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ORIGINAL ARTICLE

Immunogenicity and safety of an AS03$_A$-adjuvanted H5N1 influenza vaccine in a Taiwanese population

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Background/Purpose: A multicenter study (NCT00449670) conducted across Taiwan, Singapore, Hong Kong and Thailand evaluated the safety and manufacturing consistency of four formulations of an AS03$_A$-adjuvanted H5N1 vaccine in terms of immune response against the vaccine-homologous strain (A/Vietnam/1194/2004). This manuscript presents data from the Taiwanese population.

Methods: A total of 400 individuals, aged 18–60 years, were randomized into six groups (2:2:2:2:1:1 ratio) to receive two doses (21 days apart) of one of the four adjuvanted formulations (H5N1-AS03$_A$-groups) or one of the two nonadjuvanted formulations (H5N1-DIL-groups). Blood samples collected before vaccination (Day 0) and 21 days after each vaccine dose were analyzed using hemagglutination inhibition (HI) assay. Adverse events were recorded.

Results: All four AS03$_A$-adjuvanted formulations induced comparable immune responses against the A/Vietnam/1194/2004 strain; following the second dose, immune response in terms of HI antibodies was higher in the H5N1-AS03$_A$-groups {seroprotection rate = 91.6% [95% confidence interval (CI): 87.9–94.4]; geometric mean titer (GMT) = 177.6 (95% CI: 153.2–206.0)} compared with the H5N1-DIL-groups [seroprotection rates = 5.0% (95% CI: 1.4–12.3); GMT = 6.3 (95% CI: 5.4–7.4)]. Immune response against the heterologous A/Indonesia/05/2005 strain was also stronger in the H5N1-AS03$_A$-groups [seroprotection rate = 45.6% (95% CI: 40.0–51.4); GMT = 20.5 (95% CI: 17.8–23.7)] compared with the H5N1-DIL groups

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[seroprotection rate = 0.0% (95% CI: 0.0—4.5); GMT = 5.0 (95% CI: 5.0—5.0)]. The overall reactogenicity profile of the adjuvanted formulations was clinically acceptable.
Conclusion: The AS03$_A$-adjuvanted H5N1 influenza vaccine formulations induced stronger immune response against the vaccine-homologous and heterologous strains than the nonadjuvanted formulations. The AS03$_A$-adjuvanted H5N1 vaccine demonstrated a good immunogenicity and an acceptable safety profile in the Taiwanese population.

Introduction

In 2009, the H1N1 influenza virus emerged as the novel virus affecting the worldwide human population.[1] As of 16 May 2010, at least 18,097 deaths caused by H1N1 have been reported in more than 214 countries.[2] The World Health Organization (WHO) escalated the pandemic alert to phase 6 and health authorities worldwide aimed at mitigating the large-scale morbidity and mortality as well as the economic impact of this pandemic. Therefore, in the current scenario, the focus has shifted away from the H5N1 avian influenza virus, which was previously considered to be the virus that would possibly cause the next influenza pandemic.[3] However, the H5N1 virus continues to be a cause for concern because not only does it have the ability to start a pandemic by itself, but it can also combine with the H1N1 virus leading to devastating effects.[1]

Since its re-emergence in 2003, the H5N1 virus has caused sporadic outbreaks in humans with higher morbidity and mortality rates than observed previously.[1] This could be indicative of the fact that the health impact in case of a H5N1 influenza pandemic in the future is most likely to be severe.[1] As of 6 May 2010, 498 laboratory-confirmed cases and 294 deaths caused by H5N1 influenza infection have been recorded worldwide.[4]

To date, no cases of H5N1 influenza in humans or in poultry have been recorded in Taiwan. However, Taiwan has faced sporadic peaks in the incidence of seasonal influenza.[5] In addition, Taiwan also faces considerable threat from the H5N1 virus considering that its neighbors (China, Vietnam, Indonesia and Thailand) have recorded a high number of laboratory-confirmed H5N1 cases (cumulative: 347 cases and 237 deaths, as of 6 May 2010) and a large number of foreign workers from these countries travel to and reside in Taiwan.[4]

Timely vaccination is considered to be the most effective method of mitigating the morbidity and mortality caused by an influenza pandemic.[6] Anticipating limited availability of influenza vaccine antigens and the potential need to confer protection against the heterologous strains derived from antigenic drifts and shifts, the WHO's Strategic Advisory Group of Experts (SAGE) on immunization acknowledged the importance of those influenza vaccines formulated with oil-in-water-based adjuvants.[7] Thus, antigen-sparing through use of adjuvants and the ability to induce immune response against heterologous strains are the two essential parameters to evaluate the effectiveness of any pandemic influenza vaccine.

GlaxoSmithKline Biologicals S.A. Belgium split-virion H5N1 influenza vaccine [hemagglutinin (HA) antigen content: 3.75 μg] adjuvanted with AS03$_A$ [a tocopherol oil-in-water emulsion-based adjuvant system (11.86 mg tocopherol)] is in line with the SAGE recommendations. This vaccine has been shown to be well tolerated and has elicited strong immune response against the homologous A/Vietnam/1194/2004 strain as well as against a heterologous A/Indonesia/05/2005 strain in European populations.[8,9]

A study conducted in a large population across multiple Asian centers in Taiwan, Singapore, Hong Kong and Thailand demonstrated the vaccine's ability to induce immune response against the A/Vietnam/1194/2004 strain as well as an immune response against the heterologous A/Indonesia/05/2005 strain; the study also demonstrated manufacturing consistency across paired formulations of HA antigen and AS03$_A$ adjuvant. The overall results of this study have been published previously.[10] The current manuscript presents Taiwan-specific data and compares it with the overall results.

Materials and methods

Study design and subjects

This phase III, randomized study (NCT00449670) was conducted between March 24 and July 12, 2007, across four study regions in Asia (Taiwan, Singapore, Thailand and Hong Kong). The study could not be double-blinded because of the difference in appearance between the AS03$_A$-adjuvanted and nonadjuvanted formulations; hence, it was observer-blinded. The study evaluated the manufacturing consistency across four paired formulations (two lots of HA antigen and two lots of AS03$_A$ adjuvant) of the AS03$_A$-adjuvanted split-virion H5N1 influenza vaccine in terms of immune response against the vaccine-homologous A/Vietnam/1194/2004 strain. The vaccine's ability to induce immune response against a heterologous A/Indonesia/05/2005 strain and safety of vaccine administration were also evaluated.

In Taiwan, the study was conducted at the National Taiwan University Hospital and Taipei Veterans General Hospital. Eligible individuals aged between 18 and 60 years were randomized (2:2:2:2:1:1 ratio) using a central randomization scheme (SBIR), into six parallel study groups — four adjuvanted vaccine groups and two nonadjuvanted vaccine groups. People in the adjuvanted vaccine groups (groups H5N1-AS03$_A$) received two doses of one of the four formulations of the AS03$_A$-adjuvanted H5N1 split-virion vaccine (two production lots of H5N1 3.75 μg HA antigen mixed with two production lots of AS03$_A$ adjuvant) 21 days apart. The people in the nonadjuvanted groups (groups

H5N1-DIL) received two doses of any one of the two formulations of the nonadjuvanted H5N1 split-virion vaccine (two production lots of H5N1 3.75 μg HA antigen mixed with diluent) 21 days apart.

Volunteers were excluded if they had previously received any licensed inactivated vaccine within 2 weeks or live-attenuated vaccine within 4 weeks prior to enrollment in the study, were likely to have been exposed to the H5N1 wild-type virus, were allergic to vaccines or vaccine components, had a confirmed or suspected immunosuppressive or immunodeficient condition or were suffering from acute or chronic infections. Lactating or pregnant women were also excluded from the study.

The study protocols and associated study documents were approved by the Independent Ethics Committees of the participating centers and the Department of Health, Executive Yuan in Taiwan. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. Written informed consent was obtained from subjects or their parents/guardians prior to conducting any study-related procedures.

Vaccines

All formulations of the inactivated, split-virion $AS03_A$-adjuvanted influenza vaccine were manufactured by GlaxoSmithKline Biologicals as described previously.[8] Each dose of vaccine (Prepandrix, 0.5 mL) contained at least 3.75 μg HA of the A/Vietnam/1194/2004-like NIBRG-14 Clade 1 strain (National Institute for Biological Standards and Control Potters Bar, UK) adjuvanted with $AS03_A$ [a tocopherol oil-in-water emulsion-based adjuvant system (11.86 mg tocopherol)].[8] All formulations were made available in multidose vials. Vaccines were administered intramuscularly in the deltoid region of the nondominant arm.

Laboratory assays

Serially diluted serum samples (initial dilution of 1:10, followed by twofold serial dilution) were used to determine the hemagglutination inhibition (HI) antibody titers using standard techniques; however, modifications were done to use an equine erythrocyte suspension instead of avian erythrocytes, as described previously.[8]

Assessment of immunogenicity

Blood samples were collected before vaccination (Day 0) and 21 days after each of the two vaccine doses to assess geometric mean titers (GMT), seroprotection rates, seroconversion rates and seroconversion factors in terms of HI antibodies against the homologous A/Vietnam/1194/2004 NIBRG-14 strain and heterologous A/Indonesia/05/2005 IBCDC-RG2 strain. The HI immune response evaluations were based on the European Committee for Human Medicinal Products (CHMP) immunogenicity guidance criteria for pandemic influenza vaccines for adults aged 18–60 years: seroprotection rate >70.0%, seroconversion rate >40.0% and seroconversion factor >2.5.[11]

The seroconversion rate for HI antibodies was defined as the percentage of seronegative subjects (HI antibody titer <1:10) before vaccination with a postvaccination HI antibody titer ≥1:40 or seropositive subjects (HI antibody titer ≥1:10) before vaccination with at least a fourfold increase in HI antibody titer, after each dose. The seroconversion factor was defined as the fold increase in serum HI antibody GMTs postvaccination compared to the GMT prior to Dose 1. The seroprotection rate was defined as the percentage of subjects with a postvaccination serum HI antibody titer ≥ 1:40.

Assessment of safety and reactogenicity

Diary cards were provided to participants to record solicited local and general adverse events up to 7 days after each vaccination dose. Solicited local adverse events (pain, redness, swelling, induration and ecchymosis) and general adverse events (fatigue, fever, headache, myalgia, shivering, sweating and arthralgia) were recorded during the 7 days postvaccination follow-up period and were graded on a scale of 0–3, except fever which was graded on a scale of 0–4. Pain at injection site that hindered normal daily activities was recorded as grade 3; injection site redness, swelling, ecchymosis and induration > 100 mm was recorded as grade 3; fever > 39°C and up to 40°C was recorded as grade 3, > 40°C as grade 4; other solicited general symptoms that hindered normal day-to-day activities were recorded as grade 3 intensity. All unsolicited adverse events were recorded up to 21 days after the first dose and 30 days after the second dose. Serious adverse events (SAEs) were recorded during the entire study period; events occurring until 30 days after the second dose are reported in this manuscript.

Statistical analyses

The analyses of immunogenicity were performed on the per-protocol cohort, while the analyses of safety were performed on the total vaccinated cohort. The per-protocol cohort included people who had received at least one dose of the adjuvanted or nonadjuvanted vaccine, were compliant to the protocol and had available data for the immunogenicity end points, while the total vaccinated cohort included all vaccinated subjects with available data.

All statistical analyses were performed using SAS version 9.1 (SAS, North Carolina, USA). The 95% CIs for the group-wise GMT ratio were calculated using the ANOVA model on log-transformed titers, while all exact 95% CIs for proportions within groups were calculated using Proc StatXact 5.0 (Cytel Inc., Massachusetts, USA).

Results

Study population

A total of 1206 individuals were enrolled across the four study regions, of which 400 were enrolled in Taiwan (H5N1-$AS03_A$: $N = 319$; H5N1-DIL: $N = 81$). All individuals received at least one vaccine dose and 391 people were included in the per-protocol cohort for immunogenicity; reasons for the exclusion of nine subjects from the per-protocol cohort are presented in Fig. 1. The mean age of these 391 people was 32.2 years (standard deviation: 8.71 years; range: 18–58 years). There were more females (57.8%) than males

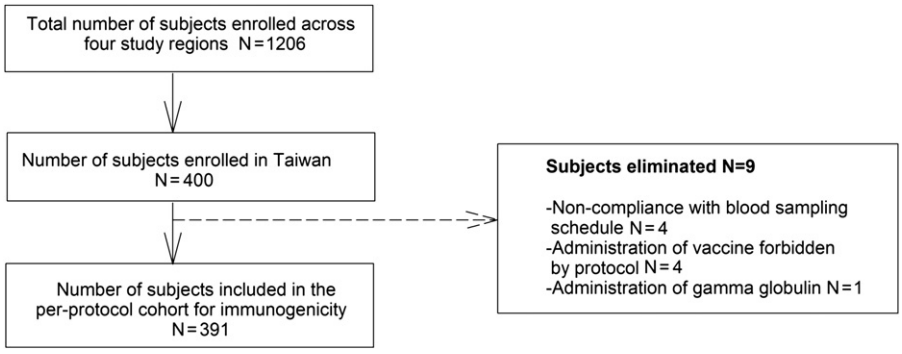

Figure 1 Consolidated Standards of Reporting Trials (CONSORT) diagram for the study.

and almost all participants were of East-Asian origin (99.8%; one person was of South-East Asian origin).

Immunogenicity

This manuscript presents the pooled immunogenicity and safety results from the H5N1-AS03$_A$ and H5N1-DIL groups.

HI antibodies against the homologous A/Vietnam/1194/2004 strain:

Less than 3.0% of participants in the H5N1-AS03$_A$ and H5N1-DIL groups had detectable levels of HI antibodies ($\geq$ 1:10) before vaccination. Following the second vaccine dose, 91.6% (95% CI: 87.9—94.4) of people in the H5N1-AS03$_A$ groups were seroprotected (Table 1) and the HI antibody GMTs for the A/Vietnam/1194/2004 strain increased progressively from 5.2 (95% CI: 5.0—5.5) before vaccination to 177.6 (95% CI: 153.2—206.0) after the second vaccine dose (Fig. 1). However, in the H5N1-DIL group, after the second vaccine dose, the seroprotection rate and HI antibody GMTs did not show an appreciable increase when compared with the prevaccination status (Table 1, Fig. 1).

Twenty-one days after the second vaccine dose, the CHMP immunogenicity guidance criteria were met for the A/Vietnam/1194/2004 strain in the H5N1-AS03$_A$ groups. None of the CHMP criteria was met in the H5N1-DIL groups.

HI antibodies against the heterologous A/Indonesia/05/2005 strain:

Before vaccination, less than 0.5% of subjects in both groups had detectable levels of HI antibodies ($\geq$ 1:10). Following the second vaccine dose, 45.6% (95% CI: 40.0—51.4) of individuals in the H5N1-AS03$_A$ group were seroprotected (Table 1) and the HI antibody GMTs for the A/Indonesia/05/2005 strain increased from 5.0 (95% CI: 5.0—5.0) before vaccination to 20.5 (95% CI: 17.8—23.7) after the second vaccine dose (Fig. 1). In comparison, after the second vaccine dose, none of the subjects in the H5N1-DIL group were seroprotected (Table 1) and the HI antibody GMT for the A/Indonesia/05/2005 strain remained at 5.0 (95% CI: 5.0—5.0) (Fig. 1).

Twenty-one days after the second vaccine dose, two of the three CHMP criteria (in terms of seroconversion rate and seroconversion factor) for the heterologous A/Indonesia/05/2005 strain were met in the H5N1-AS03$_A$ groups. None of the CHMP criteria was met in the H5N1-DIL groups (Fig. 2).

Safety and reactogenicity

A consistent trend towards higher incidence of solicited local and general symptoms during the 7 days post-vaccination follow-up period was observed in the H5N1-AS03$_A$ groups compared to the H5N1-DIL groups. Pain at the injection site [H5N1-AS03$_A$: 96.2% (95% CI: 93.5—98.0); H5N1-DIL: 37.0% (95% CI: 26.6—48.5)] and fatigue [H5N1-AS03$_A$: 75.2% (95% CI: 70.1—79.9); H5N1-DIL: 44.4% (95% CI: 33.4—55.9)] were the most frequently reported solicited local and general symptoms, respectively, in both groups. Grade 3 pain and fatigue were reported by 4.7% (95% CI: 2.7—7.6) and 5.3% (95% CI: 3.1—8.4) subjects, respectively, in the H5N1-AS03$_A$ groups. None of the participants in the H5N1-DIL groups reported solicited local or general symptoms of grade 3 intensity. The occurrence and intensity of

Table 1 Seroprotection rate, seroconversion rate and seroconversion factor for H5N1 hemagglutination inhibition antibodies against the A/Vietnam/1194/2004 and A/Indonesia/05/2005 strains 21 days after the second vaccine dose for Taiwanese population (per-protocol cohort for immunogenicity).

Strains	Parameters (CHMP criteria for adults)	H5N1-AS03$_A$ $N = 309$% (95% CI)	H5N1-DIL $N = 80$% (95% CI)
A/Vietnam/ 1194/2004 NIBRG-14	Seroprotection rate $>$ 70.0%	91.6% (87.9—94.4)	5.0% (1.4—12.3)
	Seroconversion rate $>$ 40.0%	90.9% (87.2—93.9)	3.8% (0.8—10.6)
	Seroconversion factor $>$ 2.5	33.9 (29.1—39.3)	1.2 (1.0—1.4)
A/Indonesia/ 05/2005 IBCDC-RG2	Seroprotection rate $>$ 70.0%	45.6% (40.0—51.4)	0.0% (0.0—4.5)
	Seroconversion rate $>$ 40.0%	45.6% (40.0—51.4)	0.0% (0.0—4.5)
	Seroconversion factor $>$ 2.5	4.1 (3.5—4.7)	1.0 (1.0—1.0)

CHMP = Committee for Human Medicinal Products; CI = confidence interval.

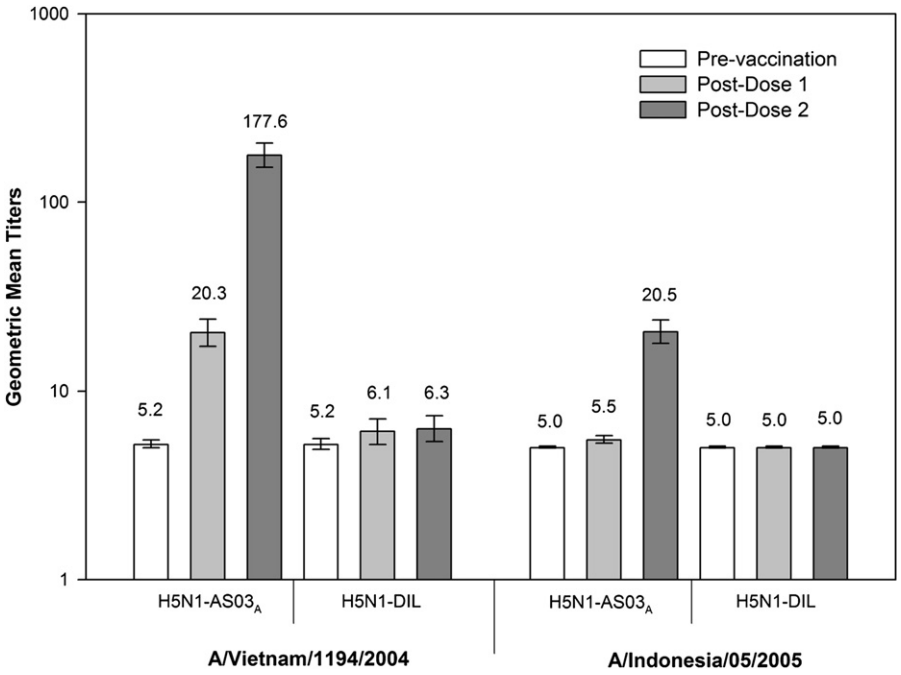

Figure 2 Geometric mean titers (GMTs) for H5N1 hemagglutination inhibition antibodies against A/Vietnam/1194/2004 and A/Indonesia/05/2005 strains, prevaccination and 21 days after each vaccine dose for Taiwanese population (per-protocol cohort for immunogenicity). [a]Error bars indicate 95% confidence intervals for each group.

other solicited local and general symptoms are presented in Table 2.

The occurrence of at least one unsolicited symptom was reported by 39.8% (95% CI: 34.4—45.4) of individuals in the H5N1-AS03$_A$ groups and 29.6% (95% CI: 20—40.8) of individuals in the H5N1-DIL groups. Unsolicited symptoms considered by the investigator to be causally related to vaccination were reported by 12.2% (95% CI: 8.8—16.3) of individuals in the H5N1-AS03$_A$ groups (39 subjects) and 2.5% (95% CI: 0.3—8.6) of individuals in the H5N1-DIL groups (two people). Dizziness was the most commonly reported unsolicited adverse event [2.8% (95% CI: 1.3—5.3); nine individuals] in the H5N1-AS03$_A$ groups, while in the H5N1-DIL groups, abdominal pain, nausea and pharyngeal pain [1.2% (95% CI: 0—6.7) of each; one participant each) were most commonly reported. Only 0.3% (95% CI: 0—1.7) of people in the H5N1-AS03$_A$ groups (one person) reported vaccine-related grade 3 unsolicited adverse events; this person reported asthenia, dizziness and lethargy. No vaccine-related grade 3 unsolicited adverse events were recorded in the H5N1-DIL groups.

During the entire study, three SAEs were recorded in three participants, all in the H5N1-AS03$_A$ groups. None of these, namely hand injury (fracture of the radius), acute appendicitis and leiomyoma of uterus, were considered by the investigators to be vaccine related. All three subjects recovered by the end of the study.

Discussion

At the onset of an influenza pandemic, the immediate availability of a large number of doses of immunogenic influenza vaccine with acceptable reactogenicity profile is of paramount importance. In addition, considering that the strain causing the pandemic in all probability could be a new strain arising from antigenic drifts or shifts in existing strains, the ability of a pandemic influenza vaccine to induce immune response against heterologous strains could be critical in limiting the spread of the virus. Hence, in addition to evaluating nonadjuvanted candidates, current vaccine development initiatives are also focusing on adjuvanted vaccines that can be both antigen sparing and can induce immune response against heterologous strains in addition to the vaccine strain.[6]

In this context, the split-virion H5N1 influenza vaccine approved by the US Food and Drug Administration (FDA) in 2007 has limitations with respect to both antigen-sparing and heterologous strain seroprotection.[12,13]

The Taiwan-specific data from the present study re-established the observations from the overall Asian population that the use of AS03$_A$ adjuvant can influence the immune responses elicited by the H5N1 vaccines; this was evident from the stronger immune response mounted by the individuals who received the AS03$_A$-adjuvanted H5N1 influenza vaccine compared to those who received the nonadjuvanted H5N1 influenza vaccine. All three CHMP criteria, were met and exceeded in subjects in the H5N1-AS03$_A$ group for the homologous A/Vietnam/1194/2004 strain, while two of the three CHMP criteria were met for the heterologous A/Indonesia/05/2005 strain.[11] In comparison, none of the CHMP criteria for either of the strains was met in subjects who received the nonadjuvanted H5N1 influenza vaccine. The immune response was further evaluated in a subset of subjects from the four study centers using a broader and more sensitive neutralizing assay which demonstrated a higher seroconversion rate for the A/Vietnam/1194/2004 and A/Indonesia/05/2005 strains in the H5N1-AS03$_A$ groups (Day 42; A/Vietnam: 96%;

Table 2 Solicited local and general adverse events recorded in the H5N1-AS03 and H5N1-DIL groups during the 7-day postvaccination follow-up period for Taiwanese population (total vaccinated cohort).

	Symptoms	Severity	H5N1-AS03$_A$ % (95% CI)	H5N1-DIL % (95% CI)
Local symptoms	Ecchymosis	All	1.6 (0.5—3.6)	0.0 (0.0—4.5)
		Grade 3	0.0 (0.0—1.1)	0.0 (0.0—4.5)
	Induration	All	8.2 (5.4—11.7)	0.0 (0.0—4.5)
		Grade 3	0.0 (0.0—1.1)	0.0 (0.0—4.5)
	Pain	All	96.2 (93.5—98.0)	37.0 (26.6—48.5)
		Grade 3	4.7 (2.7—7.6)	0.0 (0.0—4.5)
	Redness	All	6.3 (3.9—9.5)	1.2 (0.0—6.7)
		Grade 3	0.0 (0.0—1.1)	0.0 (0.0—4.5)
	Swelling	All	14.4 (10.8—18.8)	0.0 (0.0—4.5)
		Grade 3	0.3 (0.0—1)	0.0 (0.0—4.5)
General symptoms	Arthralgia	All	22.3 (17.8—27.2)	9.9 (4.4—18.5)
		Grade 3	0.9 (0.2—2.7)	0.0 (0.0—4.5)
	Fatigue	All	75.2 (70.1—79.9)	44.4 (33.4—55.9)
		Grade 3	5.3 (3.1—8.4)	0.0 (0.0—4.5)
	Fever	All	2.8 (1.3—5.3)	0.0 (0.0—4.5)
		Grade 3	0.0 (0.0—1.1)	0.0 (0.0—4.5)
	Headache	All	43.3 (37.8—48.9)	19.8 (11.7—30.1)
		Grade 3	3.4 (1.7—6.1)	0.0 (0.0—4.5)
	Myalgia	All	74.0 (68.8—78.7)	30.9 (21.1—42.1)
		Grade 3	4.1 (2.2—6.9)	0.0 (0.0—4.5)
	Shivering	All	9.1 (6.2—12.8)	1.2 (0.0—6.7)
		Grade 3	0.6 (0.1—2.2)	0.0 (0.0—4.5)
	Sweating	All	16.0 (12.1—20.5)	6.2 (2.0—13.8)
		Grade 3	0.0 (0.0—1.1)	0.0 (0.0—4.5)

CI = confidence interval.

A/Indonesia: 91.4%) compared to the H5N1-DIL groups (Day 42; A/Vietnam: 32.4%; A/Indonesia: 5.6%).[10] The vaccine was well tolerated in the Taiwanese population with no additional noticeable difference in the safety and reactogenicity profile being reported when compared to the overall study as well as to studies conducted in other populations.[8—10,14] Thus, the results of this study contribute to the existing literature on the good immunogenicity and acceptable safety data on the AS03$_A$-adjuvanted H5N1 influenza vaccine.

The Government of Taiwan has formulated and implemented its Influenza Pandemic Preparedness Plan with the aim to facilitate mitigation of an impending influenza pandemic,[5] and in this context, these Taiwan-specific data on the study vaccine are of critical importance.

This manuscript focused exclusively on the Taiwanese population to present relevant data that would be of critical importance to the healthcare authorities and decision-makers in Taiwan in the light of the continuing threat of the H5N1 influenza strain in the region. However, a possible weakness of this manuscript is that the Taiwanese study population included 400 subjects which is a comparatively smaller sample size when compared to the overall multicenter study that included 1206 participants. This sample size also did not allow for age-wise stratification. Nevertheless, ample evidence can be drawn from available literature on the safety and immunogenicity of this AS03$_A$-adjuvanted H5N1 vaccine.

In conclusion, the AS03$_A$-adjuvanted split-virion H5N1 vaccine was well tolerated and allowed antigen-sparing as observed from a stronger immune response not only against the homologous H5N1 vaccine strain but also against a heterologous H5N1 strain as compared to the nonadjuvanted formulation, with the same antigen content.

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References

1. Melidou A, Gioula G, Exindari M, Chatzidimitriou D, Diza-Mataftsi E. Influenza A (H5N1): an overview of the current situation. *Eurosurveillance* 2009;**14**:1—4.
2. World Health Organization (WHO). Pandemic (H1N1) 2009 — update 101. May 21, 2010. Global alert and response (GAR).

http://www.who.int/csr/don/2010_05_21/en/index.html [accessed 27.05.10].
3. Bartlett JG, Hayden FG. Influenza A (H5N1): will it be the next pandemic influenza? Are we ready? *Ann Intern Med* 2005;**143**: 460—2.
4. World Health Organization (WHO). Cumulative number of confirmed human cases of avian influenza A/(H5N1) reported to WHO, 6 May 2010. Global alert and response (GAR). http://www.who.int/csr/disease/avian_influenza/country/en/ [accessed 27.05.10].
5. Center for Disease Control, Department of Health, Taiwan, R.O.C. Comprehensive report of prevention strategy for influenza pandemic. *Taiwan Epidemiol Bull* 2008;**24**:853—75.
6. World Health Organization (WHO). *Options for the use of human H5N1 influenza vaccines and the WHO H5N1 vaccine stockpile*. pp. 1—3. Geneva, Switzerland: WHO Scientific Consultation; October 2007.
7. World Health Organization. Pandemic (H1N1) 2009 briefing note 2. Global alert and response (GAR). http://www.who.int/csr/disease/swineflu/notes/h1n1_vaccine_20090713/en/index.html [accessed 5.02.10].
8. Leroux-Roels I, Borkowski A, Vanwolleghem T, Dramé M, Clement F, Hons E, 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.
9. Leroux-Roels I, Bernhard R, Gerard P, Dramé M, Hanon E, Leroux-Roels G. Broad clade 2 cross-reactive immunity induced by an adjuvanted clade 1 rH5N1 pandemic influenza vaccine. *PLoS ONE* 2008;**3**(2):e1665. doi:10.1371/journal.pone.0001665. 499.
10. Chu DW-S, Hwang SJ, Lim FS, Oh HM, Thongcharoen P, Yang PC, et al. Immunogenicity and tolerability of an AS03A-adjuvanted prepandemic influenza vaccine. A phase III study in a large population of Asian adults. *Vaccine* 2009;**27**:7428—35.
11. Committee for Human Medicinal Products (CHMP). *Guideline on influenza vaccines prepared from viruses with the potential to cause a pandemic and intended for use outside of the core dossier context*, vol. 24. London: (EMEA/CHMP/VWP/263499/2006); January 2007.
12. Treanor JJ, Wilkinson BE, Masseoud F, Hu-Primmer J, Battaglia R, O'Brien D, et al. Safety and immunogenicity of a recombinant hemagglutinin vaccine for H5 influenza in humans. *Vaccine* 2001;**19**:1732—7.
13. 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.
14. Rümke HC, Bayas JM, Ramón de Juanes J, Caso C, Richardus JH, Campins M, 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.