

# Cross-clade immunogenicity and safety of an AS03<sub>A</sub>-adjuvanted prepandemic H5N1 influenza vaccine in Hong Kong

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**Objective** To present Hong Kong-specific data from a large Asian population (also involving Thailand, Singapore, and Taiwan) on safety and manufacturing consistency across four AS03<sub>A</sub>-adjuvanted H5N1 vaccine formulations in terms of immune response against the A/Vietnam/1194/2004 strain. Immunogenicity against the heterologous A/Indonesia/05/2005 strain was also assessed. NCT Number: 00449670.

**Design** Prospective, observer-blind study.

**Setting** Out-patient clinic of a tertiary hospital in Hong Kong.

**Participants** A total of 360 subjects aged 18 to 60 years were randomised into six groups to receive two doses (21 days apart) of the study vaccine.

**Interventions** One of the four adjuvanted formulations (3.75 µg H5N1 haemagglutinin [HA]+AS03<sub>A</sub>) of the vaccine (H5N1-AS03<sub>A</sub>) or one of the two non-adjuvanted (3.75 µg H5N1 [HA]) formulations of the vaccine (H5N1-DIL).

**Main outcome measures** Blood samples collected before vaccination and 21 days after each vaccine dose were analysed using haemagglutination-inhibition and neutralisation assays. Solicited, unsolicited, and serious adverse events were recorded.

**Results** Manufacturing consistency across all four vaccine formulations was demonstrated. After two doses, the AS03<sub>A</sub>-adjuvanted prepandemic influenza vaccine demonstrated high seroprotection rates against the A/Vietnam/1194/2004 strain (95.8%) and good immunogenicity against the heterologous A/Indonesia/05/2005 strain (45.7%), as compared to the non-adjuvanted vaccine (4.6% and 1.5%, respectively). The seroconversion rates induced by the adjuvanted formulations in terms of viral neutralising antibodies against the two strains were much higher than those induced by the non-adjuvanted formulations. There were no safety concerns for any of the adjuvanted vaccine formulations.

**Conclusions** The AS03<sub>A</sub>-adjuvanted H5N1 prepandemic influenza vaccine demonstrated good immunogenicity and an acceptable safety profile in Hong Kong.

## Key words

Adjuvants, immunologic; Disease outbreaks; Influenza A virus, H5N1 subtype; Influenza vaccines; Influenza, human

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## Introduction

Hong Kong reported the first-ever instance of human infection with H5N1 virus in the world in 1997. This was followed by 17 other cases in the same year, which resulted in six deaths. In 2003, two new H5N1 human cases were recorded. Since 2004, the H5N1 virus has been isolated in wild birds, and in addition cross-species transmission was observed from tigers and cats; however, no new H5N1 epidemic has ensued.<sup>1</sup> As the H5N1 influenza virus continues to circulate and cause sporadic human infections, there is a high probability of Hong Kong being vulnerable to future influenza pandemics.<sup>1-4</sup>

Vaccination with a prepandemic influenza vaccine just before or immediately after a pandemic outbreak is considered the most effective intervention to counter morbidity and mortality caused by an influenza pandemic.<sup>5-7</sup> The current strategy for prepandemic influenza vaccine development is 2-fold: cross-clade protection and antigen-sparing.<sup>5,8</sup>

## 香港流感大流行的前期疫苗AS03<sub>A</sub>佐劑的交叉分化枝抗原性及安全性

**目的** 報告四種AS03<sub>A</sub>佐劑疫苗在亞洲人口（香港、泰國、星加坡、台灣）中的安全性及製造穩定性。研究尤其針對香港人口對於流感病毒 A/Vietnam/1194/2004 品種的免疫反應，也探討對流感病毒 A/Indonesia/05/2005 異種的免疫原性（NCT編號：00449670）。

**設計** 前瞻性觀察員單盲研究。

**安排** 香港一所教學醫院的門診診所。

**參與者** 共360名18至60歲的成年人共分成6組，分別在相隔21天的時間接種兩個劑量的疫苗。

**介入治療** 參與者接種四種佐劑疫苗（3.75 μg H5N1血凝素+AS03<sub>A</sub>）的其中一種，或兩種非佐劑疫苗（3.75 μg H5N1血凝素）的其中一種。

**主要結果測量** 利用血凝抑制及中和試劑分析參與者在接種前及接種後第21天的血液樣本。所有不良反應，不論是否預見的及嚴重的，都會被記錄下來。

**結果** 四種佐劑疫苗都具製造穩定性。參與者在接種兩個劑量的疫苗後，AS03<sub>A</sub>佐劑組對流感病毒 A/Vietnam/1194/2004 品種有高的血清保護率（95.8%），並對流感病毒 A/Indonesia/05/2005 異種具高免疫原性（45.7%）；相對地，非佐劑疫苗組只有分別4.6%的血清保護率及1.5%免疫原性。病毒中和抗體方面，AS03<sub>A</sub>佐劑疫苗組比非佐劑疫苗組產生相對較高的血清保護率。所有佐劑疫苗均無安全問題。

**結論** 流感大流行的前期疫苗AS03<sub>A</sub>佐劑對這項香港研究的參與者有良好的免疫原性及可接受的安全度。

An AS03<sub>A</sub>-adjuvanted split-virion prepandemic H5N1 influenza vaccine (Prepandrix; 3.75 μg haemagglutinin [HA] antigen; AS03<sub>A</sub>: a tocopherol [11.86 mg] oil-in-water emulsion-based Adjuvant System) developed by GlaxoSmithKline (GSK) Biologicals has been found to be immunogenic with an acceptable safety profile in European populations and has also demonstrated cross-clade immunogenicity.<sup>9-11</sup>

The present study was conducted in a large Asian population in Hong Kong, Thailand, Singapore, and Taiwan to demonstrate manufacturing consistency in terms of the vaccine-homologous immune response [A/Vietnam/1194/2004 NIBRG-14 (clade 1)] across the four adjuvanted vaccine formulations as well as from the vaccine's safety profile. In addition, the cross-clade immunogenicity of the vaccine against a heterologous strain [A/Indonesia/05/2005 IBCDC-RG-2 (clade 2.1)] was also assessed.

Overall data from this study have been presented previously.<sup>12</sup> This paper presents Hong Kong-specific primary vaccination data on the immunogenicity of the AS03<sub>A</sub>-adjuvanted vaccine.

## Methods

### Study design and subjects

This multi-centre study (NCT00449670) was conducted across Singapore, Thailand, Hong Kong, and Taiwan between 24 March and 12 July 2007. The study design was observer-blind due to the difference in appearance of the AS03<sub>A</sub>-adjuvanted and non-adjuvanted vaccine. Healthy subjects 18 to 60 years of age were randomised (allocation ratio 2:2:2:2:1:1) into six groups to receive two doses (21 days apart) of either one of the four formulations of the AS03<sub>A</sub>-adjuvanted vaccine (group H5N1-AS03<sub>A</sub>) or one of the two formulations of the non-adjuvanted vaccine (group H5N1-DIL). The procedure used to derive the adjuvanted and non-adjuvanted formulations is described below under the subsection describing the vaccine. In Hong Kong, the study was conducted at the general out-patient clinic of Queen Mary Hospital according to Good Clinical Practice guidelines. All necessary approvals were obtained from the Institutional Review Board of all participating institutions and the local health authorities. Study procedures were performed after obtaining written consent from the subjects.

Subjects were excluded if they had received any licensed inactivated vaccine within 2 weeks or live-attenuated vaccine within 4 weeks prior to enrolment in the study. They were also excluded if they had previously received any prepandemic candidate vaccine or any AS03<sub>A</sub>-adjuvanted vaccine, or had been previously exposed to any H5N1 wild-type virus. Subjects with a history of hypersensitivity to vaccine components and chronic illnesses were also not considered for the study. The study excluded subjects presenting symptoms of acute disease (defined as moderate or severe illness with or without febrile illness [axillary temperature  $\geq 37.5^{\circ}\text{C}$ ]) at the time of enrolment. If a subject presented with acute disease (including febrile illness) at the time of the second dose, that dose was not administered, but he/she could continue with other study procedures (at the discretion of the investigator) and was monitored till resolution of the medical condition.

### Vaccines

The study vaccine and adjuvant were manufactured by GSK Biologicals as described previously.<sup>9</sup> The adjuvanted vaccine (0.5 mL) [Prepandrix] contained 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<sub>A</sub> (a tocopherol [11.86 mg] oil-in-water emulsion-based Adjuvant System), while the non-adjuvanted vaccine had the same constituents except AS03<sub>A</sub>. Two lots of HA antigen were combined with two lots of AS03<sub>A</sub> Adjuvant System to obtain four

adjuvanted vaccine formulations, while the same two lots of HA antigen were combined with a diluent to obtain the two controls. The vaccine doses were administered as intramuscular injections in the deltoid region of the non-dominant arm.

### Laboratory assays

Haemagglutination inhibition (HI) antibody titres against the homologous strain (A/Vietnam/1194/2004) and a heterologous strain (A/Indonesia/05/2005) were assessed using standard assay methods (cut-off for HI=1:10) modified to utilise an equine erythrocyte suspension instead of an avian erythrocyte suspension, as described previously.<sup>9</sup>

As part of the overall study, viral neutralising antibody titres were assessed in a small subset of subjects, using a standard procedure described previously, the lowest dilution tested being 1:28.<sup>12</sup> The neutralisation assay was expressed as the reciprocal of the highest dilution that achieved at least 50% neutralisation of virus growth.

### Assessment of immunogenicity

Blood samples were collected before vaccination and 21 days after each of the two vaccine doses. The seroprotection rates, seroconversion rates, and seroconversion factors for HI antibodies for the A/Vietnam/1194/2004 and A/Indonesia/05/2005 strains were calculated with their respective 95% confidence intervals (CIs).

As part of the overall assessment, in a subset of subjects the neutralising antibody titres for the A/Vietnam/1194/2004 and A/Indonesia/05/2005 strains were determined before vaccination and 21 days after the second vaccine dose.

In the absence of immunological correlates of protection against pandemic influenza strains, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) currently recommends that prepandemic candidate vaccines should meet all three of the current immunological criteria for adults aged 18 to 60 years set for licensure of seasonal influenza vaccines (ie seroconversion rate >40%, geometric mean increase >2.5, and seroprotection rate >70%).<sup>13</sup>

### Assessment of safety

Safety and reactogenicity were recorded using diary cards. Local injection site symptoms (pain, redness, swelling, induration, and ecchymosis) and general symptoms (fatigue, fever, headache, myalgia, shivering, sweating, and arthralgia) were recorded for 7 days (day 0-6) after each dose. Intensity of all symptoms was defined using a three-grade scale,

except fever, which was measured on a four-grade scale. Injection site pain that hindered normal daily activity was recorded as grade 3, while injection site redness, swelling, ecchymosis and induration of >100 mm was recorded as grade 3. Fever of >39°C and up to 40°C was recorded as grade 3 (>40°C, as grade 4), while other general symptoms (headache, fatigue, arthralgia, myalgia, shivering, and sweating) that hindered normal daily activities were recorded to be of grade 3 intensity.

All other (unsolicited) symptoms were recorded for 21 days after dose 1 and 30 days after dose 2. Unsolicited events were classified according to the Medical Dictionary for Regulatory Activities. Serious adverse events were recorded during the whole study period and events occurring until 30 days after dose 2 were logged.

### Statistical analyses

The sample size of the overall study was calculated to fulfil the primary objective of demonstrating consistency in terms of immunogenicity across the four adjuvanted vaccine formulations.<sup>12</sup> All paired vaccine formulations were considered consistent in terms of immune response against the A/Vietnam/1194/2004 strain, if the two-sided 95% CIs for the HI antibody geometric mean titre (GMT) ratios were within the pre-defined clinical limits (0.5, 2.0).

The seroconversion rate for HI antibodies was defined as the percentage of initially seronegative subjects with a post-vaccination titre of  $\geq 1:40$  or initially seropositive subjects with at least a 4-fold increase in titre, after each dose. The seroconversion rate for neutralising antibodies was defined as at least a 4-fold increase in titre, after each dose. The seroconversion factor was defined as the fold increase in serum HI antibody GMTs post-vaccination compared to that before vaccination. The seroprotection rate was defined as the percentage of subjects with a post-vaccination serum HI antibody titre of  $\geq 1:40$ .

Geometric mean titres for HI and neutralising antibodies were calculated with 95% CIs by taking the anti-log of the mean of the log titre transformations. Antibody titres below the assay cut-off were given an arbitrary value of half the cut-off for the purpose of GMT calculation. Immunogenicity analysis was performed on the per-protocol cohort, while the safety and reactogenicity analysis was performed on the total vaccinated cohort.

All statistical analyses were performed using the Statistical Analyses System (SAS) version 9.1, while all 95% CIs were calculated using Proc StatXact 5.0.

## Results

### Study population

The study enrolled 1206 subjects of which 360 subjects were from Hong Kong. Of these, 287 received any one of the four H5N1-AS03<sub>A</sub> formulations while 73 received any one of the two non-adjuvanted formulations. The mean age of subjects enrolled from Hong Kong was 32.8 years (standard deviation, 9.8 years); the subjects were predominantly male (77%) and the majority (99%) were of Asian origin.

### Immunogenicity

The consistency in the immunogenicity was demonstrated by all four adjuvanted-H5N1 formulations in terms of HI antibody response against the A/Vietnam/1194/2004 strain 21 days post-dose 2; these results having been presented previously.<sup>12</sup> Hence, the pooled results from the H5N1-AS03<sub>A</sub> and H5N1-DIL groups are presented here.

#### *Immunogenicity against the homologous A/Vietnam/1194/2004 (clade 1) strain*

Prior to vaccination, only 3.3% (95% CI, 1.5-6.2) of the subjects in the H5N1-AS03<sub>A</sub> groups and 1.5% (95% CI, 0.0-8.3) of those in the H5N1-DIL groups had detectable levels of HI antibodies ( $\geq 1:10$ ) against the A/Vietnam/1194/2004 strain. Following the first vaccine dose, the immune response against the A/Vietnam/1194/2004 strain was higher in the H5N1-AS03<sub>A</sub> groups, compared to the H5N1-DIL groups (Fig).

A marked increase in the H5N1 HI antibody GMTs against the A/Vietnam/1194/2004 strain was observed in the H5N1-AS03<sub>A</sub> groups after the second dose (202.2 [95% CI, 179.1-228.3]). In addition, the second dose also induced a high seroconversion rate (95.5% [95% CI, 92.2-97.6]), seroprotection rate (95.8% [95% CI, 92.7-97.9]), and seroconversion factor (38.0 [95% CI, 33.5-43.2]). In contrast, in the H5N1-DIL groups, the H5N1 HI antibody GMTs reached 6.5 (95% CI, 5.5-7.6), while the seroconversion and seroprotection rates (4.6% [95% CI, 1.0-12.9] each) and seroconversion factor (1.3 [95% CI, 1.1-1.5]) did not demonstrate an appreciable increase after both doses.

After the second dose of the H5N1-AS03<sub>A</sub> vaccine, all three CHMP criteria in terms of the HI antibody response against the A/Vietnam/1194/2004 strain were met (Fig). In contrast, none of the CHMP criteria were met after any dose of the H5N1-DIL vaccine.

#### *Cross-clade immunogenicity against the heterologous A/Indonesia/05/2005 (clade 2.1) strain*

Before vaccination, less than 1.0% subjects in the H5N1-

AS03<sub>A</sub> and H5N1-DIL groups had detectable levels of HI antibodies ( $\geq 1:10$ ) against the A/Indonesia/05/2005 strain. Following the first vaccine dose, the immune response against the heterologous (cross-clade) A/Indonesia/05/2005 strain was comparable in the H5N1-AS03<sub>A</sub> and H5N1-DIL groups (Fig).

However, following the second vaccine dose, the immune response to the heterologous (cross-clade) A/Indonesia/05/2005 strain was higher in the H5N1-AS03<sub>A</sub> groups than in the H5N1-DIL groups (Fig). In the H5N1-AS03<sub>A</sub> groups, the H5N1 HI antibody GMTs increased to 20.9 (95% CI, 17.7-24.7), while the seroconversion and seroprotection rates were 45.7% (95% CI, 39.6-51.9) each; the mean seroconversion factor value was 4.2 (95% CI, 3.5-4.9). In contrast, in the H5N1-DIL groups, the HI antibody GMTs remained low (5.2 [95% CI, 4.8-5.5]), while no appreciable increase in seroconversion and seroprotection rates (1.5% [95% CI, 0.0-8.3] each) and seroconversion factor (1.0 [95% CI, 1.0-1.0]) were observed.

Following the second dose of the H5N1-AS03<sub>A</sub> vaccine, two out of the three CHMP criteria for licensure of seasonal influenza vaccine, in terms of HI antibody response against the A/Indonesia/05/2005 strain were met (Fig). In contrast, none of the CHMP criteria were met after any dose of the H5N1-DIL vaccine.

### Safety and reactogenicity

There was a consistent trend towards higher frequency of solicited and unsolicited symptoms (local and general) reported during a 7-day period after each vaccination in the H5N1-AS03<sub>A</sub> groups as compared to the H5N1-DIL groups. Overall, injection site pain was the most frequently recorded solicited local symptom reported by 96.1% (95% CI, 93.1-98.0) of subjects in the H5N1-AS03<sub>A</sub> groups and 26.0% (95% CI, 16.5-37.6) of subjects in the H5N1-DIL groups (Table). Injection site pain of grade 3 intensity was reported by 9.6% (95% CI, 6.4-13.7) of subjects in the H5N1-AS03<sub>A</sub> groups. None of the subjects in the H5N1-DIL groups reported any solicited local symptom of grade 3 intensity. Frequencies of reporting of all solicited local symptoms are presented in the Table.

The most frequently reported solicited general symptom in the H5N1-AS03<sub>A</sub> groups was myalgia 61.6% (95% CI, 55.6-67.3), while in the H5N1-DIL groups it was fatigue (42.5% [95% CI, 31.0-54.6]).

At least one unsolicited symptom was reported by 43.2% (95% CI, 37.4-49.2) of subjects in the H5N1-AS03<sub>A</sub> groups and 42.5% (95% CI, 31.0-54.6) of subjects in the H5N1-DIL groups. Influenza-like illness was the most commonly reported unsolicited symptom in the H5N1-AS03<sub>A</sub> groups (8.4% [95% CI, 5.4-12.2]), while it was headache in the H5N1-DIL groups (8.2%

[95% CI, 3.1-17.0]). Unsolicited symptoms of grade 3 intensity were reported in 4.2% (95% CI, 2.2-7.2) of subjects in the H5N1-AS03<sub>A</sub> groups and 1.4% (95% CI, 0.0-7.4) in the pooled H5N1-DIL group, influenza-like illness (1.4% [95% CI, 0.4-3.5]) and irritation of the larynx (1.4% [95% CI: 0.0-7.4]) being most frequently reported in the respective groups.

Two serious adverse events were reported, both in the H5N1-AS03<sub>A</sub> group. One subject was diagnosed with acute appendicitis after the second dose, which resolved in 3 days, while another subject

died on duty in a fire accident. None of these were assessed to be causally related to vaccination.

### Discussion

The first prepandemic vaccine from GSK Biologicals, Prepandrix, was approved by the EMA in May 2008 (subsequently the licensure was extended to GSK Biologicals' pandemic vaccine, Pandemrix). The data from this study conducted in Hong Kong, Thailand, Singapore, and Taiwan were an essential component

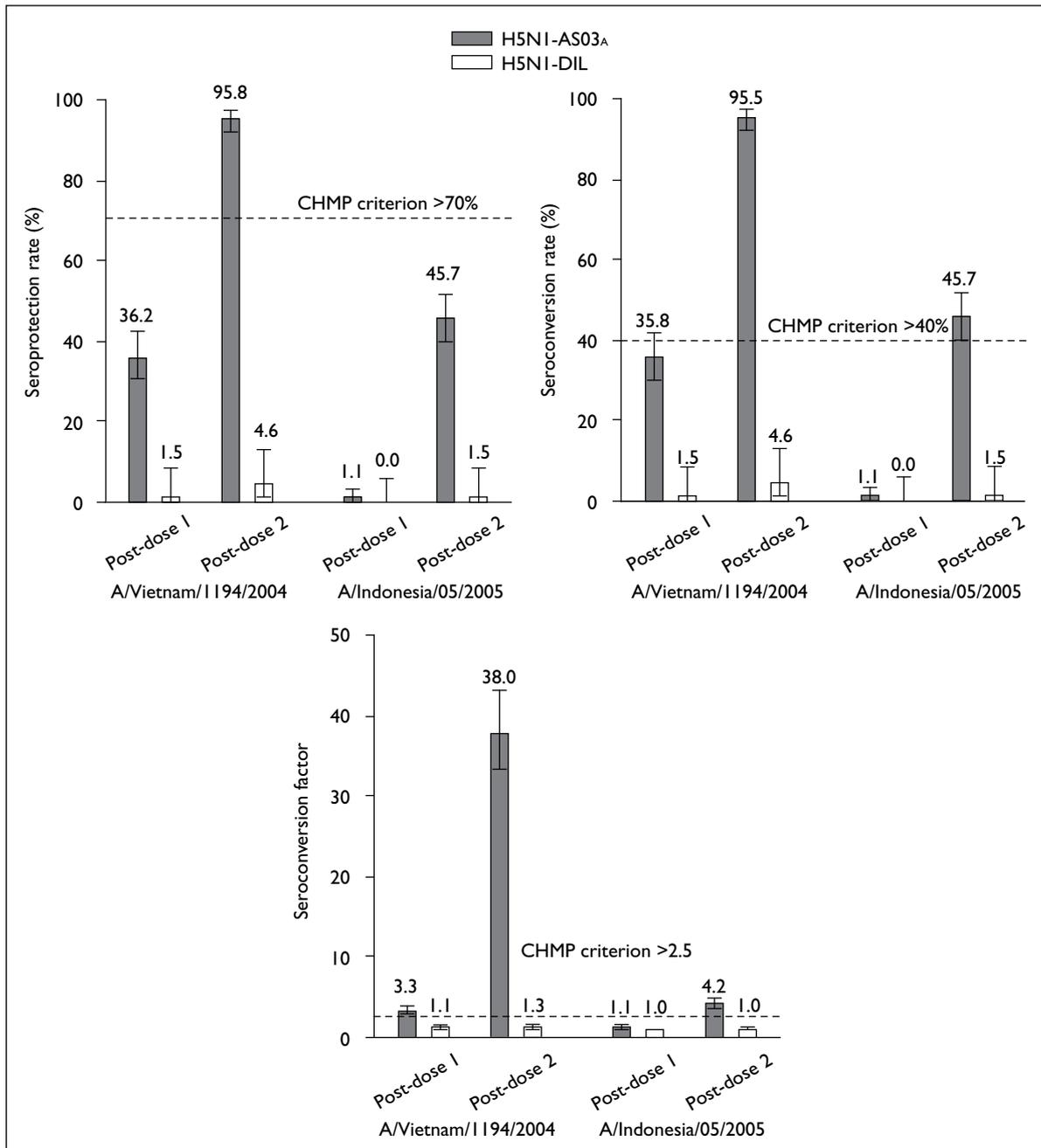


FIG. Immunogenicity of the prepandemic influenza vaccine (in terms of seroprotection rate, seroconversion rate, and seroconversion factor) against the A/Vietnam/1194/2004 (clade 1) and A/Indonesia/05/2005 (clade 2.1) strains, 21 days after each dose (per-protocol cohort for immunogenicity)

CHMP denotes Committee for Medicinal Products for Human Use

TABLE. Overall reporting of solicited local and general symptoms by subjects in the Hong Kong cohort during the 7-day post-vaccination follow-up period

Symptoms	Intensity	H5N1-AS03 <sub>A</sub> * (n=281)		H5N1-DIL† (n=73)	
		No.‡	% (95% CI§)	No.‡	% (95% CI)
Local symptoms					
Ecchymosis (mm)	All	2	0.7 (0.1-2.5)	0	0.0 (0.0-4.9)
	Grade 3	0	0.0 (0.0-1.3)	0	0.0 (0.0-4.9)
Induration (mm)	All	23	8.2 (5.3-12.0)	0	0.0 (0.0-4.9)
	Grade 3	0	0.0 (0.0-1.3)	0	0.0 (0.0-4.9)
Pain	All	270	96.1 (93.1-98.0)	19	26.0 (16.5-37.6)
	Grade 3	27	9.6 (6.4-13.7)	0	0.0 (0.0-4.9)
Redness (mm)	All	21	7.5 (4.7-11.2)	0	0.0 (0.0-4.9)
	Grade 3	1	0.4 (0.0-2.0)	0	0.0 (0.0-4.9)
Swelling (mm)	All	31	11.0 (7.6-15.3)	0	0.0 (0.0-4.9)
	Grade 3	0	0.0 (0.0-1.3)	0	0.0 (0.0-4.9)
General symptoms					
Arthralgia	All	62	22.1 (17.4-27.4)	9	12.3 (5.8-22.1)
	Grade 3	3	1.1 (0.2-3.1)	1	1.4 (0.0-7.4)
Fatigue	All	160	56.9 (50.9-62.8)	31	42.5 (31.0-54.6)
	Grade 3	15	5.3 (3.0-8.7)	2	2.7 (0.3-9.5)
Fever (axillary)	All	9	3.2 (1.5-6.0)	1	1.4 (0.0-7.4)
	Grade 3	1	0.4 (0.0-2.0)	0	0.0 (0.0-4.9)
Headache	All	105	37.4 (31.7-43.3)	14	19.2 (10.9-30.1)
	Grade 3	9	3.2 (1.5-6.0)	1	1.4 (0.0-7.4)
Myalgia	All	173	61.6 (55.6-67.3)	19	26.0 (16.5-37.6)
	Grade 3	10	3.6 (1.7-6.4)	1	1.4 (0.0-7.4)
Shivering	All	22	7.8 (5.0-11.6)	3	4.1 (0.9-11.5)
	Grade 3	1	0.4 (0.0-2.0)	1	1.4 (0.0-7.4)
Sweating	All	53	18.9 (14.5-23.9)	9	12.3 (5.8-22.1)
	Grade 3	3	1.1 (0.2-3.1)	2	2.7 (0.3-9.5)

\* This group received one of the four formulations of the AS03<sub>A</sub>-adjuvanted vaccine

† This group received one of the two formulations of the non-adjuvanted vaccine

‡ No. of subjects reporting at least one solicited symptom

§ CI denotes confidence interval

of the file documents submitted to the EMA as part of the licensure application.

Based on non-overlapping CIs, the results suggested that after two doses, the AS03<sub>A</sub>-adjuvanted split-virion H5N1 influenza vaccine induced a higher immune response against the clade 1 (A/Vietnam/1194/2004) strain with a seroconversion rate of 95.5% and a seroprotection rate of 95.8%, which was much greater than that with non-adjuvanted formulations (seroconversion and seroprotection rates in the Hong Kong population both being 4.6%). This trend was also observed in the overall study population, wherein the immune response against the A/Vietnam/1194/2004 strain was higher in subjects who received the AS03<sub>A</sub>-adjuvanted formulation of the H5N1 prepandemic influenza vaccine (seroconversion rate: 93.7%; seroprotection

rate: 94.3%) than in subjects who received the non-adjuvanted formulation (seroconversion rates: 5.6%; seroprotection rates: 10.3%).<sup>12</sup> A similar difference was observed for the immune response against the clade 2.1 (A/Indonesia/05/2005) strain in the Hong Kong population ([adjuvanted: seroconversion and seroprotection rates: 45.7%]; [non-adjuvanted: seroconversion and seroprotection rates: 1.5%]) and the overall population ([adjuvanted: seroconversion and seroprotection rates: 50.2%]; [non-adjuvanted: seroconversion and seroprotection rates: 0.4%]). As observed, the immune responses against the two strains were of the same magnitude in both populations.<sup>12</sup> As per the recommendation of the CHMP, in addition to the HI assay, samples from a subset of 350 subjects of the overall population who participated in the study (H5N1-AS03<sub>A</sub> groups,

n=279; H5N1-DIL groups, n=71) were analysed using a neutralisation assay. Following the second vaccine dose, 96.0% of subjects in the H5N1-AS03<sub>A</sub> group demonstrated a 4-fold increase in neutralising antibody titres against the vaccine homologous A/Vietnam/1194/2004 strain and 91.4% against the vaccine heterologous A/Indonesia/05/2005 strain. In comparison, in the H5N1-DIL groups, the percentages of subjects who seroconverted were much lower (A/Vietnam/1194/2004; 32.4% [95% CI, 21.8-44.5]; A/Indonesia/05/2005: 5.6% [95% CI, 1.6-13.8]).<sup>12</sup>

In the Hong Kong population, the reporting of local and general symptoms appeared to be higher following vaccination with the H5N1-AS03<sub>A</sub> formulation than following vaccination with the H5N1-DIL formulation. However, symptoms of grade 3 intensity were uncommon, except for grade 3 pain, and the overall safety profile did not give rise to any concerns. These results are in agreement with the safety results from the overall study population, wherein the same local symptoms and general symptoms (pain, myalgia and fatigue) were most frequently reported in recipients of the adjuvanted and non-adjuvanted formulations, with the former reporting comparatively higher incidence of post-vaccination solicited symptoms.<sup>12</sup> In this study, the AS03<sub>A</sub>-adjuvanted formulation of the H5N1 vaccine appeared to induce comparatively more injection site reactions (mainly pain) than the non-adjuvanted formulations. This observation has also been made in previous studies with adjuvanted H5N1 and H1N1 vaccines in different populations, though the overall safety profile of the adjuvanted formulation has been found to be clinically acceptable.<sup>9,10,14</sup>

As observed in previous studies using the same vaccine, following the second dose, the H5N1-AS03<sub>A</sub> vaccine fulfilled all three immunological CHMP criteria for the licensure of a seasonal influenza vaccine for prepandemic vaccines in terms of the HI antibody response against the clade 1 (A/Vietnam/1194/2004) strain.<sup>13</sup> The H5N1-AS03<sub>A</sub> vaccine also fulfilled two of the three CHMP licensure criteria for the heterologous clade 2.1 (A/Indonesia/05/2005) strain, following the second dose.<sup>9,11</sup>

It has been observed that a single dose of this vaccine induced immunological memory for at least 6 months and the cross-clade immunity induced by the first dose is maintained even when the second dose is derived from a different H5N1 strain.<sup>15</sup> The vaccine also offers flexibility in the interval between the two doses, which is beneficial in the context of mass vaccination and could minimise logistical difficulties related to vaccine delivery.<sup>15</sup> Considering that such effective immunogenicity results were obtained using a reduced antigen content vaccine (3.75 µg HA), this vaccine meets the World Health Organization recommendations for cross-clade

protection against different H5N1 viral strains and for antigen-sparing.<sup>5</sup> In comparison, the first split-virion influenza vaccine approved by the United States Food and Drug Administration in 2007 has limitations with respect to both of these parameters.<sup>7,16,17</sup>

Till recently, the majority of health authorities worldwide had not considered stockpiling prepandemic influenza vaccines, mainly due to uncertainties regarding the possible strain that would cause the next influenza pandemic. However, following recent preliminary reports that some vaccine trials showed putative cross-clade seroprotective efficacy in a relevant model and a long-lasting priming effect across different H5 strains after a first dose, some countries have initiated stockpiling. The Government of the Hong Kong Special Administrative Region developed its first Influenza Pandemic Plan in 2005 and is currently evaluating the possibility of stockpiling prepandemic influenza vaccines.<sup>1,18</sup>

The results from the present study provide evidence of good immunogenicity and an acceptable safety profile of the AS03<sub>A</sub>-adjuvanted prepandemic influenza vaccine that have a significant impact on the pandemic influenza preparedness strategies worldwide. This would be irrespective of which H5N1 strain of the influenza virus causes the pandemic. Currently, GSK Biologicals' AS03<sub>A</sub>-adjuvanted prepandemic influenza vaccine (Prepandrix) is the only H5N1 influenza prepandemic vaccine that is licensed for use for this indication by the EMA. In addition, the vaccine has received marketing registration approval from the Hong Kong health authority.

The results presented here are from 360 subjects who were part of the overall multi-country study that included 1206 subjects across four study sites. Hence, the number of subjects was small and precluded age-wise stratification. Nevertheless, this paper describing data from Hong Kong demonstrates agreement with the data obtained from the overall population.

## Conclusion

Data from this study demonstrate the successful development of a reduced antigen-containing AS03<sub>A</sub>-adjuvanted H5N1 prepandemic vaccine with good immunogenicity and an acceptable safety profile.

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