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Immunogenicity and safety of an AS03-adjuvanted H5N1 pandemic influenza vaccine in Korean adults: A phase IV, randomized, open-label, controlled study

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ABSTRACT

Background: AS03-adjuvanted H5N1 pandemic influenza vaccines have been assessed in an extensive clinical development program conducted in North America, Europe, and Asia including children from 6 months of age, adults, and elderly adults. We evaluated AS03-H5N1 in Korean adults 18 through 60 years of age.

Methods: This Phase IV, randomized, study was conducted to assess the immunogenicity, reactogenicity, and safety of two doses (3.75 μg of hemagglutinin antigen) of A/Indonesia/5/2005 (H5N1) adjuvanted with AS03 given 21 days apart in Korean adults. Antibody responses were assessed using hemagglutination-inhibition (HI) assays against the vaccine strain and a vaccine-heterologous strain (A/Vietnam/1194/2004) 21 days after the second dose. A control group (safety) received a licensed seasonal inactivated trivalent influenza vaccine (TIV). Reactogenicity was assessed for 7 days after each vaccination, and unsolicited adverse events were assessed for 182 days following vaccination in both study groups (NCT01730378).

Results: AS03-H5N1 was immunogenic and elicited robust HI antibody responses with seroconversion rates of 100% for the vaccine strain and 69.1% for the heterologous strain ($N = 81$). HI antibody responses fulfilled the European licensure criteria for immunogenicity (primary endpoint). The incidence of local and systemic solicited adverse events (reactogenicity) was higher with AS03-H5N1 than TIV. There was no apparent difference in the rate of unsolicited adverse events in the AS03-H5N1 and TIV groups.

Conclusion: The results indicate that AS03-H5N1 vaccine is immunogenic with reactogenicity and safety findings that are consistent with the established profile of AS03-H5N1 vaccine.

1. Introduction

The World Health Organization (WHO) reported 667 human cases of avian-origin H5N1 infection from 2003 to July 2014, of which 393 were fatal [1]. The unpredictable nature of avian-origin H5N1 influenza should not be underestimated, and the development of vaccines against influenza viruses with pandemic-potential is a public health priority.

GSK Vaccines has produced H5N1 vaccines containing the A/Vietnam or A/Indonesia antigen formulated with the oil-in-water Adjuvant System, AS03. The AS03-adjuvanted H5N1 vaccines (AS03-H5N1) are manufactured at sites in Dresden, Germany,

Abbreviations: AEs, adverse events; CBER, US Center for Biologics Evaluation and Research; CHMP, Committee for Medicinal Products for Human Use; CI, confidence interval; EMA, European Medicines Agency; FDA, US Food and Drug Administration; GMT, geometric mean titer; HA, hemagglutinin antigen; HI, hemagglutination-inhibition; MAEs, medically-attended adverse events; MGI, mean geometric increase; pIMDs, potentially immune-mediated diseases; SAE, serious adverse event; SCR, seroconversion rate; SPR, seroprotection rates; WHO, World Health Organization.

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and Quebec, Canada, and are licensed in Europe and the US (*Prepandrix*™; *Adjupanrix*™; *Pumarix*™; Q-Pan H5N1 influenza vaccine) [2,3]. AS03-H5N1 vaccines have been assessed in large studies in North America, Europe, and Asia, including children from 6 months of age, adults, and elderly adults [4–10].

Pivotal Phase 3 trials in European and Asian adults showed that two doses of AS03-H5N1 (A/Vietnam/1194/2004) vaccine containing 3.75 μg of hemagglutinin antigen (HA) was more immunogenic than non-adjuvanted vaccines [4,8,11]. Across the clinical development program, AS03-H5N1 vaccines have been shown to elicit strong, durable, cross-clade immune responses [4–8]. Furthermore, a long-term extension phase of the Asian study conducted in Taiwan, Thailand, Singapore, and Hong Kong, suggested that vaccinated populations could potentially be protected for up to three years after vaccination, which is likely to far exceed the peak of pandemic transmission [12]. The results of the long-term study showed that AS03-H5N1 vaccine may be used according to flexible prime–boost vaccination schedules, with strong cross-clade anamnestic antibody responses observed after one dose of AS03-H5N1 heterologous booster vaccine given at 6, 12, or 36 months after priming with two doses of AS03-H5N1 vaccine [12].

This Phase IV, open-label study was conducted to assess the immunogenicity and safety of a two-dose schedule of AS03-H5N1 (A/Indonesia/5/2005) vaccine in Korean adults.

2. Methods

2.1. Design and objectives

This Phase IV, randomized, open-label study evaluated the immunogenicity, reactogenicity, and safety of a two-dose primary vaccination series of AS03-H5N1 (A/Indonesia/5/2005) vaccine in adults. A safety control group received one dose of seasonal inactivated trivalent influenza vaccine (TIV). The study was multi-center and conducted in the Republic of Korea.

The main immunogenicity objective (primary outcome) was to assess if two doses of AS03-H5N1 vaccine elicited hemagglutination-inhibition (HI)-based immune responses against the vaccine strain (A/Indonesia/5/2005) which fulfilled the European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) licensure criteria for the approval of pandemic influenza vaccines [13]. The main safety objective (secondary outcome) was to evaluate solicited adverse events (AEs) and unsolicited AEs in the AS03-H5N1 group and the TIV control group.

Men and women were eligible for inclusion if they were 18 through 60 years of age at the first vaccination. Subjects were required to be in good general health with no acute illness and controlled chronic conditions. Subjects could not have received any seasonal or pandemic influenza vaccine within six months before study vaccination or during the study period. Women of child bearing potential were required to use reliable contraception.

All protocols and study documentation were approved by independent/local ethics committees in accordance with Good Clinical Practice, the Declaration of Helsinki, and regulatory requirements. (ClinicalTrials.gov NCT01730378). Subjects provided informed written consent.

2.2. Vaccines and randomization

The study vaccine was an H5N1 inactivated, split-virion recombinant influenza vaccine manufactured by GSK Vaccines in Dresden, Germany. Each dose of vaccine contained 3.75 μg HA of A/Indonesia/05/2005 adjuvanted with AS03, an oil-in-water emulsion based Adjuvant System containing 11.86 mg of α-tocopherol. The control vaccine was a licenced TIV for seasonal influenza (*Fluarix*™, GSK Vaccines) containing 15 μg of each HA that was recommended by the WHO for the 2012/13 influenza season in the Northern Hemisphere: A/Christchurch/16/10 (H1N1), A/Victoria/361/2011 (H3N2), and B/Hubei-Wujiagang/158/2009 (Yamagata lineage influenza B strain). The lot numbers were AFLSA340A (H5N1), AA03A209C (AS03), and AFLUA696A (*Fluarix*™).

Subjects were scheduled to receive two doses 21 days apart of AS03-H5N1 or one dose of TIV control vaccine, which were administered open-label in the deltoid muscle. Randomization was performed by GSK Vaccines (Rixensart, Belgium) using a blocking scheme developed in SAS® (Cary, NC, USA). Vaccines were allocated at each study site using an internet-based randomization system. Subjects were randomized 2:1 to receive AS03-H5N1 or control TIV, and a minimisation procedure was used to account for center, age strata (about 1:1 for 18–40 years and 41–60 years), and history of seasonal influenza vaccination and/or A(H1N1)pdm09 vaccine in preceding three seasons.

2.3. Immunogenicity assessments

Blood samples were taken for the evaluation of immune responses on Day 0, 21, and 42 in the AS03-H5N1 vaccine group (before and 21 days after each dose), and on Day 0 and 21 in the TIV group. In the AS03-H5N1 vaccines group, HI assays were performed using an established HI method, modified for equine rather than avian erythrocytes [14–16]. In the TIV group, HI assays against the three vaccine strains were measured using a validated method as previously described [17]. All serological testing was performed at a central GSK Vaccines laboratory.

The primary endpoint was the measurement of HI antibodies against A/Indonesia/5/2005 at Day 42 (21 days after the second vaccination) to evaluate whether two doses AS03-H5N1 vaccine elicited immune responses that fulfilled the CHMP licensure criteria for immunogenicity [13]. Secondary immunogenicity endpoints were to assess if two doses of vaccine fulfilled the US Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) licensure criteria for immunogenicity [18], and to assess HI antibody responses after one dose of AS03-H5N1 vaccine. Tertiary immunogenicity analyses were the assessment of Day 42 HI antibody responses against a vaccine-heterologous strain (A/Vietnam/1194/2004), and vaccine-homologous responses according to age (18–40 years or 41–60 years), and according to previous vaccination history (vaccinated or not vaccinated against seasonal or A(H1N1)pdm09 influenza during the previous three seasons). HI immune responses to the three strains in the TIV control vaccine were also assessed.

HI antibody parameters were Geometric Mean Titre (GMT), seroconversion rate (SCR; defined as percentage of subjects achieving an increase in HI titers from <1:10 to ≥1:40 or at least a 4-fold post-vaccination increase in HI titer from a pre-vaccination titer ≥1:10), seroprotection rate (SPR; percentage of subjects with HI titers ≥1:40 following vaccination), and Mean Geometric Increase (MGI; geometric mean of the ratio between post-vaccination and pre-vaccination reciprocal HI titers). Subjects with HI antibody titers of ≥1:10 were considered to be seropositive.

2.4. Reactogenicity and safety assessments

The secondary reactogenicity and safety endpoints were assessed in both study groups.

Solicited local and general symptoms were assessed during the 7-day post-vaccination period after each dose. Subjects recorded the occurrence and severity of solicited events on diary cards. Local (injection site) symptoms were pain, redness, and swelling,

and general symptoms were fatigue, fever, gastrointestinal symptoms, headache, joint pain, muscle aches, shivering, sweating. Fever was defined as a temperature of ≥38.0 °C (≥100.4 °F) by any route or method. All solicited local events were considered to be vaccine-related, and investigators provided causality assessments for solicited general events.

Unsolicited AEs were recorded for 21 days after each dose of AS03-H5N1 vaccine and after the single dose of TIV control vaccine. Unsolicited AEs were also recorded from Day 0 to Day 84 in the AS03-H5N1 and TIV control groups. Serious adverse events (SAEs), medically-attended adverse events (MAEs) and potential immune-mediated diseases (pIMDs) were recorded from Day 0 to Day 184. Unsolicited events were coded using the Medical Dictionary for Regulatory Activities and investigators provided causality assessments for all adverse events.

2.5. Analyses

The sample size power calculation was based on a previous study of two doses of AS03-H5N1 vaccine in 155 subjects 18 through 60 years of age in which the SPR and SCR was 98.7% and the MGI was 121.9 [19]. We calculated that a sample size of 68 evaluable subjects would allow for at least 95% power to demonstrate the primary endpoint. Allowing for a 20% drop-out or protocol violation rate, the target population was 84 subjects in the AS03-H5N1 group.

Immunogenicity data were summarized with 95% Confidence Intervals (CIs). The CHMP licensure criteria were fulfilled if the point estimate for SCR was >40%, SPR was >70%, and MGI was >2.5 [13]. CBER licensure criteria were fulfilled if the lower limits of the 95% CI for SCR was ≥40% and for SPR was ≥70% [18]. Immunogenicity was described in the per-protocol immunogenicity cohort including subjects who met the eligibility criteria, complied with the protocol, and for whom data were available at the specified evaluation time point.

Reactogenicity and safety data were tabulated with 95% CIs and were based on the total vaccinated cohort including all subjects who received at least one dose of either AS03-H5N1 or TIV.

3. Results

A total of 131 subjects were enrolled, including 84 and 47 in the AS03-H5N1 vaccine and TIV control groups, respectively (Fig. 1). The mean age (median; range) in the AS03-H5N1 vaccine group was 39.3 years (40.5 years; 18–59 years) and in the TIV control group was 40.4 years (41.0 years; 20–58 years). In the AS03-H5N1 and TIV groups, 72.6% and 72.3% of subjects, respectively, were women. All subjects were Korean with East Asian heritage. An overview of the analysis groups and reasons for withdrawal is shown in Fig. 1. The first subject was enrolled in December 2012 and the last study contact was in December 2013.

3.1. Immunogenicity

A summary of the HI antibody responses is shown in Fig. 2. Pre-vaccination, 4.9% of the AS03-H5N1 group was seropositive for the vaccine strain (A/Indonesia/5/2005). On Days 21 and 42 in the AS03-H5N1 group, the vaccine-homologous GMTs were 31.8 and 300.1, respectively. The SPR, SCR, and MGI at Day 42 fulfilled the CHMP thresholds for immunogenicity and the SPR and SCR fulfilled the CBER thresholds for immunogenicity (Fig. 2). At baseline, 65.4% of the AS03-H5N1 group were seropositive for the vaccine-heterologous strain (A/Vietnam/1194/2004). The Day 42 GMT against the heterologous strain was 54.2, and the SCR and SPR were >69.1% (Table 1).

HI responses fulfilled the primary endpoint regardless of age. In both age groups, the Day 42 vaccine-homologous SPR and SCR were 100% (Table 2). In the subgroup analysis, taking into account seasonal influenza vaccination history, the Day 42 vaccine-homologous SPR and SCR was 100% in subjects with and without influenza vaccination (Table 3). Pre-vaccination GMTs were similar

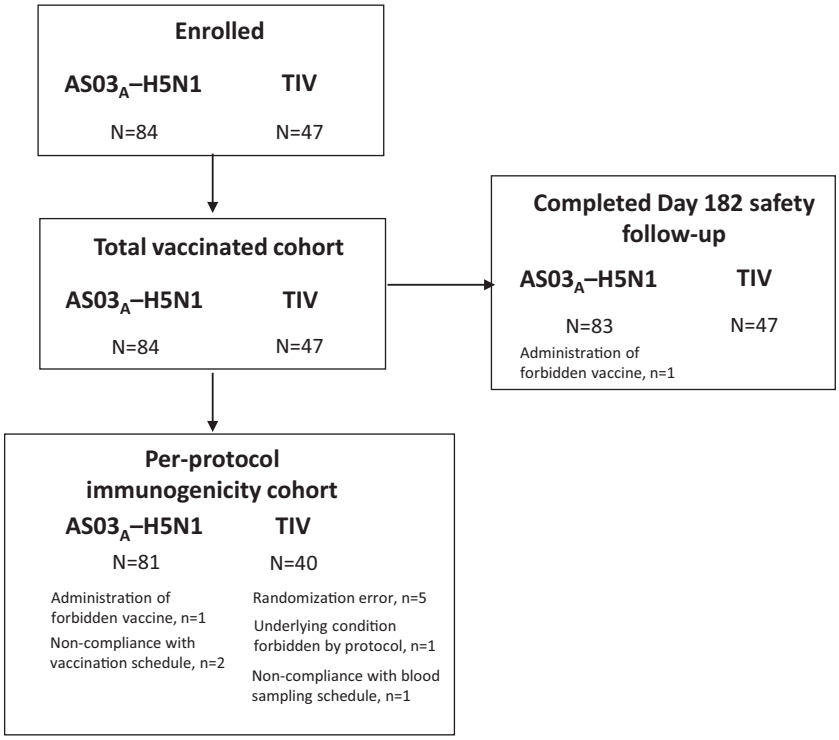

Fig. 1. Subjects flow.

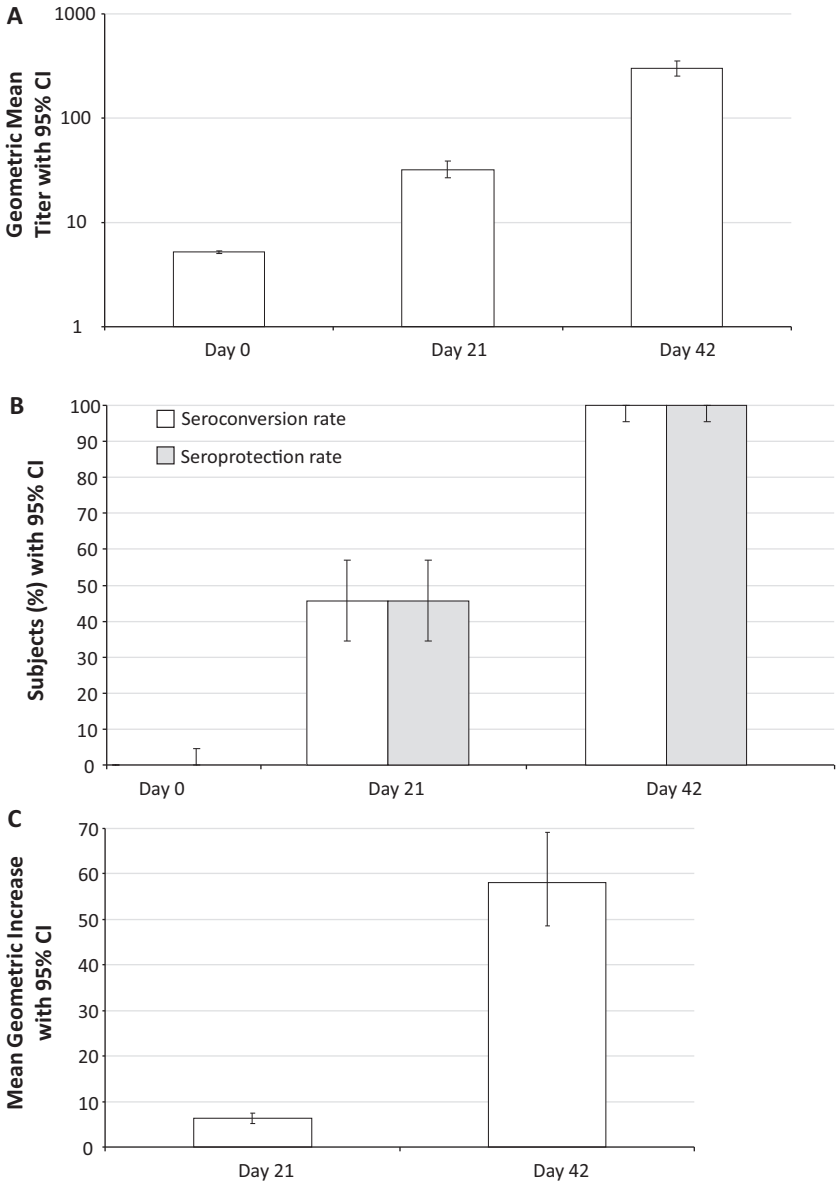

Fig. 2. Hemagglutination-inhibition antibody responses against the vaccine strain (A/Indonesia/5/2005) based on geometric mean titers (A), seroconversion rates and seroprotection rates (B), and mean geometric increase (C) after two doses of AS03-H5N1 vaccine (per-protocol immunogenicity cohort). *Note:* Seroconversion rate defined as proportion of subjects with a pre-vaccination HI antibody titer <1:10 and post-vaccination HI antibody titer ≥1:40, or subjects with at least a 4-fold increase in the post-vaccination HI antibody titer; licensure thresholds defined as a point estimate >40% (CHMP) and a lower limit of 95% CI ≥40% (CBER); Seroprotection rate defined as proportion of subjects with HI antibody titers ≥1:40; licensure threshold defined as a point estimate >70% (CHMP) and a lower limit of 95% CI ≥70% (CBER); Mean geometric increase defined as the geometric mean of the within subject ratios of reciprocal HI antibody titers for post-vaccination versus pre-vaccination; CHMP threshold defined as >2.5 increase; No CBER threshold; HI, Hemagglutination-inhibition; CHMP, European Committee for Medicinal Products for Human Use; CBER, the US Center for Biologics Evaluation and Research; CI, confidence interval.

regardless of seasonal vaccination history, and based on non-overlapping 95% CIs, the Day 42 GMTs appeared to be lower in the previously vaccinated (177.1; 95% CI: 130.0, 241.2) compared with the not previously vaccinated sub-group (374.8; 95% CI: 315.5, 445.2) (Table 3).

One dose of TIV vaccine was immunogenic. The SCRs were 82.5%, 60.0%, and 65.0% against A/H1N1, A/H3N2, and the influenza B strain, respectively (Supplemental Table 1).

3.2. Safety

3.2.1. Solicited adverse events

Local and general solicited AEs are summarized in Fig. 3. Pain was the most frequently reported local symptom, and was reported at the same rate (88.1%; 74/84) after the first and after the second doses of AS03-H5N1 vaccine. In the AS03-H5N1 group overall by subject (first and second doses combined), the frequency of pain was 95.2% (80/84), and the rate of Grade 3 pain was 9.5% (8/84). After the single dose of TIV, the frequency of pain was 68.1% (32/47), and there were no reports at Grade 3. Other local AEs were reported by <32.1% and <25.7% of subjects overall in the AS03-H5N1 (two doses) and TIV control (one dose) groups, respectively.

Muscle aches and fatigue were the most frequently reported general symptoms in both groups. In the AS03-H5N1 group overall by subject (first and second doses combined), muscle aches and fatigue were reported by 73.8% (62/84) and 64.3% (54/84) subjects, respectively. After one dose of TIV, muscle aches and fatigue were reported by 27.7% (13/47) and 29.8% (14/47) of subjects,

Table 1
Hemagglutination-inhibition antibody responses against a vaccine-heterologous strain with AS03-H5N1 vaccine (per-protocol immunogenicity cohort).

		Heterologous strain (A/Vietnam/1194/2004) *N* = 81
Seropositive at day 0, % (95% CI)		65.4 (54.0, 75.7)
GMT, value (95% CI)	Day 0	9.3 (8.2, 10.5)
	Day 21	22.5 (19.4, 26.0)
	Day 42	54.2 (48.1, 61.1)
SCR, % (95% CI)	Day 21	17.3 (9.8, 27.3)
	Day 42	69.1 (57.9, 78.9)
SPR, % (95% CI)	Day 0	2.5 (0.3, 8.6)
	Day 21	24.7 (15.8, 35.5)
	Day 42	82.7 (72.7, 90.2)
MGI, value (95% CI)	Day 21	2.4 (2.1, 2.8)
	Day 42	5.8 (5.0, 6.8)

Seropositive defined as titer ≥1:10; SCR, seroconversion rate defined as proportion of subjects with a pre-vaccination HI antibody titer <1:10 and post-vaccination HI antibody titer ≥1:40, or subjects with at least a 4-fold increase in the post-vaccination HI antibody titer; SPR, seroprotection rate defined as proportion of subjects with HI antibody titers ≥1:40; MGI, mean geometric increase defined as the geometric mean of the within subject ratios of reciprocal HI antibody titers for post-vaccination versus pre-vaccination; HI, hemagglutination-inhibition; CI, confidence interval; GMT, geometric mean titer.

Table 2
Hemagglutination-inhibition antibody responses against the vaccine strain (A/Indonesia/5/2005) by age group with AS03-H5N1 vaccine (per-protocol immunogenicity cohort).

		Age group	
		18–40 years *N* = 40	41–60 years *N* = 41
Seropositive at day 0, % (95% CI)		5.0 (0.6, 16.9)	4.9 (0.6, 16.5)
GMT, value (95% CI)	Day 0	5.2 (4.9, 5.4)	5.2 (4.9, 5.4)
	Day 21	36.6 (26.9, 49.8)	27.8 (21.6, 35.8)
	Day 42	298.6 (235.0, 379.4)	301.6 (236.8, 384.2)
SCR, % (95% CI)	Day 21	47.5 (31.5, 63.9)	43.9 (28.5, 60.3)
	Day 42	100 (91.2, 100)	100 (91.4, 100)
SPR, % (95% CI)	Day 0	0.0 (0.0, 8.8)	0.0 (0.0, 8.6)
	Day 21	47.5 (31.5, 63.9)	43.9 (28.5, 60.3)
	Day 42	100 (91.2, 100)	100 (91.4, 100)
MGI, value (95% CI)	Day 21	7.1 (5.3, 9.5)	5.4 (4.2, 6.9)
	Day 42	57.7 (45.2, 73.6)	58.3 (44.9, 75.8)

Seropositive defined as titer ≥1:10; SCR, seroconversion rate defined as proportion of subjects with a pre-vaccination HI antibody titer <1:10 and post-vaccination HI antibody titer ≥1:40, or subjects with at least a 4-fold increase in the post-vaccination HI antibody titer; SPR, seroprotection rate defined as proportion of subjects with HI antibody titers ≥1:40; MGI, mean geometric increase defined as the geometric mean of the within subject ratios of reciprocal HI antibody titers for post-vaccination versus pre-vaccination; HI, Hemagglutination-inhibition; CI, confidence interval; GMT, geometric mean titer.

respectively. There was no increase in the incidence of general symptoms after the first and second doses of AS03-H5N1 vaccine.

3.2.2. Unsolicited adverse events

The rate of unsolicited AEs during the 21 day post-vaccination period(s) was 33.3% (95% CI: 23.4, 44.5) 28/84 subjects in the AS03-H5N1 group (first and second doses combined) and 19.1% (95% CI: 9.1, 33.3) 9/47 subjects in the TIV group (single dose), which was most frequently injection site pruritus or nasopharyngitis (both 4.8%) in the AS03-H5N1 group, and pharyngitis, nasopharyngitis, dyspepsia, or rhinorrhoea (each 4.3%) in the TIV group. During the 21-day post-vaccination period(s), a Grade 3 unsolicited AE was reported by one subject in the AS03-H5N1 group (gastroenteritis) and one subject in the TIV group (nasopharyngitis). During the 21 day post-vaccination period(s), 14.3% (95% CI: 7.6, 23.6) 12/84 and 4.3% (95% CI: 0.5, 14.5) 2/47 subjects of the unsolicited AEs in the AS03-H5N1 and TIV groups, respectively, were considered by the investigator to be vaccine-related. The rate of unsolicited AEs from Day 0 to 84 was 34.5% (95% CI: 24.5, 45.7) 29/84 subjects in the AS03-H5N1 group and 25.5% (95% CI: 13.9, 40.3) 12/47 subjects in the TIV control group.

From Day 0 to 182, the rate of MAEs was 19.0% (95% CI: 11.3, 29.1) 16/84 subjects in the AS03-H5N1$_A$ group and 19.1% (95% CI: 9.1, 33.3) 9/47 subjects in the TIV group. During the study period, one subject reported 3 SAEs in the AS03-H5N1 group (appendicitis, endometriosis and right ovarian cysts), which were not considered to be vaccine-related. There were no SAEs in the TIV group. There were no pIMDs reported during the study. No subject discontinued due to an AE.

Table 3
Hemagglutination-inhibition antibody responses against the vaccine strain (A/Indonesia/5/2005) by previous influenza vaccination history with AS03-H5N1 vaccine (per-protocol immunogenicity cohort).

		No previous vaccination† *N* = 57	Previous vaccination† *N* = 24
Seropositive at day 0, % (95% CI)		1.8 (0.0, 9.4)	12.5 (2.7, 32.4)
GMT, value (95% CI)	Day 0	5.1 (4.9, 5.2)	5.5 (4.9, 6.0)
	Day 21	35.6 (28.3, 44.8)	24.4 (16.7, 35.8)
	Day 42	374.8 (315.5, 445.2)	177.1 (130.0, 241.2)
SCR, % (95% CI)	Day 21	50.9 (37.3, 64.4)	33.3 (15.6, 55.3)
	Day 42	100 (93.7, 100)	100 (85.8, 100)
SPR, % (95% CI)	Day 0	0.0 (0.0, 6.3)	0.0 (0.0, 14.2)
	Day 21	50.9 (37.3, 64.4)	33.3 (15.6, 55.3)
	Day 42	100 (93.7, 100)	100 (85.8, 100)
MGI, value (95% CI)	Day 21	7.0 (5.6, 8.8)	4.5 (3.1, 6.4)
	Day 42	74.1 (62.3, 88.0)	32.5 (23.1, 45.7)

Seropositive defined as titer ≥1:10; SCR, seroconversion rate defined as proportion of subjects with a pre-vaccination HI antibody titer <1:10 and post-vaccination HI antibody titer ≥1:40, or subjects with at least a 4-fold increase in the post-vaccination HI antibody titer; SPR, seroprotection rate defined as proportion of subjects with HI antibody titers ≥1:40; MGI, mean geometric increase defined as the geometric mean of the within subject ratios of reciprocal HI antibody titers for post-vaccination versus pre-vaccination; HI, hemagglutination-inhibition; CI, confidence interval; GMT, geometric mean titer.

† Did (yes) or did not (no) receive seasonal influenza vaccination and/or A(H1N1)pdm09 vaccine in preceding three seasons.

4. Discussion

This Phase IV open-label study in Korean adults showed that a two-dose schedule of 3.75 μg HA of H5N1 vaccine formulated with AS03 (AS03-H5N1) elicited HI antibody responses that fulfilled the CHMP and CBER licensure criteria for immunogenicity. The AS03-H5N1 vaccine was strongly immunogenic against the vaccine strain (A/Indonesia/5/2005) and a heterologous strain (A/Vietnam/1194/2004). These results are consistent with previous studies which show strong immune responses with AS03-adjuvanted H5N1 vaccines, despite the relatively weak immunogenicity of avian H5N1 in humans [8,11,20,21]. The reactogenicity and safety profile of the AS03-H5N1 vaccine was also consistent with that reported previously, including North American, European, and Asian populations [4,6,7,22].

The provision of adequate vaccine coverage in the event of the emergence and global spread of a highly pathogenic H5N1 virus represents a major manufacturing and logistical challenge. A key strategy for pandemic preparedness is the formulation of vaccines with an adjuvant to decrease the amount of antigen needed per dose ('antigen sparing'), which will increase the number of doses available early in the response; the batches of vaccine and adjuvant can be stockpiled to be deployed in a pre-pandemic setting [23–26]. The stockpiled vaccines may be subtype-matched but not strain-matched to the emerging virus, and can be used to prime the population in advance of the manufacture of a booster vaccine

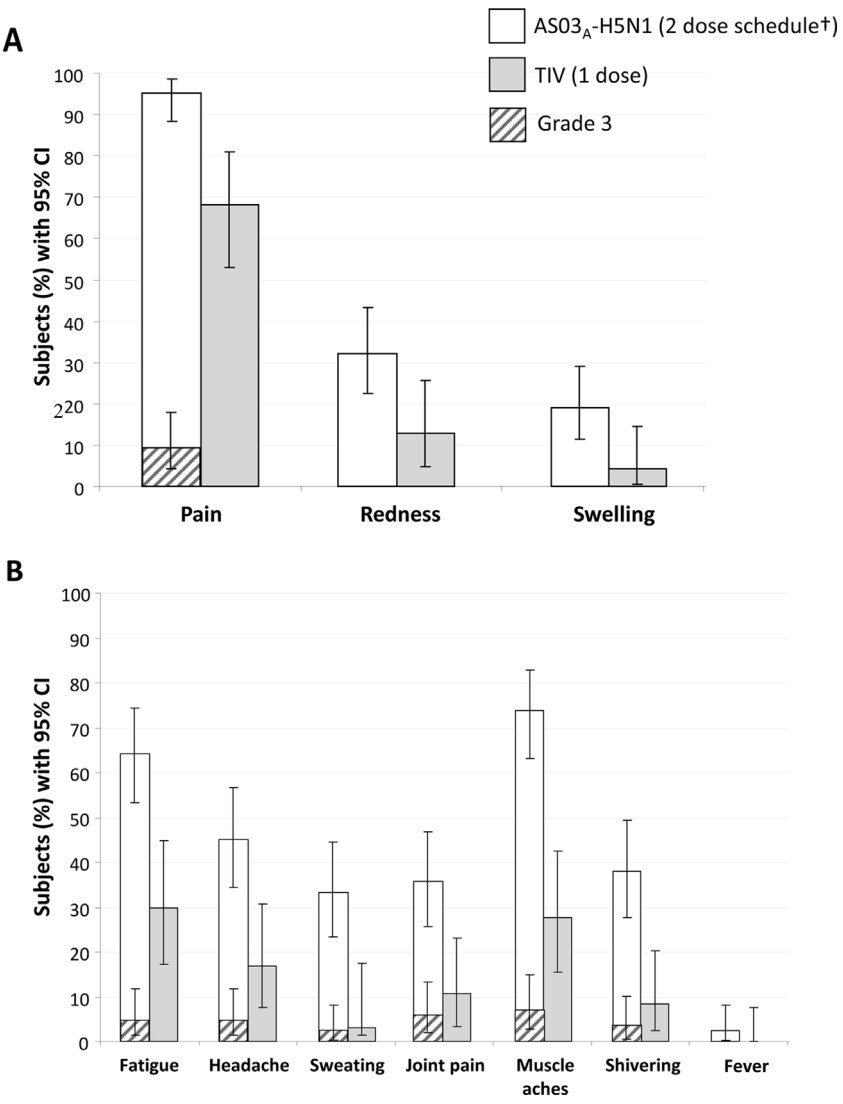

Fig. 3. Solicited local adverse events (A) and general adverse events (B) during the 7-day post-vaccination period in the total vaccinated cohort. *Note:* † Overall AEs for dose 1 and 2; Grade 3 events defined as severe events which prevent daily activities; Grade 3 redness and swelling defined as diameter >100 mm; Grade 3 fever defined as a temperature >39 °C. CI, confidence interval; TIV, inactivated trivalent influenza vaccine.

against the pandemic strain [23–26]. The successful implementation of a pandemic vaccination strategy using stockpiled vaccine will depend upon the availability of vaccines that provide some degree of cross-reactive immunogenicity, and therefore can be used according to various prime–boost strategies.

Previous studies have shown that two doses of AS03-H5N1 vaccine containing 3.75 μg of HA are immunogenic against the vaccine strain and that up to 36 months after initial vaccination, a further single dose of booster AS03-H5N1 vaccine against a drifted strain elicits strong immune responses against the priming and drifted strains [12,26]. We now show that AS03-H5N1 vaccine provides robust HI antibody responses against vaccine homologous and heterologous strains in Korean adults. Sub-group analyses showed that post-vaccination HI GMTs were lower in subjects who had received one or more seasonal influenza vaccinations within the previous 3 seasons compared with those who had not (177.1 versus 374.8, respectively). This finding is consistent with previous studies which reported lower immune responses following pandemic influenza vaccination in subjects who had previously received seasonal influenza vaccine compared with those who had not [27–30]. Despite the lower immune response in subjects having previously received seasonal flu vaccines in our study, SCRs and SPRs were 100% against the vaccine strain regardless of vaccination history, and immunogenicity met the regulatory acceptance criteria.

We observed baseline seropositive (HI titer ≥1:10) rates of 4.9% for A/Indonesia and 65.4% for A/Vietnam, although GMTs were low, and most subjects who were seroposotive at baseline had titers close to the assay cut-off. Exposure to H5N1 virus is unlikely and is reported to have a low impact on sero-epidemiology of H5N1 [31,32]. In South Korea, for example, during the 2003/04 outbreak of influenza in ducks and chickens (A/goose/Guangdong/1/96 lineage; clade 2.5), among a sample of 2,512 bird cullers, there were only 9 confirmed/suspected cases of the prevalent H5N1 virus, suggesting that poultry-to-human transmission is low even among high-risk workers [31]. The relatively high baseline seropositivity rate for A/Vietnam suggests that cross-reacting antibodies with a related or unrelated antigen may lead to some H5N1 antibody cross-reactivity in the lower range.

The reactogenicity and safety profile of AS03-H5N1 vaccine in our study was consistent with that reported previously [4,6,7,22]. After the first dose of AS03-H5N1 vaccine, we observed a higher rate of injection site reactions and systemic reactions compared with

one dose of TIV, and this finding is consistent with previous studies showing that adjuvanted vaccine is typically more reactogenic than non-adjuvanted vaccine. The short- and longer-term AE profiles were similar between the AS03-H5N1 and TIV control groups, with 19% of each group reporting a medically-attended event up to 182 days after vaccination. One subject reported an SAE (AS03-H5N1 group) which was not considered to be vaccine-related, and no subject withdrew from the study due to an AE.

Epidemiological data currently available to GSK suggest an increased risk of narcolepsy following vaccination with the H1N1 vaccine *Pandemrix*™ [33–37]. No cases of narcolepsy were detected in this study of an H5N1 vaccine.

In conclusion, AS03-H5N1 vaccine was immunogenic in Korean adults up to 60 years of age with HI antibody responses that fulfilled CHMP licensure criteria for immunogenicity. The reactogenicity and safety profile of AS03-H5N1 vaccine was consistent with that previously reported throughout the extensive clinical development program of this vaccine.

5. Trademarks

Prepandrix, *Adjupanrix*, *Pumarix*, *Fluarix* and *Pandemrix* are trademarks of the GSK group of companies.

Financial disclosure

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Conflict of interest statement

AS, DV, and PI are employees of the GSK group of companies and own stock/stock options/restricted shares in GSK. MD is an employee of the GSK group of companies. The remaining authors have nothing to disclose.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.04.027

References

[1] World Health Organization. Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003–2014; 2014, 27 July.
[2] Food and Drug Administration. FDA approves first adjuvanted vaccine for prevention of H5N1 avian influenza; 2013. ⟨http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm376444.htm⟩ [accessed 20 November 2014].
[3] US Food and Drug Administration. H5N1 Influenza Virus Vaccine, manufactured by Sanofi Pasteur, Inc. Questions and Answers; 2013. ⟨http://www.fda.gov/BiologicsBloodVaccines/Vaccines/QuestionsaboutVaccines/ucm080753.htm⟩ [accessed 20 November 2014].
[4] Chu DW, Hwang SJ, Lim FS, et al. Immunogenicity and tolerability of an AS03(A)-adjuvanted prepandemic influenza vaccine: a phase III study in a large population of Asian adults. Vaccine 2009;27:7428–35, http://dx.doi.org/10.1016/j.vaccine.2009.07.102.
[5] Díez-Domingo J, Garcés-Sanchez M, Baldó J-M, et al. Immunogenicity and safety of H5N1 A/Vietnam/1194/2004 (clade 1) AS03-adjuvanted pre-pandemic candidate influenza vaccines in children aged 3 to 9 years: a phase II, randomized, open, controlled study. Pediatr Infect Dis J 2010;29:e35–46, http://dx.doi.org/10.1097/INF.0b013e3181daf921.
[6] Langley JM, Frenette L, Ferguson L, et al. Safety and cross-reactive immunogenicity of candidate AS03-adjuvanted prepandemic H5N1 influenza vaccines: a randomized controlled phase 1/2 trial in adults. J Infect Dis 2010;201:1644–53, http://dx.doi.org/10.1016/j.vaccine.2012.03.046.
[7] Langley JM, Risi G, Caldwell M, et al. Dose-sparing H5N1 A/Indonesia/05/2005 pre-pandemic influenza vaccine in adults and elderly adults: a phase III, placebo-controlled, randomized study. J Infect Dis 2011;203:1729–38, http://dx.doi.org/10.1093/infdis/jir172.
[8] Leroux-Roels I, Borkowski A, Vanwolleghem T, et al. Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine: a randomised controlled trial. Lancet 2007;370:580–9, http://dx.doi.org/10.1016/S0140-6736(07)61297-5.
[9] Nolan T, Izurieta P, Lee BW, et al. Heterologous Prime–boost vaccination using an AS03B-adjuvanted influenza A(H5N1) vaccine in infants and children <3 years of age. J Infect Dis 2014;210:1800–10, http://dx.doi.org/10.1093/infdis/jiu359.
[10] Vaughn D, Seifert H, Hepburn A, et al. Safety of AS03-adjuvanted inactivated split virion A(H1N1)pdm09 and H5N1 influenza virus vaccines administered to adults: Pooled analysis of 28 clinical trials. Hum Vaccines Immunother 2014;10:2942–57, http://dx.doi.org/10.4161/21645515.2014.972149. Published online: 21 Nov.
[11] Leroux-Roels I, Bernhard R, Gerard P, Drame 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:e1665, http://dx.doi.org/10.1371/journal.pone.0001665.
[12] Gillard P, Chu DW, Hwang SJ, et al. Long-term booster schedules with AS03A-adjuvanted heterologous H5N1 vaccines induces rapid and broad immune responses in Asian adults. BMC Infect Dis 2014;14:142, http://dx.doi.org/10.1186/1471-2334-14-142.
[13] European Medicines Agency. European Committee for Proprietary Medicinal Products. Note for guidance on harmonisation of requirements for influenza vaccines. ⟨http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf⟩ [accessed 20 November 2014]. in press.
[14] Kendal A, Pereira M, Skehel J. Hemagglutination inhibition. In: Kendal AP, Pereira MS, Skehel JJ, editors. Concepts and procedures for laboratory-based influenza surveillance. Atlanta, GA: Centers for Disease Control and Prevention and Pan-American Health Organization; 1982. p. B17–35.
[15] Rowe T, Abernathy RA, Hu-Primmer J, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol 1999;37:937–43.
[16] Stephenson I, Wood JM, Nicholson KG, Charlett A, Zambon MC. Detection of anti-H5 responses in human sera by HI using horse erythrocytes following MF59-adjuvanted influenza A/Duck/Singapore/97 vaccine. Virus Res 2004;103:91–5, doi: S0168170204001182.
[17] Hehme N, Künzel W, Petschke F, Türk G, Raderecht C, van Hoecke C, et al. Ten years of experience with the trivalent split-influenza vaccine, Fluarix™. Clin Drug Invest 2002;22:751–69.
[18] US Food and Drug Administration. Clinical data needed to support the licensure of pandemic influenza vaccines; 2007. ⟨http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074794.htm⟩ [accessed 20 November 2014].
[19] Yang PC, Yu CJ, Chang SC, et al. Safety and immunogenicity of a split-virion AS03A-adjuvanted A/Indonesia/05/2005 (H5N1) vaccine in Taiwanese adults. J Formos Med Assoc 2012;111:333–9, http://dx.doi.org/10.1016/j.jfma.2011.02.006.
[20] Bresson JL, Perronne C, Launay O, et al. Safety and immunogenicity of an inactivated split-virion influenza A/Vietnam/1194/2004 (H5N1) vaccine: phase I randomised trial. Lancet 2006;367:1657–64, http://dx.doi.org/10.1016/S0140-6736(06)68656-X.
[21] 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;354:1343–51, http://dx.doi.org/10.1056/NEJMoa055778.
[22] Rumke HC, Bayas JM, de Juanes JR, et al. Safety and reactogenicity profile of an adjuvanted H5N1 pandemic candidate vaccine in adults within a phase III safety trial. Vaccine 2008;26:2378–88, http://dx.doi.org/10.1016/j.vaccine.2008.02.068.

[23] Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. Nature 2006;442:448–52, http://dx.doi.org/10.1038/nature04795.

[24] Jennings LC, Monto AS, Chan PK, Szucs TD, Nicholson KG. Stockpiling prepandemic influenza vaccines: a new cornerstone of pandemic preparedness plans. Lancet Infect Dis 2008;8:650–8, http://dx.doi.org/10.1016/S1473-3099(08)70232-9.

[25] Godeaux O, Izurieta P, Madariaga M, Drame M, Li P, Vaughn DW. Immunogenicity and safety of AS03-adjuvanted H5N1 influenza vaccine prepared from bulk antigen after stockpiling for 4 years. Vaccine 2015;33:2189–95, http://dx.doi.org/10.1016/j.vaccine.2014.07.062. Jul 30 [Epub ahead of print].

[26] Gillard P, Caplanusi A, Knuf M, et al. An assessment of prime–boost vaccination schedules with AS03A -adjuvanted prepandemic H5N1 vaccines: a randomized study in European adults. Influenza Other Respir Viruses 2013;7:55–65, http://dx.doi.org/10.1111/j.1750-2659.2012.00349.x.

[27] Leroux-Roels I, Roman F, Forgus S, et al. Priming with AS03(A)-adjuvanted H5N1 influenza vaccine improves the kinetics, magnitude and durability of the immune response after a heterologous booster vaccination: an open non-randomised extension of a double-blind randomised primary study. Vaccine 2010;28:849–57, http://dx.doi.org/10.1016/j.vaccine.2009.10.017. Epub 2009 Oct 14.

[28] Nolan T, Richmond PC, Formica NT, et al. Safety and immunogenicity of a prototype adjuvanted inactivated split-virus influenza A (H5N1) vaccine in infants and children. Vaccine 2008;26:6383–91, http://dx.doi.org/10.1016/j.vaccine.2008.08.046.

[29] Plennevaux E, Sheldon E, Blatter M, Reeves-Hoche MK, Denis M. Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomised controlled phase 2 trials. Lancet 2010;375:41–8, http://dx.doi.org/10.1016/S0140-6736(09)62026-2.

[30] Roman F, Vaman T, Kafeja F, Hanon E, Van Damme P. AS03(A)-adjuvanted influenza A (H1N1) 2009 vaccine for adults up to 85 years of age. Clin Infect Dis 2010;51:668–77, http://dx.doi.org/10.1086/655830.

[31] Kwon D, Lee JY, Choi W, Choi JH, Chung YS, Lee NJ, et al. Avian influenza a (H5N1) virus antibodies in poultry cullers, South Korea, 2003–2004. Emerg Infect Dis 2012;18:986–8, http://dx.doi.org/10.3201/eid1806.111631.

[32] Toner ES, Adalja AA, Nuzzo JB, Inglesby TV, Henderson DA, Burke DS. Assessment of serosurveys for H5N1. Clin Infect Dis 2013;56:1206–12, http://dx.doi.org/10.1093/cid/cit047.

[33] Duffy J, Weintraub E, Vellozzi C, DeStefano F. Narcolepsy and influenza A(H1N1) pandemic 2009 vaccination in the United States. Neurology 2014;83:1823–30, http://dx.doi.org/10.1212/WNL.0000000000000987.

[34] European Centre for Disease Control and Prevention. Narcolepsy in association with pandemic vaccination; a multi-country European epidemiological investigation; 2012. ⟨http://www.ecdc.europa.eu/en/publications/Publications/Vaesco%20report%20FINAL%20with%20cover.pdf⟩ [accessed 13 March 2015].

[35] Miller E, Andrews N, Stellitano L, Stowe J, Winstone AM, Shneerson J, et al. Risk of narcolepsy in children and young people receiving AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine: retrospective analysis. BMJ 2013;346:f794, http://dx.doi.org/10.1136/bmj.f794.

[36] Nohynek H, Jokinen J, Partinen M, Vaarala O, Kirjavainen T, Sundman J, et al. AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. PLoS ONE 2012;7:e33536, http://dx.doi.org/10.1371/journal.pone.0033536.

[37] Partinen M, Saarenpaa-Heikkila O, Ilveskoski I, Hublin C, Linna M, Olsen P, et al. Increased incidence and clinical picture of childhood narcolepsy following the 2009 H1N1 pandemic vaccination campaign in Finland. PLoS ONE 2012;7:e33723, http://dx.doi.org/10.1371/journal.pone.0033723.