Cross-Clade Immunogenicity and Antigen-Sparing with an AS03$_A$-Adjuvanted Prepandemic Influenza Vaccine in a Thai Population

Prasert Thongcharoen MD*,
Prasert Auewarakul MD*, Yanee Hutagalung MD**,
Gary Ong MMed (Public Health), Dip (Fam Med)**, Paul Gillard MD**,
Mamadou Drame MSc**, Hans L Bock MD**

** Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand*
*** GlaxoSmithKline Biologicals, Wavre, Belgium*

Objective: *The present study (NCT00449670) in Asian subjects (18-60 years) evaluated the manufacturing consistency of four formulations of 3.75mg AS03$_A$-adjuvanted H5N1 influenza vaccine, in terms of post-immunization Hemagglutination Inhibition (HI) titers against the A/Vietnam/1194/2004 and A/Indonesia/05/2005 strains. The immunogenicity and safety of the vaccine in the Thai population are reported herein.*

Material and Method: *Subjects were randomized (2:2:2:2:1:1) between four vaccine groups and two control groups to receive two doses of either the AS03$_A$-adjuvanted or non-adjuvanted H5N1 vaccine formulations, 21 days apart. Sera were assayed for HI antibody titers against the two strains.*

Results: *After the second dose of AS03$_A$-adjuvanted vaccine, 94.2% subjects in the H5N1-AS03$_A$ groups seroconverted and 94.9% subjects were seroprotected against the A/Vietnam/1194/2004 strain. Cross-clade immune response against the A/Indonesia/05/2005 strain was observed. All vaccine formulations had an acceptable safety profile.*

Conclusion: *This antigen-sparing AS03$_A$-adjuvanted influenza vaccine could be a suitable candidate for combating and mitigating future influenza pandemics.*

Keywords: *H5N1, Thailand, Adjuvant System, Influenza, Prepandemic*

J Med Assoc Thai 2011; 94 (8): 916-26
Full text. e-Journal: *http://www.mat.or.th/journal*

Avian influenza viruses have been responsible for three major pandemics in the twentieth century; the first one in 1918 (caused by an H1N1 strain), followed by two others in 1957 (H2N2 virus) and 1968 (H3N2)[1-3]. All three pandemics caused substantial morbidity and mortality, worldwide[1-4]. Since then, the avian influenza viruses have continued to diversify, mainly due to their inherent ability to undergo rapid antigenic mutations, which have led to their largely uncontrolled spread across different geographical regions. Genetic studies based on phylogenetic analysis of the hemagglutinin (HA) gene have revealed that at least 10 clades of H5N1 viruses have emerged since 2000, Clades 1 and 2 being the predominant ones[5-8]. This antigenic variability poses a constant challenge for influenza vaccine development.

The World Health Organization (WHO) had previously predicted that the H5N1 avian influenza virus could be the most likely candidate to cause the next influenza pandemic[9]. However, true to the characteristics of influenza viruses, a new H1N1 swine influenza strain has emerged and is responsible for the influenza pandemic that has so far (as of 4 April 2010) caused at least 17,700 deaths in humans[10]. While the focus is on combating the H1N1 virus, the H5N1 virus continues to pose a serious threat (493 laboratory-confirmed cases and 292 deaths (as of 9 April 2010)[11] considering its ability to cause a pandemic by itself or in combination with the newly emerged strain and is one of the potential candidates for causing the next influenza pandemic of the twenty-first century[12]. Two factors add to this concern. Firstly, today's world

Correspondence to:
Thongcharoen P, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Prannok Rd, Bangkok Noi, Bangkok 10700, Thailand.
Phone: 0-2866-2575, Fax: 0-2419-8406
E-mail: siptc@mahidol.ac.th

population is largely naive to this highly pathogenic form of avian influenza virus and secondly, this virus has already spread across Asia, Africa, Middle East and Europe[9].

Thailand, home to more than 60 million people, has experienced seven outbreaks of avian influenza virus in poultry (the first one being in 2004)[13]. As of April 9, 2010, 25 laboratory-confirmed human cases of H5N1 influenza have been reported in Thailand, with the total death toll amounting to 17[11]. Adding to the concern is the fact that all of Thailand's immediate neighbors-Myanmar, Laos, Cambodia and Malaysia-have experienced avian influenza outbreaks either in poultry or in humans or both[14]. Thus, in addition to internal avian influenza outbreaks, Thailand could also be exposed to risk from uncontrolled outbreaks in the neighboring countries and, in turn, become a risk for these countries. Serious concerns have been raised about the spread of H5N1 influenza viruses from Asia to other regions of the world[15-17] and with the ever-increasing rate of air travel and urbanization, the virus could spread even faster and containing it would become even more challenging.

The most effective measure for limiting morbidity and mortality caused by influenza pandemics is through vaccination just before or immediately after the identified start of a pandemic[18]. In previous studies, two doses of various adjuvanted and non-adjuvanted candidate (pre) pandemic avian influenza vaccines with a hemagglutinin (HA) antigen content ranging between 10 mg and 90 mg per dose, were found to be immunogenic against the vaccine-homologous strain[19–23].

An $AS03_A$-adjuvanted prepandemic H5N1 influenza vaccine (containing 3.75 mg HA) developed by GlaxoSmithKline Biologicals has been found to be immunogenic against the vaccine-homologous strain, with an acceptable safety profile in previous studies conducted in Europe. Furthermore, the vaccine has successfully demonstrated cross-clade immunogenicity against drifted strains[15,24].

The present study assessed the consistency in the immune response of four paired formulations of the $AS03_A$-adjuvanted (a tocopherol [11.86 mg] oil-in-water emulsion-based Adjuvant System) prepandemic H5N1 influenza vaccine against the vaccine-homologous A/Vietnam/1194/2004 (Clade 1) strain in a large Asian population in four regions (Thailand, Singapore, Taiwan and Hong Kong). The cross-clade immune response induced against the A/Indonesia/05/2005 (Clade 2.1) strain and safety of the vaccine were also assessed. The overall results of the present study have been presented in the primary manuscript[25]. The present paper presents Thailand-specific data on the immunogenicity and safety of the vaccine.

Material and Method

Study design and subjects

This primary vaccination phase of this phase III, randomized study was conducted between March 24 and July 12, 2007, in six study centers in Taiwan, Singapore, Thailand, and Hong Kong (109630/NCT: 00449670). The present study design was overall observer-blind due to the different appearance of the $AS03_A$-adjuvanted and non-adjuvanted formulations. In Thailand, the present study was conducted at Siriraj Hospital, Mahidol University.

Written informed consent was obtained from subjects prior to conducting any study-related procedures. The present study protocols and associated study documents were approved by the Independent Ethics Committees of the participating centers. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki.

Subjects aged between 18 and 60 years were randomized into six parallel study groups (four vaccine groups and two control groups), in the allocation ratio 2:2:2:2:1:1. The vaccine groups received two doses of any one of the four formulations of the split-virion $AS03_A$ Adjuvant System-based study vaccine (derived by mixing two commercial production lots of 3.75 μg H5N1 HA antigen and two commercial production lots of $AS03_A$ Adjuvant System); the two doses were administered 21 days apart. The control groups received two doses of any one of the two formulations of the non-adjuvanted split-virion vaccine (3.75 μg H5N1 HA antigen) mixed with a diluent, administered 21 days apart. Blood samples were collected prior to the first vaccine dose and 21 days after both vaccine doses.

At the time of enrolment, subjects who had received any licensed inactivated vaccine within two weeks, any live-attenuated vaccine within four weeks prior to enrolment in the present study, any pandemic influenza candidate vaccine previously or had had a previous contact with the H5N1 wild type virus, were excluded. Subjects were also excluded if they had a history of allergic reactions or hypersensitivity towards vaccines, any serious chronic or acute disease (with or without fever) at the time of enrolment. Female volunteers who were lactating or pregnant were also excluded from the present study.

Vaccines

All formulations of the inactivated, split-virion $AS03_A$-adjuvanted prepandemic influenza vaccine were manufactured by GlaxoSmithKline Biologicals, as described previously[15,24]. The $AS03_A$-adjuvanted H5N1 influenza vaccine (*Prepandrix*□, 0.5 mL) 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 $AS03_A$ (composed of squalene [10.69 milligrams], DL-α-tocopherol [11.86 milligrams] and polysorbate 80 [4.86 milligrams])[15]. All formulations were made available in multi-dose vials. Vaccines were administered intramuscularly in the deltoid region of the non-dominant arm.

Laboratory assays

After an initial dilution of 1:10, the serum samples were serially diluted by two-fold. The Hemagglutination Inhibition (HI) assay was carried out as per standard procedures, except for the modification of using equine erythrocytes instead of avian erythrocytes[26].

A neutralizing antibody assay was performed on a subset of subjects' samples[25]. It involved incubating serially diluted serum samples with influenza virus and adding the serum/virus mixture onto living cells. Viral neutralization was confirmed using a subsequent hemagglutination step, *i.e.* the supernatants of the cells that were infected in the presence of serum/virus mixture were incubated with red blood cells to identify the presence of influenza virus via hemagglutination.

All dilutions were performed in triplicates; the first serum dilution was 1:20. Once full neutralization of the virus in all three wells was reached for the first dilution, the neutralizing titer (endpoint titer) was calculated as the reciprocal of the highest dilution achieved at least 50% neutralization of virus growth (neutralizing dose 50 [ND_{50}]) by applying the Reed and Muench method[27], which gave the cut-off titer of 1:28. Any value below this cut-off was considered negative.

Assessment of immunogenicity

Blood samples were assayed for determining HI antibody titers against the A/Vietnam/1194/2004 strain (Clade 1) and cross-clade immunogenicity against the A/Indonesia/05/2005 strain (Clade 2.1). The HI antibody titers were expressed as the reciprocal of the highest dilution that completely inhibited hemagglutination.

The seroprotection rates, seroconversion rates, and seroconversion factors for the A/Vietnam/1194/2004 and A/Indonesia/05/2005 strains were calculated with a 95% confidence interval (CI), prior to Dose 1 and 21 days post Dose 1 and Dose 2.

The immunogenicity cut-offs (seroprotection rates > 70%, seroconversion rates > 40% and seroconversion factor > 2.5) were based on correlates of sufficient immune response required for the licensure of seasonal influenza vaccines for adults (18-60 years) and expected by the Committee for Human Medicinal Products (CHMP) to be met for prepandemic vaccines[28,29].

In line with the CHMP requirement[28], sera samples from a small subset of subjects from the overall study were assessed (21 days post Dose 2) for neutralizing antibody titers against the A/Vietnam/1194/2004 and A/Indonesia/05/2005 strains.

Assessment of safety and reactogenicity

The safety and reactogenicity analyses were performed on the total vaccinated cohort. All solicited injection site local symptoms (pain, redness, swelling, induration and ecchymosis) and general symptoms (arthralgia, fatigue, fever, headache, myalgia, shivering, and sweating) were recorded using diary cards during the 7-day follow-up period after each vaccine dose.

Intensity of solicited local injection site reactions was graded on a 4-point (0-3) scale. For reactions such as redness and swelling, diameters > 100 mm were considered to be of Grade 3 intensity. Pain was classified as Grade 3 if it prevented normal daily activity. All solicited general symptoms were graded on a 3-point scale depending on the level of interference with normal daily activities, except for fever, which was graded on a scale of 4. Grade 3 fever was defined as axillary temperature ≥ 39°C and ≤ 40°C, while temperatures > 40°C were rated Grade 4.

All unsolicited symptoms were recorded for 21 days after Dose 1 and 30 days after Dose 2. Serious adverse events (SAEs) were recorded throughout the present study period; events occurring up to 30 days after the second dose are reported in the present study.

Statistical analyses

The seroconversion rate for HI antibodies was defined as the percentage of seronegative subjects (HI antibody titer < 1:10) before vaccination, with a post-vaccination HI antibody titer ≥ 1:40 or seropositive subjects (HI antibody titer ≥ 1:10) before vaccination, with at least a four-fold increase in HI antibody titer,

after each dose. The seroconversion rate for neutralizing antibodies was defined as at least a four-fold increase in titer after each dose. The increase in geometric mean antibody titer (GMT) was defined as the fold increase in serum HI antibody GMTs post-vaccination compared to the GMT prior to Dose 1, otherwise known as seroconversion factor. The seroprotection rate was defined as the percentage of subjects with a post-vaccination serum HI antibody titer $\geq$ 1:40.

GMTs for HI antibodies were calculated with 95% confidence intervals (CI) by taking the anti-log of the mean of the log titer transformations. Antibody titers below the assay cut-off were taken as half the cut-off (arbitrarily) for the purpose of GMT calculation.

Immunogenicity analyses were performed on the per-protocol cohort, which consisted of subjects without any protocol violations and with assay results available. The HI GMT ratio (with 95% CI) between vaccine groups was computed using the one-way analysis of variance (ANOVA) model on natural log-transformed titers. The safety and reactogenicity analysis was performed on the total vaccinated cohort.

All statistical analyses were performed using Statistical Analyses System (SAS) version 9.1, while all 95% CI for proportions within a group were calculated using Proc StatXact 5.0.

Results

Demographics

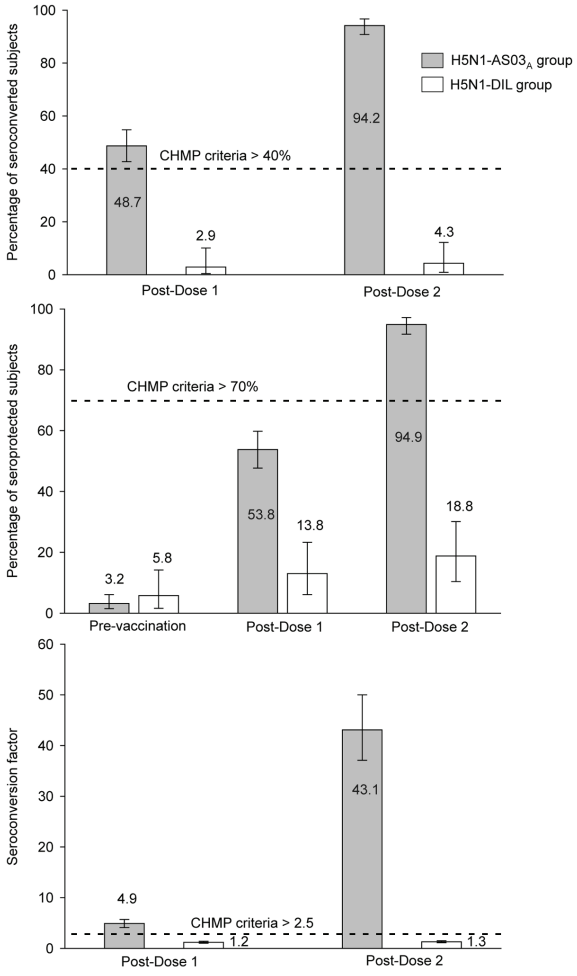
A total of 1206 healthy subjects were enrolled in the present study; of these 350 healthy subjects were enrolled in Thailand and randomized to receive any one of the four adjuvanted vaccine formulations (n = 280) or any one of the two non-adjuvanted formulations (n = 70). The mean age of subjects was 35.7 $\pm$ 10.42 years (range: 18-59 years). The study population consisted predominantly of females (70.9%). All the subjects were of Asian-South East Asian heritage.

Immunogenicity

As consistency across paired formulations in terms of immunogenicity against the A/Vietnam/1194/2004 strain 21 days after Dose 2 was confirmed in the overall study and presented previously[25], immunogenicity results from the H5N1-AS03$_A$ and H5N1-DIL groups are presented here.

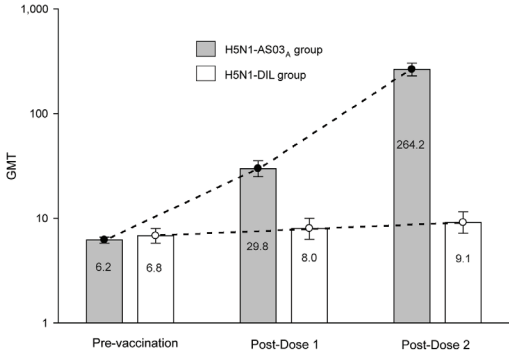
Hemagglutination inhibition assay

Immunogenicity against the A/Vietnam/1194/2004 (homologous) strain: Before vaccination, few subjects in the H5N1-AS03$_A$ and H5N1-DIL groups (14.7% and 18.8%, respectively) had detectable levels of antibodies (HI antibody titer $\geq$ 1:10) against the A/Vietnam/1194/2004 strain. Following two doses of the AS03$_A$-adjuvanted vaccine, 95.3% subjects in the H5N1-AS03$_A$ groups were seropositive for HI antibody against the A/Vietnam/1194/2004 strain. In addition, after the second dose of the AS03$_A$-adjuvanted vaccine, 94.2% subjects in the H5N1-AS03$_A$ groups seroconverted, while 94.9% subjects were seroprotected against the A/Vietnam/1194/2004 strain;



Bars indicate 95% confidence intervals for each group

Fig. 1 Seroconversion rates, seroprotection rates and seroconversion factor for H5N1 HI antibodies against the A/Vietnam/1194/2004 (homologous) strain, across adjuvanted and non-adjuvanted groups, after two doses (Per protocol cohort for immunogenicity)



Bars indicate 95% confidence intervals for each group

Fig. 2 GMTs for H5N1 HI antibodies against the A/Vietnam/1194/2004 (homologous) strain, after two doses (Per protocol cohort for immunogenicity)

the seroconversion factor was 43.1 (Fig. 1). The HI antibody GMT after Dose 2 (264.2) was considerably higher than that after Dose 1 (29.8) (Fig. 2). In the H5N1-DIL groups, even after the second dose, only 30.4% subjects were seropositive and GMTs remained low at 9.1.

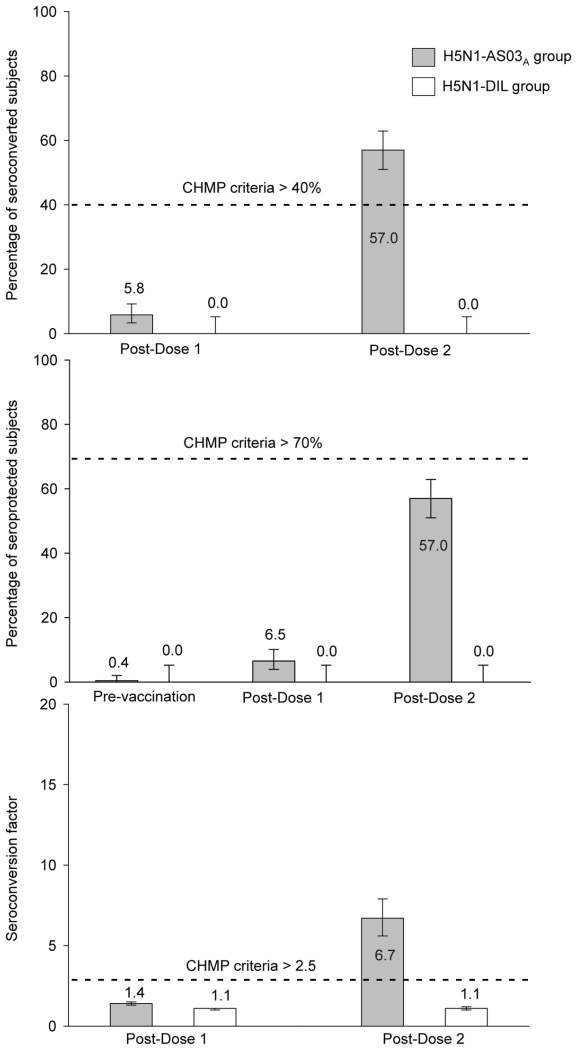
In comparison, in the H5N1-DIL groups, seroconversion rates, seroprotection rates, and seroconversion factor (4.3%, 18.8%, and 1.3, respectively) were very low compared to the H5N1-AS03$_A$ groups.

Cross-clade immunogenicity against the A/Indonesia/05/2005 (heterologous) strain: In addition to demonstrating an immune response against the vaccine (A/Vietnam/1194/2004) strain, the AS03$_A$-adjuvanted split-virion vaccine also successfully induced cross-clade immunogenicity against the heterologous A/Indonesia/05/2005 strain. Prior to vaccination, less than 3.0% subjects in the H5N1-AS03$_A$ and H5N1-DIL groups were seropositive for HI antibodies against the A/Indonesia/05/2005 strain.

Following Dose 2 of the AS03$_A$-adjuvanted split-virion vaccine, the seroconversion rates and seroprotection rates (both 57.0%) in the H5N1-AS03$_A$ groups were much higher than after Dose 1 (5.8% and 6.5%, respectively) (Fig. 3). In addition, the HI antibody GMT for the A/Indonesia/05/2005 strain improved considerably (34.2) across the H5N1-AS03$_A$ groups.

CHMP criteria

Homologous (A/Vietnam/1194/2004) strain: Following Dose 2 of the AS03$_A$-adjuvanted split-virion vaccine, the H5N1-AS03$_A$ groups met all three CHMP licensure criteria for adults [in terms of seroconversion rate > 40%, seroprotection rate > 70%, seroconversion factor > 2.5 for HI antibodies against the A/Vietnam/1194/2004 strain], for licensure of seasonal influenza vaccines and expected by the CHMP to be fulfilled for prepandemic vaccines[28,29] (Fig. 2). The H5N1-DIL groups could not achieve any of the CHMP criteria, after any of the vaccine doses (Fig. 2).



Bars indicate 95% confidence intervals for each group

Fig. 3 Seroconversion rates, seroprotection rates and seroconversion factor for H5N1 HI antibodies against the A/Indonesia/05/2005 (heterologous) strain, across adjuvanted and non-adjuvanted groups, after two doses (Per protocol cohort for immunogenicity)

Heterologous (A/Indonesia/05/2005) strain: Following two doses of the $AS03_A$-adjuvanted split-virion vaccine, the CHMP criteria for seroconversion rate and seroconversion factor for HI antibodies against the A/Indonesia/05/2005 strain were achieved (Fig 3). Despite not meeting the CHMP criterion for seroprotection rate against the A/Indonesia/05/2005 strain in the H5N1-$AS03_A$ groups after Dose 2, a good seroprotection rate (57.0%) was observed. None of the non-adjuvanted formulations (H5N1-DIL groups) demonstrated any cross-clade immunogenicity against the A/Indonesia/05/2005 strain and these formulations did could not meet any of the CHMP criteria.

Neutralization assay (in a subset of subjects from the Asian study population)

Results of the neutralization assay conducted on a small subset of subjects indicated that 21 days post Dose 2, the majority of subjects in the adjuvanted groups seroconverted in terms of neutralizing antibodies against the A/Vietnam/1194/2004 (96.0%) and A/Indonesia/05/2005 strains (91.4%). In comparison, in the non-adjuvanted groups, the percentage of subjects who seroconverted was much lower (A/Vietnam/1194/2004: 32.4%; A/Indonesia/05/2005: 5.6%)[25].

Safety and reactogenicity

In the present study, all vaccine formulations ($AS03_A$-adjuvanted and non-adjuvanted) had an acceptable safety profile. The incidence of local and general symptoms (solicited and unsolicited) in the H5N1-$AS03_A$ groups (72.4% and 71.3%, respectively) demonstrated a consistent higher trend when compared to the H5N1-DIL groups (10.0% and 45.7%, respectively). However, Grade 3 local and general symptoms were reported by $\leq$ 4.3% subjects in the H5N1-$AS03_A$ groups. In the H5N1-DIL groups, $\leq$ 1.4% subjects reported Grade 3 symptoms.

Pain at injection site was the most frequently reported solicited local symptom in the H5N1-$AS03_A$ groups (68.1% subjects; Grade 3 = 1.8% subjects) and the H5N1-DIL groups (8.6% subjects) during the 7-day post-vaccination follow-up period. Myalgia and fatigue were the most frequently reported solicited general symptoms in the H5N1-$AS03_A$ groups (Myalgia: 58.4% subjects; Grade 3 = 1.8%; Fatigue 43.4% subjects; Grade 3 = 1.1%). In the H5N1-DIL groups, fatigue (27.1% subjects), headache (25.7% subjects) and myalgia (22.9% subjects) were most frequently reported during the 7-day post-vaccination follow-up period. None of the subjects in the H5N1-DIL groups reported any Grade 3 local or general symptoms.

Forty-three subjects (15.4%) in the H5N1-$AS03_A$ groups and 16 subjects (22.9%) in the H5N1-DIL groups reported at least one unsolicited adverse event. None of the subjects in the H5N1-$AS03_A$ and H5N1-DIL groups reported unsolicited adverse events of Grade 3 intensity. Nasopharyngitis was the most frequently reported (11 subjects; 3.9%) unsolicited adverse event in the H5N1-$AS03_A$ groups, while in the H5N1-DIL groups, headache and rash (two subjects each; 2.9%) were most frequently reported. Eleven subjects in the H5N1-$AS03_A$ groups and two subjects in the H5N1-DIL groups reported unsolicited symptoms that were determined by the investigator to be causally-related to vaccination. Injection site pruritis (four subjects; 1.4%) was the most common vaccine-related unsolicited adverse event in the H5N1-$AS03_A$ groups, while it was injection site urticaria and rash (one subject each; 1.4%) in the H5N1-DIL groups. None of these was of Grade 3 intensity.

Two subjects in the H5N1-$AS03_A$ groups reported serious adverse events. One female subject reported endocervical polyp with bleeding and another reported head injury with lacerated scalp wound. Investigators determined both cases to be causally-unrelated to vaccination and both subjects recovered.

Discussion

The results of the present study confirm that the 3.75 μg HA H5N1 prepandemic vaccine using the $AS03_A$ adjuvant system, scores well on two essential parameters-antigen-sparing (through use of Adjuvant Systems) and cross-clade immunity. Compared with the non-adjuvanted H5N1 formulation, the $AS03_A$-adjuvanted H5N1 formulation induced a significantly better immune response against the vaccine homologous strain (A/Vietnam/1194/2004; Clade 1) and also demonstrated strong cross-clade immunogenicity against a heterologous strain (A/Indonesia/05/2005; Clade 2.1), unlike the non-adjuvanted formulation. This indicates that the $AS03_A$-adjuvanted H5N1 vaccine is most likely to mitigate even those avian influenza pandemics caused by heterologous influenza strains arising as a result of antigen drifts from the vaccine strain.

The CHMP guidelines for licensure of pandemic influenza vaccines for adults recommend that all candidate prepandemic vaccines meet the immunological criteria for licensure of seasonal

influenza vaccines for adults (18-60 years)[28,29]. The $AS03_A$-adjuvanted H5N1 prepandemic vaccine investigated in this study met and exceeded all three CHMP licensure criteria for the vaccine homologous A/Vietnam/1194/2004 strain after administration of two doses of the vaccine. For the heterologous A/Indonesia/05/2005 strain, two of the three CHMP criteria (for seroconversion rate and seroconversion factor were met after two doses of the adjuvanted vaccine), while, an appreciable increase in the seroprotection rate was observed between Dose 1 and Dose 2 (from 6.5% to 57.0%). In contrast, the non-adjuvanted H5N1 formulations did not meet any of the CHMP criteria.

In addition to the ability to confer strong seroprotection and cross-clade immunity, it is imperative that a prepandemic vaccine also has an acceptable safety and reactogenicity profile. There were no safety concerns on the vaccine in the present study, including that in the Thai subset population. The frequencies of reporting of all symptoms in the present study are in line with those reported in the overall study (which was adequately powered)[25] as well as in previous studies conducted using the same $AS03_A$-adjuvanted H5N1 vaccine with various antigen contents[15,24].

South-East Asia has long been susceptible to H5N1 avian influenza outbreaks, especially in poultry. Although since its re-emergence in 2004, the H5N1 avian influenza virus has predominantly been limited to poultry and migratory birds, the virus has also crossed the species barrier and occasional cases of zoonotic transmission to humans have been reported[13]. In Thailand, the H5N1 outbreaks between 2004 and 2008 have mostly been in poultry, which required culling and disposal of massive numbers of infected birds in the country[30,31]. However, sporadic outbreaks have led to at least 25 cases of H5N1 in humans, majority of which were fatal[13,15,32,33]. Several genetic characterization studies have been conducted to assess the predominant H5N1 strains circulating in Thailand during these outbreaks[13,34,35]. These studies have reported that the majority of the strains circulating throughout Thailand in 2004-2005 belonged to Clade 1, and were genetically comparable to the strains in the Thai-Vietnamese lineage[13,34]. However, a few strains isolated in 2006-2007 from north-eastern provinces in Thailand belonged to Clade 2, 3 and 4 and were genetically comparable to strains circulating in south-east People's Republic of China (Fujian strains)[13,34,35]. Furthermore, during the H5N1 outbreaks of 2008, the majority of the circulating strains were found to have mutated, while a few belonged to Clade 1[13]. A previous report from the World Health Organization (WHO), has indicated that the Clade 2 viruses, which were initially limited to poultry, have also increasingly contributed to the number of human cases in 2005-2006[36]. Whilst H5N1 prepandemic vaccines will be updated to contain the currently circulating strain as notified by the WHO,[37] the fact evident from these reports nevertheless illustrates that H5N1 viruses are in continual evolution in Thailand and it would be beneficial to have H5N1 prepandemic vaccines that can confer cross-clade protection even if they contain the latest circulating strain. As part of its Pandemic Preparedness Plan Thailand aims to ensure availability and supply of pandemic influenza vaccine for its citizens in the time of need[14] and in this scenario, stockpiling of a sufficient number of doses of licensed prepandemic vaccines is of paramount importance. Recent success of adjuvanted vaccines in demonstrating cross-clade neutralizing antibody response have opened up the option of stockpiling adequate numbers of prepandemic influenza vaccine doses and effectively prime the population prior to or immediately after the onset of a pandemic[4].

Very few licensed prepandemic vaccines are currently available for stockpiling. The United States Food and Drug Administration (USFDA)-approved split-virion influenza vaccine has several limitations in terms of antigen-sparing and immunogenicity[20,21]. In comparison, GlaxoSmithKline Biologicals' split-virion, inactivated, $AS03_A$-adjuvanted, A/Vietnam/1194/2004 NIBRG-14, approved by the European Medicines Agency (EMEA) in April 2008 has a low antigen content (3.75 mg) and has demonstrated a strong immunogenicity profile against the homologous A/Vietnam/1194/2004 strain as well as the heterologous A/Indonesia/05/2005 strain, with an acceptable safety profile. Thus, despite the worldwide pandemic mitigation and response activities in response to the 2009 H1N1 influenza emergence, the frequent H5N1 outbreaks and constantly evolving H5N1 strains in Thailand and in the South-East Asian region warrants attention and in this context, this $AS03_A$-adjuvanted H5N1 prepandemic vaccine could be a suitable candidate for mitigating future H5N1 avian influenza pandemics.

In the light of the continuing threat of the H5N1 influenza strain in the region, the present study aimed to present relevant immunogenicity and safety data on this vaccine specifically in the Thai population

that would aid the healthcare authorities and decision-makers in Thailand. The results presented here are from 350 subjects in Thailand who were part of the overall multi-center study. The sample size of the Thai sub-population did not allow for age-wise stratification. Of note, neutralization titers were measured in a subset of the overall Asian population.

The results of the present study add on to the existing literature on the robust immunogenicity and good safety of GlaxoSmithKline Biologicals' $AS03_A$-adjuvanted H5N1 prepandemic vaccine.

Conclusion

In the present study, the $AS03_A$-adjuvanted H5N1 prepandemic influenza vaccine (3.75 mg HA of A/Vietnam/1194/2004 split-virion) has demonstrated strong immunogenicity to the homologous A/Vietnam/1194/2004 strain. The vaccine was well-tolerated in the 18-60 year old Thai sub-population in the large Phase III trial. Of pivotal importance, cross-clade immunogenity against a heterologous strain (A/Indonesia/05/2005 strain) was also demonstrated. Hence, this vaccine could be an ideal candidate for minimizing the consequences of any impending influenza pandemic.

Acknowledgement

The authors want to thank Avishek Pal for medical writing and Ming-Tung Lim and Dr. Roselynn Tien for publication coordination during the preparation of this manuscript.

GlaxoSmithKline Biologicals was the funding source and was involved in all stages of the study conduct and analysis. GlaxoSmithKline Biologicals also took charge of all costs associated with the development and the publishing of the present manuscript. The corresponding author had full access to the data and had □nal responsibility to submit for publication.

Hans L. Bock, Mamadou Dram□, Paul Gillard, Yanee Hutagalung and Gary Ong are/were employees of the GlaxoSmithKline Group of Companies at the time of the study and manuscript preparation.

Potential conflicts of interest

Prasert Thongcharoen had received honoria and/or travel support from GlaxoSmithKline for scientific meetings. Prasert Auewarakul has no conflict of interest.

Prepandrix is a trademark of the GlaxoSmith-Kline group of companies.

References

1. Skeik N, Jabr FI. Influenza viruses and the evolution of avian influenza virus H5N1. Int J Infect Dis 2008; 12: 233-8.
2. Sellwood C, Asgari-Jirhandeh N, Salimee S. Bird flu: if or when? Planning for the next pandemic. Postgrad Med J 2007; 83: 445-50.
3. Goodman C, Mukherjee D, Faulkner E. How effective would antiviral vaccination and antiviral drug prevention and treatment strategies be for reducing the impact of the next influenza pandemic? [database on the Internet]. Copenhagen: WHO Regional Office for Europe; 2006 [cited 2007 Nov 20]. Available from: http://www.euro.who.int/__data/assets/pdf_file/0003/74658/E88034.pdf
4. Jennings LC, Monto AS, Chan PK, Szucs TD, Nicholson KG. Stockpiling prepandemic influenza vaccines: a new cornerstone of pandemic preparedness plans. Lancet Infect Dis 2008; 8: 650-8.
5. Chen H, Smith GJ, Li KS, Wang J, Fan XH, Rayner JM, et al. Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. Proc Natl Acad Sci U S A 2006; 103: 2845-50.
6. Salzberg SL, Kingsford C, Cattoli G, Spiro DJ, Janies DA, Aly MM, et al. Genome analysis linking recent European and African influenza (H5N1) viruses. Emerg Infect Dis 2007; 13: 713-8.
7. World Health Organization. Global Influenza Program Surveillance Network. Evolution of H5N1 avian influenza viruses in Asia. Emerg Infect Dis 2005; 11: 1515-21.
8. World Health Organization (WHO). Global Alert and Response (GAR). Continuing progress towards a unified nomenclature system for the highly pathogenic H5N1 avian influenza viruses [database on the Internet]. Updated March 2009 [cited 2009 Mar 10]. Available from: http://www.who.int/csr/disease/avian_influenza/guidelines/nomenclature/en/
9. Li WC, Huang YC. Development of vaccines against influenza A virus (H5N1). Chang Gung Med J 2007; 30: 294-304.
10. World Health Organization. Pandemic (H1N1) 2009, update 59 [database on the Internet]. 2009 [cited 2010 Apr 16]. Available from: http://www.who.int/csr/don/2010_04_09/en/index.html
11. World Health Organization. Cumulative number of confirmed human cases of avian influenza A/(H5N1) [database on the Internet]. 09 April 2010 [cited 2010 Apr 16]. Available from: http://

www.who.int/csr/disease/avian_influenza/country/cases_table_2010_04_09/en/index.html
12. Melidou A, Gioula G, Exindari M, Chatzidimitriou D, Diza-Mataftsi E. Influenza A(H5N1): an overview of the current situation. Euro Surveill 2009; 14: pii:19216.
13. Suwannakarn K, Amonsin A, Sasipreeyajan J, Kitikoon P, Tantilertcharoen R, Parchariyanon S, Chaisingh A, Nuansrichay B, Songserm T, Theamboonlers A, Poovorawan Y. Molecular evolution of H5N1 in Thailand between 2004 and 2008. Infect Genet Evol 2009; 9: 896-902.
14. The Second National Strategic Plan for Prevention and Control of Avian Influenza and Preparedness for Influenza Pandemic (B.E. 2551-2553) (A.D. 2008-2010). Sub-committee for the Second National Strategic Plan for Prevention and Control of Avian Influenza and Preparedness for Influenza Pandemic. Bangkok: Veterans Press; 2007.
15. Leroux-Roels I, Borkowski A, Vanwolleghem T, Drame 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.
16. World Health Organization. H5N1 avian influenza: timeline [database on the Internet]. Geneva: WHO; 2006 [cited 2008 Sep 1]. Available from: http://www.who.int/csr/disease/avian_influenza/timeline.pdf
17. Longini IM Jr, Nizam A, Xu S, Ungchusak K, Hanshaoworakul W, Cummings DA, et al. Containing pandemic influenza at the source. Science 2005; 309: 1083-7.
18. World Health Organization. Options for the use of human H5N1 influenza vaccines and the WHO H5N1 vaccine stockpile [database on the Internet]. WHO scientific consultation. Geneva, Switzerland 1-3 October 2007 [cited 2008 Nov 12]. Available from: http://www.who.int/csr/resources/publications/WHO_HSE_EPR_GIP_2008_1/en/index.html
19. Lin J, Zhang J, Dong X, Fang H, Chen J, Su N, et al. Safety and immunogenicity of an inactivated adjuvanted whole-virion influenza A (H5N1) vaccine: a phase I randomised controlled trial. Lancet 2006; 368: 991-7.
20. 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.
21. 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.
22. Bresson JL, Perronne C, Launay O, Gerdil C, Saville M, Wood J, et al. Safety and immunogenicity of an inactivated split-virion influenza A/Vietnam/1194/2004 (H5N1) vaccine: phase I randomised trial. Lancet 2006; 367: 1657-64.
23. Nicholson KG, Colegate AE, Podda A, Stephenson I, Wood J, Ypma E, et al. Safety and antigenicity of non-adjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a randomised trial of two potential vaccines against H5N1 influenza. Lancet 2001; 357: 1937-43.
24. Leroux-Roels I, Bernhard R, Gerard P, Drame 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: e1665.
25. Chu DW, Hwang SJ, Lim FS, Oh HM, Thongcharoen P, Yang PC, et al. Immunogenicity and tolerability of an AS03(A)-adjuvanted prepandemic influenza vaccine: a phase III study in a large population of Asian adults. Vaccine 2009; 27: 7428-35.
26. Stephenson I, Wood JM, Nicholson KG, Charlett A, Zambon MC. Detection of anti-H5 responses in human sera by HI using horse erythrocytes following MF59-adjuvanted influenza A/Duck/Singapore/97 vaccine. Virus Res 2004; 103: 91-5.
27. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. Am J Hyg 1938; 27: 493-7.
28. 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. EMEA/CHMP/VWP/263499/2006 [database on the Internet]. January 2007 [cited 2009 Mar 9]. Available from: http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500003804
29. Committee for Human Medicinal Products (CHMP). Guideline on dossier structure and content for pandemic influenza vaccine marketing authorization application (revision). EMEA/CPMP/VEG/4717/2003-Rev.1 [database on the Internet]. December 2008 [cited 2009 Mar 17]. Available from: http://www.ema.europa.eu/ema/pages/

includes/document/open_document.jsp?webContentId=WC500003869
30. Auewarakul P. The past and present threat of avian influenza in Thailand. In: Lu Y, Essex M, Roberts B, editors. Emerging infections in Asia [database on the Internet]. 2008 [cited 2009 Jan 20]. Available from: http://www.springer.com/978-0-387-75721-6
31. Chunsuttiwat S. Response to avian influenza and preparedness for pandemic influenza: Thailand's experience. Respirology 2008; 13 (Suppl 1): S36-40.
32. Oshitani H, Kamigaki T, Suzuki A. Major issues and challenges of influenza pandemic preparedness in developing countries. Emerg Infect Dis 2008; 14: 875-80.
33. World Health Organization. Media centre. Projected supply of pandemic influenza vaccine sharply increases [database on the Internet]. 2007 [cited 2009 Mar 18]. Available from: http://www.who.int/mediacentre/news/releases/2007/pr60/en/index.html
34. Amonsin A, Chutinimitkul S, Pariyothorn N, Songserm T, Damrongwantanapokin S, Puranaveja S, et al. Genetic characterization of influenza A viruses (H5N1) isolated from 3rd wave of Thailand AI outbreaks. Virus Res 2006; 122: 194-9.
35. Chutinimitkul S, Songserm T, Amonsin A, Payungporn S, Suwannakarn K, Damrongwatanapokin S, et al. New strain of influenza A virus (H5N1), Thailand. Emerg Infect Dis 2007; 13: 506-7.
36. World Health Organization Global Influenza Program Surveillance Network. Global Influenza Program Surveillance Network. Evolution of H5N1 avian influenza viruses in Asia. Emerg Infect Dis 2005; 11: 1515-21.
37. European Medicines Agency. Summary of Product Characteristics. *Prepandrix*□, H5N1 prepandemic influenza vaccine [database on the Internet]. 2009 [cited 2010 May 7]. Available from: http://www.ema.europa.eu/humandocs/PDFs/EPAR/prepandrix/emea-combined-h822en.pdf

ภูมิคุ้มกันข้ามสายพันธุ์จากการใช้วัคซีนไข้หวัดใหญ่ชนิดแพร่ระบาดที่มีแอนติเจนปริมาณน้อยร่วมกับสารเสริมภูมิคุ้มกัน เอเอสโอ 3 (AS03$_A$-adjuvanted) ในประชากรไทย

ประเสริฐ ทองเจริญ, ประเสริฐ เอื้อวรากุล, ญาณี ฮูทะกาลุง, Gary Ong, Paul Gillard, Mamadou Dram, Hans L Bock

วัตถุประสงค์: *การศึกษา (NCT00449670) ในคนเอเชียอายุระหว่าง 18-60 ปี เพื่อประเมินความสม่ำเสมอของกระบวนการผลิตวัคซีนไข้หวัดใหญ่ชนิดแพร่ระบาดที่มีแอนติเจนในปริมาณ 3.75 ไมโครกรัม ร่วมกับสารเสริมภูมิคุ้มกันชนิดเอเอสโอ 3 (AS03$_A$-adjuvanted) 4 สูตร โดยประเมินการตอบสนองของภูมิคุ้มกันชนิด Hemagglutination Inhibition (HI) titers หลังฉีดวัคซีนต่อการป้องกันไข้หวัดใหญ่สายพันธุ์ A/Vietnam/1194/2004 และ A/Indonesia/05/2005 การตอบสนองของภูมิคุ้มกันและความปลอดภัยของวัคซีนในคนไทยได้นำมาเสนอในบทความนี้*

วัสดุและวิธีการ: *ผู้เข้าร่วมการวิจัยจะถูกสุ่มแบ่งเป็นหกกลุ่ม โดย 4 กลุ่มได้รับวัคซีนที่มีสารเสริมภูมิคุ้มกันชนิดเอเอสโอ 3 และ 2 กลุ่มได้รับวัคซีนที่ไม่มีสารเสริมภูมิคุ้มกันในอัตราส่วน 2:2:2:2:1:1 โดยจะได้รับวัคซีน 2 ขนาดยาห่างกัน 21 วัน และมีการเก็บตัวอย่างเลือดสำหรับหาระดับแอนติบอดี HI ต่อเชื้อไข้หวัดทั้งสองสายพันธุ์*

ผลการศึกษา: *หลังจากที่ผู้เข้าร่วมวิจัยได้รับวัคซีนที่มีสารเสริมภูมิคุ้มกันชนิดเอเอสโอ 3 (H5N1-AS03$_A$) เข็มที่ 2 มีร้อยละ 94.2 ของผู้ที่ได้รับวัคซีนในกลุ่มมีภูมิขึ้นตอบสนองต่อเชื้อสายพันธุ์ในวัคซีน และร้อยละ 94.9 มีภูมิขึ้นในระดับที่ป้องกัน (seroprotected) ต่อเชื้อ A/Vietnam/1194/2004 strain และยังสามารถป้องกันข้ามสายพันธุ์ไปยังสายพันธุ์ A/Indonesia/05/2005 ได้อีกด้วย ในการศึกษานี้พบว่าวัคซีนมีความปลอดภัยในเกณฑ์ที่ยอมรับได้ในทุกกลุ่มที่ได้รับวัคซีน*

สรุป: *วัคซีนไข้หวัดใหญ่ชนิดแพร่ระบาดที่มีแอนติเจนปริมาณน้อยร่วมกับสารเสริมภูมิคุ้มกันเอเอสโอ 3 (AS03$_A$-adjuvanted) นั้นน่าจะเหมาะสมกับการใช้ป้องกันการระบาดของไข้หวัดใหญ่ H5N1 หากเกิดมีการระบาดในอนาคต*