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A Phase 2/3 double blinded, randomized, placebo-controlled study in healthy adult participants in Vietnam to examine the safety and immunogenicity of an inactivated whole virion, alum adjuvanted, A (H5N1) influenza vaccine (IVACFLU-A/H5N1)

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ABSTRACT

Background: A global shortfall of vaccines for avian influenza A(H5N1) would occur, especially in low- and-middle income countries, if a pandemic were to occur. To address this issue, development of a pre-pandemic influenza vaccine was initiated in 2012, leveraging a recently established influenza vaccine manufacturing capacity in Vietnam.

Methods: This was a Phase 2/3, double-blinded, randomized, placebo-controlled study to test the safety and immunogenicity of IVACFLU-A/H5N1 vaccine in healthy adults. Phase 2 was a dose selection study, in which 300 participants were randomized to one of the three groups (15 mcg, 30 mcg, or placebo). Safety and immunogenicity were assessed in all participants. In Phase 3, 630 participants were randomized to receive the IVACFLU-A/H5N1 vaccine dose selected in Phase 2 (15 mcg, n = 525) or placebo (n = 105). Safety was assessed in all Phase 3 participants and immunogenicity was measured in a subset of participants.

Results: The vaccine was well tolerated and most of the adverse events were mild and of short duration. Mild pain at the injection site was the most common adverse event seen in 60 percent of participants in the vaccine group in Phase 3. In Phase 2, both 15 mcg and 30 mcg doses were immunogenic, so the lower dose was selected for further testing in Phase 3. In Phase 3 overall seroconversion rates were 68 percent for hemagglutination inhibition (HI), 51 percent for microneutralization (MN) and 56 percent for single radial hemolysis (SRH). The seroprotection rates were 44 percent for HI, 41 percent for MN and 55 percent for SRH. The GMT ratio was 5.31 and 3.7 for HI and MN respectively; GMA was 4.75 for the SRH.

Conclusion: The IVACFLU A/H5N1 was safe and immunogenic. Development of this pandemic avian influenza vaccine is a welcome addition to the limited global pool of these vaccines.

ClinicalTrials.gov register NCT02612909.

1. Introduction

From the first reported case of bird-to-human transmission in Hong Kong in 1997, to its subsequent reemergence in 2003, avian influenza A(H5N1) has gradually spread across the world, threatening lives wherever it circulates [1]. It has caused widespread infection of poultry in parts of Asia, Africa, and the Middle East. Avian A(H5N1) influenza infection in people is associated with high mortality: globally, 454 deaths were reported in 860 cases between 2003 and 2018 [2]. Vietnam alone reported 64 deaths in 127 cases during that period. In an increasingly interconnected

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world, avian A(H5N1) influenza remains a potential pandemic threat to humans worldwide. Vaccination is an effective public health intervention strategy to contain the infection; thus, licensed vaccine products and facilities to manufacture them quickly are critical to prepare against a potential avian influenza pandemic outbreak.

In 2005, when A(H5N1) spread through Asia and into Europe, Africa and the Middle East, it became clear that the global supply of vaccines was inadequate and large parts of the world—especially low-resource countries—lacked equitable access to pandemic influenza vaccines. To address this problem, the World Health Organization (WHO) in 2006 launched a Global Action Plan on Influenza Vaccines (GAP) with the objective of expanding the development of influenza vaccines to counter the threat of influenza pandemics [3]. Since 2009, PATH, in partnership with Biomedical Advanced Research and Development Authority (BARDA) of the US Department of Health and Human Services and WHO, has contributed to GAP by providing technical assistance to many vaccine manufacturers in developing countries.

The Institute of Vaccines and Medical Biologicals (IVAC) in Vietnam received funding and technical assistance from PATH, BARDA, and WHO to develop and license a pandemic avian influenza A (H5N1) inactivated vaccine. This product is a whole virion, alum adjuvanted vaccine candidate that was previously evaluated in Phase 1 clinical trial [4]. In that Phase 1 study 75 participants were randomized to receive two doses of low-dose vaccine (7.5 mcg HA), high-dose vaccine (15 mcg HA), or placebo. In this paper, we present the results from a Phase 2/3 study to evaluate safety and immunogenicity of IVAC's A(H5N1) vaccine (IVACFLU-A/H5N1)) in adults.

2. Methods

2.1. Study design and implementation

This was a Phase 2/3, double-blind, randomized, placebo-controlled study conducted from March 2016 to August 2017 by the National Institute of Hygiene and Epidemiology (NIHE) in Hanoi, Vietnam. The study protocol was approved by WHO's Ethics Review Committee (ERC) and NIHE's Institutional Review Board (IRB). The Vietnamese Ministry of Health (MOH) ethics committee also approved the protocol. The trial was conducted according to the principles of the Declaration of Helsinki, Good Clinical Practice, and Vietnamese regulatory requirements [5]. The study is registered in the National Institutes of Health ClinicalTrials.gov register (NCT02612909). All the participants provided written informed consent prior to enrollment.

This Phase 2/3 study was designed to evaluate the safety and immunogenicity of two doses of IVACFLU-A/H5N1 vaccine given 21 days apart. All participants were healthy (based on medical history and physical examination) male and female adults, 18–60 years of age, literate, and willing to complete diary cards. Major exclusion criteria included participation in another clinical trial involving any vaccine or therapy within the previous three months, receipt of any non-study vaccine within four weeks prior to enrollment, current or recent (within two weeks of enrollment) acute illness with or without fever, chronic administration of immunosuppressive agents, history of asthma or hypersensitivity after previous administration of any vaccine, pregnancy, and lactation. The investigator used a standard scale to grade AEs, which were defined as mild, moderate, or severe if resulting in no limitation, some limitation, or an inability to perform normal daily activities, respectively. An SAE was defined as an AE that met one of the following conditions like death; was life-threatening; required inpatient hospitalization or prolongation of existing hospitalization; resulted in congenital anomaly/birth defect; resulted in a persistent or significant disability or incapacity; and important medical events.

The study was conducted in two stages. Phase 2 was a dose selection study and enrolled approximately 300 participants at one site (Khanh Hoa). Phase 3 was a pivotal trial that assessed the dose selected in Phase 2, in 630 participants according to local and international immunogenicity criteria [5,6,7].

For the Phase 2 portion, participants were randomized to one of three groups (15 mcg IVACFLU-A/H5N1 vaccine, 30 mcg IVACFLU-A/H5N1 vaccine, or placebo) at a 1:1:1 ratio, and safety and immunogenicity were assessed in all 300 participants. Full evaluation of vaccine safety continued through Day 91; however, an interim assessment of the safety and immunogenicity data collected through Day 43 helped determine whether to proceed to Phase 3 and at what dose.

The study was paused while the immunogenicity and safety data through Day 43 were analyzed and safety data was reviewed by the study Data Safety Monitoring Board (DSMB). Phase 3 initiation was dependent on demonstrating an HI response titer of $\geq$ 1:40 in $\geq$ 60 percent of vaccine recipients in at least one of the two Phase 2 IVACFLU-A/H5N1 vaccine groups. Based on the review by DSMB, NIHE and the MOH ERCs, 15 mcg of IVACFLU A/H5N1 vaccine dose was selected for further evaluation in Phase 3.

In the Phase 3 study, a total of 630 participants were randomized at two sites (Khanh Hoa and Hai Phong) to receive 15 mcg of IVACFLU-A/H5N1 vaccine (n = 525) or placebo (n = 105). The sample size of the vaccine group was driven by statistical considerations and local Vietnamese MOH guidance for Influenza vaccine clinical trials [5]. The placebo group was incorporated in the study to blind the safety assessments by participants and investigators. Safety was assessed in all participants and immunogenicity was measured in a subset of approximately 200 participants receiving IVACFLU-A/H5N1 vaccine and 40 participants receiving placebo at the Hai Phong study site. Statistical analysis was done for safety parameters; however, no statistical analysis was performed for immunogenicity end points with placebo as comparator because licensure requirements are based on point estimates of immune responses [5].

2.2. Investigational vaccine product

The investigational vaccine was an inactivated whole virion monovalent A(H5N1) influenza vaccine produced in embryonated eggs, inactivated with formaldehyde, and formulated with aluminum hydroxide. The virus seed strain NIBRG-14 was provided by National Institute for Biological Standards and Control (United Kingdom). In NIBRG-14 the HA and NA genes are from A/Vietnam/1194/2004 (H5N1) virus and other genes for internal proteins are from A/PR/8/34 (H1N1) virus. IVACFLU-A/H5N1 was formulated to contain either 15 mcg HA and 0.6 mg of aluminum hydroxide adjuvant per 0.5 mL dose, or 30 mcg HA and 0.6 mg of aluminum hydroxide adjuvant per 0.5 mL dose. It was filled in single dose vials and stored at +2 °C to +8 °C. The Phase 2 lots were manufactured in February 2015 and the HA content was measured by SRID using potency reagents from the NIBSC [Influenza Antigen (NIBRG-14), NIBSC code: 09/184, version 3.0, dated 12 Feb 2010 &; Influenza Antiserum, NIBSC code: 04/214, version 5.0, dated 24 Aug 2011]; 19.6 mcg HA/dose for 15 mcg/dose fill and 34.05 mcg HA/dose for 30 mcg/dose fill. The Phase 3 lots were manufactured in June 2016 and had SRID of 18.27 mcg HA/dose for 15 mcg/dose using the same potency reagents that were used to test Phase 2 vaccine. The placebo was phosphate buffered saline (PBS) manufactured by IVAC and provided in 0.5 mL single-dose vials.

There was a slight difference in the physical appearance between vaccine and placebo because IVACFLU-A/H5N1 vaccine

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used aluminum hydroxide as an adjuvant and the placebo was PBS. To blind the vaccinator and study participants, a nurse was responsible for withdrawing study product from vials according the randomization schedule in a separate room/closed private space, then masking the syringe before handing it over to the vaccinator. This nurse did not participate in any safety evaluation of study participants. All vaccine and the placebo were administered in the deltoid muscle of the non-dominant arm using a 23–25 gauge hypodermic needle. The vial labeling was done at IVAC before study vaccine and placebo were shipped to the study site. IVACFLU-A/H5N1 vaccine and placebo vials were packaged and labeled in such a way that they had similar external appearance.

2.3. Immunogenicity assessment

Serum samples were collected at baseline (Day 1, before vaccination), on day of second vaccination (Day 22, before vaccination) and 21 days after second vaccination (Day 43) in the Phase 2 study. In the Phase 3 study, serum samples were collected at baseline (Day 1) and 21 days after second vaccination (Day 43). The sera samples were stored at −20 °C before they were analyzed at NIHE in Vietnam for hemagglutination inhibition (HI) and VisMederi, srl in Italy (for microneutralization [MN] and single radial hemolysis [SRH]). In the Phase 2 study, HI and MN assays and in the Phase 3 study HI, MN, and SRH were used to evaluate immunogenicity. A previously described method was used for HI (utilizing horse-derived RBCs), MN, and SRH assays [8,9,10,11,12].

Seroprotection in HI and MN assay was defined as an HI and MN titer $\geq$ 1:40 after the second vaccination on Day 43; and in the SRH assay as area of $\geq$ 25 mm^2 after the second vaccination on Day 43. Note that use of the term seroprotection for pandemic influenza vaccines is debatable, as the antibody titers that correspond to correlate of protection have not been defined. Seroconversion in HI and MN assay was defined as percentage of participants achieving an increase in HI and MN titer from <1:10 pre-vaccination to $\geq$1:40 post-vaccination (Day 43), or at least a four-fold post-vaccination increase in titer from a pre-vaccination titer $\geq$1:10; and in the SRH assay as area of $\geq$25 mm^2 after immunization in case of negative baseline sample ($\leq$4 mm^2), or 50 percent increase in SRH area if baseline sample was >4 mm^2.

2.4. Safety assessment

Participants were kept under close observation for 30 min after vaccination to evaluate for any immediate reactogenicity events. Participants were provided with a diary card to record any solicited injection-site and systemic reactions occurring during a seven-day post injection period. Solicited injection-site reactions evaluated were redness, swelling, induration, pain, and tenderness. Solicited systemic reactions assessed were fever, fatigue/malaise, generalized muscle aches, joint aches/pains, chills, nausea, vomiting, and headache. Unsolicited adverse events were recorded for 21 days post each vaccination. All serious adverse events (SAEs) were recorded over the entire study period (Days 1–91). Additional safety assessments included clinical laboratory evaluations (hematology, serum chemistry, hepatitis B & C, for Phase 2 only), physical examinations, and evaluation of vital signs.

Safety was monitored routinely throughout the study (generally weekly) by the Protocol Safety Review Team (PSRT), which included the investigator, PATH medical officers, the IVAC medical officer, and the contract research organization (CRO) medical monitor. Blinded safety reports produced by the CRO were reviewed by the PSRT. The Phase 2 study had an additional Data Safety Monitoring Board (DSMB) that was composed of independent vaccine and infectious disease experts and a biostatistician. The DSMB was responsible for reviewing Phase 2 data and providing a recommendation on moving the vaccine to Phase 3 study. For Phase 3, no formal DSMB review was planned. However, the PSRT continued to conduct blinded safety reviews.

2.5. Statistical considerations

This was an operationally seamless Phase 2/3 trial with a primary objective to evaluate the immunogenicity and safety of two dose levels of IVACFLU-A/H5N1 (15 mcg vs. 30 mcg in the Phase 2 study), and to select the optimal of the two dose levels to evaluate further for potential licensure (Phase 3 study). The sample size for this study was selected for the primary immunogenicity analysis and the safety analysis to satisfy Vietnamese MOH guidance for influenza vaccine clinical trial [5]. The Vietnamese MOH guidance on serological immune response requirements for influenza vaccine is a modification of the EMEA/CHMP serological criteria for assessing seasonal influenza for licensure. The sample size for Phase 2 of the study was approximately 300 participants (100 per group); for Phase 3, it was 630 participants (525 for the IVACFLU-A/H5N1 group and 105 for the placebo group). Overall, approximately 600 participants received the selected dose of study vaccine.

Categorical variables were summarized by frequencies and percentages and continuous variables were summarized by means, standard deviations, medians, interquartile ranges, minima, and maxima. Immunogenicity data was presented as point estimates and exact 95 percent confidence intervals for the proportions meeting immunogenicity endpoints. Immunogenicity analysis was done in per protocol population for all participants and by age group (18–40 and 41–60 years of age). Per protocol (PP) population was defined as participants who received all doses of vaccine as scheduled and had a valid baseline and a post-vaccination immunogenicity measure with no major protocol violations that could potentially interfere with the immunogenicity assessment of the study vaccine. Safety analysis was done in full analysis (FA) population that included all participants who were randomized and received a study vaccination. For the solicited injection-site and systemic adverse events, the Cochran-Mantel-Haenszel (CMH) test at two-sided 0.05 alpha was used to compare the treatment groups. The CMH analysis was conducted using STATXACT (Cytel Studio 9.0). No statistical testing was performed for unsolicited AEs including SAEs.

3. Results

3.1. Phase 2 clinical study

3.1.1. Disposition of participants

A total of 300 participants were randomized (placebo group, n = 100; 15 mcg vaccine group, n = 100; 30 mcg vaccine group, n = 100) in the study (Fig. 1a). All randomized participants received a first dose of study products and all but eight participants (placebo, n = 2; 15 mcg vaccine group, n = 5; 30 mcg vaccine group, n = 1) received a second dose of study products. Seven participants voluntarily withdrew and one participant (30 mcg vaccine group) had a positive pregnancy test prior to the second dose. The pregnant participant was followed throughout pregnancy and delivered a healthy baby.

3.1.2. Demographic and other baseline characteristics

Of the 300 participants, 66 percent were female and 34 percent were male (Supplementary Table 1). Age ranged from 18 to 59 years; the mean age was 39.7 years. All participants reported their ethnicity as Kinh. None of the reported medical histories were considered exclusionary. No noteworthy differences were

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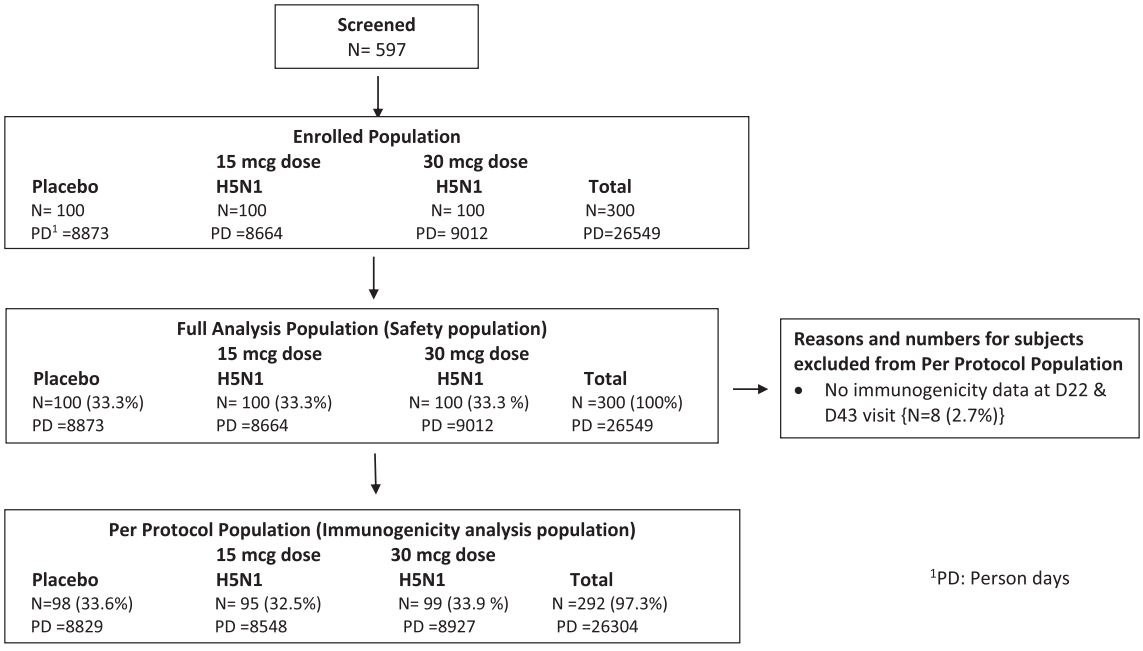

Fig. 1a. Overall Study Status by Treatment Group (phase 2).

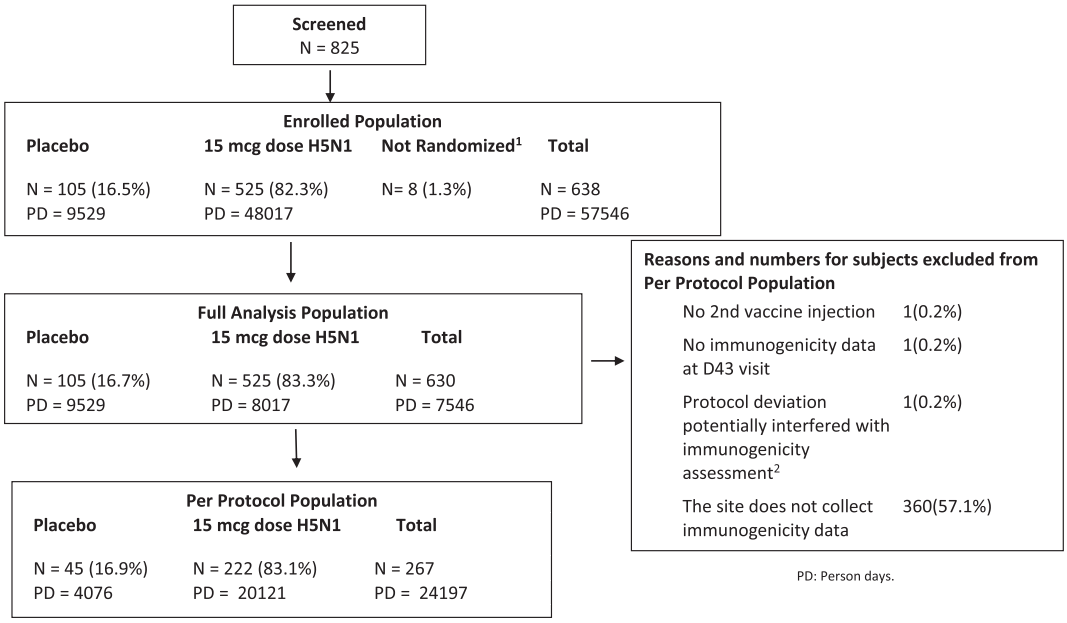

Fig. 1b. Overall Study Status by Treatment Group (phase 3).

observed between groups with regards to baseline vital signs or physical examination results.

3.1.3. Immunogenicity assessment

In the Phase 2 study, immunogenicity was assessed by HI and MN assays. The proportion of participants achieving an HI and MN titer ≥1:40 (seroprotection levels) after each dose is summarized in Fig. 2 (Panel A). Seroprotection levels in the HI assay were attained for both IVACFLU-A/H5N1 vaccine dose groups on Day 43 with 83.2 percent of participants in the 15 mcg vaccine group and 81.8 percent of participants in the 30 mcg vaccine group achieving a HI titer ≥1:40. On Day 43, a MN (neutralizing) antibody titer ≥1:40 was achieved for 44.2 percent of participants in the 15 mcg vaccine group and 31.3 percent of participants in the 30 mcg vaccine group. No participant had an HI titer or MN titer ≥1:40 on Day 1.

The proportions of participants with at least a four-fold increase in HI titers (seroconversion) on Day 43 were comparable in the IVACFLU-A/H5N1 vaccine groups. The seroconversion rates were high with 92.6 percent of participants in the 15 mcg vaccine group and 93.9 percent of participants in the 30 mcg vaccine group seroconverting. In the MN assay, proportion of participants with at least a four-fold increase in neutralizing antibody titers at Day 43 were comparable in the IVACFLU-A/H5N1 vaccine groups (60 percent of participants in the 15 mcg vaccine group and 63.6 percent of participants in the 30 mcg vaccine group) (Fig. 2; Panel A).

Fig. 2 (Panel B) summarizes the Geometric Mean Titer Ratio (GMTR), which is defined as the ratio between GMTs on Day

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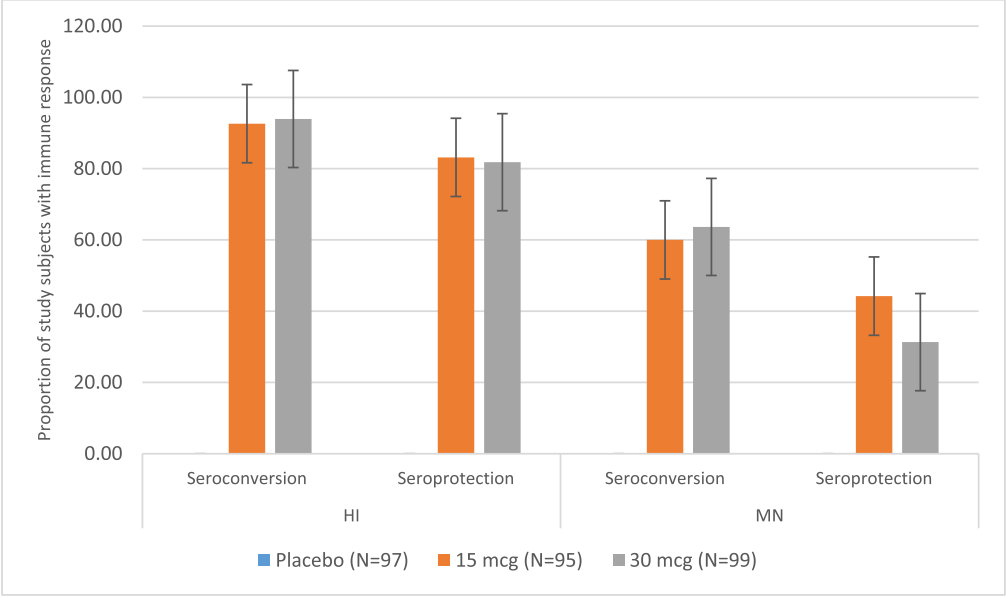
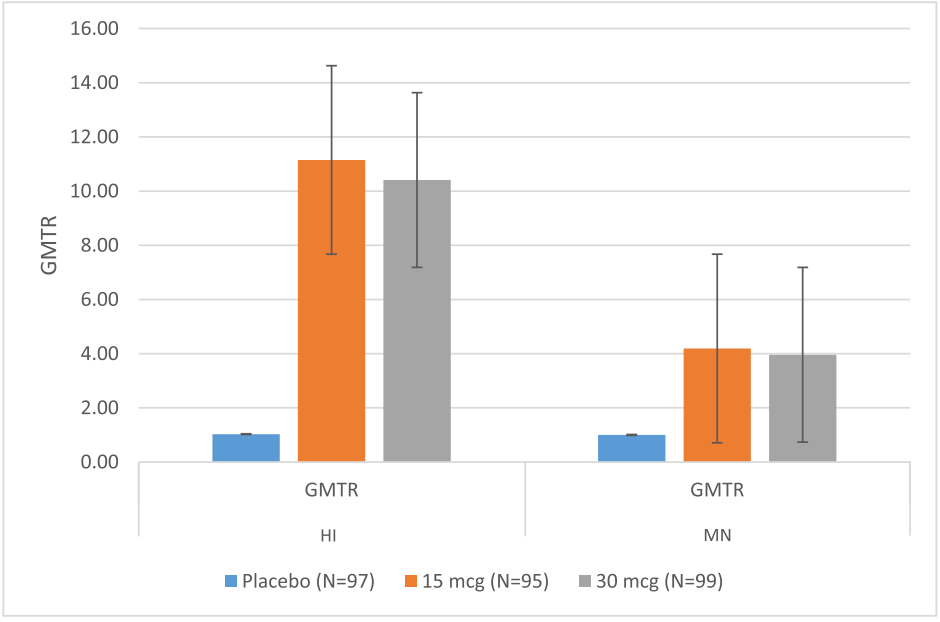
A) Proportion (and 95% CI) of participants Seroconverting* and reaching Seroprotection** levels on Day 43



* Seroconversion defined as at least a four-fold increase in post-vaccination titer on Day 43;
** Seroprotection defined as an HI and MN titer ≥1:40 after the second vaccination on Day 43

B) Geometric Mean Titer Ratio (GMTR)* and 95% CI on day 43 with respect to day 1 in HI and MN assay



*GMTR: Geometric Mean of Titer of Day 43/Day 1 as determined by HI and MN assays

Fig. 2. Immune responses in Phase 2 study.

43 and Day 1. No statistical difference was observed in the HI GMTRs for the 15 mcg and 30 mcg vaccine groups on Day 43 (11.15 and 10.41, respectively). Similarly, the neutralizing antibody GMTRs were comparable for the 15 mcg and 30 mcg vaccine groups at Day 43 (4.19 and 3.96, respectively).

The serum antibody titer responses were lower in the MN assay as compared to HI assay. Based on the pre-decided immunogenicity criteria, a dose of 15 mcg was selected for further evaluation in Phase 3 study.

3.1.4. Safety assessment

Frequency of solicited events (immediate and at Day 7) after each dose are summarized in Table 1. Within 30 min following the second injection, solicited injection-site reactions were reported for approximately 2 percent of participants across groups.

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The immediate reactogenicity events reported were pain or tenderness (injection-site) and joint pain and fever (systemic). All the events were mild in severity. Over the seven-day period following the first injection and second injection, higher proportions of participants receiving vaccine experienced solicited injection-site and systemic reactions as compared to placebo (Table 1). Mild pain and tenderness were the most commonly observed injection-site solicited events; mild fatigue and headache were the most commonly observed systemic solicited events (Supplementary Tables 2 and 3). Within 21 days following the first injection and second injection, the proportion of participants reporting unsolicited AEs were comparable in the 15 mcg and 30 mcg vaccine and placebo group. Common AEs reported across groups were conjunctivitis, upper abdominal pain, nasopharyngitis, varicella, cough, oropharyngeal pain, and pruritus. Most of the AEs were mild in severity. One participant in the 15 mcg vaccine group had an SAE (tonsillitis). There were no significant abnormalities noted in the clinical laboratory tests and vital signs.

3.2. Phase 3

3.2.1. Disposition of participants

Based on the results of the Phase 2 study, 15 mcg of IVACFLU-A/H5N1 was selected for further evaluation in the Phase 3 study. A total of 630 participants were randomized (placebo group, n = 105; 15 mcg vaccine group, n = 525) in the Phase 3 study (Fig. 1b). The Phase 3 study was conducted at two sites and blood samples for immunogenicity analysis were collected at one of the sites (n = 270). A total of 267 participants were included in the PP population for evaluation of immunogenicity. All randomized participants received first dose of study products and all but seven participants (placebo, n = 2; 15 mcg vaccine group, n = 5) received the second dose of study products. The reasons for study product discontinuation included pregnancy (n = 4), AE (n = 2), and voluntary withdrawal (n = 1). All but one participant completed the final visit for Phase 3 (Day 91). There were two additional pregnancies detected after the second vaccination. Six participants in total had positive pregnancy tests after enrollment in the study: five participants were in the vaccine group and one participant was in the placebo group. Of the six participants, three decided to undergo elective abortion and three participants delivered a healthy term baby.

3.2.2. Demographic and other baseline characteristics

Of the 630 participants randomized in the study, 55.9 percent were female and 44.1 percent were male (Supplementary Table 4). The mean age was 40.1 years and ranged from 19 to 60 years. All but one participant (15 mcg vaccine group) reported their ethnicity as Kinh. No significant difference was observed between the groups. None of the reported medical histories was considered exclusionary. No clinically significant differences were observed between groups with regard to baseline vital signs or physical examination results.

3.2.3. Immunogenicity assessment

In the Phase 3 study, immunogenicity was assessed by HI, MN, and SRH assays. Fig. 3 (Panel A) summarizes the seroprotection data across three assays. The proportion of participants with HI titer $\geq$ 1:40 (seroprotection levels) on Day 43 (21 days after second dose) was 44.1 percent in the IVACFLU-A/H5N1 vaccine group. In the MN assay; the proportion of participants with neutralizing antibodies $\geq$1:40 on Day 43 was 41 percent; and in the SRH assay the proportion of participants with SRH area $\geq$25 mm^2 was 54.9 percent in the IVACFLU-A/H5N1 vaccine group. In all the three assays, a higher proportion of participants in the 18–40 year age group reached seroprotection levels as compared to the 41–60 year age group (Fig. 3; Panel A).

Fig. 3 (Panel A) also summarizes the seroconversion data across three assays. The proportion of participants in the vaccine group with at least a four-fold increase in post-injection HI titers (seroconversion) and MN titers on Day 43 was 67.6 percent and 51.4 percent respectively. In the SRH assay, the proportion of participants seroconverting [area of $\geq$25 mm^2 after immunization in case of negative baseline sample ($\leq$4mm^2) or 50 percent increase in SRH area if baseline sample is >4 mm^2] was 55.9 percent. In all the assays, seroconversion rates were higher in the younger 18–40 year age group as compared to the 41–60 year age group.

Fig. 3 (Panel B) summarizes the GMT ratio across three assays. The HI and MN GMTR for the vaccine group on Day 43 with respect to Day 1 were 5.3 and 3.7 respectively. The SRH geometric mean area (GMA) ratio for the vaccine group on Day 43 was 4.8. Like other immunological parameters, the ratios were better in the younger age group.

3.2.4. Safety assessment

The frequency of solicited events (immediate and at Day 7) after each dose is summarized in Table 2. Within 30 min following vaccination, solicited injection-site and systemic reactions were reported for approximately <2 percent of participants across groups. Reported immediate reactogenicity events included pain and swelling (injection-site) and joint pain and tiredness (systemic). All events were mild in severity. Over the seven-day period following the first and second injections, a higher proportion of participants in the vaccine group displayed solicited injection-

Table 1
Immediate reactogenicity and 7-day solicited adverse events – Phase 2.

Immediate reactogenicity (within 30 min)				
Local or Systemic Reactogenicity	Placebo n (%) (95% CI)	15 mcg vaccine n (%) (95% CI)	30 mcg vaccine n (%) (95% CI)	p- values
1st injection, n	100	100	100	
Any local reaction	0 (0.0) 0.00–3.62)	1 (1.0) (0.03–5.45)	1 (1.0) (0.03–5.45)	0.66
Any systemic reaction	1 (1.0) (0.03 - 0.45)	1 (1.0) (0.03–5.45)	0 (0.0) (0.00–3.62)	0.66
2nd injection, n	98	95	99	
Any local reaction	2 (2.0) (0.25 - 0.18)	2 (2.1) (0.26–7.40)	2 (2.0) (0.25–7.11)	0.97
Any systemic reaction	0 (0.0) (0.00 - 0.69)	1 (1.1) (0.03–5.73)	0 (0.0) (0.00–3.66)	1.00
7-Day Reactogenicity (over a 7-Day Period Post-injection)				
1st injection, n	100	100	100	
Any local reaction	22 (22.0)(14.33–1.39)	83 (83.0) (74.18–9.77)	87 (87.0) (78.80–92.89)	<0.0001
Any systemic reaction	52 (52.0) (41.78–2.10)	66 (66.0) (55.85–5.18)	83 (83.0) (74.18–89.77)	<0.0001
2nd injection, n	98	95	99	
Any local reaction	12 (12.2) (6.49–41)	44 (46.3) (36.02–6.85)	50 (50.5) (40.27–60.71)	<0.0001
Any systemic reaction	29 (29.6) (20.79–9.66)	31 (32.6) (23.36–3.02)	37 (37.4) (27.85–47.67)	0.24

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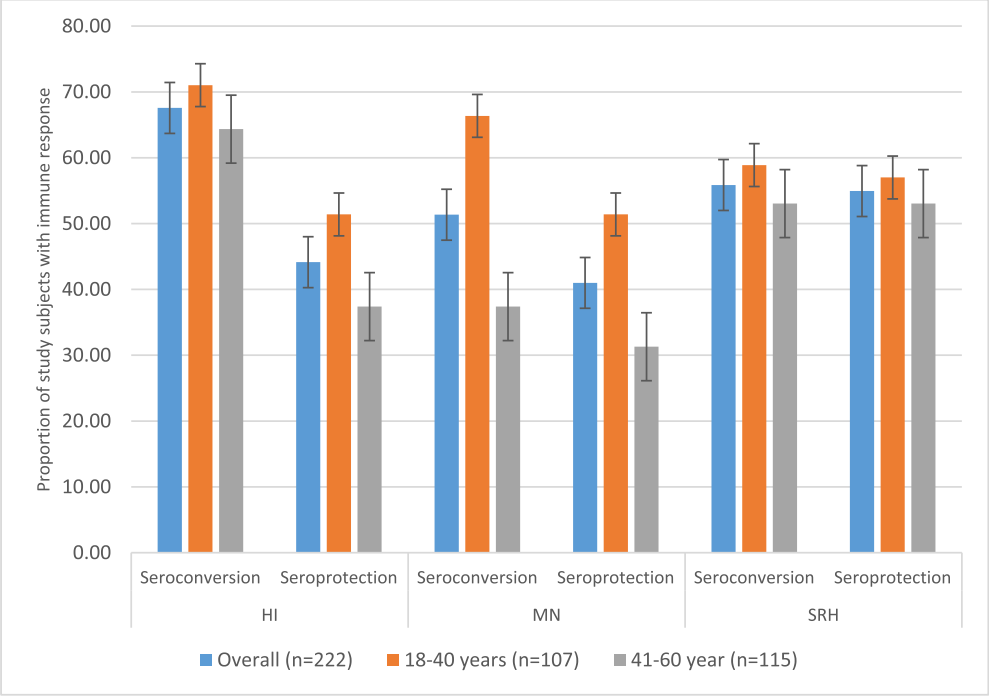
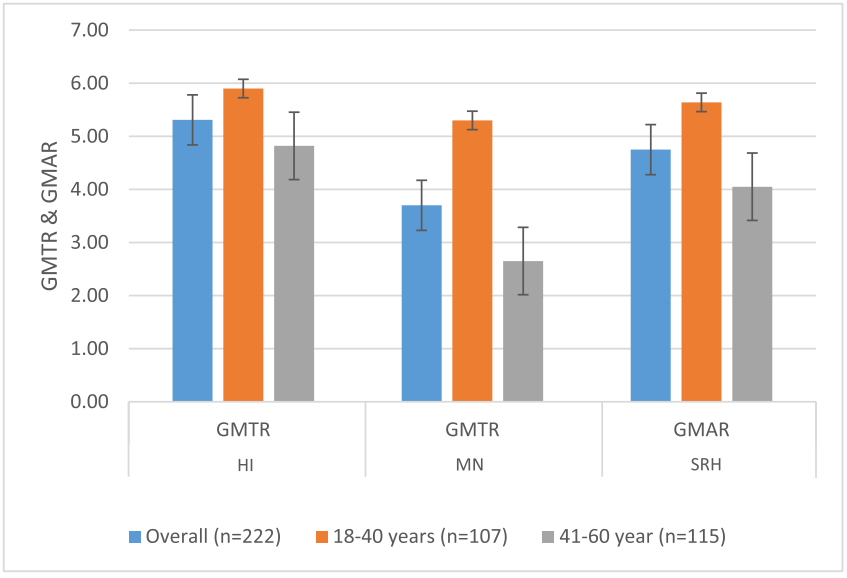
A) Proportion (and 95% CI) of participants in the vaccine group Seroconverting* and reaching Seroprotection** levels on Day 43



* Seroconversion defined as at least a four-fold increase in post-vaccination titer on Day 43 and in the SRH assay as area of ≥25 mm^2 after immunization in case of negative baseline sample (≤4 mm2), or 50% increase in SRH area if baseline sample is >4 mm^2;
**Seroprotection defined as an HI and MN titer ≥1:40 after the second vaccination on Day 43 and in the SRH assay as area of ≥25 mm^2 after the second vaccination on Day 43

B) Geometric Mean Titer Ratio (GMTR)* and 95% CI and Geometric Mean Area Ratio (GMAR)** and 95% CI on Day 43 with respect to Day 1



*GMTR: Geometric Mean of Titer of Day 43/Day 1 as determined by HI and MN assays;
** GMAR: Geometric Mean Area of Day 43/Day 1 as determined by SRH assay

Fig. 3. Immune responses in Phase 3 study.

site and systemic reactions as compared to those in the placebo group (Table 2). Mild pain and tenderness were the most common injection-site solicited events after first dose of vaccine. Mild fatigue, headache, and generalized muscle aches were the most common systemic solicited events observed after vaccination (Supplementary Tables 5 and 6).

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Table 2
Immediate reactogenicity and 7-day solicited adverse events – Phase 3.

Immediate reactogenicity (within 30 min)			
Local or Systemic Reactogenicity	Placebo n (%) (95% CI)	15 mcg vaccine n (%) (95% CI)	p-values
1st injection, n	105	525	
Any local reaction	0 (0.0) (0.00–3.45)	1 (0.2) (0.00–1.06)	1.00
Any systemic reaction	0 (0.0) (0.00–3.45)	0 (0.0) (0.00–0.70)	1.00
2nd injection, n	103	520	
Any local reaction	2 (1.9) (0.24–6.84)	3 (0.6) (0.12–1.68)	0.19
Any systemic reaction	2 (1.9) (0.24–6.84)	2 (0.4) (0.05–1.38)	0.13
7-Day Reactogenicity (over a 7-Day Period Post-injection)			
1st injection, n	105	525	
Any local reaction	19 (18.1) (11.26–26.81)	409 (77.9) (74.11–81.38)	<0.0001
Any systemic reaction	36 (34.3) (25.30–44.19)	286 (54.5) (50.11–58.80)	0.0002
2nd injection, n	103	520	
Any local reaction	15 (14.6) (8.39–22.88)	227 (43.7) (39.34–48.04)	<0.0001
Any systemic reaction	19 (18.4) (11.49–27.30)	129 (24.8) (21.15–28.75)	0.20

Within 21 days following vaccination, the proportion of participants who reported unsolicited AEs were comparable between the vaccine and placebo groups. Common unsolicited AEs reported were oropharyngeal pain, upper respiratory tract infection, and pharyngitis. The majority of events reported were mild in severity and none of them was considered to be related to study products. Twelve SAE were reported: eight in the vaccine group and four in the placebo group. None of the SAEs was considered related to the study product. There were no laboratory tests in the Phase 3 study.

4. Discussion

Sustainable and equitable supply of vaccines is an integral part of any strategy to avert the spread of infection in case of a pandemic. Vaccine manufacturers in low- and middle-income countries have been receiving technical support from WHO and BARDA to develop capabilities to produce local stockpiles of vaccines against pandemic influenza. Vietnamese MOH-affiliated vaccine manufacturer IVAC has been one of the recipients of this assistance. This study evaluated safety and immunogenicity of an IVAC avian influenza vaccine (IVACFLU-A/H5N1) under a single Phase 2/3 protocol. The Phase 2 trial was a dose selection study to select the optimal dose between 15 and 30 mcg of vaccine.

The 15 mcg was immunogenic and safe in the Phase 3 study. The vaccine was well tolerated and most of the adverse events were mild and of short duration. Pain and tenderness at the site of injection was the most common adverse event. The immune responses in the Phase 3 study were lower compared to the immunogenicity elicited in the Phase 2 study. The vaccine met the criteria for seroconversion and GMT-fold rise, per Vietnamese MOH guidance on influenza vaccine clinical trial, for both age groups in all the assays [5]. The consistently high seroconversion rates reflected in all three assays were especially reassuring. The criteria stated in the MOH guidance for clinical evaluation of influenza vaccines is based on performance of the vaccine in HI and SRH assay. However, the seroprotection criteria was not met in any of the assays. Thus, two of the three criteria were met. These results were similar to a previous study of a non-adjuvanted split A/H5N1 vaccine, in which licensure criteria were met for seroconversion and GMT-fold rise but not for seroprotection [13].

The reduced antibody titers measured by HI in the Phase 3 trial compared to the equivalent 15 mcg vaccine dose in the Phase 2 group was an unexpected finding. While we lack the data to pinpoint the determining factor with certainty, a few reasons might account for the difference. Variation in vaccine potency was excluded based on review of the study product potency (SRID) methodology, execution, and results of vials maintained in the stability studies (data not shown). Serological testing was relatively consistent for MN while a significant drop was observed in HI. Possible explanations may be variability in HI testing and population differences.

HI, particularly for hemagglutinin of avian origin, can be particularly variable based on methodology, operator and type or batch of red blood cells used and, in this case, seemed to be particularly sensitive in identifying immunogenicity anti-H5 in the Phase 2 study [14,15]. Ideally, the phase 2 and 3 samples should have been re-tested simultaneously in the laboratory to understand the accuracy of differences; however inadequate sera samples at the end of the study prevented this re-testing. While neutralization has no official seroprotection cut-off and criteria for vaccine licensure are linked to HI and SRH only, MN adds significant value to the analyses because of its ability to detect low-titer functional antibodies. Importantly the elicitation of a strong neutralizing antibodies response was confirmed by the results of MN assay. Nevertheless, MN also showed some decrease from Phase 2 to Phase 3, albeit less pronounced as compared to HI.,

Another possibility for a variation in results could be that the populations studied in Phase 2 and Phase 3 were not similar. The immunogenicity of Phase 3 was carried out only on a subset of participants, all coming from a single recruitment site at Hai Phong, while for Phase 2 the only recruitment site was Khanh Hoa. North and central Vietnam have different climate conditions, and evidence exists of differential influenza circulation in tropical and subtropical regions in south-east Asia [16]. Prior exposure to other influenza A viruses, such as H1N1, could potentially affect response to H5N1 via common epitopes on group 1 hemagglutinin and N1 neuraminidase, although this cannot be further investigated with the data generated by this study. Different socio-economic conditions and other factors could further contribute to the list of potential variables in response to influenza vaccination, particularly in seronegative participants studied in independent trials [17,18].

One limitation of this study was the skewed randomization ratio of 5:1 for vaccine and placebo in the Phase 3 study. The smaller placebo group constrained a definite comparison of safety data, though this did not influence the interpretation of immunogenicity data. There has been debate about the optimal protective titers for the pandemic vaccine, as the criteria of HI titer of 1:40 for protecting 50 percent of individuals in a population is based on experience of seasonal influenza viruses [19,20]. The EMEA and FDA guidelines for licensure of influenza vaccines have been conventionally based on the knowledge gained from studies on seasonal influenza vaccines [6,7]. The MOH guidelines have been adapted from the EMA guidelines and in fact provide a concession for lower (<1:40) HI titer to be considered as seroprotective for pandemic influenza vaccines. Some studies have also used lower titers (1:32 or 1:20) for HI and neutralizing antibodies to characterize the immune response to pandemic vaccines and have demonstrated a statistical correlation between MN titer cut-off of 1:20 and SRH area of 25 mm^2 [9,21,22]. In the current study, overall 55.41 percent (95%CI; 48.61–62.06) attained a MN titer of $\geq$ 1:20. The corresponding age-wise numbers were 72.90 percent (95 percent CI; 63.45–81.04) for 18–40 year and 39.13 percent (95 percent CI; 30.16–48.67) 41–60 year age-group (Supplementary Table 7). Given the pivotal role that vaccines play in pre-pandemic

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preparedness strategy and relative global short supplies, regulatory authorities have been liberal with approvals for pandemic influenza A(H5N1) vaccines. With level of antibodies required for protection against severe infection and mortality against avian influenza not known, vaccines with modest immunogenicity (seroprotection rates of 44 percent and seroconversion rates of 43 percent) have been licensed in the past [23,24].

Avian influenza vaccines are known to be inherently less immunogenic as compared to vaccines derived from human strains [25,26]. This is in part due to the naïve status of the subject, as demonstrated by lack of HI or MN antibody titers in placebo or pre-vaccination samples. Various strategies have been proposed to increase the immunogenicity of avian influenza vaccines—such as using adjuvants, increasing the antigen content, administering multiple doses, and prime boosting. Multiple dose approach may not work in case of a rapidly spreading and evolving pandemic. Prime boost homologous regimens have been shown to elicit robust immune response in previous studies [27,28]. This phenomenon has also been seen in administration of heterologous vaccines [28,29]. In the current Phase 3 study, at baseline, all but three participants were immunologically naïve to A(H5N1)—i.e. most of the population had not been primed with previous exposure to A(H5N1) virus. Though more than half of the vaccinated participants did not reach seroprotection levels as per definition based on seasonal influenza, the great majority of them exhibited at least a two-fold seroresponse level, indicative of priming (Supplementary Table 8). A potential approach to counter pandemic threats from A(H5N1) influenza may include priming the population with an A(H5N1) vaccine and then boosting with a dose of the pandemic vaccine at the start of a pandemic.

There has been a consistent improvement in the production capacity of pandemic vaccines since the initiation of GAP. The estimated annual global production capacity has increased from 1.5 billion doses in 2006 to 6.4 billion doses in 2016 with the added dose-sparing afforded by oil-emulsion adjuvants [30]. However, in the event of a pandemic, two doses of vaccine may be required to illicit an adequate immune response, resulting in an insufficient supply of vaccine to meet the needs of the global population. Global production capacity will be further augmented as vaccines from other manufacturers in low- to -middle-income countries near their clinical development. The influenza vaccine production facility at IVAC can be used in the event of a pandemic to produce vaccines to be used at a national level meaning that Vietnam is not solely reliant on imported or donated vaccines. Furthermore, IVACFLU-A/H5N1 can be stockpiled as a part of pandemic preparedness plan to counter the threat posed by future influenza pandemics and can be deployed at a short notice in the event of an outbreak. Successful development of IVACFLU-A/H5N1 will contribute substantively to the global pool of pandemic influenza vaccine and marks an important milestone to combat avian influenza in Vietnam and the rest of Asia.

Declaration of Competing Interest

Vien Chinh Chien and Nguyen Thi Lan Phuong, are employed with Institute of Vaccine and Medical Biologics (IVAC), which manufactured the vaccine. Other authors have no financial/personal relationships that may be considered as potential competing interests.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.11.059.

References

[1] Claas ECJ, Osterhaus ADME, van Beek R, De Jong JC, Rimmelzwaan GF, Senne DA, et al. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet 1998;351:472–7.
[2] World Health Organization. Influenza. Cumulative number of confirmed human cases of avian influenza A(H5N1) reported to WHO. 2 March 2018. http://www.who.int/influenza/human_animal_interface/2018_03_02_tableH5N1.pdf?ua=1 (Accessed on 20 Mar 2018).
[3] World Health Organization. Global pandemic influenza action plan to increase vaccine supply Available on: http://apps.who.int/iris/bitstream/10665/69388/1/WHO_IVB_06.13_eng.pdf (Accessed on 20 Mar 2018).
[4] Phan TL, Ho VT, Vu MH, Nguyen TN, Duong HT, Holt R, et al. Clinical testing of an inactivated influenza A(H5N1) vaccine candidate in a double-blinded, placebo-controlled, randomized trial in healthy adults in Vietnam. Vaccine 2016;34(45):5449–56.
[5] Guidance on clinical trial of influenza vaccine. Ministry of Health, Vietnam, 2013.
[6] European Agency for the Evaluation of Medical Products - Human Medicines Evaluation Unite. Note for guidance on clinical evaluation of new vaccines. London, May 1999; CPMP/EWP/463/97. Available from http://www.eudra.org/emea.html.
[7] Food and Drug Administration. Licensure of pandemic influenza vaccines: Demonstration of effectiveness. FDA Briefing document on vaccine and related Biological Products Advisory Committee meeting. February 29, 2012.
[8] Manual for the laboratory diagnosis and virological surveillance of influenza. WHO Global Influenza Surveillance Network, 2011; Available on http://apps.who.int/iris/bitstream/10665/44518/1/9789241548090_eng.pdf (accessed on 22/Mar/2018).
[9] Ehrlich HJ, Müller M, Oh HM, Tambyah PA, Joukhadar C, Montomoli E, Fisher D, Berezuk G, Fritsch S, Löw-Baselli A, Vartian N, Bobrovsky R, Pavlova BG, Pöllabauer EM, Kistner O, Barrett PN; Baxter H5N1 Pandemic Influenza Vaccine Clinical Study Team. A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. N Engl J Med. 2008; 358(24): 2573-84. http://doi.org/10.1056/NEJMoa073121.
[10] Finney DJ. Statistical Methods in Biological Assay, 2nd ed., Griffin, London, England, 196.
[11] Schild GC, Pereira MS, Chakraverty P. Single-radial-haemolysis: a new method for the assay of antibody to influenza haemagglutinin. Bull World Health Organization 1975;52:43–50.
[12] Wood JM, Schild GC, Newman RW, Seagroatt V. An improved single-radial-immunodiffusion technique for the assay of influenza haemagglutinin antigen: application for potency determinations of inactivated whole virus and subunit vaccines. J Biol Stand 1977;5:237–47.
[13] 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(9523):1657–64.

[14] Trombetta CM, Perini D, Mather S, Temperton N, Montomoli E. Overview of serological techniques for influenza vaccine evaluation: past. Present Future Vaccines (Basel) 2014;2(4):707–34.
[15] Jia N, Wang SX, Liu YX, Zhang PH, Zuo SQ, Lin-Zhan Dang RL, et al. WC. Increased sensitivity for detecting avian influenza-specific antibodies by a modified hemagglutination inhibition assay using horse erythrocytes. J Virol Methods. 2008;153(1):43–8.
[16] Saha S, Chadha M, Al Mamun A, Rahman M, Sturm-Ramirez K, Chittaganpitch M, et al. Influenza seasonality and vaccination timing in tropical and subtropical areas of southern and south-eastern Asia. Bull World Health Organ. 2014 May 1;92(5):318–30.
[17] Virk RK, Gunalan V, Tambyah PA. Influenza infection in human host: challenges in making a better influenza vaccine. Expert Rev Anti Infect Ther. 2016;14(4):365–75.
[18] Brodin P, Jojic V, Gao T, Bhattacharya S, Angel CJ, Furman D, et al. Variation in the human immune system is largely driven by non-heritable influences. Cell 2015 Jan 15;160(1–2):37–47.
[19] de Jong JC, Palache AM, Beyer WE, Rimmelzwaan GF, Boon AC, Osterhaus AD. Haemagglutination-inhibiting antibody to influenza virus. Dev Biol (Basel). 2003;115:63–73.
[20] Potter CW, Oxford JS. Determinants of immunity to influenza infection in man. Br Med Bull. 1979;35(1):69–75.
[21] van der Velden MV, Geisberger A, Dvorak T, Portsmouth D, Fritz R, Crowe BA, et al. Safety and immunogenicity of a vero cell culture-derived whole-virus H5N1 influenza vaccine in chronically ill and immunocompromised patients. Clin Vaccine Immunol. 2014 Jun;21(6):867–76.
[22] Nolan T, Richmond PC, Formica NT, Hoschler K, Skeljo MV, Stoney T, et al. Safety and immunogenicity of a prototype adjuvanted inactivated split-virus influenza A (H5N1) vaccine in infants and children. Vaccine. 2008;26(50):6383–91.
[23] 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(13):1343–51.
[24] Prescribing information, Influenza Virus Vaccine, H5N1, Sanofi Pasteur, Available on https://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM112836.pdf (site accessed on 29 Mar 2018).
[25] Couch RB, Patel SM, Wade-Bowers CL, Nino D. A randomized clinical trial of an inactivated avian influenza A (H7N7) vaccine. PLoS ONE 2012;7:e49704.
[26] Keitel WA, Atmar RL. Vaccines for pandemic influenza: summary of recent clinical trials. Curr. Top. Microbiol. Immunol. 2009;333:431–51.
[27] Banzhoff A, Gasparini R, Laghi-Pasini F, Staniscia T, Durando P, Montomoli E, et al. MF59-adjuvanted H5N1 vaccine induces immunologic memory and heterotypic antibody responses in non-elderly and elderly adults. PLoS ONE 2009;4(2):e4384. https://doi.org/10.1371/journal.pone.0004384.
[28] Gillard P, Chu DW, Hwang SJ, Yang PC, Thongcharoen P, Lim FS, et al. Long-term booster schedules with AS03A-adjuvanted heterologous H5N1 vaccines induces rapid and broad immune responses in Asian adults. BMC Infect Dis. 2014 Mar;15(14):142.
[29] Winokur PL, Patel SM, Brady R, Chen WH, El-Kamary SS, Edwards K, et al. Safety and immunogenicity of a single low dose or high dose of clade 2 influenza A(H5N1) inactivated vaccine in adults previously primed with clade 1 influenza A(H5N1) vaccine. J Infect Dis. 2015 Aug 15;212(4):525–30.
[30] McLean KA, Goldin S, Nannei C, Sparrow E, Torelli G. The 2015 global production capacity of seasonal and pandemic influenza vaccine. Vaccine. 2016 Oct 26;34(45):5410–3.

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