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Long-term immunogenicity of an AS03-adjuvanted influenza A(H1N1)pdm09 vaccine in young and elderly adults: An observer-blind, randomized trial ☆☆

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ABSTRACT

Background: This study (NCT00979602) evaluated the immunogenicity and relative protective efficacy of one dose of influenza A(H1N1)pdm09 vaccine with or without AS03 (an α-tocopherol oil-in-water emulsion based Adjuvant System).
Methods: Four thousands and forty-eight healthy adults aged ≥18 years were randomized (1:1) to receive one dose of either the adjuvanted split virion (3.75 μg hemagglutinin antigen [HA]/AS03) or non-adjuvanted (15 μg HA) vaccine. Hemagglutination inhibition [HI] antibody response was evaluated before vaccination and at Days 21, 42 and 182 (Month 6). Safety of the study vaccines was evaluated during the entire study duration.
Results: At Day 21, both study vaccines induced HI immune responses meeting the US regulatory criteria in subjects 18–64 years (seroprotection rate [SPR]: 98.0% [97.1–98.6]; seroconversion rate [SCR]: 89.7% [88.0–91.2] in the AS03-adjuvanted group; SPR: 91.4% [89.9–92.8]; SCR: 74.6% [72.3–76.9] in the non-adjuvanted group) and >64 years of age (SPR: 86.0% [82.5–89.0]; SCR: 75.3% [71.1–79.2] in the AS03-adjuvanted group; SPR: 69.1% [64.6–73.3]; SCR: 56.7% [52.0–61.3] in the non-adjuvanted group). The AS03-adjuvanted vaccine induced higher HI geometric mean titers than the non-adjuvanted vaccine at all time points. At Month 6, only subjects 18–64 years of age from both vaccine groups still met the US regulatory criteria (SPR: 82.1% [80.0–84.1]; SCR: 62.3% [59.6–64.8] in the AS03-adjuvanted group; SPR: 75.3% [72.9–77.5]; SCR: 53.7% [51.0–56.4] in the non-adjuvanted group). Protective efficacy was not evaluated due to low number of RT-qPCR-confirmed A(H1N1)pdm09 influenza cases. Through Month 12, 216 serious adverse events (in 157 subjects: 84 in the AS03-adjuvanted and 73 in the non-adjuvanted group) and 12 potentially immune mediated diseases (5 in the AS03-adjuvanted and 7 in the non-adjuvanted group) were reported.
Conclusion: A single dose of either adjuvanted or non-adjuvanted influenza A(H1N1)pdm09 vaccine induced protective HI antibody levels against the A/California/7/2009 strain that persisted through Month 6 in the 18–64 years population.

Abbreviations: ATP, according to protocol; BARDA, Biomedical Advanced Research and Development Authority; BMI, body mass index; CBER, Center for Biologics Evaluation & Research; CHMP, Committee for Medicinal Products for Human Use; CI, confidence interval; GMFR, geometric mean fold rise; GMT, geometric mean titer; HA, hemagglutinin; HI, hemagglutination inhibition; HHS, United States Department of Health and Human Services; ILIs, Influenza-Like-Illnesses; pIMDs, potential immune mediated diseases; RVP, respiratory viral panel; SAEs, serious adverse events; SCR, seroconversion rate; SD, standard deviation; SPR, seroprotection rate; TVC, total vaccinated cohort; VEI, vaccine effectiveness improvement; WHO, World Health Organization.

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1. Introduction

Mass immunization is considered to be an effective prophylactic method of mitigating influenza pandemic-associated morbidity and mortality [1–4]. Due to the novel antigenic characteristics of the swine-origin influenza A(H1N1) 2009 pandemic virus [influenza (A(H1N1)pdm09] [5,6], the seasonal influenza vaccines available at the time of the 2009–2010 H1N1 pandemic were unlikely to confer protection against the novel virus [5,7,8].

The World Health Organization (WHO) encouraged the development and use of adjuvanted influenza A(H1N1)pdm09 vaccines [9,10], with the aim of dose-reduction, antigen-sparing and to potentially provide broader vaccine efficacy against drifted strains through cross-reactive immunity [11]. Based on the experience of developing a pre-pandemic A/H5N1 influenza vaccine utilizing AS03 (an α-tocopherol oil-in-water emulsion based Adjuvant System) [12,13] that was well-tolerated and highly immunogenic in adults [14–16], an AS03-adjuvanted influenza A(H1N1)pdm09 vaccine with 3.75 μg hemagglutinin (HA) content was developed [17–19].

This large-scale, randomized study in subjects ≥18 years of age assessed whether one dose of AS03-adjuvanted 3.75 μg HA influenza A(H1N1)pdm09 vaccine elicited immune response that met the US and European regulatory criteria. Additionally, non-inferiority and superiority of this vaccine protective efficacy versus a non-adjuvanted 15 μg HA influenza A(H1N1)pdm09 vaccine were evaluated.

2. Materials and methods

2.1. Study design and participants

In this phase III, observer-blind, randomized study (NCT00979602), adults ≥18 years of age were enrolled across 25 centers in the US and 13 in Canada between November 2009 and December 11, 2009. They were randomized (allocation ratio 1:1) to receive one dose of either a monovalent AS03-adjuvanted 3.75 μg HA A/California/7/2009 pandemic influenza vaccine or a non-adjuvanted 15 μg HA A/California/7/2009 pandemic influenza vaccine. The enrolment stratification was by age (1:1:1:1; 18–30 years, 31–40 years, 41–64 years, ≥65 years). The subjects and study personnel involved in evaluating end points were blinded to the intervention administered. Double blinding was not possible because the vaccine preparation required mixing of AS03 and A(H1N1)pdm09 antigen from two vials. Randomization was performed using a central, internet-based system that balanced groups with respect to center, age strata and previous seasonal influenza vaccination.

Adults were excluded from enrolment: if they had a history of physician-confirmed A(H1N1)pdm09 influenza infection or vaccination, those who received any vaccination other than a seasonal influenza vaccine within 30 days preceding study start, those with confirmed or suspected immunosuppressive or immunodeficient conditions, diagnosed with or undergoing treatment for cancer, and/or with a history of allergic/anaphylactic reactions following previous influenza vaccination. In addition, laboratory screening was performed to exclude those with results outside of protocol-specified normal ranges. The following safety laboratory parameters were tested to evaluate the participants' eligibility: hepatic aminotransferases, total and direct bilirubin, alkaline phosphatase, creatinine, serum urea nitrogen, hemoglobin, hematocrit, white blood cell count and platelet count.

Active surveillance of influenza-like infections (ILIs: defined as fever ≥38.0 °C/100.4 °F or new or worsening myalgia accompanied by new or worsening cough or sore throat) was done during study visits and through bi-weekly telephonic contact through Day 385 (12 months after the initially planned administration of the second study vaccine dose). Additionally, the subjects were instructed to contact the study sites if they develop any ILI symptoms. Once the study site had been notified of a possible ILI episode, a visit for nasal and throat swab sample collection was scheduled within 5 days of symptom onset and before initiating any antimicrobial/influenza antiviral therapy. If an ILI episode was reported more than 5 days after onset, no swab specimen was collected.

Written informed consent was obtained from all subjects prior to conducting any study-related procedures. The study was conducted in accordance with the Good Clinical Practice guidelines, the Declaration of Helsinki and local regulations. All study-related documents were approved by institutional review boards.

2.2. Study vaccines

The influenza A(H1N1)pdm09 vaccine was a monovalent, inactivated, split-virion antigen suspension (A/California/07/2009 strain) adjuvanted with AS03 (*Arepanrix*™, a trademark of GlaxoSmithKline Vaccines) or administered as plain antigen. The H1N1 viral seed for the vaccine was prepared as per WHO recommendations [20]. AS03 is an oil-in-water emulsion based Adjuvant System containing squalene (10.69 mg per dose), DL-α-tocopherol (11.86 mg) and polysorbate 80 (4.86 mg). The AS03-adjuvanted influenza A(H1N1)pdm09 vaccine doses were prepared by mixing the A(H1N1)pdm09 antigen and AS03 (1:1) from separate multi-dose vials. 0.5 ml of the assigned study vaccine was administered into the deltoid muscle within 30 min after mixing the antigen and the adjuvant.

2.3. Study objectives and end points

The first co-primary objective of the study was to evaluate HI antibody responses 21 days after vaccination in the AS03-adjuvanted vaccine group based on the Center for Biologics Evaluation and Research (CBER) and Committee for Medicinal Products for Human Use (CHMP) criteria for pandemic influenza vaccines in adults [21,22].

At least 360 RT-qPCR-confirmed A/California influenza cases were required to evaluate the second co-primary objective on non-inferior protective efficacy followed by superiority. As only three RT-qPCR-confirmed A/California influenza cases were diagnosed during the study, descriptive analyses of the influenza attack rate and vaccine efficacy improvement (VEI) were computed only for ILI and pneumonia cases.

The study also assessed whether the non-adjuvanted 15 μg HA influenza A(H1N1)pdm09 vaccine elicited immune responses that met the US and European regulatory criteria, 21 days after vaccination and whether these criteria were met for either study vaccines at Day 42 (in a small subset of subjects) and at Day 182 (Month 6).

2.4. Laboratory assays

Hemagglutination inhibition (HI) antibody levels in serum samples were assessed at GlaxoSmithKline Vaccines central laboratory using a validated in-house assay [cut-off: ≥1:10] that used chicken erythrocytes, as previously described [23]. The A/California/7/2009 strain was used as the antigen strain.

RT-qPCR was performed on viral RNA from the clinical samples as described previously [24]. Viral load values were quantified and the sample was considered positive when the measured viral load was equal to or above the assay cut-off [24].

2.5. Immunological assessment

Serum samples were collected before vaccination (Day 0), at Days 21, 42 (in a subset of subjects) and 182 (Month 6) for assessment of humoral immune response and for clinical chemistry and hematology assessments at Days 0, 7 and 21.

The immunological assessment was based on HI antibody seroconversion rates (SCR), seroprotection rate (SPR) and geometric mean fold rise (GMFR), against the vaccine homologous strain.

Post hoc exploratory analyses included the assessment of possible correlation of HI antibody response with body mass index (BMI) and with previous influenza vaccination history. Further assessments were performed to identify the respiratory viruses isolated from swab samples from ILI cases using xTAG Respiratory Viral Panel (RVP) Fast assay (Luminex Molecular Diagnostics Inc., Toronto, Canada) [25,26].

2.6. Safety and reactogenicity assessment

Subjects used diary cards to record the solicited local and general symptoms occurring within 7 days following vaccination and the unsolicited adverse events occurring within 42 days following vaccination. Potential immune-mediated diseases (pIMDs: subset of AEs that include both autoimmune diseases and other inflammatory and/or neurologic disorders which may/may not have an autoimmune etiology) and serious adverse events (SAEs) were recorded throughout the study period. The intensity of all solicited adverse events except fever was graded on a scale of (0–3), Grade 1 being those that did not interfere with normal activities and Grade 3 being those that prevented normal activities (Grade 3 redness and swelling: diameter >100 mm; Grade 3 fever: temperatures $\geq$39.0–$\leq$40.0 °C. Fever was graded on a scale of (0–4), Grade 4 being temperatures >40.0 °C. Based on clinical judgment, the investigators assessed whether the AEs/SAEs were potentially related/not related to the study vaccine.

Serum samples for the analysis of clinical safety laboratory parameters were collected at Days 7 and 21. The following laboratory parameters were tested: hepatic aminotransferases, total and direct bilirubin, alkaline phosphatase, creatinine, serum urea nitrogen, hemoglobin, hematocrit, white blood cell count and platelet count.

2.7. Statistical analyses

The sample size was calculated taking into consideration the co-primary objectives. Overall, 1900 evaluable subjects (1800 for VEI evaluation) in each of the two treatment groups (accounting for 5% and 10% drop-out rates for the co-primary objectives) was estimated to provide a power of 91.85% to meet the co-primary objectives, assuming 90%/74% as reference for SPR/SCR in subjects 18–64 years and >64 years of age, respectively, 40% vaccine efficacy for the non-adjuvanted influenza A(H1N1)pdm09 vaccine, and an attack rate of 20% in subjects who do not receive any H1N1 vaccine (PASS 2005; one-sided test, one-sided alpha = 2.5%).

The SCR, SPR and GMFR and incidence rates of solicited and unsolicited adverse events were calculated with 95% confidence interval (CI). The analyses of immunogenicity were performed on the according to protocol (ATP) cohort which included evaluable subjects meeting eligibility criteria and adhering to protocol-defined procedures. A Cox regression model, including the vaccine group as a fixed effect, age and baseline antibody titer as covariates was used to estimate the VEI for the any ILI cases and any pneumonia cases (the first event was considered if multiple events were reported by a subject). All statistical analyses were performed using Statistical Analysis Software (SAS) version 9.1.

3. Results

3.1. Study population

A total of 5660 subjects were screened, 4048 received vaccine, and 3770 completed the study through Day 385. The reasons for withdrawals and elimination of subjects from the analyses at different time points are presented in Fig. 1.

The mean age of subjects in the TVC at the time of vaccination in the 18–64 years age group was 37.4 years (range: 18–64 years); >64 years age group was 71.2 years (range: 65–92 years). Overall, 59.2% and 56.3% of subjects in the respective two age groups were female and the majority of subjects were Caucasians (86.9% and 93.0%, respectively).

3.2. Immune response

Co-primary objectives: The first co-primary objective was met. A single dose of the AS03-adjuvanted 3.75 μg HA influenza A(H1N1)pdm09 vaccine elicited HI immune responses in the 18–64 years and >64 years age groups that met the CBER regulatory criteria at Day 21 (Table 1). The CHMP criteria were met in the 18–60 years and >60 years age groups (data not presented).

The second co-primary objective was not evaluated as only three RT-qPCR-confirmed A/California influenza cases were identified (AS03-adjuvanted: 1; non-adjuvanted: 2).

Secondary objectives: In the Day 42 subset (N = 192) which received the AS03-adjuvanted 3.75 μg HA influenza A(H1N1)pdm09 vaccine, the CBER criteria were met in the 18–64 years age group and >64 years age group (Table 1). At Day 182 (Month 6), the CBER criteria were met only for subjects 18–64 years of age (Table 1). Subjects >64 years of age had a LL of the 95% CI for SPR of 47.7%, thus not fulfilling the CBER criteria at this time point.

At Day 21, a single dose of the non-adjuvanted 15 μg HA influenza A(H1N1)pdm09 vaccine elicited HI immune responses in subjects 18–64 years and >64 years of age that met the CBER regulatory criteria (Table 1). Only those in the 18–64 years age group met the CBER criteria at Day 42 and at Day 182 (Month 6). At this time points subjects >64 years of age had a LLs of the 95% CI for SPR of 43.2 and 38.6%, respectively and LLs of the 95% CI for SCR of 27.0% and 22.6%, respectively, thus not fulfilling the CBER criteria.

The CHMP criteria were met at Day 21 and Day 42 in the 18–60 years and >60 years age groups for both study vaccines. At Day 182, the CHMP criteria were met in the 18–60 years age group but not in the >60 years age group for both study vaccines (data not presented).

HI antibody GMTs in both age groups were higher at all post-vaccination time points for those who received the AS03-adjuvanted influenza A(H1N1)pdm09 vaccine compared to those who received the non-adjuvanted vaccine; GMTs were generally lower in the >64 years compared to the 18–64 years age group at all time points (Table 1). Persistence of HI antibody response at Day 182 (Month 6) was observed for both study vaccines, although at lower levels compared to that observed at Day 21 (Table 1). Overall, the immune response against the vaccine homologous strain appeared to decrease with advancing age (Fig. 2/Web-appendix Table 1).

Post hoc exploratory analyses showed that HI antibody responses were mostly comparable across healthy weight, overweight and obese subjects. No clear patterns emerged due to the modest number of subjects in the underweight category (Web-appendix Table 2). A higher HI antibody was observed among influenza vaccine-naïve subjects, compared with those with previous seasonal influenza vaccination, in terms of HI antibody GMTs and GMFRs (Web-appendix Table 2).

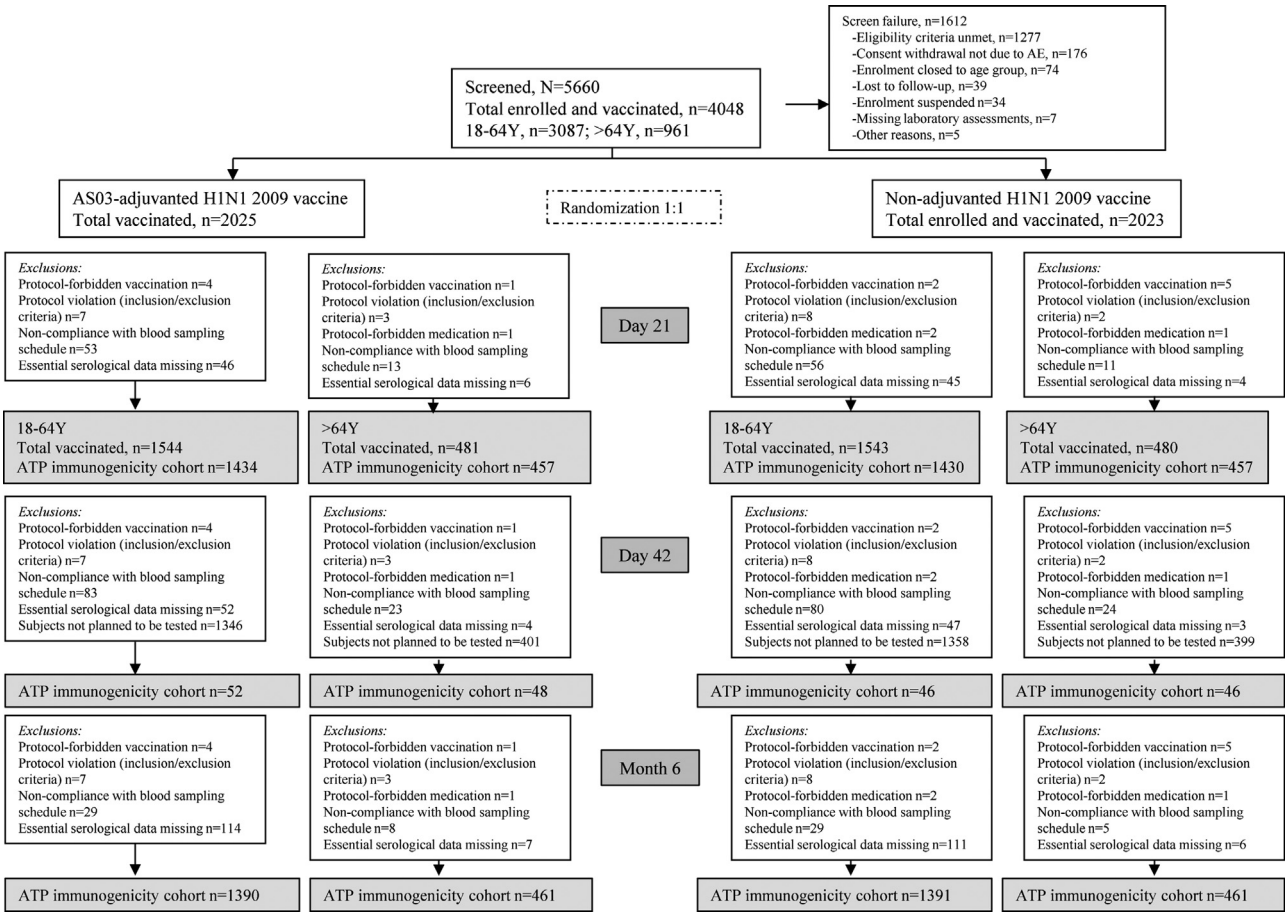

Fig. 1. Participant flow diagram.

Table 1
Hemagglutination inhibition antibody response to the A/California/7/2009 (H1N1) strain in the 18–64 years and >64 years age groups at all time points (according to protocol cohort for immunogenicity).

Immune response	Time point	AS03$_A$/3.75 μg HA[a]				Non-adjuvanted 15 μg HA			
			18–64 years		>64 years		18–64 years		>64 years
		N[c]	Percentage or value (95% CI[b])	N	Percentage or value (95% CI)	N	Percentage or value (95% CI)	N	Percentage or value (95% CI)
Seroconversion rate	Day 21	1426	89.7% (88.0–91.2)	454	75.3% (71.1–79.2)	1414	74.6% (72.3–76.9)	455	56.7% (52.0–61.3)
	Day 42	52	98.1% (89.7–100)	48	70.8% (55.9–83.0)	46	78.3% (63.6–89.1)	46	41.3% (**27.0**–56.8)
	Day 182 (Month 6)	1383	62.3% (59.6–64.8)	458	34.9% (30.6–39.5)	1380	53.7% (51.0–56.4)	458	26.6% (**22.6**–30.9)
Seroprotection rate	Pre-vaccination	1428	24.5% (22.3–26.8)	454	13.4% (10.4–16.9)	1420	24.4% (22.2–26.7)	456	17.1% (13.8–20.9)
	Day 21	1432	98.0% (97.1–98.6)	457	86.0% (82.5–89.0)	1424	91.4% (89.9–92.8)	456	69.1% (64.6–73.3)
	Day 42	52	98.1% (89.7–100)	48	75.0% (60.4–86.4)	46	93.5% (82.1–98.6)	46	58.7% (**43.2**–73.0)
	Day 182 (Month 6)	1388	82.1% (80.0–84.1)	461	51.4% (**46.7**–56.1)	1391	75.3% (72.9–77.5)	459	43.1% (**38.6**–47.8)
Geometric mean titer	Pre-vaccination	1428	14.7 (13.8–15.7)	454	10.9 (10.0–11.9)	1420	14.7 (13.7–15.7)	456	12.0 (11.0–13.2)
	Day 21	1432	396.2 (373.8–419.9)	457	128.6 (114.6–144.3)	1424	217.6 (203.3–232.9)	456	75.2 (65.9–85.9)
	Day 42	52	276.5 (207.1–369.0)	48	105.9 (70.2–159.9)	46	170.0 (117.7–245.4)	46	47.2 (32.1–69.3)
	Day 182 (Month 6)	1388	109.5 (102.3–117.1)	461	37.1 (33.2–41.5)	1391	83.3 (77.5–89.5)	459	29.2 (26.0–33.0)
Geometric mean fold rise	Day 21	1345	27.7 (25.7–29.9)	535	12.5 (11.2–13.9)	1338	15.5 (14.2–16.8)	531	6.4 (5.7–7.1)
	Day 42	50	26.8 (20.0–35.8)	50	11.1 (7.7–16.0)	44	13.7 (9.1–20.6)	1304	5.9 (5.4–6.4)
	Day 182 (Month 6)	1302	7.7 (7.2–8.4)	539	3.5 (3.2–3.9)	48	4.0 (2.9–5.5)	534	2.5 (2.3–2.8)

Bolded value = did not meet CBER criteria; SCR: percentage of subjects with pre-vaccination titer <1:10 and post-vaccination titer ≥1:40, or pre-vaccination titer >1:10 and at least four-fold increase in post-vaccination titer, SPR: percentage of subjects with a post-vaccination titer ≥1:40; GMFR: post-vaccination fold increase in geometric mean titers (GMTs) in terms of HI antibodies against the vaccine homologous strain; Center for Biologics Evaluation and Research (CBER) criteria in adults <65 years of age: lower bound of 95% confidence interval [CI] for HI antibody SCR: ≥40% and SPR: ≥70%; CBER criteria in adults ≥65 years of age: lower bound of 95% CI for HI antibody for SCR: ≥30% and SPR: ≥60%; Committee for Medicinal Products for Human Use (CHMP) criteria in adults 18–60 years of age: point estimates for HI antibody SCR: >40%, SPR: >70% GMFR: >2.5 [data not presented]; CHMP criteria in adults >60 years of age: point estimates for HI antibody SCR: >30%, SPR: >60% GMFR: >2 [data not presented].

[a] HA = hemagglutinin.

[b] CI = confidence interval.

[c] *N* = number of subjects with available results.

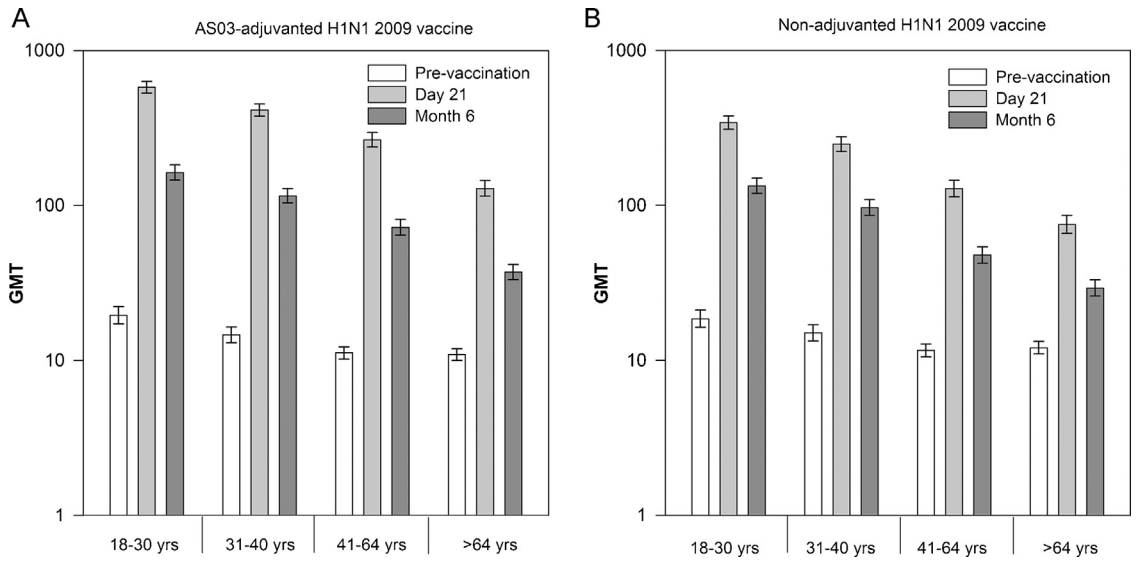

Fig. 2. Geometric mean titers for influenza A(H1N1)pdm09 hemagglutination inhibition antibodies by age strata at Days 21 (A) and 182 (Month 6) (B) (according to protocol cohort for immunogenicity). Footnotes: HI, hemagglutination inhibition; GMTs, geometric mean titers; ATP, according to protocol.

Table 2
Attack rate and vaccine efficacy increase for ILI cases and vaccine efficacy increase for pneumonia cases occurring during the post study starts periods, Day 0–Day 385 (Month 12) and Day 14–Day 385 (Month 12) (according to protocol cohort for efficacy).

				AR[a]	VEI[b]
Day 0–Day 385 (Month 12)					
Event type	Group	*N*	*n*	% (95% CI[e])	% (95% CI)
ILI[c]	$AS03_A$/3.75 μg HA[d]	1950	171	8.77 (7.55–10.11)	11.44 (−8.85–27.95)
	Non-adjuvanted 15 μg HA	1954	191	9.77 (8.49–11.18)	–
Pneumonia	$AS03_A$/3.75 μg HA	1950	8	0.41 (0.18–0.81)	62.52 (15.19–83.44)
	Non-adjuvanted 15 μg HA	1954	21	1.07 (0.67–1.64)	–
Day 14–Day 385 (Month 12)					
Event type	Group	*N*	*n*	% (95% CI)	% (95% CI)
ILI	$AS03_A$/3.75 μg HA	1950	164	8.41 (7.22–9.73)	7.41 (−14.54–25.15)
	Non-adjuvanted 15 μg HA	1954	176	9.01 (7.77–10.36)	–
Pneumonia	$AS03_A$/3.75 μg HA	1950	8	0.41 (0.18–0.81)	58.69 (5.35–81.97)
	Non-adjuvanted 15 μg HA	1954	19	0.97 (0.59–1.51)	–

N = number of subjects in each group without missing values; *n* = number of subjects reporting at least one event in each group; attack rate = percentage of subjects reporting at least one ILI case; VEI = relative risk of ILI and pneumonia cases in subjects who received the AS03-adjuvanted 3.75 μg HA influenza A(H1N1)pdm09 vaccine versus subjects who received the non-adjuvanted 15 μg HA influenza A(H1N1)pdm09 vaccine.

[a] AR = attack rate.
[b] VEI = vaccine efficacy increase.
[c] ILI = influenza-like infection.
[d] HA = hemagglutinin.
[e] CI = confidence interval.

Relative efficacy outcomes: The attack rates and VEIs for ILI cases and VEI for pneumonia cases Day 0–Day 385 (Month 12) and Day 14–Day 385 (Month 12) are presented in Table 2. For the efficacy analysis of ILIs, 429 ILI cases (195 cases [10.01%] and 234 cases [11.95%] in the AS03-adjuvanted and non-adjuvanted treatment groups) were reported and 337 were sampled during the study. Of these, 290 samples were tested and only three cases (0.7%) of A(H1N1)pdm09 were confirmed by RT-qPCR (one and two cases respectively). The incidence of ILI cases was comparable between the two treatment groups, except through Day 28 (10 versus 18 [Day 0–Day 14] and 10 versus 23 [Day 14–Day 28] ILI cases in the respective treatment groups). Twenty-nine pneumonia cases were reported during the entire study period: 8 [0.41%] and 21 [1.07%] cases in the AS03-adjuvanted and non-adjuvanted treatment groups, respectively. Of these, 2 cases were diagnosed during the first 14 days post-vaccination in the non-adjuvanted vaccine group, 1 between Days 14 and 28 in the non-adjuvanted vaccine group, and 26 between Days 28 and 365 (8 in the AS03-adjuvanted group and 18 in the non-adjuvanted group). The VEI was 100% from Day 0 to Day 28, 62.52% (95% CI: 15.19–83.44) from Day 0 to Day 385 (Month 12) and 58.69% (95% CI: 5.35–81.97) from Day 14 through Day 385 (Month 12).

Respiratory viruses: Rhinovirus, identified from 74 (25.5%) nasopharyngeal swabs, was the most frequently determined respiratory virus (Table 3).

3.3. Safety and reactogenicity

Solicited adverse events: Pain at the injection site was the most frequently reported solicited local adverse event. It was reported for 82.1% and 29.9% of subjects in the 18–64 years age group who received the AS03-adjuvanted and the non-adjuvanted influenza A(H1N1)pdm09 vaccine, respectively ($p < 0.0001$) and for 56.7% and 9.4% of subjects in the >64 years group who received the adjuvanted and the non-adjuvanted influenza A(H1N1)pdm09 vaccine, respectively ($p < 0.0001$) (Fig. 3/Web-appendix Table 3). Additionally, in both age groups, a statistically significant higher percentage of subjects receiving the adjuvanted vaccine reported

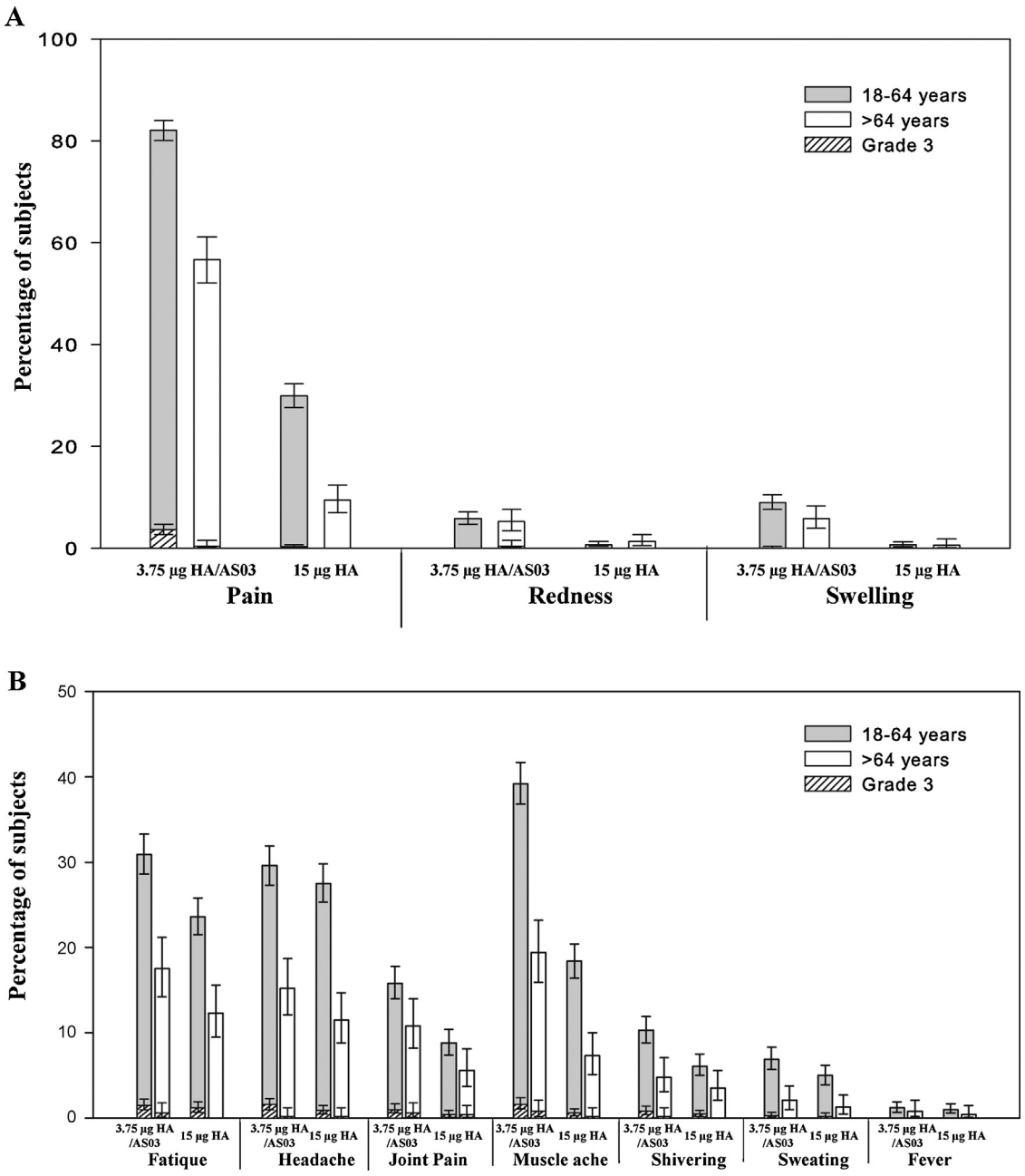

Fig. 3. The incidence of solicited local (A) and general (B) adverse events reported during the 7-day post-vaccination period (total vaccinated cohort).

redness and swelling compared with non-adjuvanted vaccine group ($p < 0.05$ for both). Muscle ache (AS03-adjuvanted/non-adjuvanted: 18–64 years: 39.2%/18.4%, $p < 0.0001$; >64 years: 19.4%/7.3%, $p < 0.0001$), fatigue (AS03-adjuvanted/non-adjuvanted: 18–64 years: 30.9%/23.6%, $p < 0.0001$; >64 years: 17.5%/12.3%, $p = 0.03$) and headache (AS03-adjuvanted/non-adjuvanted: 18–64 years: 29.6%/27.5%, $p = 0.21$; >64 years: 15.2%/11.5%, $p = 0.11$) were the most frequently reported solicited general adverse events (Fig. 3/Web-appendix Table 3). In the 18–64 years age group, a higher percentage on subjects receiving the adjuvanted vaccine reported joint pain, shivering and sweating compared with non-adjuvanted group ($p < 0.05$ for all). In the >64 years group joint pain was reported by a higher percentage of subjects receiving adjuvanted vaccine compared with the subjects receiving the non-adjuvanted vaccine ($p = 0.005$). In this age group, no statistically significant differences were observed between vaccine groups in terms of shivering, sweating and fever ($p > 0.05$). Solicited local and general adverse events of Grade 3 intensity were reported for $\leq$3.6% of subjects. In the 18–64 years age group, the incidence of pain at the injection site, joint pain and muscle aches of Grade 3 intensity was significantly higher in the adjuvanted vaccine group compared with the non-adjuvanted group ($p < 0.0001$ for pain at the injection site; $p = 0.03$ for joint pain; $p = 0.006$ for muscle aches). The incidence of other solicited symptoms of Grade 3 intensity in this age group, as well as in the >64 years age group, was not statistically significant different between the adjuvanted and the non-adjuvanted vaccine groups ($p > 0.05$). Reporting of solicited adverse events was higher in the 18–64 years age group.

Unsolicited adverse events: A total of 181 subjects (4.5%; AS03-adjuvanted: 18–64 years: 87 [5.6%], >64 years: 14 [2.9%]; non-adjuvanted: 18–64 years: 65 [4.2%], >64 years: 15 [3.1%]) reported at least one unsolicited adverse event causally related

Table 3
Distribution of other respiratory viruses identified on swab samples collected during ILIs (total vaccinated cohort).

		n[a]	%
Number of samples tested		290	100.0
No virus detected		162	55.9
One virus detected	Rhinovirus	74	25.5
	Human metapneumovirus (hMPV)	14	4.8
	Human Coronavirus HKU1	13	4.5
	Respiratory syncytial virus (RSV)	5	1.7
	Human Coronavirus 229E	5	1.7
	Human Coronavirus OC43	4	1.4
	Parainfluenzavirus 3	3	1.0
	Parainfluenzavirus 4	3	1.0
	Parainfluenzavirus 1	3	1.0
	Adenovirus	2	0.7
	Influenza B	1	0.3
	Bocavirus	1	0.3
Two viruses detected	Parainfluenzavirus 1 + Bocavirus	1	0.3

[a] *n* = number of subjects/samples in a given category.

to vaccination, during the 42-day post-vaccination follow-up period.

Overall, 216 SAEs were reported in 157 subjects (3.9%) through Day 385 (Month 12) (Web appendix Table 4); 84 subjects in the AS03-adjuvanted treatment group, 18–64 years: 2.9%, >64 years: 8.1%, and 73 subjects in the non-adjuvanted treatment group, 18–64 years: 2.1%, >64 years: 8.5%. Two of these events, intestinal obstruction (AS03-adjuvanted treatment group) and multiple sclerosis (non-adjuvanted treatment group) were considered by the investigator to be possibly related to study vaccine and were also considered pIMDs. Through Day 385 (Month 12), 12 pIMDs according to the predefined list of pIMD preferred terms were reported, with 5 and 7 in AS03-adjuvanted and non-adjuvanted influenza treatment groups, respectively. Seven fatal SAEs were reported, 6 and 1 in AS03-adjuvanted and non-adjuvanted treatment groups, respectively. All were assessed by investigators as not related to vaccination. A detailed description of all fatal SAEs is provided in Web appendix Table 5. Overall, 32 samples had laboratory values for the hematological and biochemical parameters outside the normal laboratory reference range at Days 7 and 21. Of these, 14 were from subjects in the adjuvanted vaccine group and 18 were from subjects in the non-adjuvanted vaccine group.

4. Discussion

Data from this large, controlled study in adults 18 years of age and older demonstrated that a single dose of AS03-adjuvanted or non-adjuvanted influenza A(H1N1)pdm09 vaccine elicited strong HI immune responses 21 days later that met the CHMP and the more stringent CBER criteria for pandemic influenza vaccines. The HI antibody response persisted through six months after vaccination for both vaccines, although the CBER criteria were met only in the 18–64 years age group and CHMP criteria in the 18–60 years age group.

The co-primary objective concerning relative vaccine efficacy against influenza was not evaluated due to the small number of RT-qPCR-confirmed H1N1/09 influenza cases. The low number of cases observed may be partially due to the timing of the study; the start of study vaccination followed the peak of A(H1N1)pdm09 virus transmission in the US and Canada by a week or more (last week of October, 2009), by which time A(H1N1)pdm09 circulation had diminished considerably. Published estimates of AS03-adjuvanted influenza A(H1N1)pdm09 vaccine effectiveness against influenza range from 62.0% to 100.0% [27–30].

Overall, the incidence of ILI cases was comparable between the two groups, except in the first 28 days after vaccination (20 versus 41 ILI cases in the AS03-adjuvanted and non-adjuvanted treatment groups, respectively). This study was not sufficiently powered to detect statistical significance in this analysis.

The data for elderly subjects from the present study are in agreement with observations made in previous studies that one dose of the AS03-adjuvanted 3.75 μg HA influenza A(H1N1)pdm09 vaccine may be insufficient to meet CBER criteria at 6 months in elderly [31] and two doses of vaccine administered 21 days apart induce long-term persistence of HI antibodies at putatively protective levels [32–34]. Nicholson et al. demonstrated that two doses of a different AS03-adjuvanted influenza A(H1N1)pdm09 vaccine elicited HI immune responses that persisted at seroprotective levels in >70% of subjects ≥65 years of age, up to six months after vaccination, although at lower levels compared to younger adults ($p < 0.0001$) [34].

Similar to other observations [11,17,18,35–40], our results showed that previous seasonal vaccination appeared to negatively influence the strength of the immune response elicited by the influenza A(H1N1)pdm09 vaccines, especially in terms of long-term immunogenicity. There are conflicting reports on whether previous seasonal influenza vaccination increases the risk of subsequently contracting A(H1N1)pdm09 infection requiring medical attention [30,41]. The effect of BMI on immune response was also studied. Consistent with previous trials [42,43], in the present study, high BMI did not appear to impair HI antibody response shortly after vaccination. However, Sheridan et al. reported a decrease in HI antibody titers in obese subjects 12 months after vaccination [43], an observation also made in the present study.

The reactogenicity and safety profile was in agreement with available data in adults and children [19,32,44]. The frequency of solicited local adverse events in this study was higher in the AS03-adjuvanted versus the non-adjuvanted treatment group and the frequency of solicited adverse events were comparatively lower in the >64 years age group. Previous clinical trials of influenza A(H1N1/)pdm09 vaccines [17,45,2,46] comparing safety outcomes between adjuvanted and non-adjuvanted vaccines reported similar observations, with higher frequency of both local and general adverse events with adjuvanted vaccines compared with non-adjuvanted vaccines. In our study, we did not observe any differences between the two vaccine groups in terms of SAEs considered as possibly related to vaccination (1 in each group). Although an imbalance in the number of fatal SAEs was observed between the adjuvanted and non-adjuvanted group (6 versus 1), none were considered to be related to vaccination and they all occurred in subjects with a relevant medical history.

A gradual decrement in the HI antibody GMTs elicited by both study vaccines against the A(H1N1)pdm09 vaccine strain in older subjects was observed and this could be attributed to "immunosenescence" [47,48]. A decreasing trend with advancing age was also observed in the frequency of solicited adverse events.

A possible limitation of this study was the absence of blood samples collection for assessment of the immune response after Day 182 (Month 6). This period of six months was anticipated to cover the period of transmission of influenza virus during one season. A recently published study enrolling 240 subjects randomized to receive one or two doses of the same adjuvanted vaccine and followed up to 12 months, showed that regulatory criteria were met 6 months after the administration of the last vaccine dose in subjects aged 18–60 years receiving either one or two vaccine doses and in subjects aged >60 years receiving two vaccine doses [49]. At Day 385 (Month 12) the regulatory criteria were still met only in subjects aged 18–60 years who received two vaccine doses.

In conclusion, a single dose of either adjuvanted or non-adjuvanted influenza A(H1N1)pdm09 vaccines elicited protective

levels of HI antibodies against the vaccine homologous A/California/7/2009 strain that persisted up to Day 182 (Month 6) in the 18–64 years population. Adjuvantation potentially offers the opportunity for antigen-sparing, making this AS03-adjuvanted influenza A(H1N1)pdm09 vaccine a candidate to help meet the demands for the large number of vaccine doses required to mitigate pandemic influenza.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2013.07.007.

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