Vaccine (2008) **26**, 2378–2388

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/vaccine

Safety and reactogenicity profile of an adjuvanted H5N1 pandemic candidate vaccine in adults within a phase III safety trial

Hans C. Rümke[a,*], José-María Bayas[b], José-Ramón de Juanes[c], Covadonga Caso[d], Jan Hendrik Richardus[e], Magda Campins[f], Lars Rombo[g], Xavier Duval[h], Viktor Romanenko[i], Tino F. Schwarz[j], Rustem Fassakhov[k], Francisco Abad-Santos[l], Frank von Sonnenburg[m], Mamadou Dramé[n], Roland Sänger[n], W. Ripley Ballou[n]

[a] *VAXINOSTICS BV, University Vaccine Center Rotterdam Nijmegen, Beursplein 37, PO Box 30142, 3001 DC Rotterdam, The Netherlands*
[b] *Hospital Clinic, Barcelona, Spain*
[c] *Hospital 12 de Octubre, Madrid, Spain*
[d] *Hospital Clínico San Carlos, Madrid, Spain*
[e] *Municipal Public Health Service Rotterdam-Rijnmond, Rotterdam, The Netherlands*
[f] *Hospital Vall d'Hebron, Pso. Vall d'Hebron 119-129, 08035 Barcelona, Spain*
[g] *Infektionskliniken, Mälarsjukhuset, Eskilstuna, Sweden*
[h] *Centre d'Investigation Clinique, Hôpital Bichat Claude Bernard, 75018 Paris, France*
[i] *City Children's Hospital N40, Ekaterinburg, Russian Federation*
[j] *Stiftung Juliusspital, Würzburg, Germany*
[k] *Kazan Research Institute of Epidemiology and Microbiology, Kazan, Russian Federation*
[l] *Hospital de La Princesa, Madrid, Spain*
[m] *Ludwig Maximilians Universität, München, Germany*
[n] *GlaxoSmithKline Biologicals, Rixensart, Belgium*

Received 26 October 2007; received in revised form 18 February 2008; accepted 27 February 2008
Available online 27 March 2008

KEYWORDS
H5N1 pandemic influenza;
Adjuvanted vaccine;
Safety;
Phase III clinical trial

Summary A multicentre, randomized, phase III clinical trial in 5071 healthy adults was conducted to evaluate the safety and reactogenicity of a 15 μg HA dose of a candidate oil-in-water emulsion-based adjuvant system (AS)-adjuvanted split-virion H5N1 (AS-H5N1) vaccine compared to a licensed seasonal influenza vaccine, *Fluarix*™.[1] Stringent criteria were used to evaluate adverse events and reactogenicity profile. Overall, 96.7% of the 5071 vaccinated subjects completed the study. Significantly more participants in the AS-H5N1 vaccine group reported general or local adverse events. Pain was the most common symptom in both treatment groups. Less

[1] *Fluarix*™ is a trademark of GlaxoSmithKline group of companies.

* Corresponding author. Tel.: +31 10 205 5554; fax: +31 10 205 5551.
E-mail address: rumke@vaxinostics.com (H.C. Rümke).

0264-410X/$ – see front matter
doi:10.1016/j.vaccine.2008.02.068

than 1% of subjects withdrew from the study due to adverse events and no withdrawals were due to serious adverse events related to vaccination. The safety and reactogenicity profile of the AS-H5N1 candidate vaccine can be considered clinically acceptable in the context of its use against pandemic influenza.

Introduction

Since its emergence in domestic poultry in 1996, H5N1 avian influenza A virus has given rise to growing concern as the most likely candidate to cause the next influenza pandemic [1]. The basis of this concern is the high pathogenicity of the H5N1 virus in humans, as reflected by 227 deaths (62.9% fatality rate) among the 361 cases reported to the World Health Organization up to 15 February 2008 [2]. H5N1 avian influenza infections in migratory waterfowl and domestic poultry were initially confined to South East Asia, but since 2005, infection has spread rapidly westward to affect domestic poultry and wild bird populations in Asia, Africa and Europe [3,4]. Occasional transmission of H5N1 virus to humans and the continuing antigenic evolution of the virus raise the prospect of the virus adapting to humans [4—6].

It is widely accepted that vaccines (pre-pandemic and pandemic) can play a crucial role in the first-line defence against pandemic influenza [4,5,7]. Seasonal influenza vaccines currently in use against inter-pandemic strains are unlikely to be effective for use in a pandemic in an H5N1-naïve population [8]. Therefore, there is an urgent need for a safe and immunogenic H5N1 vaccine. Both the European Medicines Agency's Committee for Human Medicinal Products (CHMP) and the US Department of Health and Human Services' Food and Drug Administration (FDA) have issued guidelines to aid the rapid licensing of pandemic influenza vaccines, including proposals for developing 'mock-up' vaccines [9—13]. These prototype vaccines would establish the manufacturing process, antigenic content and adjuvant where appropriate in preparation for production of a final pandemic vaccine once the pandemic virus strains have been identified [10,13].

Influenza vaccines that can be administered before or immediately after the onset of a pandemic (so-called pre-pandemic vaccines) are likely to play a major role in substantially reducing the overall infection rate and consequent morbidity and mortality of pandemic influenza within the population [14]. The antigenic diversity of circulating H5N1 virus means that an important key requirement of a pre-pandemic vaccine is to provide cross-protective immunity against antigenically distinct viruses [15]. Non-adjuvanted H5 vaccines are poorly immunogenic and require a high antigenic content and two immunizations to elicit sufficient levels of immunity [16—22]. A two-dose regimen with alum-adjuvanted 30 μg split-virion H5N1 vaccine resulted in a greater immune response compared to its non-adjuvanted counterpart with no apparent safety issues; encouraging responses were also observed with lower antigen doses [16]. The need for high doses is of concern since, to maximize the impact of pre-pandemic vaccination at individual and collective levels, several billion doses are likely to be needed to match global demand [5]. Pre-pandemic vaccines that contain lower antigenic content (known as having an antigen-sparing characteristic) without compromising broad-spectrum immunogenicity are needed to meet demands. H5N1 vaccines incorporating modern adjuvanted technologies are key to satisfying the dual requirements of an 'antigen-sparing' strategy coupled with broad-spectrum immunogenicity.

A candidate pandemic inactivated split virus H5N1 influenza vaccine adjuvanted with a novel proprietary oil-in-water emulsion-based adjuvant system (AS) has been developed [23]. In a dose-ranging study assessing the immunogenicity of this vaccine in 394 adults (aged 18—60 years), adjuvanted doses of 3.8 μg up to 30 μg of haemagglutinin induced immune responses against the homologous recombinant vaccine strain that met or exceeded FDA and EMEA licensure criteria [23]. The 3.8 μg haemagglutinin adjuvanted dose also induced cross-immunity against heterologous strains from clade 2 (H5N1-A/Indonesia/5/2005, H5N1-A/turkey/Turkey/1/2005, A/Anhui/1/2005) in 75—85% of subjects [23,24]. Since antigen-sparing strategies are the key to increasing production capacity and meeting demands for large volumes of vaccine, the 3.8 μg HA adjuvanted antigen formulation represents a potentially promising candidate for use in a wide-scale pre-pandemic vaccination programme.

The current study was conducted to compare the safety and reactogenicity of this candidate H5N1 split-virion adjuvanted vaccine with that of the licensed seasonal influenza vaccine *Fluarix*™ in a large cohort of healthy adult volunteers, including subjects over 60 years of age. For this purpose we selected a haemagglutinin dose of 15 μg to ensure that appropriate safety data are available in the event that a higher dose of H5N1 vaccine may be required in subjects with declining immunity, such as the elderly.

Materials and methods

Study participants

This was a multicentre, randomized, observer-blind phase III clinical trial conducted in 2006, in seven European countries (Estonia, France, Germany, the Netherlands, Russia, Spain and Sweden) involving healthy male and female volunteers aged ≥18 years (e-track: 107064/107217; ClinicalTrials.gov number NCT00319098). Females of child-bearing potential were required to use adequate contraception for 30 days prior to first vaccination and for 2 months after completion of vaccination. Exclusion criteria included administration of other licensed influenza vaccines 2—4 weeks prior to the start of the study; receipt of immunosuppressants or an immunodeficiency condition; hypersensitivity to vaccines or allergy to any component of the vaccine; acute or chronic medical condition; and use of any investigational or non-registered drug or vaccine other than study vaccine within

Table 1 Comparison of vaccine safety definitions issued by the European Committee for Proprietary Medicinal Products (CPMP), the Federal Drug Administration (FDA) and those used in this study

	CPMP/EVM/EFPIA	FDA	Current study
Redness/erythema			
Grade 1	1—20 mm	25—50 mm	1—20 mm
Grade 2	21—50 mm	51—100 mm	21—50 mm
Grade 3	>50 mm	>100 mm	>50 mm
Induration	Graded as above	Not documented	Graded as above
Swelling	Graded as above	20—50 mm	Graded as above
Ecchymosis	Graded as above	Not documented	Graded as above
Local pain			
Grade 1		No interference with activity/easily tolerated	
Grade 2		Interferes with normal activity	
Grade 3		Prevents normal activity	
Fatigue/myalgia	Graded as for pain	Graded as for pain	Graded as for pain
Malaise	Graded as for pain	Not documented	Graded as for pain
Shivering	Graded as for pain	Not documented	Graded as for pain
Sweating	Graded as for pain	Not documented	Graded as for pain
Arthralgia	Graded as for pain	Not documented	Graded as for pain
Nausea/vomiting	Not included	Graded as for pain	Not included
Diarrhoea	Not included	Graded on frequency of loose stools	Not included
Temperature			
Grade 1	>38 °C	38.0—38.4 °C	37.5 to ≤38.0 °C
Grade 2		38.5—38.9 °C	38.1 to ≤39.0 °C
Grade 3		39.0—40.0 °C	>39.0 °C

30 days of start of study. The study was conducted according to Good Clinical Practice Guidelines, the 1996 Declaration of Helsinki and the local ethical rules and regulations of each country. All eligible subjects provided written informed consent before inclusion in the study.

Study vaccines

The monovalent, inactivated, split-virion influenza H5N1 vaccine was manufactured by GlaxoSmithKline (GSK) Biologicals at Sächsisches Serumwerk (SSW), in Dresden, Germany. The vaccine was developed from an H5N1 reassortant reference strain derived from the H5N1 A/Vietnam/1194/2004 strain (NIBRG-14 strain, developed by National Institute for Biological Standards and Control) and considered suitable for use as a pandemic candidate vaccine by CHMP [10]. The candidate H5N1 influenza vaccine was an adjuvant system-adjuvanted monovalent split-virion vaccine and each dose contained 15 μg of haemagglutinin. The AS is an oil-in-water emulsion-based adjuvant system manufactured by GSK (Rixensart, Belgium) [23]. The two components of the vaccine were mixed at the time of administration, which was carried out with either a 25G1 or 23G1 needle (length: 25 mm). *Fluarix*™, the control vaccine, is a licensed trivalent, inactivated, split-virion seasonal influenza vaccine that does not contain adjuvant [25—27]. It was administered with a pre-filled syringe with sealed 25G5/5 needle (length: 16 mm). It is currently registered in over 100 countries. Each 0.5 ml dose of *Fluarix*™ for the Southern Hemisphere 2006 influenza season used in the trial contained 15 μg of each of the haemagglutinin antigens of A/New Caledonia/20/99 (H1N1), A/California/7/2004 (H3N2) and B/Malaysia/2506/2004 viruses.

Study procedures

Eligible individuals were randomized into two groups to receive AS-H5N1 or *Fluarix*™/placebo with a ratio of 3:1 using a standard Statistical Analysis System (SAS® v8.02, SAS Institute Inc., Cary, NC, USA) programme with a blocking scheme to ensure balance between the treatment groups. Subjects in each group were further stratified by age: 18—60 years and over 60 years. The AS-H5N1 group received two doses of the vaccine given 21 days apart. The control group received one dose of *Fluarix*™ (day 0) and one dose of placebo (day 21). The vaccines were administered by intramuscular injection into the deltoid muscle of the non-dominant arm followed by 30 min of close observation.

The primary objective of the study was to evaluate the safety and reactogenicity of the AS-H5N1 vaccine; the secondary objective was to evaluate the immunogenicity of the AS-H5N1 influenza vaccine 21 days after each vaccination. According to protocol adverse events were defined as any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medical product. Adverse events were monitored over 7 days after each vaccination for solicited symptoms and over 21 days after the first vaccination and 30 days after the second vaccination for unsolicited symptoms. Serious adverse events were recorded prospectively throughout the study period, ending at the day 180 follow-up visit for all subjects. The

criteria used for reporting and grading adverse events were stringent and differed to varying degrees from those proposed by the EMEA Committee for Proprietary Medicinal Products (CPMP) [28], with modifications later agreed by the European Vaccine Manufacturers (EVM) and the European Federation of Pharmaceutical Industries and Associations (EFPIA) and the FDA [29] guidelines (Table 1).

The occurrence and severity of adverse events were self-recorded on diary cards provided to the participants. Solicited adverse events listed in the diary cards were divided into injection-site symptoms (pain, redness, swelling, induration and ecchymosis) and general symptoms (arthralgia, fatigue, fever, headache, muscle aches, shivering and sweating). The diameters of any injection-site redness, swelling, induration and ecchymosis and daily body temperature (axillary) were recorded. The intensities of other adverse events were recorded according to a three-grade scale: 'easily tolerated' ('on touch' for injection-site pain), 'interferes with normal activity' ('when limb is moved' for injection-site pain) and 'prevents normal activity'. All safety and reactogenicity observations were reviewed by the investigator following interviews with participants and were transcribed onto symptom sheets in the clinical study report. An assessment of causality was made by the investigator for solicited general and unsolicited adverse events. Any analgesics and/or antipyretics taken by the subject to relieve the symptoms (local and/or general) during the 21-day follow-up period after each vaccination were also recorded by the investigator.

Statistical analysis

The primary analysis was based on all vaccinated participants in the study. The sample size was based on the primary objective to evaluate the safety and reactogenicity of the AS-H5N1 vaccine. With 3600 evaluable subjects in the AS-H5N1 group, there was at least 95% chance of detecting one adverse event with an occurrence rate of 0.1%. With 1200 evaluable subjects in the *Fluarix*™ group there was a 95% chance of detecting two adverse events with an occurrence rate of 0.5%. The percentage of subjects with at least one general adverse event (solicited and unsolicited) and with any adverse event during the solicited follow-up was tabulated with exact 95% confidence intervals (95% CI) after each vaccination and overall. The same tabulations were performed for grade 3 adverse events, for adverse events related to vaccination and for grade 3 adverse events related to vaccination.

Role of funding source

GSK Biologicals was the funding source and was involved in all stages of the study conduct and analysis. GSK Biologicals also took in charge all cost associated to the development and the publishing of the present manuscript. The corresponding author had full access to the data and had final responsibility to submit for publication.

Results

Participants

From April to June 2006, the study enrolled 5075 subjects, of whom 5071 were randomized to receive either two doses of the AS-H5N1 vaccine ($n = 3802$) or one dose of *Fluarix*™ followed by one dose of placebo ($n = 1269$) (Fig. 1). The baseline characteristics of the two treatment groups were

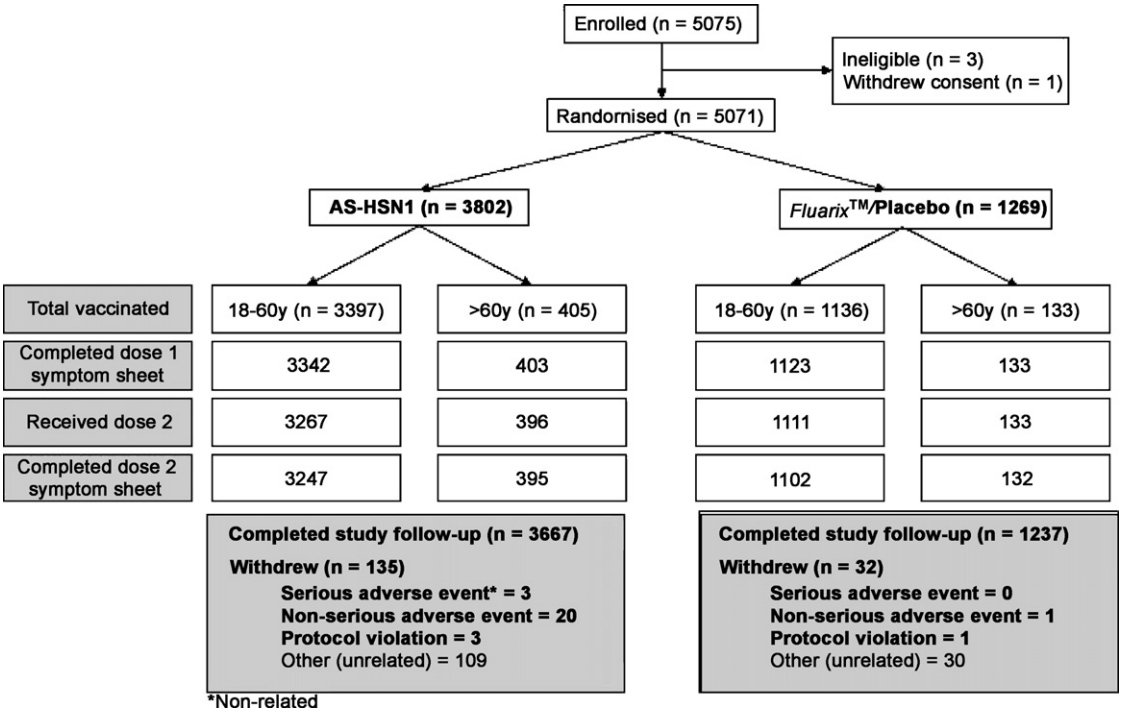

Figure 1 Subject disposition.

similar with regards to mean age, gender and ethnic distribution (Table 2). The age-stratified groups were also balanced in terms of age and gender. The overall mean age was 38.4 ± 15.4 years, 10.6% of subjects were over 60 years of age, female to male ratio was 1.39, and 95.1% of the cohort was of White Caucasian origin. A total of 167 (3.3%) subjects prematurely withdrew from the study, of whom 24 (0.5%) withdrew due to adverse events: three subjects from the AS-H5N1 group withdrew due to a serious adverse event that was considered unrelated to vaccination (one from cellulitis in the contra-lateral arm, one from inguinal hernia and one from necrotising fasculitis of the leg, possibly due to a wound infection). Twenty-one subjects reported non-serious adverse events leading to early withdrawal; these could not be distinguished from symptoms reported by participants who completed the study (Fig. 1).

Overall, 4904 of 5071 (96.7%) subjects completed the study (3667 of 3802 [96.5%] in the AS-H5N1 group and 1237/1269 [97.5%] in the *Fluarix*™/placebo group). Detailed analysis of safety relied on the completion of symptom sheets, which were completed by 5001 subjects who received dose 1 and 4876 who received dose 2.

Overall incidence of adverse events

After the first vaccination, significantly more participants reported symptoms in the AS-H5N1 group compared with the control group and this observation was noted more frequently in the younger age group (93.7% [95% CI: 92.8—94.5] versus 79.4% [95% CI: 76.9—81.8] in the 18—60 years age group ($p < 0.0001$); 76.2% [95% CI: 71.7—80.3] versus 57.1% [95% CI: 48.3—65.7] in the >60 years age group). A negligible number of participants (9 of 5071 [0.2%]) withdrew after the first dose but before the second dose due to adverse reactions. After the second dose of AS-H5N1 vaccine, the incidence of symptoms was lower compared to after the first dose (84.3% [95% CI: 83.0—85.5] in the 18—60 years age group; 69.4% [95% CI: 64.6—73.9] in the >60 years age group). In the control group, symptoms were reported by 40.2% (95% CI: 37.3—43.2) of subjects aged 18—60 years and 34.1% (95% CI: 26.1—42.8) of subjects >60 years old after placebo vaccination.

Serious adverse events up to day 51 were reported in 11 of 3802 (0.3%) and 6 of 1269 (0.5%) subjects in the AS-H5N1 and control groups, respectively. All serious adverse events were considered unrelated to vaccination by the investigators and all resolved during the study. No adverse events related to vaccination were observed after 6 months of follow-up.

Solicited local symptoms

Among solicited local symptoms, pain was the most frequently reported adverse event in both treatment groups and age-strata within the 7-day period following vaccination (Table 3). There was a significantly higher incidence of pain in subjects receiving the AS-H5N1 vaccine compared with *Fluarix*™ or placebo ($p < 0.0001$). After the first dose of AS-H5N1 vaccine, pain was reported more frequently in the 18—60 years age group (87.6%) than in the >60 years group (57.8%), but the incidence of pain was reduced in all groups following the second dose. Induration and swelling were

Table 2 Summary of demographic characteristics per age-stratum (total vaccinated cohort)

	AS-H5N1		*Fluarix*™/placebo		Total ($n = 5071$)
	18—60 ($n = 3397$)	>60 ($n = 405$)	18—60 ($n = 1136$)	>60 ($n = 133$)	
Mean age, years (S.D.)	34.8 (12.24)	67.3 (6.01) (range 61—93)	35.4 (12.51)	66.6 (5.20) (range 61—85)	38.4 (15.4)
Female, *n* (%)	2018 (59.4)	187 (46.2)	691 (60.8)	56 (42.1)	2958 (58.2)
Race					
African/African-American	47 (1.4)	1 (0.2)	6 (0.5)	0	54 (1.1)
Asian	40 (1.2)	0	15 (1.3)	0	55 (1.1)
White Caucasian/European	3212 (94.6)	396 (97.8)	1081 (95.2)	133 (100.0)	4822 (95.1)
Other	98 (2.9)	8 (2.0)	34 (3.0)	0	140 (2.8)

Table 3 Incidence of all solicited local symptoms per age stratum reported at any day 0—6 days post-vaccination

Symptom	Dose 1% (95% CI)				Dose 2% (95% CI)			
	AS-H5N1		*Fluarix*™		AS-H5N1		Placebo	
	18—60 (n = 3342)	>60 (n = 403)	18—60 (n = 1123)	>60 (n = 133)	18—60 (n = 3247)	>60 (n = 395)	18—60 (n = 1102)	>60 (n = 132)
Ecchymosis	6.3 (5.5—7.2)	4.0 (2.3—6.4)	3.5 (2.5—4.7)	2.3 (0.5—6.5)	4.4 (3.8—5.2)	4.1 (2.3—6.5)	2.8 (1.9—4.0)	2.3 (0.5—6.5)
Induration	26.2 (24.7—27.7)	18.1 (14.5—22.2)	17.7 (15.5—20.1)	12.8 (7.6—19.7)	22.6 (21.2—24.1)	19.0 (15.2—23.2)	3.7 (2.7—5.0)	3.8 (1.2—8.6)
Pain	87.6 (86.4—88.7)	57.8 (52.8—62.7)	64.5 (61.6—67.3)	27.1 (19.7—35.5)	75.5 (74.0—77.0)	50.4 (45.3—55.4)	15.7 (13.6—18.0)	6.1 (2.7—11.6)
Redness	27.2 (25.7—28.8)	27.8 (23.5—32.4)	25.0 (22.5—27.7)	25.6 (18.4—33.8)	25.2 (23.7—26.8)	26.6 (22.3—31.2)	8.4 (6.9—10.2)	12.1 (7.1—18.9)
Swelling	24.3 (22.9—25.8)	21.8 (17.9—26.2)	15.7 (13.6—17.9)	11.3 (6.5—17.9)	22.1 (20.7—23.6)	22.5 (18.5—27.0)	3.8 (2.8—5.1)	3.0 (0.8—7.6)

reported more frequently in the AS-H5N1 vaccine group than in the control group. No significant difference was detected between the treatment groups in the incidence of redness at the injection site after the first dose. Compared with induration, swelling and redness, ecchymosis was less frequent in both vaccine groups (Table 3). There was a low incidence of grade 3 local symptoms in both vaccine groups and age-strata (Table 4), although the 18—60 years age group experienced more frequent grade 3 pain, induration, redness and swelling with the AS-H5N1 vaccine than with *Fluarix*™. No differences were observed in the duration of symptoms between the two vaccine groups, which generally ranged from 2 to 3 days (apart from ecchymosis, which ranged from 4 to 6 days).

Solicited general symptoms

Most solicited general symptoms were considered to be associated with vaccination. The most commonly observed general symptoms in both the AS-H5N1 and control groups and in the two age groups were fatigue, headache and myalgia. In the AS-H5N1 group general symptoms, most notably fatigue, headache, myalgia and shivering, were more frequent in the 18—60 years age group compared with those >60 years (Table 5). This age dependency also occurred in the control group for fatigue, headache and myalgia. All general symptoms occurred more frequently in the AS-H5N1 compared with the control group, with the exception of fever and sweating, which tended to be more common with *Fluarix*™ in the >60 years age group. All general symptoms resolved within 1—7 days. In the 18—60 years age group, resolution of symptoms was similar for both the AS-H5N1 and control groups, but in older individuals (>60 years) there was a trend for symptoms to persist longer in the control group.

In both treatment groups, grade 3 solicited general symptoms were infrequent (0.2—2.7%), although the incidence of grade 3 arthralgia, fatigue, headache and myalgia was higher with AS-H5N1 than with *Fluarix*™ in the 18—60 years age group (Table 6). In contrast, in the >60 years age group, these effects tended to occur more frequently with *Fluarix*™ than with AS-H5N1.

Solicited symptoms using FDA criteria

The incidences of all local symptoms and fever using the FDA functional scale for redness, swelling and fever are summarized in Table 7. There was no significant difference in the incidence of pain as determined according to the adverse events stringent criteria defined in the protocol of the study (Table 3) and the FDA criteria (Table 7). However, using the FDA functional scale the incidences of redness, swelling and fever were lower. In the 18—60 years age group, the incidence of redness with the AS-H5N1 vaccine was reduced from 27.2 and 25.2% to 10.2 and 10.6% for doses 1 and 2, respectively. Similarly, the incidence of swelling was reduced using the FDA scale. Most notably, the incidence of fever with the AS-H5N1 vaccine was 3.8% versus 8.1% (18—60 years age group), compared with 0.7% versus 2.5% (>60 years age group) for dose 1, according to the FDA and the study criteria, respectively. For dose 2, the incidence of fever was 4.3% versus 8.6% (18—60 years age group) compared with

Table 4 Incidence of grade 3 solicited local symptoms per age stratum reported during days 0—6 post-vaccination

Symptom	Dose 1% (95% CI)				Dose 2% (95% CI)			
	AS-H5N1		*Fluarix*™		AS-H5N1		Placebo	
	18—60 (*n* = 3341)	>60 (*n* = 403)	18—60 (*n* = 1123)	>60 (*n* = 133)	18—60 (*n* = 3246)	>60 (*n* = 395)	18—60 (*n* = 1102)	>60 (*n* = 132)
Ecchymosis	0.2 (0.1—0.5)	0.2 (0.0—1.4)	0 (0.0—0.3)	0 (0.0—2.7)	0.1 (0.0—0.3)	0.3 (0.0—1.4)	0.1 (0.0—0.5)	0 (0.0—2.8)
Induration	2.9 (2.3—3.5)	1.5 (0.5—3.2)	0.9 (0.4—1.6)	0 (0.0—2.7)	2.5 (2.0—3.1)	1.8 (0.7—3.6)	0 (0.0—0.3)	0 (0.0—2.8)
Pain	5.1 (4.3—5.9)	0.7 (0.2—2.2)	0.5 (0.2—1.2)	0 (0.0—2.7)	2.4 (1.9—3.0)	0.3 (0.0—1.4)	0.2 (0.0—0.7)	0 (0.0—2.8)
Redness	3.8 (3.2—4.5)	3.0 (1.5—5.1)	1.5 (0.9—2.4)	0 (0.0—2.7)	5.2 (4.4—6.0)	6.3 (4.1—9.2)	0.1 (0.0—0.5)	0 (0.0—2.8)
Swelling	4.0 (3.4—4.7)	3.0 (1.5—5.1)	1.1 (0.6—1.9)	0.8 (0.0—4.1)	3.0 (2.4—3.6)	2.8 (1.4—4.9)	0 (0.0—0.3)	0 (0.0—2.8)

Table 5 Incidence of solicited general symptoms per age-stratum reported during days 0—6 post-vaccination

Symptom	Dose 1% (95% CI)				Dose 2% (95% CI)			
	AS-H5N1		*Fluarix*™		AS-H5N1		Placebo	
	18—60 (*n* = 3341)	>60 (*n* = 403)	18—60 (*n* = 1123)	>60 (*n* = 133)	18—60 (*n* = 3246)	>60 (*n* = 395)	18—60 (*n* = 1102)	>60 (*n* = 132)
Arthralgia	18.7 (17.4—20.1)	14.1 (10.9—17.9)	8.9 (7.3—10.7)	9.8 (5.3—16.1)	15.8 (14.6—17.1)	11.9 (8.9—15.5)	3.7 (2.7—5.0)	0 (0.0—2.8)
Fatigue	41.4 (39.7—43.1)	19.9 (16.1—24.1)	25.3 (22.8—27.9)	15.0 (9.4—22.3)	34.1 (32.5—35.8)	19.5 (15.7—23.7)	13.4 (11.5—15.6)	10.6 (5.9—17.2)
Fever	8.1 (7.2—9.1)	2.5 (1.2—4.5)	2.1 (1.4—3.2)	4.5 (1.7—9.6)	8.6 (7.6—9.6)	4.1 (2.3—6.5)	1.4 (0.8—2.2)	3.8 (1.2—8.6)
Headache	34.7 (33.1—36.3)	19.6 (15.8—23.8)	24.5 (22.0—27.1)	13.5 (8.2—20.5)	28.8 (27.2—30.4)	17.5 (13.9—21.6)	14.1 (12.1—16.3)	6.1 (2.7—11.6)
Myalgia	40.0 (38.3—41.7)	23.1 (19.1—27.5)	21.3 (18.9—23.8)	9.0 (4.7—15.2)	32.2 (30.6—33.8)	18.2 (14.5—22.4)	6.1 (4.7—7.7)	7.6 (3.7—13.5)
Shivering	14.4 (13.3—15.7)	3.5 (1.9—5.8)	6.0 (4.7—7.5)	2.3 (0.5—6.5)	12.0 (10.9—13.2)	3.8 (2.1—6.2)	2.2 (1.4—3.2)	3.8 (1.2—8.6)
Sweating	14.4 (13.2—15.6)	9.4 (6.8—12.7)	8.5 (6.9—10.2)	12.8 (7.6—19.7)	13.6 (12.5—14.8)	10.4 (7.6—13.8)	5.4 (4.1—6.9)	9.8 (5.3—16.3)

Table 6 Incidence of grade 3 solicited general symptoms per age-stratum reported during days 0—6 post-vaccination

Symptom	Dose 1% (95% CI)				Dose 2% (95% CI)			
	AS-H5N1		*Fluarix*™		AS-H5N1		Placebo	
	18—60 (*n* = 3341)	>60 (*n* = 403)	18—60 (*n* = 1123)	>60 (*n* = 133)	18—60 (*n* = 3246)	>60 (*n* = 395)	18—60 (*n* = 1102)	>60 (*n* = 132)
Arthralgia	1.4 (1.0—1.9)	0.2 (0.0—1.4)	0.3 (0.1—0.8)	0.8 (0.0—4.1)	1.4 (1.0—1.9)	0.8 (0.2—2.2)	0.2 (0.0—0.7)	0 (0.0—2.8)
Fatigue	2.7 (2.2—3.3)	0.7 (0.2—2.2)	0.4 (0.1—0.9)	1.5 (0.2—5.3)	2.5 (2.0—3.1)	0.5 (0.1—1.8)	0.5 (0.1—1.8)	0.8 (0.0—4.1)
Fever	0.2 (0.1—0.4)	0 (0.0—0.9)	0 (0.0—0.3)	0 (0.0—2.7)	0.3 (0.1—0.6)	0.3 (0.0—1.4)	0 (0.0—0.3)	0 (0.0—2.8)
Headache	1.8 (1.4—2.3)	0.2 (0.0—1.4)	0.5 (0.2—1.2)	1.5 (0.2—5.3)	1.8 (1.4—2.3)	0.5 (0.1—1.8)	0.7 (0.3—1.4)	0 (0.0—2.8)
Myalgia	2.3 (1.8—2.9)	0.2 (0.0—1.4)	0.4 (0.1—1.0)	1.5 (0.2—5.3)	1.9 (1.4—2.4)	1.0 (0.3—2.6)	0.5 (0.1—1.1)	0 (0.0—2.8)
Shivering	1.0 (0.7—1.4)	0 (0.0—0.9)	0.2 (0.0—0.6)	0 (0.0—2.7)	0.9 (0.6—1.3)	0.3 (0.0—1.4)	0 (0.0—0.3)	0 (0.0—2.8)
Sweating	0.5 (0.3—0.8)	0.7 (0.2—2.2)	0.1 (0.0—0.5)	0.8 (0.0—4.1)	1.0 (0.7—1.4)	1.8 (0.7—3.6)	0.2 (0.0—0.7)	0.8 (0.0—4.1)

Table 7 Incidence of solicited local symptoms[a] and fever reported during days 0—6 post-vaccination using the FDA functional scale

Symptom	Dose 1% (95% CI)				Dose 2% (95% CI)			
	AS-H5N1		*Fluarix*™		AS-H5N1		Placebo	
	18—60 (*n* = 3341)	>60 (*n* = 403)	18—60 (*n* = 1123)	>60 (*n* = 133)	18—60 (*n* = 3246)	>60 (*n* = 395)	18—60 (*n* = 1102)	>60 (*n* = 132)
Pain	87.6 (86.4—88.7)	57.8 (52.8—62.7)	64.5 (61.6—67.3)	27.1 (19.7—35.5)	75.5 (74.0—77.0)	50.4 (45.3—55.4)	15.7 (13.6—18.0)	6.1 (2.7—11.6)
Redness	10.2 (9.2—11.2)	10.7 (7.8—14.1)	6.9 (5.5—8.6)	6.0 (2.6—11.5)	10.6 (9.6—11.7)	13.4 (10.2—17.2)	0.5 (0.1—1.1)	1.5 (0.2—5.4)
Swelling	11.1 (10.1—12.2)	10.7 (7.8—14.1)	5.6 (4.3—7.1)	3.8 (1.2—8.6)	9.1 (8.1—10.1)	8.6 (6.0—11.8)	0.5 (0.2—1.2)	0.8 (0.0—4.1)
Fever	3.8 (3.2—4.5)	0.7 (0.2—2.2)	1.1 (0.6—1.9)	2.3 (0.5—6.5)	4.3 (3.6—5.0)	2.3 (1.0—4.3)	0.5 (0.1—1.1)	0.0 (0.0—2.8)

[a] Ecchymosis and induration are not documented under FDA guidelines.

2.3% versus 4.1% (>60 years age group), according to the FDA and the study criteria, respectively.

Unsolicited symptoms

In both age-strata, the incidence of unsolicited symptoms within 21 days of the first dose or 30 days of the second dose was similar in both the AS-H5N1 and control groups; 1570 of 6664 (23.6% [95% CI: 22.5—24.6]), 244 of 1136 (21.5% [95% CI: 19.1—24.0]) and 180 of 1111 (16.2% [95% CI: 14.1—18.5]) in the 18—60 years age group and 151 of 801 (18.9% [95% CI: 16.2—21.7]), 19 of 133 (14.3% [95% CI: 8.8—21.4]) and 23 of 133 (17.2% [95% CI: 11.3—24.8]) in the >60 years age group for the AS-H5N1, *Fluarix*™ and placebo vaccinations, respectively. The most common unsolicited symptoms were injection-site pruritus (1.8% of the AS-H5N1 and 0.8% of the *Fluarix*™ groups), malaise (1.7% of the AS-H5N1 and 0.5% of the *Fluarix*™ groups) and nausea (2.1% of the AS-H5N1 and 0.9% of the *Fluarix*™ groups). Local lymph node swelling (lymphadenopathy), lymph node pain and lymphadenitis were occasionally observed in the AS-H5N1 group (1.35% after dose 1 and 0.86% after dose 2). Vaccine-related unsolicited grade 3 adverse events were observed at a similar frequency in both groups (3.5% >60 years, 5.8% 18—60 years in the AS-H5N1 group and 3.0% >60 years and 5.6% 18—60 years in the *Fluarix*™/placebo group).

Discussion

This phase III study of a large cohort of 5071 participants focused on safety and tolerability following vaccination with two doses of an AS-adjuvanted candidate H5N1 vaccine containing 15 μg haemagglutinin in comparison with one dose of a licensed seasonal influenza vaccine (*Fluarix*™) followed by one dose of placebo. The immunogenicity findings (data not shown) were consistent with those from a previous study [23]. The AS-H5N1 vaccine had a good safety profile despite the use of antigenic content four times higher than the lowest dose found to be fully immunogenic in a dose-ranging study in adults aged 18—60 years [23]. The majority of vaccinated subjects experienced some vaccine-related adverse events, but these were of mild to moderate intensity and of short duration. There were no serious adverse events related to vaccination and the majority (96.7%) of participants in the AS-H5N1 group completed the study. Less than 1% of subjects withdrew from the study with adverse events and there were no withdrawals due to serious adverse events related to vaccination. The unsolicited adverse event profile was consistent with expectations for the study population and was similar between AS-H5N1 and control groups.

The incidences of all solicited adverse events were higher with the adjuvanted H5N1 vaccine than in the control group. As observed in other studies of adjuvanted H5N1 vaccines [16,19,22], the most common symptom in all treatment groups was pain at the injection site, although the incidence was significantly higher in the AS-H5N1 group. The incidence of grade 3 adverse events was low in the AS-H5N1 group, although they occurred more frequently in the 18—60 years age group. Grade 3 adverse events were of short duration and resolved within 3 days in most instances. A decrease in adverse events was observed on second vaccination compared with first dose. A negligible number of participants (9 of 5071 [0.2%]) withdrew after the first dose but before the second dose due to adverse reactions. Many of the symptoms, including ecchymosis, induration, pain, fatigue, fever, headache, myalgia and shivering, were more common in the 18—60 years age group. The lower reactogenicity reported for the vaccine in the elderly (>60 years) population as compared to younger adults (18—60 years) is consistent with other published studies [26,27].

In general, the safety profile of the candidate adjuvanted H5N1 vaccine is similar to those of other adjuvanted vaccines [16,19,22]. The use of adjuvants to boost the immunogenicity of influenza vaccines has previously been reported to result in more frequent injection-site reactions [16,19,20,30]. In phase I studies with MF59-adjuvanted H5N3 and H9N2 vaccines, an increase in injection-site pain associated with the adjuvant was observed [20,30]. More extensive clinical experience with MF59-adjuvanted vaccines showed that these induced more symptoms than conventional non-adjuvanted seasonal influenza vaccines [31].

There is a lack of standardization in the reporting of vaccine safety and reactogenicity across studies and across regions. Guidelines for the evaluation of vaccine toxicity issued by the CPMP [28] and the FDA [29] differ in their definition and grading of adverse events. Comparison of safety and reactogenicity data across studies with different candidate pandemic vaccines would benefit from a common approach. In the absence of standardized criteria, we have reported data analysed according to the most rigorous definitions, using GSK in-house criteria, which are similar to those agreed between CPMP and European Vaccine Manufacturers in association with the European Federation of Pharmaceutical Industries Association. In order to facilitate comparison with other studies we also analysed solicited local symptoms according to the FDA criteria. Using the FDA grading for redness, swelling and fever, the incidence of these symptoms was decreased by 50% in the AS-H5N1 vaccine group.

Safety assessment of vaccines under development for pandemic use must carefully weigh the risk—benefit balance in the context of potentially high mortality from the disease. Increased adverse events observed with MF59-adjuvant seasonal influenza vaccines were considered to be clinically acceptable for annual vaccination of the elderly in an inter-pandemic context [31]. Given the substantial benefit of an effective influenza vaccine in the face of a high risk of serious illness and mortality during a pandemic, some increase in transient adverse effects could be balanced by an increase in immunogenicity, improving protection and increasing coverage through dose reduction. In the current study, the safety and reactogenicity profile of the 15 μg HA dose of candidate adjuvanted H5N1 vaccine, determined according to the stringent criteria applied in this large clinical trial, can be considered clinically acceptable in the context of its use against pandemic influenza.

Acknowledgements

The authors are indebted to all the investigators, clinicians, nurses and laboratory technicians at the study sites, in particular—*Estonia*: Dr. Airi Poder (Tartu University Clinics,

Tartu, Estonia) and Dr. Kai Zilmer (West Tallinn Central Hospital, Tallinn, Estonia). *France*: Dr. Anne Kervella (Medicis/Larime Lagord, Lagord, France), Dr. David Gendre (Medicis/Larime Poitiers, Poitiers, France), Dr. Serge Fitoussi (Medicis/Cémax, Rouen, France), Dr. Evelyne Guenole (Therapharm recherches, Caen Cedex 4, France), Dr. Francois Raffi (CHU Hôtel Dieu, Nantes, France), Dr. L. Alavoine and Dr. A. Ankotche (Inserm CIC007, France). *Germany*: Dr. Helen Arievich (Medars GmbH, Berlin, Germany), Dr. Tilman Dreykluft (Klinische Forschung, Berlin, Germany), Dr. Alexander Deines (Kfh-Klinische Forschung, Hannover, Germany), Dr. Christine Grigat (Clinical Research Institute, Hamburg, Germany), Dr. Susanne Mindt-Pruefert (Klinische Forschung, Hamburg, Germany), Dr. Thomas Horacek (KliFo Center GmbH, Witten, Germany), Prof. Dr. Wolfgang Jilg (Universitätsklinik, Regensburg, Germany), Dr. Olga Maus (Zentrum für Therapiestudien, Leipzig, Germany), Dr. Hans-Detlev Stahl (ClinPharm International GmbH & Co. KG, Leipzig, Germany), Dr. Reiner Lehmann (Klinische Forschung Berlin-Mitte, Berlin, Germany), Dr. Anton Strobel (MTS Meditel GmbH, München, Germany), Dr. Elke Witczak (ClinPharm International GmbH, Dresden, Germany), Dr. Han Folesky (CRU Berlin, Berlin, Germany) and Dr. Hanns-Gerd Dammann (Klinische Forschung Schwerin, Schwerin, Germany). *The Netherlands*: Dr. Michiel van Beek (Vaxinostics, Rotterdam, the Netherlands), Dr. Nanoesjka van Gorcum (Vaxinostics, Rotterdam, the Netherlands), Dr. Tessa Pols (Vaxinostics, Rotterdam, the Netherlands), Mr. Hendrik-Jan Vos (Vaxinostics, Rotterdam, the Netherlands), Dr. Henk Visser (Municipal Public Health Service Rotterdam-Rijnmond, Rotterdam, the Netherlands) and Mr. Sander van Ravensteijn (Municipal Public Health Service Rotterdam-Rijnmond, Rotterdam, the Netherlands). *Russian Federation*: Dr. Olga Reshetko (City out-patient clinic N20, Saratov, Russian Federation), Dr. Tatana Golosova (City Hospital No. 5, Novokuznetsk, Russian Federation) and Dr. Vitaliy Fomin (Ekaterinburg Clinical Hospital No. 40, Ekaterinburg, Russian Federation). *Spain*: Dr. Teresa del Campo (Fundación Jimenez Diaz, Madrid, Spain), Dr. Antonio Salazar (C.S. Pública de Valencia, Valencia, Spain) and Dr. Enrique Ortega (Consorcio Hospital Universitario de Valencia, Valencia, Spain). *Sweden*: Dr. Erik Ekwall (Infektionskliniken, Karolinska Universitetssjukhuset Huddinge, Stockholm, Sweden). We also appreciate support and contributions throughout the study of the sponsor's project staff, in particular Astrid Borkowski for study management, Pilar García-Corbeira, Ruprecht Schmidt-Ott, Leif Wannerud, Nathalie Clyti, Rogier Doets, Paul de Jonge, Mikhail Sherbakov, Sven Tarum for study coordination and Yamina Lainé for preparation of the study protocol and related study documentation. Finally we would like to thank Meditech Media Ltd., UK for editorial assistance in preparing the manuscript and Isabelle Camby (XPePharma SA, Belgium) for coordination.

This study was supported by GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium.

References

[1] World Health Organization. Responding to the avian influenza pandemic threat: recommended strategic actions. WHO; 2005. Available at http://www.who.int/csr/resouces/publications/influenza/WHO_CDS_CSR_GIP-05-8-EN.pdf [accessed February 2008].

[2] World Health Organization. Cumulative number of confirmed cases of avian influenza A/(H5N1) reported to WHO. Available at http://www.who.int/csr/disease/avian_influenza/country/cases_table_2007_10_12/en/index.html [accessed February 2008].

[3] Webster RG, Peiris M, Chen H, Guan Y. H5N1 outbreaks and enzootic influenza. Emerg Infect Dis 2006;12(1):3—8.

[4] Webster RG, Govorkova EA. H5N1 influenza—continuing evolution and spread. N Engl J Med 2006;355(21):2174—7.

[5] Osterhaus AD. Pre- or post-pandemic influenza vaccine? Vaccine 2007;25(27):4983—4.

[6] Yang Y, Halloran ME, Sugimoto JD, Longini Jr IM. Detecting human-to-human transmission of avian influenza A (H5N1). Emerg Infect Dis 2007;13(9):1348—53.

[7] Subbarao K, Joseph T. Scientific barriers to developing vaccines against avian influenza viruses. Nat Rev Immunol 2007;7(4):267—78.

[8] Wood JM. Selection of influenza vaccine strains and developing pandemic vaccines. Vaccine 2002;20(Suppl 5):B40—4.

[9] European Committee for Medicinal Products for Human Use (CHMP). Guidance on influenza vaccines prepared from viruses with the potential to cause a pandemic and intended for use outside of the core dossier content. EMEA/CHMP/VWP/263499/2006; 2007.

[10] European Committee for Proprietary Medicinal Products (CPMP). Guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application. CPMP/VEG/4717/03; 2004.

[11] European Committee for Medicinal Products for Human Use (CHMP). Guidance on clinical evaluation of new vaccines. EMEA/CHMP/VWP/164653/2005; 2005.

[12] European Committee for Medicinal Products for Human Use (CHMP). Concept paper on the revision of guideline on dossier structure and content for pandemic influenza marketing authorisation application (CPMP/VEG/4717/03). EMEA/CHMP/166042/2007; 2005.

[13] US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for industry: clinical data needed to support the licensure of pandemic influenza vaccines. Available at http://www.fda.gov/cber/gdlns/panfluvac.htm [accessed February 2008].

[14] Poland GA. Vaccines against avian influenza—a race against time. N Engl J Med 2006;354(13):1411—3.

[15] Stephenson I, Gust I, Pervikov Y, Kieny MP. Development of vaccines against influenza H5. Lancet Infect Dis 2006;6(8):458—60.

[16] Bresson JL, Perronne C, Launay O, Gerdil C, Saville M, Wood J, et al. Safety and immunogenicity of an inactivated split-virion influenza A/Vietnam/1194/2004 (H5N1) vaccine: phase I randomised trial. Lancet 2006;367(9523):1657—64.

[17] Hehme N, Engelmann H, Künzel W, Neumeier E, Sänger R. Pandemic preparedness: lessons learnt from H2N2 and H9N2 candidate vaccines. Med Microbiol Immunol 2002;191(3—4):203—8.

[18] Hehme N, Engelmann H, Kuenzel W, Neumeier E, Saenger R. Immunogenicity of a monovalent, aluminum-adjuvanted influenza whole virus vaccine for pandemic use. Virus Res 2004;103(1—2):163—71.

[19] Lin J, Zhang J, Dong X, Fang H, Chen J, Su N, et al. Safety and immunogenicity of an inactivated adjuvanted whole-virion influenza A (H5N1) vaccine: a phase I randomised controlled trial. Lancet 2006;368(9540):991—7.

[20] Nicholson KG, Colegate AE, Podda A, Stephenson I, Wood J, Ypma E, et al. Safety and antigenicity of non-adjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a randomised trial of two potential vaccines against H5N1 influenza. Lancet 2001;357(9272):1937—43.

[21] Treanor JJ, Wilkinson BE, Masseoud F, Hu-Primmer J, Battaglia R, O'Brien D, et al. Safety and immunogenicity of a recombinant hemagglutinin vaccine for H5 influenza in humans. Vaccine 2001;19(13—14):1732—7.
[22] Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. N Engl J Med 2006;354(13):1343—51.
[23] Leroux-Roels I, Borkowski A, Vanwolleghem T, Drame M, Clement F, Hons E, et al. Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine. Lancet 2007;370(9587):580—9.
[24] Leroux-Roels I, Bernhard R, Gérard P, Dramé M, Hanon E, Leroux-Roels G. Broad clade 2 cross-reactive immunity induced by an adjuvanted clade 1 rH5N1 pandemic influenza vaccine. PLoS One 2008;3(2):e1665.
[25] Treanor JJ, Campbell JD, Brady RC, Keitel WA, Drame M, Jain VK, et al. Rapid licensure of a new, inactivated influenza vaccine in the United States. Hum Vaccin 2005;1(6):239—44.
[26] Hehme NW, Künzel W, Petschke F, Turk G, Raderecht C, van Hoeke C, et al. Ten years of experience with the trivalent split-influenza vaccine, *Fluarix*™. Clin Drug Invest 2002;22(11):751—69.
[27] Rose GW, Cooper CL. *Fluarix*™, inactivated split-virus influenza vaccine. Expert Opin Biol Ther 2006;6(3):301—10.
[28] European Committee for Proprietary Medicinal Products (CPMP). Note for guidance on harmonisation of requirements for influenza vaccines. CPMP/BWP/214/96; 1997.
[29] US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials (Draft guidance 2005). Available at http://www.fda.gov/cber/gdlns/toxvac.htm [accessed February 2008].
[30] Banzhoff A, Nacci P, Podda A. A new MF59-adjuvanted influenza vaccine enhances the immune response in the elderly with chronic diseases: results from an immunogenicity meta-analysis. Gerontology 2003;49(3):177—84.
[31] Atmar RL, Keitel WA, Patel SM, Katz JM, She D, Sahly H, et al. Safety and immunogenicity of nonadjuvanted and MF59-adjuvanted influenza A/H9N2 vaccine preparations. Clin Infect Dis 2006;43(9):1135—42.