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Characterization and long-term persistence of immune response following two doses of an AS03$_A$-adjuvanted H1N1 influenza vaccine in healthy Japanese adults

Hideyuki Ikematsu,[1,*] Hideaki Nagai,[2] Masahiro Kawashima,[2] Yasunobu Kawakami,[1] Kazuyoshi Tenjinbaru,[3] Ping Li,[4] Karl Walravens,[5] Paul Gillard[5] and François Roman[5]

[1]Department of Clinical Trials, Center for Advanced Medical Innovation, Kyushu University; Fukuoka, Japan; [2]National Hospital Organization Tokyo National Hospital; Kiyose-city, Tokyo, Japan; [3]GlaxoSmithKline; Tokyo, Japan; [4]GlaxoSmithKline Biologicals; King of Prussia, Philadelphia, PA USA; [5]GlaxoSmithKline Biologicals; Wavre, Belgium

Keywords: adjuvant, AS03, H1N1, long-term, pandemic, persistence

Abbreviations: AESI, adverse event of special interest; ATP, according to protocol; 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 titre; HA, hemagglutinin; HI, hemagglutination inhibition; pIMD, potential immune-mediated disease; RBC, red blood cell; SAE, serious adverse event; SCR, seroconversion rate; SPR, seroprotection rate; TVC, total vaccinated cohort; VRR, vaccine response rate; WHO, World Health Organization

Background: Long-term persistence of immune response and safety of two doses of an A/California/07/2009 H1N1 pandemic influenza vaccine adjuvanted with AS03 (an α-tocopherol oil-in-water emulsion-based Adjuvant System) administered 21 d apart was evaluated in Japanese adults [NCT00989612].

Methods: One-hundred healthy subjects aged 20–64 y (stratified [1:1] into two age strata 20–40 y and 41–64 y) received 21 d apart, two doses of AS03-adjuvanted 3.75 μg haemagglutinin (HA) H1N1 2009 vaccine. Immunogenicity data by haemagglutination inhibition (HI) assay six months after the first vaccine dose (Day 182) and microneutralization assay following each of the two vaccine doses (Days 21 and 42) and at Day 182 are reported here.

Results: Persistence of strong HI immune response was observed at Day 182 that met the US and European regulatory thresholds for pandemic influenza vaccines (seroprotection rate: 95%; seroconversion rate: 93%; geometric mean fold-rise: 20). The neutralizing antibody response against the A/Netherlands/602/2009 strain (antigenically similar to vaccine-strain) persisted for at least up to Day 182 (vaccine response rate: 76%; geometric mean titer: 114.4) and paralleled the HI immune response at all time points. No marked difference was observed in HI antibody persistence and neutralising antibody response between the two age strata. The vaccine had a clinically-acceptable safety profile.

Conclusion: Two priming doses of H1N1 2009 pandemic influenza vaccine induced an immune response persisting for at least six months after the first vaccine dose. This could be beneficial in evaluating the importance and effect of vaccination with this AS03-adjuvanted pandemic influenza vaccine.

The H1N1 2009 epidemic in Japan started off as isolated outbreaks in small clusters between May and July 2009. The number of cases rose steadily from mid-August 2009 and peaked in November 2009.[1] An estimated 20 million cases (as of February 5, 2010)[2] and 202 deaths related to H1N1 2009 (as of the end of H1N1 2009 pandemic)[3] were recorded in Japan. The majority of infections were recorded in school children and young adults, with the hospitalization rates being highest in children aged 5–9 y.[4,5] Although, adults appeared to be less susceptible to the H1N1 clinical disease, H1N1 2009 related fatality peaked in adults aged 40–49 y in addition to children aged < 10 y (as of August 10, 2010).[3,5]

Clinical effectiveness of neuraminidase inhibitors, zanamivir and oseltamivir has been reported.[6-8] These drugs are able to mitigate morbidity and mortality caused by an influenza pandemic. However, mass immunization is an effective intervention against pandemic influenza. Identifying the necessity to make available a large number of vaccine doses worldwide and the potential for cross-reactive immunity, the World Health Organization (WHO) supported the development of adjuvanted pandemic influenza vaccines in parallel with non-adjuvanted vaccines.[9,10] A H1N1 2009 pandemic vaccine utilizing 3.75 μg A/California/07/2009 (H1N1)v-like haemagglutinin (HA) antigen

*Correspondance to: Hideyuki Ikematsu; Email: hikematsu@camiku.kyushu-u.ac.jp, ikematsu@gray.plala.or.jp
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adjuvanted with AS03 Adjuvant System (an α-tocopherol oil-in-water emulsion-based Adjuvant System) was developed based on GlaxoSmithKline Biologicals' previous experience with the AS03-adjuvanted prepandemic H5N1 vaccine.[11-13] This H1N1 2009 vaccine has demonstrated strong immunogenicity (fulfilling the US and European regulatory guidance criteria for pandemic influenza vaccines) and a clinically acceptable safety profile in different populations.[14,15]

In an open-label, single group study (NCT00989612) in Japanese adults aged 20–64 y, two doses of this H1N1 2009 pandemic influenza vaccine administered 21 d apart was found to be well-tolerated and highly immunogenic (all subjects seroconverted/ were seroprotected 21 d after the second vaccine dose), achieving the US and European regulatory guidance criteria for pandemic influenza vaccines in adults.[16] This manuscript presents follow-up data from the same population (stratified into 20–40 y and 41–64 y) on the persistence of humoral immune response in terms of HI antibody titers against the vaccine-homologous strain six months after primary vaccination with two doses of this AS03-adjuvanted H1N1 2009 vaccine (at Day 182), as well as neutralising antibody titers against the vaccine-homologous strain following each of the two doses and six months later (Days 21 and 42; Day 182). Data on the safety profile of the vaccine up to Day 182 is also presented here.

Results

Study population. The six month follow-up phase of this study was completed on April 19, 2010 (up to Day 182).

All 100 subjects who were part of the primary assessment and had received two doses of the H1N1 2009 vaccine completed the study up to Day 182 and were included in the according to protocol (ATP) cohort for persistence. The median age of subjects at the time of enrolment was 40.5 y (range: 21 to 59 y); 64% of subjects were female and all were of Japanese heritage.

Immunogenicity. *HI antibody immune response.* The haemagglutination inhibition (HI) immune response against the H1N1 2009 strain after six months after the first vaccine dose (Day 182) is presented in **Table 1**. The seroprotection rate (SPR) was 95%, seroconversion rate (SCR) was 93%, with a corresponding geometric mean titer (GMT) of 175.1 and geometric mean fold rise (GMFR) of 20. These values still met and exceeded the Center for Biologics Evaluation and Research (CBER) and Committee for Medicinal Products for Human Use (CHMP) guidance criteria for pandemic influenza vaccines. There was no appreciable difference in HI antibody persistence between the two age strata (overlapping 95% confidence intervals [CIs]). It is to be noted that the samples from Day 0, Day 21 and Day 42 were tested at the same time, while the Day 182 samples were tested later without an assessment of variability from earlier time points. Due to potential assay variability, a comparative interpretation of the HI response at Day 182 with earlier time points should be done with caution.

Neutralizing antibody response. Prior to receiving vaccination, 51% of subjects were seropositive for neutralising antibodies against the A/Netherlands/602/09 strain and the corresponding geometric mean titers (GMT) was low (8.5). Twenty-one days after the first vaccine dose (Day 21), the GMT rose to 136.9, with a vaccine response rate (VRR) of 74%. Following the second vaccine dose, these values increased to 305.8 and 96%, respectively. Six months after the first vaccine dose, persistence of neutralizing antibody response against the A/Netherlands/602/09 strain was evident (overall, GMT of 114.4 and VRR of 76%). No difference in neutralizing immune response was observed between the two age strata at any of the time points (overlapping 95% CIs) (**Table 2**). The proportion of subjects with antibody titers equal or above different threshold of positivity have been presented. The reverse cumulative curves for neutralizing antibodies 21 d after each of the two vaccine doses and at Day 182 (**Fig. 1**) and the neutralizing antibody titers for all time points (**Table 3**) showed a large proportion of subjects with titers equal or above the thresholds of 1:8, 1:16, 1:32 and 1:64, for six months after the first vaccine dose.

Safety and reactogenicity. Overall, at least one unsolicited symptom was reported in 46 subjects (20–40 y: 21 subjects; 41–64 y: 25 subjects) during the 84 d post-vaccination follow-up period, of which 18 were considered to be vaccine-related.

Diarrhea, nasopharyngitis and headache (five subjects each) were the most frequently reported unsolicited symptoms. Of these, four cases of diarrhea and one case of headache were considered to be causally related to vaccination. One subject reported an unsolicited symptom of Grade 3 intensity (urticaria) which required medical attention and was unrelated to vaccination.

Table 1. Immune response in terms of haemagglutination inhibition antibodies against the vaccine homologous A/California/07/2009 strain at Day 182 (ATP cohort for persistence)

Age strata	Time point	Seroprotection rates		Seroconversion rates		Geometric mean titers		Geometric mean fold rise	
		N	% (95% CI)	N	% (95% CI)	N	Value (95% CI)	N	Value (95% CI)
Overall	Pre-vaccination	100	6 (1.9–13.6)	–	–	100	8.8 (7.3–10.5)	–	–
	Day 182	100	95 (88.7−98.4)	100	93.0 (86.1−97.1)	100	175.1 (144.2−212.7)	100	20.0 (16.8−23.8)
20−40	Pre-vaccination	50	6 (1.3–16.5)	–	–	50	8.9 (7.1–11.1)	–	–
years	Day 182	50	98.0 (89.4−99.9)	50	96.0 (86.3−99.5)	50	182.6 (141.1−236.4)	50	20.6 (16.0−26.5)
41−64	Pre-vaccination	50	6 (1.3–16.5)	–	–	50	8.6 (6.8–10.9)	–	–
years	Day 182	50	92.0 (80.8−97.8)	50	90.0 (78.2−96.7)	50	167.9 (124.5−226.5)	50	19.4 (15.1−25.1)

N, number of subjects with available results; CI, confidence interval; ATP, according to protocol.

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

Age strata	Time point	Vaccine response rates		Geometric mean titers	
		N	% (95% CI)	N	Value (95% CI)
Overall	Pre-vaccination	100	–	100	8.5 (7.1–10.2)
	Day 21	100	74.0 (64.3–82.3)	100	136.9 (97.0–193.3)
	Day 42	100	96.0 (90.1–98.9)	100	305.8 (242.5–385.6)
	Day 182	100	76.0 (66.4–84.0)	100	114.4 (89.3–146.5)
20−40 y	Pre-vaccination	50	–	50	8.7 (6.6–11.4)
	Day 21	50	78.0 (64.0–88.5)	50	146.6 (90.1–238.6)
	Day 42	50	96.0 (86.3–99.5)	50	336.6 (247.8–457.1)
	Day 182	50	84.0 (70.9–92.8)	50	133.5 (97.3–183.1)
41−64 y	Pre-vaccination	50	–	50	8.4 (6.6–10.7)
	Day 21	50	70.0 (55.4–82.1)	50	127.8 (77.1–211.9)
	Day 42	50	96.0 (86.3–99.5)	50	277.7 (194.3–397.0)
	Day 182	50	68.0 (53.3–80.5)	50	98.0 (66.6–144.3)

N, number of subjects with available results; CI, confidence interval; ATP, according to protocol.

No potential immune mediated diseases (pIMD) or adverse events of special interest (AESIs) were recorded during the study period. Three serious adverse events (SAEs) were reported in two subjects during the entire study period. One male subject aged 44 y presented with ureteric calculi, approximately four months after the second vaccine dose which was resolved within three days and a female subject aged 36 y had a viral infection and pharyngeal ulceration approximately four and half months after the second vaccine dose which resolved in seven and 11 d, respectively; none of the SAEs were considered by the investigators to be vaccine-related. No fatalities were reported.

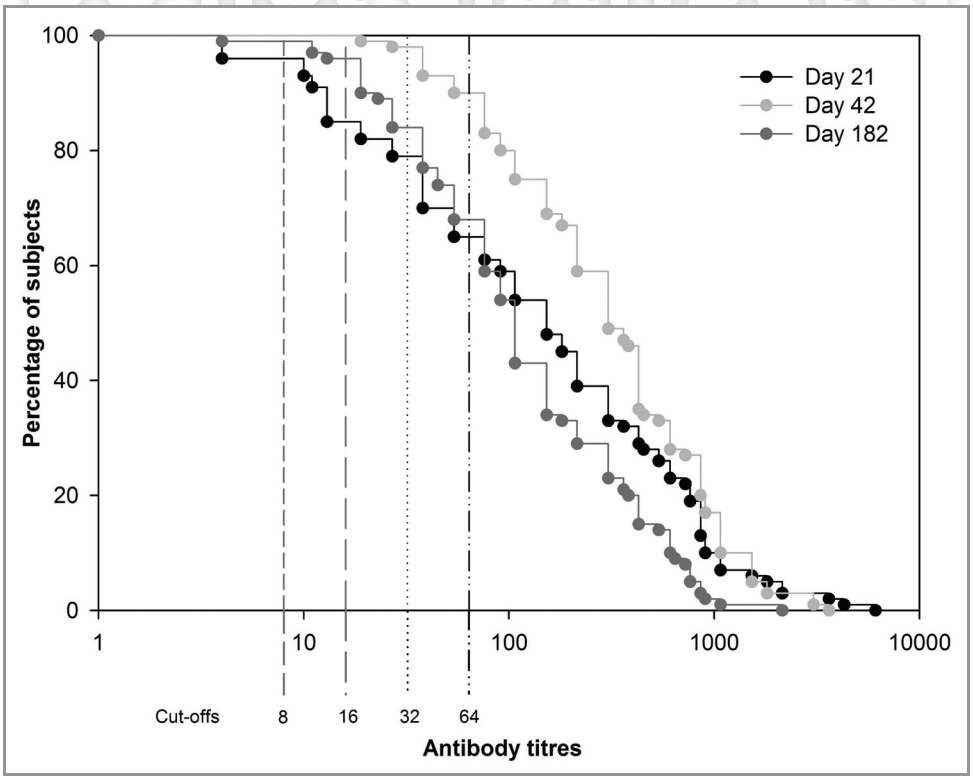

Figure 1. Reverse cumulative curves for neutralising antibody responses 21 d after each of the two vaccine doses (Days 21 and 42) and six months after the first vaccine dose (Day 182) (ATP cohort for immunogenicity). ATP, according to protocol.

Table 3. Percentage of subjects aged 20−64 y with neutralizing antibodies titers ≥ 1:8, ≥ 1:16, ≥ 1:32 and ≥ 1:64 against the A/Netherlands/602/09 strain [antigenically homologous to the vaccine strain] at all time points (ATP cohort for immunogenicity)

Time point		≥ 1:8	≥ 1:16	≥ 1:32	≥ 1:64
	N	% (95% CI)			
Pre-vaccination	100	51.0 (40.8−61.1)	20.0 (12.7−29.2)	12.0 (6.4−20.0)	6.0 (2.2−12.6)
Day 21	100	96.0 (90.1−98.9)	85.0 (76.5−91.4)	79.0 (69.7−86.5)	65.0 (54.8−74.3)
Day 42	100	100 (96.4−100)	100 (96.4−100)	98.0 (93.0−99.8)	90.0 (82.4−95.1)
Day 182	100	99.0 (94.6−100)	96.0 (90.1−98.9)	84.0 (75.3−90.6)	68.0 (57.9−77.0)

N, number of subjects with available results; CI, confidence interval; ATP: according to protocol

Discussion

This is the first study assessing the persistence of immunological response against the A/California/07/2009 strain in Asian adults, six months after vaccination with the AS03-adjuvanted H1N1 2009 pandemic influenza vaccine.

Persistence of HI immune response against the A/California/07/2009 strain was observed for six months after the first vaccine dose (SPR: 95%; SCR: 93%); the CHMP and CBER guidance criteria for pandemic influenza vaccines were met and exceeded at Day 182. The observations from this study is in agreement with available data from studies in other adult populations that reported that the immune response induced by two doses of the 3.75 μg HA AS03-adjuvanted H1N1 2009 vaccine persists for six months after vaccination.[17,18] These observations are important for assessment of disease management strategies in the context of the WHO recommendations for the post-pandemic period which stresses on continuous vigilance, surveillance and disease management of circulating influenza strains.[19]

A previous head-to-head comparison study in UK between a similar AS03-adjuvanted H1N1 2009 vaccine and a non-adjuvanted whole-virion H1N1 2009 vaccine in adults (including those aged ≥ 65 y) reported that a single dose of the adjuvanted vaccine was sufficient to induce immune responses meeting the US and European regulatory criteria while two doses of the whole-virion vaccine were required. In addition, a large proportion of the participants were found to have protective levels of antibodies against the vaccine strain even six months after vaccination with two doses (although age-related decline was evident), indicating that pandemic influenza vaccines can potentially confer immunity against successive waves of the same virus.[20] This is in agreement with previous studies using the AS03-adjuvanted H1N1 2009 vaccine that have demonstrated substantial benefits in terms of induction of rapid, strong and long-lasting immune responses.

Theoretically, neutralization assays can capture a broad range of anti-influenza antibody activities and are able to interrupt several steps of the infectious life cycle of the virus. In contrast, HI assays are largely restricted to measuring the receptor-binding blocking activity of antibodies.[21] However, many different neutralising assays with different variations in protocols and expression of endpoints have been described[22] and it is likely that the biological activity of antibodies measured by these different assays is variable. The assay used in this study is characterized by a short incubation time between the virus and the tested serum. Although the extent of method-specific variation in neutralisation titer and its clinical significance is unknown, assay validation demonstrated good assay specificity (97% with 95% CI: 91.48–99.38%) and a good correlation with the HI assay (r = 0.64) (unpublished GSK data). The study demonstrated strong neutralising antibody response as evident from the high VRRs following each of the two vaccine doses and persistence of high VRRs for six months after the first vaccine dose. Neutralizing antibody responses observed in the study population confirmed the robust immunogenicity of the vaccine and persistence of anti-A/California/07/2009-like antibodies. Overall, the vaccine had a clinically acceptable safety profile in the study population.

The epidemiology and characteristics of the H1N1 2009 virus in Japan has been similar to that observed in other countries in the northern hemisphere and the trends in incidence mirrored those observed worldwide. Although there were fewer laboratory-confirmed H1N1 2009 cases after February–March 2010, and the last reported death due to H1N1 2009 in the pandemic period was in July 2010,[3] the virus continued to circulate in the post-pandemic phase, making it essential to investigate whether pandemic vaccination programs led to long-term persistence of immune response against the H1N1 2009 virus.

The present study advances information on the safety, immunogenicity and long-term immunological persistence of this AS03-adjuvanted H1N1 2009 pandemic influenza vaccine in an Asian population. Contrary to the observations made by Nicholson et al. using a similar vaccine in adults including the elderly,[20] no age-related declined immunological response was observed at Day 42 in the present study, and the data indicated that the immunological response was persistent up to Month 6 in both age strata (20–40 and 41–64 y). The safety profile of the vaccine in Asian adults was comparable to previous reports and no-Asia-specific safety concerns were reported. Thus, the data obtained from this study provides a holistic worldwide dimension to the safety and immunogenicity profile of the study vaccine observed across different populations, now including this Japanese population.

In conclusion, this study presents novel data on persistence of immunological response against the H1N1 2009 virus in adults and on neutralising antibody response induced by this H1N1 2009 pandemic influenza vaccine. It was established that following two doses of a 3.75 μg HA AS03-adjuvanted H1N1 2009 pandemic influenza vaccine in adults aged 20–64 y, immune response against the vaccine homologous A/California/07/2009 strain persisted for at least six months after the first vaccine dose. The immunological response met the US and

European guidance criteria for pandemic influenza vaccines up to six months after the first vaccine dose. These results will be beneficial in evaluating the importance and effect of vaccination with this AS03-adjuvanted pandemic influenza vaccine.

Materials and Methods

Study design and subjects. In the primary phase, 100 healthy Japanese adults aged 20–64 y without history of clinically-confirmed influenza infection or previous vaccination with a novel H1N1 2009 vaccine or any seasonal influenza vaccination within 14 d prior to study start were enrolled to receive 21 d apart, two doses of a monovalent AS03-adjuvanted 3.75 μg HA A/California/07/2009 pandemic influenza vaccine. The subjects were further stratified by age (stratification ratio: 1:1) into 20–40 y and 41–64 y age strata.

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.

Study vaccine. The H1N1 2009 pandemic influenza vaccine was a monovalent, inactivated, split-virion antigen adjuvanted with $AS03_A$ (*Arepanrix*™, a trademark of the GlaxoSmithKline group of companies). The H1N1 viral seed for the vaccine was prepared from the reassortant virus NYMC X-179A (New York Medical College, New York) generated from the A/California/07/2009 strain, as recommended by the WHO.[23] $AS03_A$ is an oil-in-water emulsion-based Adjuvant System containing α-tocopherol (11.86mg tocopherol).

Both vaccine doses were administered intramuscularly at alternate deltoid muscles sides.

Immunogenicity assessments. Blood samples were collected before vaccination, 21 d after each of the two vaccine doses and six months after the first vaccine dose.

Serum samples collected six months after the first vaccine dose (Day 182) were tested at GSK Biologicals Central Laboratory using a validated in-house HI assay [cut-off: ≥ 1:10] that used chicken erythrocytes as previously described.[24]

The viral microneutralisation assay was performed on serum samples collected at all time points at Viroclinics Biosciences (Rotterdam, The Netherlands).[25] The sera were subjected to heat treatment at 56°C for 30 min and then tested in triplicate. The assay used a constant amount of A/Netherlands/602/2009 pandemic H1N1 Influenza virus (A A/California/07/2009-like virus) mixed with serial 2-fold dilutions of serum samples. The mixture of virus and serum was added to Madin-Darby Canine Kidney (MDCK) cell cultures (10^4 cells per well) and incubated for one hour at 37°C, following which the virus-antibody mixture was removed from the wells by aspiration, cells were fed with fresh culture medium and further incubated for 6 d at 37°C. After the incubation period, the well supernatants were transferred into 96 well plates and a suspension of turkey red blood cells (RBCs) was added to it; following an incubation for 60 min at 4°C, the culture supernatants (virus replication) were visualized by haemagglutination of RBCs. The 50% neutralisation titer of a serum was calculated by the Reed and Muench method.[26] The assay cut-off was 1:8.

The evaluation of outcome measures of immune response was based on the immunogenicity criteria for pandemic influenza vaccines in adults as required by the CHMP: point estimates for HI antibody SCR: > 40%, SPR: > 70% and GMFR: > 2.5 and CBER: lower bound of 95% CI for HI antibody for SCR: ≥ 40% and SPR: ≥ 70%.[27,28] SPR was defined as percentage of subjects with a post-vaccination titer ≥ 1:40, SCR as percentage of subjects with pre-vaccination titer < 1:10 and post-vaccination titer ≥ 1:40 or pre-vaccination titer > 1:10 and at least 4-fold increase in post-vaccination titer and GMFR as post-vaccination fold increase in GMTs for HI antibodies. For neutralising antibodies, immunological assessments were based on the VRRs defined as percentage of subjects with either a pre-vaccination titer < 1:8 and a post-vaccination titer ≥ 1:32, or a pre-vaccination titer ≥ 1:8 and at least a 4-fold increase in post-vaccination titer.

Safety and reactogenicity assessments. Unsolicited adverse events were recorded up to 84 d following the first vaccine dose; pIMD (which are a subset of adverse events that include both autoimmune diseases and other inflammatory and/or neurologic disorders which may or may not have an autoimmune etiology), AESI and SAEs occurring during the entire study period were recorded.

Statistical analyses. The analyses of immunogenicity in terms of HI antibodies at Day 182 were performed on the per-protocol cohort for persistence, analyses of immunogenicity in terms of neutralising antibodies at all time points were performed on the per-protocol cohort for immunogenicity and the analyses of safety were performed on the total vaccinated cohort (TVC). The according to protocol cohort for immunogenicity included all subjects who received both vaccine doses and met all protocol-defined eligibility criteria and procedures and for whom data was available at Days 21 and 42. The according to protocol cohort for persistence included all subjects who received both vaccine doses and met all protocol-defined eligibility criteria and procedures and for whom data was available at Days 21, 42 and 182. The TVC included all vaccinated subjects for whom data was available. For the purpose of GMT calculations, antibody titers below the cut-off value of each assay were substituted by half of the cut-off value.

Disclosure of Potential Conflicts of Interest

H.I. and H.N. were the principal investigators of this study and disclose having received honoraria/paid expert testimony and travel grants from the commercial entity that sponsored the study. M.K. and Y.K. disclose having no conflict of interest. All participating institutions received compensation for study involvement. P.G., F.R., K.W., K.T. and P.L. are employees of GlaxoSmithKline Biologicals. P.G. and F.R. report ownership of stock options.

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All authors participated in the implementation of the study including substantial contributions to conception and design, the gathering of the data, or analysis and interpretation of the data. All

authors were involved in the drafting of the article or revising it critically for important intellectual content, and final approval of the manuscript.

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Trademark Statement

Arepanrix is a trademark of GlaxoSmithKline group of companies. Zanamivir *(Relenza)* is a trademark of GlaxoSmithKline group of companies. Oseltamivir *(TamiFlu)* is a trademark of Roche. ClinicalTrials.gov Identifier: NCT00989612

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