NCT00492063 plus a subset (5:5:3:3) of elderly (≥ 65y) randomized to receive influenza vaccine alone or concomitantly with PCV23 (Pneumo 23)	Poland (northern; 2007–08)	Adults (18–60), elderly (≥61)	H1N1: A/ Solomon Islands/ 3/2006 H3N2: A/ Wisconsin/67/ 2005 B: B/Malaysia/ 2506/2004	Agrippal/ Agriflu	CHMP criteria	Non-randomized part of study. Group 1: one dose of CCIV to subjects of group 1 from NCT00306527. Group 2: one dose of CCIV to subjects of group 2 from NCT00306527. Group 3: one dose of CCIV to subjects of group 3 from NCT00306527. Group 4: one dose of TIV to subjects of group 4 from NCT00306527. Randomized part of study (+ PCV23). Group 1: one dose of CCIV. Group 2: one dose of CCIV and PCV23. Group 3: one dose of TIV. Group 4: one dose of TIV and PCV23	Non-randomized part of study. Groups 1–3: 940 (549 + 391)[‡] Group 4: 313 (169 + 144)[‡] Total: 1253 (718 + 535)[‡] Randomized part of study. Group 1: 90 Group 2: 78 Group 3: 57 Group 4: 44 Total: 269	Immunogenicity: In adults, CCIV met all CHMP criteria for H1N1 and H3N2 and GMR and SCR criteria for B strain. TIV did not meet any criterion for B strain. In the elderly, CCIV met all CHMP criteria for H1N1 and H3N2 and GMR and SPR criteria for B strain. TIV met all CHMP criteria for H1N1 and H3N2 and GMR criterion for B strain. Safety: CCIV judged safe. Compared with TIV, a statistically higher percentage of injection site pain in the elderly receiving CCIV. Compared with TIV+PCV23, a statistically higher percentage of local and systemic reactions in the elderly receiving CCIV+PCV23	[55]
00645411 [68]	Multicenter randomized active-controlled observer-blind non-inferiority[§]	US, Finland, Croatia, Hungary, Lithuania, Italy, Romania (northern; 2007–08)	Children and adolescents (cohorts 1 and 2: 9–17, cohort 3: 3–8)	H1N1: A/ Solomon Islands/ 3/2006 H3N2: A/ Wisconsin/67/ 2005	Fluvirin	CBER and CHMP criteria	Cohorts 1 and 2: one dose of either CCIV or TIV Cohort 3: one doses of either	Cohort 1: 305 (151 + 154)[¶] Cohort 2: 669 (505 +164)[¶] Cohort 3: 2630 (1608 + 1022)[¶]	Immunogenicity: In children, both CCIV and TIV met at least one CHMP criterion for each strain using both cell culture- and	[52]

[†]See the main text for more detailed information.
[‡]Number of participants is reported as total (adults + elderly).
[§]Combined II/III Phase.
[¶]Number of participants is reported as total (CCIV + TIV).
[#]Phosphate-buffered saline (0.5 ml).
[††]Registered as multicenter; the required sample size, however, was reached at one center.
CBER: Center for biological evaluation and research; CCIV: Cell culture-derived inactivated vaccine; CHMP: Committee for medicinal products for human use; GMR: Geometric mean ratio; ILI: Influenza-like illness; NCT: National clinical trial; SCR: Seroconversion rate; SPR: Seroprotection rate; TIV: Trivalent inactivated vaccine.

Table 3. Summary information on published Phase III clinical trials (cont.).

NCT ID	Study design	Study location (hemisphere, season)	Study population (age, years)	Vaccine strain-like composition	Comparator TIV vaccine	Immunogenicity and efficacy endpoints	Schedule	Sample size on enrollment	Main results[†]	Ref.
				B: B/Malaysia/2506/2004			CCIV or TIV on days 1 and 29	Total: 3604 (2264 + 1340)[¶]	egg-derived antigens. In adolescents, both CCIV and TIV met all CHMP criteria for each strain using both cell culture- and egg-derived antigens. Safety: CCIV judged safe. No clinically significant difference between CCIV and TIV	
00630331 [69]	Multicenter randomized (1:1:1) active- and placebo-controlled[#] observer-blind	US, Finland and Poland (northern; 2007–08)	Adults (18–49)	H1N1: A/Solomon Islands/3/2006 H3N2: A/Wisconsin/67/2005 B: B/Malaysia/2506/2004	Agrippal/Agriflu	Vaccine efficacy against both vaccine-like and non-vaccine-like strains (6-month influenza surveillance); CBER and CHMP criteria	One dose of CCIV, TIV or placebo	CCIV: 3828 TIV: 3676 Placebo: 3900 Total:11,404	Efficacy: both CCIV and TIV met CBER criteria. CCIV efficacy against vaccine-like and all circulating strains was 83.8% and 69.5%, respectively. Immunogenicity: both CCIV and TIV met all CBER and CHMP criteria. Safety: CCIV judged safe. No clinically significant difference between CCIV and TIV	[22]
01422512 [70]	Multicenter[††] open-label uncontrolled	Germany (northern; 2011–12)	Adults (18–60), elderly (≥ 61)	H1N1: A/California/7/2009 H3N2: A/Perth/16/2009 B: B/Brisbane/60/2008	–	CHMP criteria	1 dose of CCIV	Adults: 62 Elderly: 64 Total: 126	Immunogenicity: CCIV met at least one CHMP criterion for all strains in both adults and the elderly. Safety: CCIV judged safe. No serious adverse events	[57]

[†]See the main text for more detailed information.
[‡]Number of participants is reported as total (adults + elderly).
[§]Combined II/III Phase.
[¶]Number of participants is reported as total (CCIV + TIV).
[#]Phosphate-buffered saline (0.5 ml).
[††]Registered as multicenter; the required sample size, however, was reached at one center.
CBER: Center for biological evaluation and research; CCIV: Cell culture-derived inactivated vaccine; CHMP: Committee for medicinal products for human use; GMR: Geometric mean ratio; ILI: Influenza-like illness; NCT: National clinical trial; SCR: Seroconversion rate; SPR: Seroprotection rate; TIV: Trivalent inactivated vaccine.

Table 3. Summary information on published Phase III clinical trials (cont.).

NCT ID	Study design	Study location (hemisphere, season)	Study population (age, years)	Vaccine strain-like composition	Comparator TIV vaccine	Immunogenicity and efficacy endpoints	Schedule	Sample size on enrollment	Main results[†]	Ref.
01640314 [71]	Multicenter, open-label, uncontrolled	Germany (northern; 2012–13)	Adults (18–60), elderly (≥ 61)	H1N1: A/California/7/2009pdm09 H3N2: A/Victoria/361/2011 B: B/Wisconsin/1/2010	–	CHMP criteria	One dose of CCIV	Adults: 63 Elderly: 63 Total: 126	Immunogenicity: CCIV met all CHMP criteria for all strains in both adults and the elderly. Safety: CCIV judged safe. No serious adverse events	[56]

[†]See the main text for more detailed information.
[‡]Number of participants is reported as total (adults + elderly).
[§]Combined II/III Phase.
[¶]Number of participants is reported as total (CCIV + TIV).
[#]Phosphate-buffered saline (0.5 ml).
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CBER: Center for biological evaluation and research; CCIV: Cell culture-derived inactivated vaccine; CHMP: Committee for medicinal products for human use; GMR: Geometric mean ratio; ILI: Influenza-like illness; NCT: National clinical trial; SCR: Seroconversion rate; SPR: Seroprotection rate; TIV: Trivalent inactivated vaccine.

with those of the HI assay, except for the GMR criterion in the adult group, in which GMR was slightly lower (2.24) than the required threshold.

In another German study [57], SPR of at least 87% against the three strains in the HI assay were observed among participants, with little difference between adults and the elderly. In the adult group, the GMR criterion was fulfilled for all strains, while the SCR criterion was not achieved against the B strain (35%). Analogously, GMR against the B strain was only 1.89 among the elderly, while SCR met the CHMP criterion for only the H1N1 strain. In the SRH assay, adults met all CHMP criteria for H1N1 and H3N2 strains, while with regard to the B strain, the GMR criterion was not met. The SRH assay performed in the elderly group revealed that GMR against H3N2 and B strains and SCR against the B strain were not met, while other criteria were.

Figure 1 summarizes immunogenicity data – GMR, SPRs and SCRs with 95% CIs determined in the HI assay – from the above-described adults and elderly subjects in Phase III trials.

Safety & tolerability

Across all trials, CCIV was judged safe and well tolerated among both adults and the elderly; almost all solicited local and systemic reactions were of mild or moderate severity. No SAEs were judged to be vaccine-related [22,53–57]. As shown in Figure 2, pain was the most prevalent local adverse event across the Phase III trials; its frequency was somewhat age-dependent, being higher among non-elderly adults. Fatigue and headache were generally the most common systemic events and showed the same age pattern. In one trial [56], a relatively high frequency of myalgia was reported in both adults and the elderly. The relative frequency of fever ≥38°C did not exceed 1%.

Generally, when comparing the safety and tolerability profiles of CCIV with those of traditional egg-based vaccines, no clinically significant differences emerged, although statistical significance was noted for some adverse reactions. Szymszakiewicz-Multanowska *et al.* [54] reported a significantly higher incidence ($p < 0.05$) of injection-site pain, which was of mild-to-moderate severity, among subjects vaccinated with CCIV than with TIV in both adults (22 vs 17%) and the elderly (9 vs 5%). The same research group [55] documented a higher rate of solicited local and systemic reactions, especially injection-site pain, in the elderly receiving a concomitant PCV23 dose; this increase was, however, independent from the type of influenza vaccine.

Regulatory affairs

CCIV was granted approval by the EMA [18] in 2007 and by the FDA [19] in 2012 for use in adults aged ≥18 years. This means that CCIV may be commercialized in all Member States of the EU, plus countries of the European Economic Area (Norway, Switzerland and Iceland) and the US.

Post-marketing surveillance

During the 2012–13 flu vaccination campaign in the Spanish region of Castile-León, 12,806 doses of Optaflu were

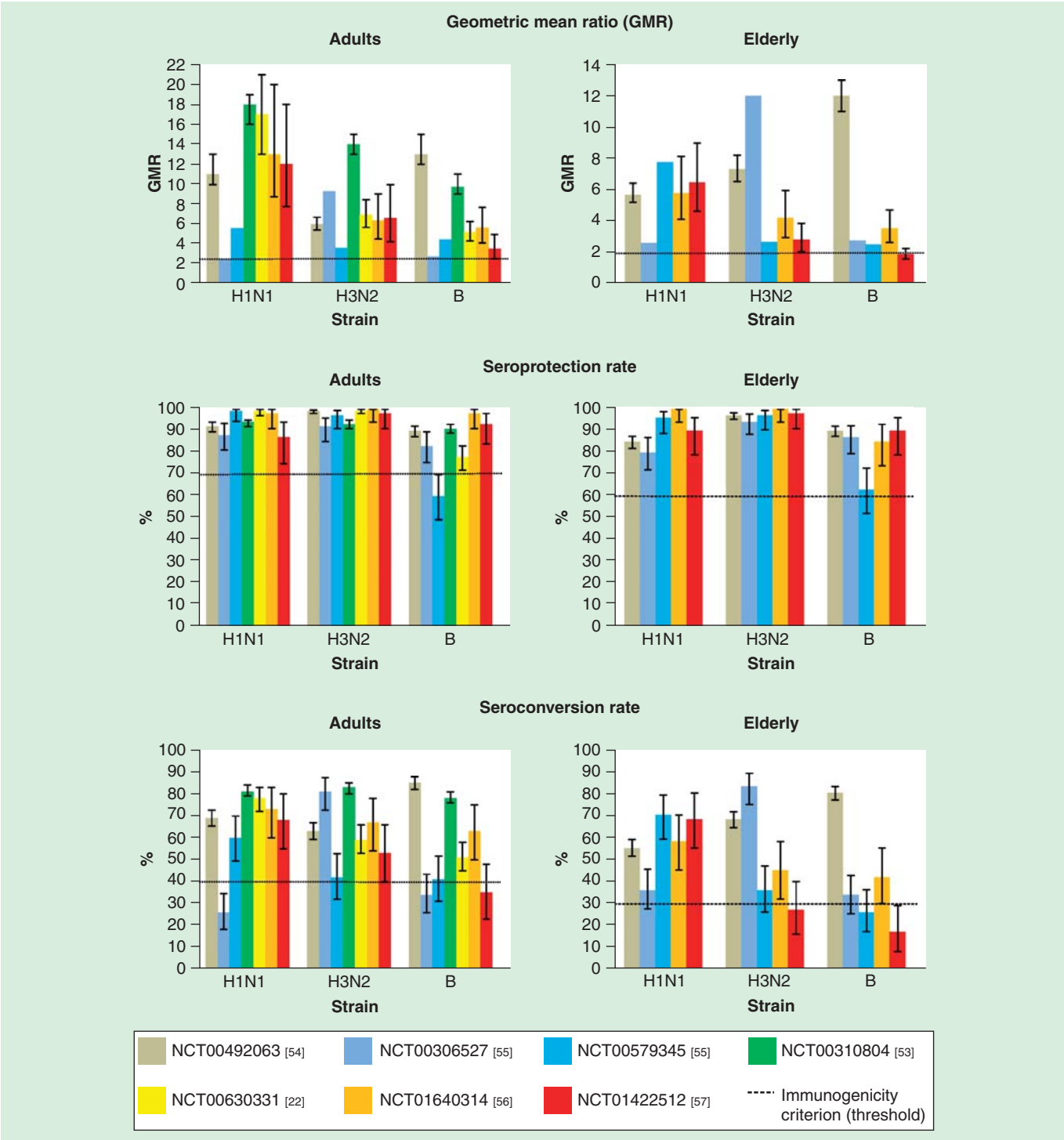

Figure 1. Three-week post-vaccination immunogenicity parameters elicited by cell culture-derived inactivated vaccine in adults and the elderly in Phase III trials.
Figure legend refers to the trial identification number (reference).

administered to subjects aged over 18 years; most vaccine doses (about 75%) were administered to the subjects over 65 years of age. There were no notifications of any adverse events in the Regional Center of Pharmacovigilance [58].

Conclusion

The available evidence has proved that CCIV is an adequate alternative to conventional egg-based vaccines. During its development program, more than 10,000 CCIV doses were

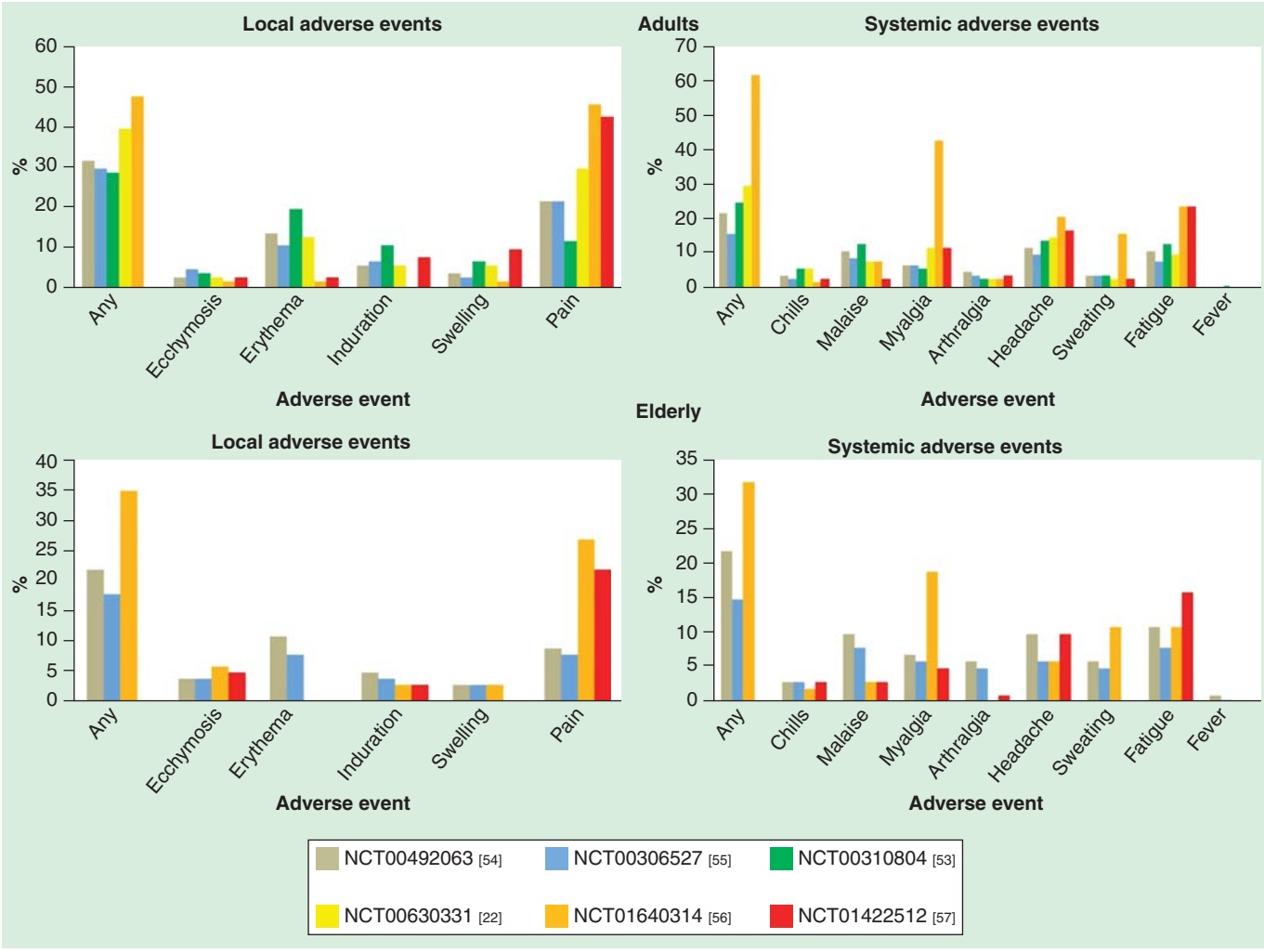

Figure 2. Frequency of local and systemic adverse events solicited in adults and the elderly immunized with cell culture-derived inactivated vaccine in Phase III trials.
Figure legend refers to the trial identification number (reference).

administered; the clinical data reviewed in the present paper suggest that CCIV is immunogenically non-inferior to conventional egg-based inactivated vaccines and meets existing regulatory criteria, and that its consecutive production lots are bioequivalent [16,22,42,51–57]. Good immunogenicity has been found in different age classes, including children and adolescents [52], adults [16,22,42,51,53–57] and the elderly [16,42,54–57]. In the clinical trials reviewed here, the use of cell-derived antigens produced higher HI titers than egg-derived antigens in vaccinees with CCIV. A probable explanation for these observations lies in the relatively high genetic and antigenic stability (no egg-adaptive mutations) of viruses grown in the suspension cell line [16,52]. The duration of vaccine-induced immunity, which is another key parameter, is sufficiently long; indeed, CCIV has been shown to compare well with TIV in terms of antibody persistence up to 6 months [53]. The safety and tolerability profiles of CCIV are adequate and generally similar to those of TIV. The few available data on post-marketing surveillance confirm the robust safety of CCIV.

No study has established any safety concern regarding the MDCK 33016PF continuous cell line. Specifically, CCIV is safe to use in individuals with an allergy to dogs [47,48]. Moreover, highly standardized and reproducible technology and the strict application of good manufacturing practices, including several steps for virus inactivation, reduce the risk of microbiological contamination [24,25].

Expert commentary

Influenza vaccine shortage is not a rare event. The availability of an alternative substrate, such as MDCK cells, may potentially prevent vaccine shortages and their disastrous effects and provide equal access to immunization. Moreover, cell culture-based technology has advantages over egg-based technology in terms of time-saving and flexibility [33].

Although CCIV is being increasingly used in routine immunization practice, its current market share is relatively small. This could be due to the fact that the vaccine is recommended only for those ≥18 years old [18,19]. However, given that egg

allergy is much more prevalent among young children, this age group could gain substantial benefits from expanding the current age indication; indeed, a large pediatric trial [52] found CCIV to be safe, well-tolerated and immunogenic. Another explanation of CCIV's limited market share is that stakeholders in general may be less familiar with CCIV than with traditional vaccines [59]. Providing information on alternative options may be profitable, since this would increase the choices available to healthcare consumers. For example, the absence of egg allergens, antibiotics and preservatives in CCIV could be a constructive argument for influenza immunization among so-called 'vaccine-hesitant' individuals.

In the elderly, the concomitant administration of influenza and pneumococcal vaccines is a common practice. Although CCIV is safe and well-tolerated in this age group when administered alone [16,42,54–57], co-administration of CCIV with PCV23 may be associated with a higher rate of mild-to-moderate local and systemic reactions than TIV + PCV23 [55]. Additional immunization risk communication strategies would be beneficial.

To conclude, for decades egg-based vaccines have faced three major challenges: suboptimal cross-protection; immunogenicity, especially among the elderly and the length of manufacturing time. CCIV and other vaccines produced with continuous cell lines have made real progress with regard to the third point; the other two are still to be addressed.

Five-year view

It has often been speculated that cell culture-derived flu vaccines will substitute egg-derived ones in the near future on account of their several advantages. More recently, however, it has been claimed [60] that cell-derived influenza vaccines offer only modest benefits, and indeed some multinational pharmaceutical companies have abandoned this new technology [60]. Moreover, other promising novel approaches, such as the use of HA stalk antibodies, are under preclinical and clinical development [17].

High-quality, cost–effectiveness analyses and health technology assessment (HTA) reports on CCIV will be useful to policy-makers and healthcare planners, given the increased demand for effective influenza vaccines, their highly competitive market and the ongoing financial crisis in some countries. This is particularly relevant to the EU, where policy divergences among member states persist.

According to the latest WHO position paper [4], pregnant women should be the first priority group for seasonal influenza vaccination; there are, however, no data on the safety of CCIV administered during pregnancy. An ongoing cohort study [61] will address the issue of CCIV safety in pregnant women and their offspring.

Quadrivalent vaccine formulations may have the advantage of expanding protection by covering two influenza B lineages, thus reducing morbidity from influenza B [11]. It is therefore plausible that future research will be directed toward quadrivalent cell culture-derived vaccines, and that CCIV will become quadrivalent. For instance, a quadrivalent cell-based vaccine candidate, GC3106, has already been investigated in a Phase I/IIa trial [62]. A quadrivalent formulation of CCIV would be beneficial, as in trials aimed at evaluating vaccine effectiveness most laboratory-confirmed influenza cases have been due to a B virus [22,53].

To date, only one report on CCIV post-marketing surveillance is available [58]; continuous monitoring and reporting of adverse events will further elucidate the vaccine safety and tolerability profiles.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Key issues

- In comparison with egg-based technology, Madin–Darby canine kidney-based technology offers advantages of flexibility, a higher virus isolation rate and virus immutability, a lower risk of microbial contamination, smaller amounts of excipients and preservatives, and the possibility of being used in egg-allergic subjects.
- Flucelvax®/Optaflu® is the first cell culture-derived seasonal influenza vaccine to gain approval from both the EMA and the FDA by meeting the requirements for seasonal inactivated influenza vaccines imposed by both organizations.
- The Madin–Darby canine kidney 33016PF suspension cell line used to manufacture Flucelvax/Optaflu is safe: tumorigenicity, oncogenicity and hypersensitivity reactions among dog-allergic individuals are improbable.
- Across eleven Phase I-III clinical trials conducted so far, Flucelvax/Optaflu has proved safe, well-tolerated and immunogenic.
- The safety, tolerability, efficacy and immunogenicity profiles of Flucelvax/Optaflu are generally comparable to those of conventional egg-based trivalent inactivated vaccines.
- Flucelvax/Optaflu can be safely co-administered with pneumococcal vaccines in the elderly, though a slightly higher frequency of mild-to-moderate adverse reactions in comparison with egg-based inactivated vaccines has been documented.
- The robustness of the vaccine safety profile has been confirmed by a post-marketing surveillance study, which recorded no vaccine-related adverse events.

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