

Evaluation of immune response following one dose of an AS03_A-adjuvanted H1N1 2009 pandemic influenza vaccine in Japanese adults 65 years of age or older

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Abbreviations: ATP, according-to-protocol; CBER, Center for Biologics Evaluation & Research; CDC, Centers for Disease Control and Prevention; CHMP, Committee for Medicinal Products for Human Use; CI, confidence interval; GMFR, geometric mean fold rise; GMT, geometric mean titre; HA, hemagglutinin; HI, hemagglutination inhibition; MAE, medically-attended adverse event; MHLW, Ministry of Health, Labour and Welfare; pIMD, potential immune-mediated disease; SAE, serious adverse event; SCR, seroconversion rate; SPR, seroprotection rate; TVC, total vaccinated cohort; VRR, vaccine response rate; WHO, World Health Organization

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Objective: This study assessed the immunogenicity, long-term persistence of immune response and safety of a single dose of an A/California/07/2009 H1N1 pandemic influenza vaccine adjuvanted with AS03 (α -tocopherol and squalene based oil-in-water emulsion Adjuvant System) in subjects ≥ 65 y of age (NCT01114620).

Results: At Day 21, the HI immune response met all three European guidance criteria [seroconversion rate (SCR): 60.0%; seroprotection rate (SPR): 64.0%; geometric mean fold rise (GMFR): 10.2] and the US guidance criterion for SCR. At month 6, the HI immune response against the A/California/07/2009 H1N1 strain persisted but at levels lower than that observed at Day 21 (SCR: 38.8%; SPR: 42.9%; HI antibody geometric mean titer: 27.6); the European regulatory guidance criteria for SCR and GMFR were still met. Overall, the vaccine was well-tolerated.

Methods: In this open-label, single group study, 50 subjects received one dose of the 3.75 μ g hemagglutinin (HA) AS03-adjuvanted H1N1 2009 vaccine. Immunogenicity assessments were made before vaccination, 21 days and six months after vaccination using hemagglutination inhibition (HI) and microneutralization assays. Immunogenicity end points were based on US and European regulatory criteria.

Conclusion: A single dose of the 3.75 μ g HA AS03-adjuvanted H1N1 2009 pandemic vaccine induced immune responses against the vaccine strain that met the European regulatory guidance criteria at day 21 in the elderly Japanese population; the immune response persisted at lower levels at month 6. No safety concerns were identified. These results suggest that two vaccine doses might be useful for the elderly population to improve antibody induction and persistence.

Introduction

The influenza pandemic of 2009 caused by the novel, swine-origin influenza A H1N1 2009 reaffirmed the unpredictable nature of influenza viruses.¹ This triple re-assortant influenza virus was characterized by a unique combination of genes from both North American and Eurasian swine lineages hitherto unidentified in human or swine populations.^{1,2} Due to its genetic divergence from the circulating seasonal H1N1 influenza viruses, the existing seasonal influenza vaccines were thought unlikely to confer protection against the influenza A H1N1 2009 virus.^{1,3,4}

The Center for Disease Control and Prevention (CDC) estimates indicated that contrary to the epidemiological pattern observed for seasonal influenza which is associated with high morbidity and mortality in the elderly, the number of influenza A H1N1 2009 cases and associated hospitalizations in the elderly population ≥ 65 y in the US were lower than that reported in adults and children; however, influenza A H1N1 2009 related complications led to a high mortality rate in those ≥ 65 y of age, which although lower than that in younger adults, was comparable to that in children.⁵ The trends in the epidemiology of the H1N1 2009 pandemic in Japan were similar to these observations, where despite the infection rate in those ≥ 60 y of age being

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Table 1. Immune response in terms of HI antibodies against the vaccine homologous A/California/7/2009 strain at all evaluation time points (ATP cohort for immunogenicity)

Age group	N	Seroprotection rate	Seroconversion rate	Geometric Mean titer	Geometric Mean Fold Rise
		% (95% CI)	% (95% CI)	Value (95% CI)	Value (95% CI)
Pre-vaccination	50	4.0% (0.5–13.7)	–	6.6 (5.5–7.9)	–
Day 21	50	64.0% (49.2–77.1)	60.0% (45.2–73.6)	67.2 (46.1–98.0)	10.2 (6.9–14.9)
Month 6	49	42.9% (28.8–57.8)	38.8% (25.2–53.8)	27.6 (19.2–39.8)	4.2 (3.0–6.0)

N, number of subjects with available results; CI, confidence interval.

lower compared with that in the younger age groups, the case-fatality rate was relatively high among elderly adults, especially those with underlying medical conditions.^{6,7}

Immunization is considered to be the most effective prophylactic approach to mitigate pandemic influenza associated illness and death.^{8–10} In order to effectively combat the H1N1 2009 influenza pandemic, the World Health Organization (WHO) endorsed the use of adjuvanted pandemic influenza vaccines in parallel with unadjuvanted vaccines to meet the vaccine dose requirements and to confer cross-reactive immunity.^{11,12} Consequently, a number of H1N1 2009 pandemic influenza vaccines at different doses with or without adjuvants were developed.¹³ Two meta-analyses of trials evaluating these vaccines in various age groups have reported that the adjuvanted vaccines elicited higher immune responses than the non-adjuvanted vaccines and did not give rise to any safety concerns.^{14,15}

Based on the experience of developing a pre-pandemic H5N1 influenza vaccine with an AS03 Adjuvant System (an Adjuvant System containing α -tocopherol and squalene in an oil-in-water emulsion) which was found to be highly immunogenic and well-tolerated in different populations,^{16–18} an AS03-adjuvanted H1N1 2009 pandemic influenza vaccine with 3.75 μ g hemagglutinin (HA) content was developed. This vaccine has been shown to be highly immunogenic (fulfilling the US and European regulatory guidance criteria for pandemic influenza vaccines) with a clinically acceptable safety and reactogenicity profile in adults.^{19,20} A previous study in Japan reported similar results following a single dose of the AS03-adjuvanted 3.75 μ g HA H1N1 2009 vaccine in adults 20–64 y of age.²¹

As a post-licensure commitment to the Japanese health authorities [Ministry of Health, Labor and Welfare (MHLW)] for granting exceptional approval for the AS03-adjuvanted H1N1 2009 vaccine, this study was conducted to evaluate the immunogenicity and safety of the vaccine in older adults \geq 65 y of age. The objective of the study was to demonstrate that a single dose of this H1N1 2009 vaccine could induce a humoral immune response in terms of H1N1 hemagglutination inhibition (HI) antibody titers meeting the US and European regulatory guidance criteria. In addition, the study also assessed the persistence of this immune response at month 6 and the safety of the vaccine in this age group.

Results

Study population. The study was conducted between May 2010 and November 2010. All 50 subjects who were enrolled received one dose of the H1N1 2009 vaccine [Total vaccinated cohort (TVC)] and completed the study up to month 6; all subjects were included in the according-to-protocol (ATP) cohort for immunogenicity at day 21. One subject was excluded from the ATP cohort for immunogenicity at month 6 due to protocol violations. This 69 y-old male subject had received an investigational treatment for diabetes during the study; however, this protocol violation remained unreported until data validation for the month 6 time point, resulting in the inclusion of this subject in the ATP cohort for the day 21 analyses.

The mean age of subjects at the time of vaccination was 69.8 y (range: 65 to 80 y). The male to female ratio was 58.0%:42.0% and all subjects were of Japanese heritage.

Immunogenicity. HI immune response. Before vaccination, 24.0% of subjects were seropositive for HI antibodies against the vaccine homologous strain and the corresponding HI antibody geometric mean titer (GMT) was 6.6. Twenty-one days after vaccination, the seropositivity rate increased to 96.0% and the GMT to 67.2. All three Committee for Medicinal Products for Human Use (CHMP) criteria [seroprotection rate (SPR), seroconversion rate (SCR), geometric mean fold rise (GMFR)] for pandemic influenza vaccines in older adults were met (64.0%, 60.0% and 10.2, respectively). In addition, the Center for Biologics Evaluation and Research (CBER) criterion for SCR was met but not the criterion for the percentage of subjects with HI antibody titers \geq 1:40 (Table 1).

Six months from the first vaccine dose (month 6), the HI immune response against the H1N1 2009 strain persisted but at levels lower than that observed at day 21. Seropositivity rates persisted at 81.6%, with the corresponding HI antibody GMT of 27.6. The CHMP guidance criteria for SCR and GMFR were still met (38.8% and 4.2, respectively). The two CBER criteria were unmet at month 6. As the samples from month 6 and the samples from days 0 and 21 were tested by HI assay at different times, the potential variation of biologic assays over time must be taken into account.

Microneutralization assay. Before vaccination, 38.0% of subjects had seropositive levels of neutralizing antibodies against the A/Netherlands/602/09 strain. Twenty-one days after

vaccination (day 21), the seropositivity rate rose to 82.0%, with a vaccine response rate (VRR) of 40.0%. Six months later (month 6), persistence of neutralizing antibody response against the A/Netherlands/602/09 strain was evident—seropositivity rate of 83.7% and VRR of 30.6%. The neutralizing antibody GMTs are presented in Table 2.

Safety and reactogenicity. During the 7-day period following vaccination, at least one solicited or unsolicited adverse event assessed by the investigator to be vaccination-related was reported for 74.0% of the subjects (local: 70.0%; general: 38.0%). Pain at the injection site (reported for 66.0% of subjects) and fatigue and muscle ache (both reported for 20.0% of subjects) were the most frequently reported solicited local and general adverse events, respectively during the 7-day post-vaccination follow-up period. None of the subjects reported any solicited local adverse events of Grade 3 intensity. Fatigue and headache of Grade 3 intensity occurring on day 4 following vaccination was reported in 2.0% of subjects (one subject for each symptom) (Figs. 1 and 2).

Ten subjects (20.0%) reported at least one unsolicited adverse event during the 42-day post-vaccination follow-up period. One unsolicited adverse event, upper respiratory tract inflammation of Grade 2 intensity was considered by the investigator to be causally related to vaccination. A total of seven subjects (14.0%) experienced at least one medically attended adverse event (MAE) during the 42-day post-vaccination follow-up period.

Overall, serious adverse events (SAEs) were reported for four subjects (8.0%). A male subject 77 years of age reported relapsed liver cancer 27 days after vaccination (original diagnosis was in 2005, but stage and other details are not known) and another male subject 80 years of age was diagnosed with multiple myeloma 33 days after vaccination. Both subjects required hospitalization and the events remained unresolved at study conclusion. Two other subjects required hospitalization for the treatment of cataract and vertigo (onset 118 and 84 days after vaccination, respectively); both events resolved before study conclusion. None of these serious adverse events were considered by the investigator to be related to vaccination. No potential immune-mediated diseases (pIMDs) were identified.

Discussion

The number of doses of H1N1 2009 pandemic influenza vaccine required to induce an optimal immune response in the elderly adult population (a population at lower risk for H1N1 disease)⁵ continues to be a topic of discussion. In this assessment of an AS03-adjuvanted H1N1 2009 pandemic influenza vaccine, a single dose induced strong HI immune responses 21 days later that met all three CHMP criteria for pandemic influenza vaccines in elderly adults; the CBER criterion for SCR was also met at day 21 but not the criterion for the percentage of subjects with HI antibody titers $\geq 1:40$. In a study conducted in the UK, where 71 elderly subjects ≥ 65 years of age were enrolled in a larger study, a two dose regimen with an AS03-adjuvanted 3.75 μg HA formulated as split virion vaccine ($n = 37$) or unadjuvanted 7.5 μg HA formulated as whole-virion vaccine ($n = 34$) was recommended for elderly adults as European regulatory criteria were

Table 2. Immune response in terms of neutralizing antibodies against the A/Netherlands/602/09 strain [antigenically homologous to the vaccine strain] at all evaluation time points (ATP cohort for immunogenicity)

Time point	Vaccine Response Rates		Geometric Mean titers
	N	% (95% CI)	Value (95% CI)
Pre-vaccination	50	–	7.7 (5.8–10.1)
Day 21	50	40.0% (26.4–54.8)	29.3 (18.6–46.0)
Month 6	49	30.6% (18.3–45.4)	20.4 (14.4–28.7)

N, Number of subjects with available results; CI, Confidence interval

not met in this age stratum following one dose of 3.75 μg HA adjuvanted with AS03.²² A study in the US in which 257 subjects ≥ 65 years of age received the AS03-adjuvanted 3.75 μg HA vaccine, 21 days following a single vaccine dose, European and US regulatory criteria were met.²³ In another study in the ≥ 65 years population, a single dose of unadjuvanted 7.5 μg HA vaccine also induced potentially protective immune responses.²⁴

The HI antibody response in the elderly study population in the present study persisted to six months after vaccination though at lower levels than at day 21, as evident from the high seropositivity rates (81.6%). The CHMP criteria for SCR and GMFR were still met at month 6. This is in agreement with observations made in two separate studies in the US and UK, which reported that in subjects ≥ 65 years of age two doses of the AS03-adjuvanted 3.75 μg HA H1N1 2009 vaccine were necessary to induce long-term persistence of HI antibody.^{22,23}

The evidence for an effective immune response induced by this AS03-adjuvanted H1N1 2009 vaccine in the elderly population is not well-established in Japan. However, data for Japanese adults under age 65 years is available from studies conducted just before the mass vaccination programs with adjuvanted and non-adjuvanted formulations of H1N1 2009 vaccine.^{21,25} These studies reported that the immune response after two doses of AS03-adjuvanted 3.75 μg HA H1N1 2009 vaccine in Japanese adults was much higher than that observed after two doses of non-adjuvanted 15 μg HA H1N1 2009 vaccine, although the immune response after a single dose of either the adjuvanted or the non-adjuvanted formulations was comparable. The above studies suggested that two doses of AS03-adjuvanted vaccine may improve the persistence of immune response in the elderly Japanese population.

The vaccine induced a strong neutralizing antibody response in the elderly Japanese population in the present study, as evident from the VRRs at day 21. The neutralizing antibody response decreased at month 6, similar to HI antibody persistence, as the VRR at month 6 was lower than that at day 21, as was the observation for the SPR and SCR for HI antibody response at month 6.

Overall, no safety concerns related to vaccination were identified in this study population ≥ 65 years of age. The majority of the solicited symptoms reported were of mild nature ($< 2.0\%$

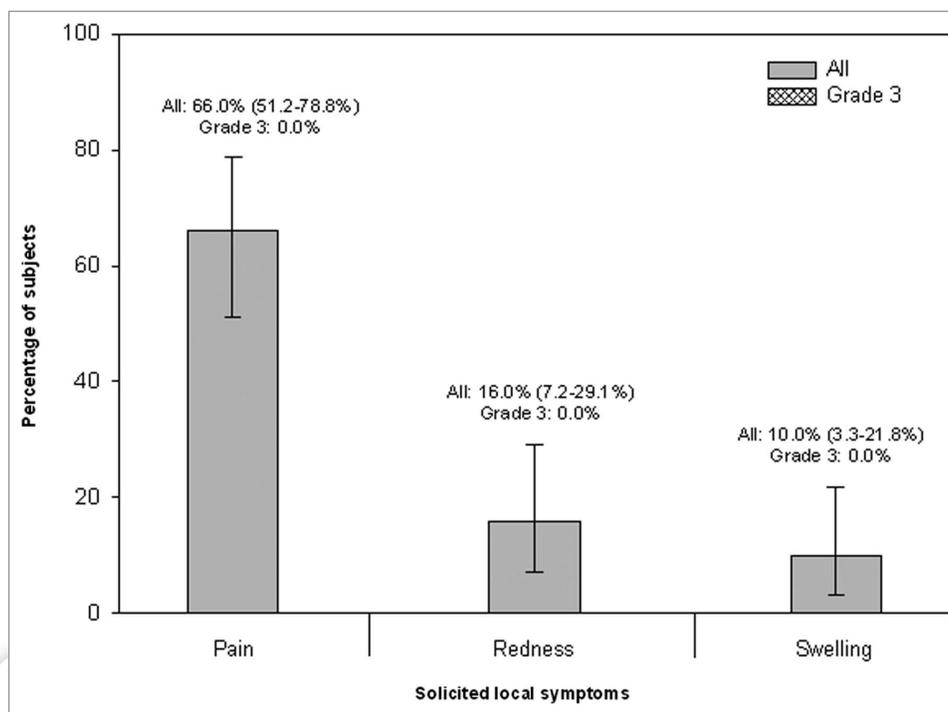


Figure 1. Percentage of subjects reporting solicited local adverse events during the 7-d post-vaccination follow-up period (Total vaccinated cohort).

were of Grade 3 intensity). A comparable observation was made in a previous study in Japanese adults 20–64 years of age using a similar vaccine.²¹ The incidence of solicited adverse events, especially fever in the present study population of elderly adults was lower than that observed in previous studies in younger adults and children.^{21,28,29} In a previous study in children 6 months to 10 years of age, following two doses of an AS03-adjuvanted H1N1 pandemic influenza vaccine, the HI antibody titers were found to be the highest in children with fever $\geq 38.0^{\circ}\text{C}$, which indicated a possible correlation between the immunogenicity and reactogenicity profiles of the vaccine.³⁰ In contrast, post hoc exploratory analyses in this study indicated that there was no apparent correlation between GMT (for HI or neutralizing antibodies) and injection site pain (data not shown).

This study had certain drawbacks. First, it was a single-center study and the sample size was modest, which could be a plausible reason for not meeting the CBER regulatory guidance criteria following a single dose of the study vaccine. However, this relatively small sample size was proposed in the absence of a suitable reference study in the elderly Japanese population and taking into consideration the commitment to provide timely clinical evidence on the H1N1 2009 vaccine to the regulatory agencies at the time of the ensuing pandemic. And second, in the absence of a non-adjuvanted control group, no direct comparisons of immunogenicity or reactogenicity may be made between adjuvanted and non-adjuvanted vaccine formulations.

Since 2001, mass vaccination programs in Japan focused on two target groups – the ≥ 65 year-old population and those 60–64 years of age with chronic disorders of heart, kidney and lung.³¹ In this context, long-term persistence data in the

elderly population from this study will complement existing literature on immune response immediately following primary vaccination.

Materials and Methods

Study design and subjects. This phase IV, open-label, single center study (NCT01114620) trial took place in the context of fulfilling a post-licensure commitment to Japanese health authorities. Older adults ≥ 65 years of age residing in Japan and without a history of previous receipt of a pandemic H1N1 vaccine or any investigational or non-registered medicinal product within 30 days of study start or diagnosed with cancer or under treatment for cancer for three years preceding study start were enrolled to receive a single dose of a monovalent AS03-adjuvanted 3.75 μg HA A/California/7/2009 pandemic influenza vaccine. Serum samples were collected before vaccination and seven days after vaccination for hematological and biochemical assessments. For immunological assessments, serum samples were those collected before vaccination, 21 days (day 21) and six months after vaccination (month 6). Telephone contact was made with vaccinated subjects 42 days and 84 days after vaccination to collect information on the safety profile of the vaccine.

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 an Institutional Review Board.

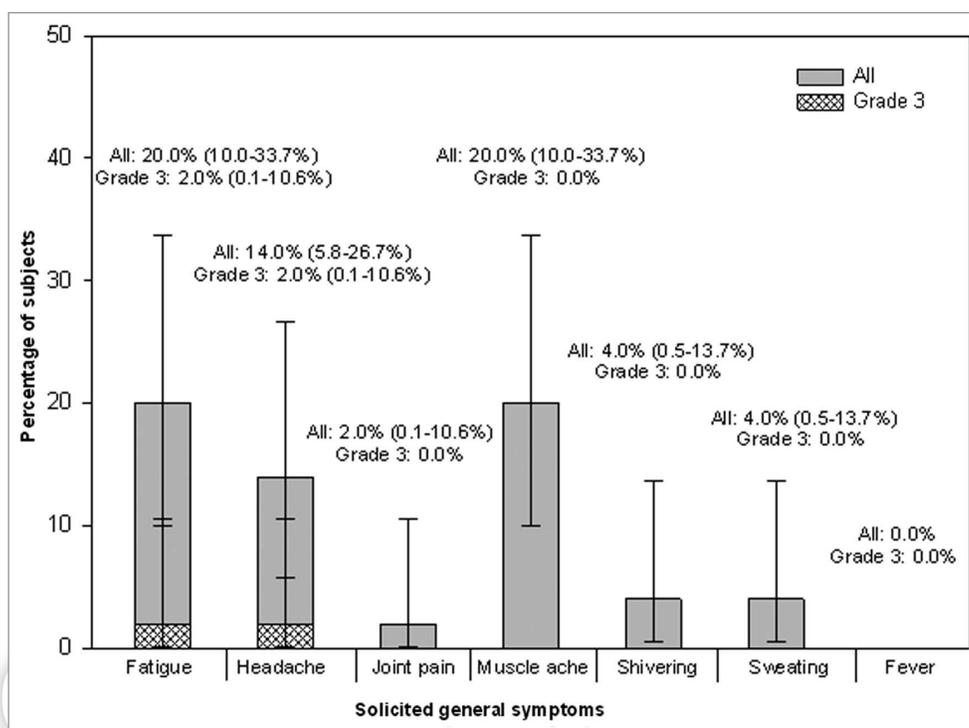


Figure 2. Percentage of subjects reporting solicited general adverse events during the 7-d post-vaccination follow-up period (Total vaccinated cohort).

Study vaccine. The H1N1 2009 pandemic influenza vaccine was a monovalent, inactivated, split-virion antigen adjuvanted with AS03_A (*Arepanrix*TM, a trademark of GlaxoSmithKline group of companies). The H1N1 viral seed for the vaccine was prepared from the reassortant virus NYMC X-179A (New York Medical College) generated from the A/California/07/2009 strain, as recommended by the WHO.¹¹ AS03_A is an Adjuvant System containing α -tocopherol and squalene in an oil-in-water emulsion (squalene 10.69 mg, DL- α -tocopherol 11.86 mg and polysorbate 80 4.86 mg). The antigen and Adjuvant System were made available in multi-dose vials, which were mixed before vaccination. The vaccine was administered into the deltoid muscle.

Immunogenicity assessments. Serum samples collected before vaccination, 21 days after the vaccine dose (day 21) and six months later (month 6) were tested at GSK Vaccines Central Laboratory using a validated in-house hemagglutination inhibition (HI) assay [cut-off: $\geq 1:10$] that used chicken erythrocytes as described previously.³²

The serum samples collected at all time points were tested using a viral microneutralization assay as previously described,^{4,33} and using a strain that is antigenically similar to the vaccine strain (A/Netherlands/602/09) at Viroclinics Biosciences (Rotterdam).³⁴ The neutralizing antibody titers were expressed as the reciprocal of the highest dilution achieving at least 50% neutralization of viral growth [neutralizing dose 50 (ND₅₀)] by applying the Reed and Muench method,³⁵ which gave the cut-off value of 1:8.

The primary immunological assessments were based on the geometric mean titers (GMTs), seroprotection rate (SPR; percentage of subjects with HI antibody titers $\geq 1:40$), seroconversion

rate (SCR; percentage of subjects with either a pre-vaccination HI antibody titers $\leq 1:10$ and post-vaccination titers $\geq 1:40$ or a pre-vaccination HI antibody titers $\geq 1:10$ and at least a 4-fold increase in post-vaccination HI antibody titers) and geometric mean fold rise (GMFR; fold increase in post-vaccination HI antibody GMTs compared with pre-vaccination) in terms of HI antibodies and on the vaccine response rates (VRRs; percentage of subjects with at least a 4-fold increase in post-vaccination neutralizing antibody titers compared with pre-vaccination) in terms of neutralizing antibodies against the vaccine homologous strain.

The immunological outcomes were assessed in terms of the Center for Biologics Evaluation and Research [CBER; lower limit of the 95% confidence interval (CI) for HI antibody SCR: $\geq 40\%$ and SPR: $\geq 70\%$] and Committee for Medicinal Products for Human Use (CHMP; point estimates for HI antibody SCR: $> 40\%$, SPR: $> 70\%$ and GMFR: > 2.5) guidance criteria for pandemic influenza vaccines.^{36,37}

Safety and reactogenicity assessments. Diary cards were used to record the solicited local and general adverse events occurring within 7 days following vaccination, the unsolicited adverse events and medically-attended adverse events (MAEs) occurring within 42 days following vaccination; potential immune-mediated diseases (pIMD) and serious adverse events (SAEs) were recorded during the entire study period up to month 6. The intensity of all solicited adverse events except fever was graded on a standard 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); fever was graded on a 0–4 scale, Grade 3 being axillary temperatures ≥ 39.0 – $\leq 40.0^\circ\text{C}$ and Grade 4 being axillary temperatures $> 40.0^\circ\text{C}$.

Statistical analyses. A sample size of 50 subjects accounting for a 10.0% drop-out rate (45 evaluable subjects) gave 99.9% power to meet the primary objective to fulfil the CBER and CHMP criteria. The reference values for power calculation were chosen based on the results of the most recent study [NCT00985088] using a similar AS03-adjuvanted 3.75 µg HA H1N1 2009 vaccine (SCR = 70.0%, SPR = 88.0% and GMFR = 8.0).

The analyses of immunogenicity were performed on the according-to-protocol (ATP) cohort for immunogenicity which included subjects who received vaccination as per protocol, complied with all protocol-defined procedures and for whom the immunological results (both HI and neutralizing antibody) were available at the given time points (day 21 and month 6); the analyses of safety were performed on the total vaccinated cohort (TVC) which included all subjects with documented vaccination.

Conclusion

The data from this study showed that a single dose of the AS03-adjuvanted 3.75 µg HA H1N1 2009 pandemic influenza vaccine induced HI antibody response against the vaccine homologous A/California/7/2009 strain in subjects ≥ 65 y of age, that met the European regulatory guidance criteria for pandemic influenza vaccines. The HI immune response persisted at lower levels compared with day 21, six months after vaccination. Similar trends were observed for the neutralizing antibody response against the vaccine strain at both time points. Overall, the vaccine did not give rise to any safety concerns in this study population.

Disclosure of Potential Conflicts of Interest

Dr H.I. was the principal investigator. All participating institutions received compensation for study involvement. Drs K.T., A.M., D.V. and P.L. are employees of GlaxoSmithKline Biologicals.

Financial Disclosure Statement

GlaxoSmithKline Biologicals was the funding source and was involved in all stages of the study conduct and analysis

(ClinicalTrials.gov Identifier: NCT01114620). GlaxoSmithKline Vaccines also took in charge all costs associated with the development and the publishing of the present manuscript.

Trademark Statement

Arepanrix is a trademark of the GlaxoSmithKline group of companies.

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