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Safety and immunogenicity of a virus-like particle pandemic influenza A (H1N1) 2009 vaccine in a blinded, randomized, placebo-controlled trial of adults in Mexico

Constantino López-Macías[a,*], Eduardo Ferat-Osorio[a], Alejandra Tenorio-Calvo[a,c], Armando Isibasi[a], Juan Talavera[b], Oscar Arteaga-Ruiz[a], Lourdes Arriaga-Pizano[a], Somia P. Hickman[d], María Allende[d], Kathy Lenhard[d], Steven Pincus[d], Kevin Connolly[d], Ramadevi Raghunandan[d], Gale Smith[d], Gregory Glenn[d,**]

[a] Medical Research Unit on Immunochemistry, Specialities Hospital of the National Medical Centre "Siglo XXI" Mexican Social Security Institute (IMSS), Mexico City, Mexico
[b] Epidemiology Research Unit, Specialities Hospital of the National Medical Centre "Siglo XXI" IMSS, Mexico City, Mexico
[c] Biomedicine and Molecular Biotechnology Program, Biochemistry Department, National School of Biological Sciences, National Polytechnic Institute, Mexico City, Mexico
[d] Novavax, Inc., 9920 Belward Campus Drive, Rockville, MD 20850, USA

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ABSTRACT

Virus-like particles (VLPs) can be rapidly developed from influenza virus genetic sequences in order to supply vaccine after the onset of a pandemic. The safety and immunogenicity of one or two doses of a recombinant A (H1N1) 2009 influenza VLP vaccine was evaluated in a two-stage, Phase 2, randomized, double-blind, placebo-controlled study conducted in 4563 healthy adults, 18–64 years of age, during the H1N1 2009 pandemic in Mexico. In Part A, 1013 subjects were randomized into four treatment groups (5 μg, 15 μg, or 45 μg hemagglutinin [HA] VLP vaccine or placebo) and vaccinated 21 days apart, with sera collected on Days 1, 14 and 36 for hemagglutination inhibition (HAI) testing. After review of safety and immunogenicity data from Part A, additional subjects were immunized with a single dose of 15 μg VLP vaccine ($N = 2537$) or placebo ($N = 1011$) and assessed for safety in Part B. Results showed the H1N1 2009 VLP vaccine was safe and well-tolerated. Systemic solicited events were similar between placebo and VLP vaccinated groups with no vaccine-related serious adverse events. Dose response trends for solicited local adverse events were observed, with higher incidences of local pain, swelling, tenderness, and redness reported in the higher VLP dose groups (15 μg and 45 μg) compared to the placebo and 5 μg VLP groups following both vaccinations. Although the majority of local AEs were mild in severity, a dose trend in events of moderate or greater severity was also noted for these solicited events. The VLP vaccine groups demonstrated robust HAI immune responses after a single vaccination, with high rates of seroprotection (≥40 HAI titer) in 82–92% of all subjects and in 64–85% of subjects who were seronegative at the time of immunization. HAI geometric mean titers (GMTs), geometric mean ratios (GMRs) and seroconversion rates were also all statistically higher in the VLP groups compared to placebo for both post-baseline time points. Based on these data, additional clinical trials are in development to evaluate influenza vaccine candidate antigens manufactured using Spodoptera frugiperda (Sf9)/baculovirus-based VLP technology.

1. Introduction

The 2009–2010 H1N1 influenza pandemic provided a serious challenge to the global health and vaccine infrastructure. US public health experts reviewed national and global responses to the pandemic outbreak and concluded non-egg based, recombinant influenza vaccines should be pursued and developed further as there were many shortfalls in embryonated egg-based influenza vaccine manufacturing systems [1]. Recombinant, insect cell produced virus-like particle manufacturing is emerging as one such technology with the potential to meet the challenges posed by an influenza pandemic.

Recombinant influenza vaccines under development include hemagglutinin (HA) subunit vaccine [2] and the virus-like particle (VLP) vaccines. Influenza VLPs are made by co-expression of influenza HA, neuraminidase (NA), and matrix 1 (M1) proteins

* Corresponding author at: Coordinación de Investigación en Salud 4º piso Bloque B Unidad de Congresos, Centro Médico Nacional Siglo XXI. Av. Cuauhtémoc 330 Col. Doctores C.P. 06020, México, D.F., México. Tel.: +52 55 5627 6915.
** Corresponding author. Tel.: +1 240 268 2065; fax: +1 240 268 2100.
E-mail addresses: constantino@sminmunologia.org (C. López-Macías), gglenn@novavax.com (G. Glenn).

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which assemble into enveloped particles that mimic the morphology of influenza virus, but contain no genetic material and are non-infectious [2]. The influenza HA, NA and M1 genes are synthesized and cloned into baculovirus, which is then used to infect Spodoptera frugiperda (Sf9) insect cells. The virus-encoded proteins are expressed within the cells, assemble and bud off as virus-like particles mimicking the virus structure expressing the relevant epitopes in a particle empty of any viral genetic material [3].

VLPs produced in SF9 insect cell-derived vaccines have been used extensively in preclinical settings and have been shown to induce functional, protective antibodies to seasonal and pandemic influenza and other pathogens. Recombinant influenza VLP vaccines being developed by Novavax, Inc. (Rockville, MD) have been shown to induce protection in ferrets immunized with seasonal [2], avian H9N2 [4,5], highly pathogenic H5N1 [6], pandemic 1918 H1N1, and pandemic 2009 A/California/04/2009 (H1N1) [7,8] influenza strains. Recombinant VLP/particle vaccines have been shown to be protective against other viral pathogens such as the Severe Acute Respiratory Syndrome Virus (SARS, [9]) and Respiratory Syncytial Virus (RSV) [10]. Recombinant trivalent influenza VLP vaccines genetically engineered with HA and NA genes from influenza H1N1, H3N2, and B recommended strains for the 2005–2006, 2008–2009, and 2009–2010 influenza seasons have been well-tolerated and immunogenic in phase 2 clinical trials. Licensed VLP vaccines such as the vaccine developed against the human papilloma virus, produced by insect or other manufacturing platforms, indicate that the VLP technology represents a proven approach to immunization [11].

A novel influenza A (H1N1) triple-reassortant virus with swine, human and avian genes was first identified in April 2009 as the cause of human respiratory disease in Mexico. By June 11, 2009, there were almost 30,000 confirmed cases reported in 74 countries and the World Health Organization (WHO) declared an influenza pandemic [12]. WHO and the United States (US) Centers for Disease Control and Prevention (CDC) recommended that A/California/7/2009 (H1N1)-like virus be used as a monovalent vaccine during the 2009 pandemic as well as for the 2010–2011 flu season [13]. Results reported at a WHO meeting in 2010 indicated the pandemic influenza H1N1 2009 vaccines elicited potentially protective antibody responses in a high proportion of older children and adults within 2 weeks of administration of a single dose of vaccine [14]. Efficacy studies of H1N1 2009 inactivated vaccines have confirmed these vaccines are protective and that hemagglutination inhibition (HAI) seroprotection predicts prevention of influenza infection and illness. For example, a study in China of over 95,000 children and adults immunized with a split-virion A (H1N1) 2009 vaccine was 87.3% effective in preventing confirmed influenza infection in school-age children [15].

In spite of this success in the development of an immunogenic and efficacious pandemic influenza vaccine, the first doses of H1N1 2009 vaccines were not available in the US until October 5, 2009, 5 months after the epidemic had reached the US and Mexico, where the pandemic was first recognized in April 2009. Mexico did not obtain a licensed vaccine to start its own vaccination program until November 27, 2010, which was at the end of the second pandemic wave and after most of the 72,000 confirmed cases and 1300 deaths had occurred [16]. The August 2010 report by the US President's Council of Advisors on Science and Technology reviewed the US response and concluded that a vaccine made by recombinant DNA technology using genetic sequences should be a priority for development as the technology could potentially reduce the time required to supply vaccine should another influenza pandemic occur [1]. Thus, clinical evaluation of the safety and immunogenicity of an insect cell-produced influenza VLP vaccine will be an important step towards achieving this goal.

In this report, the safety and immunogenicity of a recombinant VLP vaccine was assessed in adults 18–64 years old, in the midst of the H1N1 2009 pandemic in Mexico. In the first stage of the trial, safety and immunogenicity were evaluated in subjects immunized on Days 1 and 22 with 5 μg, 15 μg, or 45 μg H1N1 VLP vaccine and compared to placebo. The second stage assessed safety only in a larger number of subjects randomized to receive a single 15 μg dose of the H1N1 VLP vaccine or placebo.

2. Materials and methods

2.1. Vaccine

Influenza A/California/04/2009 (H1N1) VLPs were produced in Spodoptera frugiperda (Lepidoptera, Fall Armyworm) Sf9 cells infected with recombinant insect baculovirus Autographa californica Nuclear Polyhedrosis Virus (AcNPV) expressing HA, NA and M1 genes. The HA and NA protein sequences were derived from influenza A/California/04/2009 (H1N1), GenBank accession number ACP41105.1 and ACP41107.1, respectively. The M1 protein sequence was from the avian influenza A/Indoneisa/05/2005 (H5N1), GenBank accession number ABI36004.1. The influenza genes were codon optimized for expression in insect cells and biochemically synthesized, inserted into a pFastBac1 baculovirus transfer vector (Invitrogen, Calsbad, CA) and cloned into Escherichia coli. HA, NA, and M1 genes were under transcriptional control of the baculovirus AcMNPV polyhedrin promoter at the 5′ end and poly (A) sequence at the 3′ end. A recombinant baculovirus containing the three influenza genes was generated using the Bac-to-Bac baculovirus expression system (Invitrogen). Recombinant bacmid DNA was purified and transfected into Sf9 insect cells, and a single recombinant baculovirus that expressed HA, NA, and M1 was identified, plaque-purified, and then amplified for use in the manufacture of the influenza A (H1N1) 2009 VLPs.

The cGMP manufacture of recombinant influenza VLPs was conducted in a 100 L Wave Bioreactor (GE Healthcare) with Sf9 cells infected with the recombinant baculovirus. H1N1 VLPs were harvested after 72 h using pre-sterilized, disposable tangential flow filtration (TFF) assemblies for clarification, concentration and diafiltration. Concentrated VLPs were then separated from host contaminants, baculoviruses, and nucleic acids using ion exchange chromatography. After concentration by ultrafiltration and inactivation of residual baculovirus with 0.2% beta propiolactone, final purification was accomplished in the following steps: gel filtration chromatography, sucrose gradient ultracentrifugation, diafiltration in PBS, and 0.22 μm filtration. The sterile purified H1N1 2009 VLPs were stored at 2–8 °C and were considered stable when stored at this temperature for at least 1 year.

The particle size of the purified VLP was measured by dynamic light scattering (Malvern Instruments) and purity by scanning densitometry of SDS-PAGE gels. HA content in purified VLPs was measured using a single radial immunodiffusion (SRID) assay based on reference standards from US Food and Drug Administration Center for Biologics Evaluation and Research (CBER). Two separate lots were manufactured as described above and used separately in Part A and Part B of the study as described below.

The purified influenza A (H1N1) VLPs were pleomorphic, largely spherical enveloped particles (as shown by dynamic light scattering and transmission electron microscopy [8]), with a mean particle diameter of 144.5 nm and 39.3% HA, 8.6% NA, and 42.4% M1 composition (as measured by scanning densitometry). An avian influenza M1 was used in place of the native H1N1 M1 to increase yields.

The (H1N1) 2009 Influenza VLP vaccine was formulated to contain 5 μg, 15 μg and 45 μg recombinant HA per 0.5 mL dose as measured using the SRID assay. The vaccine was in a neutral pH,

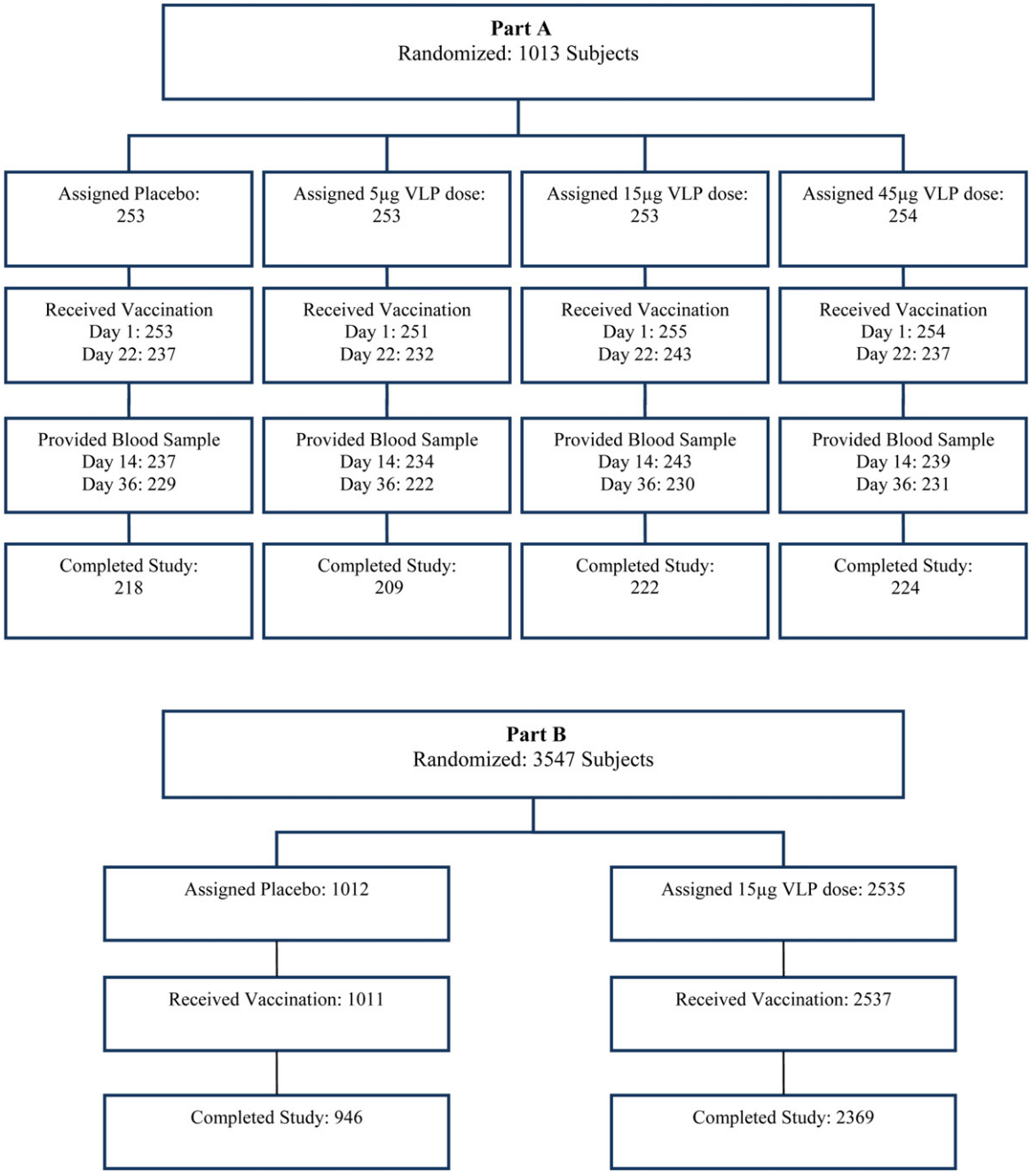

Fig. 1. Subject flow and disposition.

phosphate buffer formulation and filled into individual sterile vials for injection.

2.2. Study design

This Phase 2, randomized, double-blind, placebo-controlled study was designed to evaluate the safety, tolerability, and immunogenicity of 3 dose levels (5 μg, 15 μg, and 45 μg HA) of the 2009 pandemic A/California/04/2009 H1N1 influenza VLP vaccine as compared with a placebo, in healthy adults, aged 18–64 years. The study was conducted in two parts: Part A was designed to evaluate the safety and immunogenicity of the H1N1 influenza VLP vaccine over a dose range, and to select a dose for use in an expanded safety evaluation in Part B. In Part A, 1013 subjects were randomly assigned to one of four treatment groups, in an approximately 1:1:1:1 ratio (5 μg, 15 μg, and 45 μg VLP vaccine or placebo groups), to receive two intramuscular (IM) injections with the study vaccine, 21 days apart (Fig. 1). After the first 511 subjects were enrolled, enrollment was halted until a safety review by the Data and Safety Monitoring Board (DSMB) was conducted. The DSMB was composed of international experts in the fields of vaccinology, epidemiology, biostatistics and infectious diseases, and provided independent safety oversight during study conduct. As no significant safety concerns were identified during this review, enrollment continued until all 1013 subjects were enrolled into Part A. Based on an interim safety and immunogenicity analysis, an additional 3547 (2537 active and 1011 placebo) subjects were enrolled in Part B of the study and received a single injection on Day 1 with the placebo or 15 μg VLP (second lot) vaccine (Fig. 1).

Table 1
Demographic characteristics of subjects in Parts A and B.

Characteristic	Part A						Part B	
	Placebo (*N* = 253)	5 μg dose (*N* = 251)	15 μg dose (*N* = 255)	45 μg dose (*N* = 254)	All VLP (*N* = 760)	All Groups (*N* = 1013)	Placebo (*N* = 1011)	15 μg dose (*N* = 2537)
Age years								
Mean (SD)	34 (13)	36 (14)	35 (13)	35 (14)	35 (14)	35 (13)	35 (12)	35 (12)
Range	18–64	18–64	18–64	18–65[a]	18–65[a]	18–65[a]	18, 64	18, 65[a]
Gender *n* (%)								
Male	104 (41)	118 (47)	92 (36)	106 (42)	316 (42)	420 (42)	365 (36)	956 (38)
Female	149 (59)	133 (53)	163 (64)	148 (58)	444 (58)	593 (59)	646 (64)	1581 (62)

[a] One subject in the 45 μg HA group in Part A and two subjects in the 15 μg HA group in Part B were >64 years old at the time of enrollment.

2.3. Subjects and study procedures

Subjects were enrolled at a single site in Mexico City, between October 19, 2009 and March 5, 2010. Study approval was obtained from the Mexico Ministry of Health through the Federal Commission for Protection Against Sanitary Risk (COFEPRIS) and by The Mexican Social Security Institute (IMSS) through the National Commission of Scientific Research composed by Scientific, Ethics and Biosafety Committees in accordance with the International Conference on Harmonization (ICH) guidelines for Good Clinical Practices (GCP) and with the Mexico Emergency Use Regulatory Requirements. Written informed consent was obtained from each subject according to current GCP guidelines. The clinical research organization (CRO) ICON Clinical Research, monitored the study.

Eligible subjects were between 18 and 64 years of age, healthy, with no acute disease on the day of vaccination (defined as moderate or severe illness with or without fever $\geq$38 °C), and no immunization with any live vaccine within four weeks, or inactivated vaccine within two weeks of receiving VLP vaccination. Women of childbearing potential were required to have a negative pregnancy test at the time of screening and before each vaccination. All subjects provided signed Informed Consent prior to any study procedures being performed.

2.4. Safety assessments

All subjects were observed for 30 min after vaccination for reactogenicity and safety evaluation. In addition, safety assessments were performed at all in-clinic visits (i.e., on Days 14, 22, and 36 for Part A and on Day 22 for Part B) and telephone contact visits (i.e., monthly after the Day 36 visit through 6 months following the second vaccination [Day 202] for Part A; and on the Days 2/3 and 14 visits and monthly after the Day 22 visit through 6 months following vaccination [Day 181]) for review of AEs, serious AEs (SAEs), significant new medical conditions (SNMCs), and concomitant medications. Also in Part A, subjects maintained a symptom diary for seven days following each vaccination for daily recording of solicited events (SEs). The SEs measured included: body temperature, chills, muscle pain, joint pain, diarrhea, nausea, vomiting, headache, fatigue, cough, difficulty breathing, difficulty swallowing, hoarseness, chest tightness, sore throat, redness in eyes, and facial swelling for systemic reactions; and local pain, bruising, tenderness, redness and swelling for injection site reactions.

All AEs were coded according to the MedDRA adverse event dictionary (Version 12.1) [17] and graded for severity using a standard scale. Vaccine-emergent AEs were defined as starting or worsening after vaccine administration. Vaccine-related AEs were defined as those AEs possibly related to study vaccine.

2.5. Immunogenicity assessments

Immunogenicity assessments were only conducted in Part A, on sera collected at baseline (prior to immunization on Day 1), Day 14 and Day 36 (14 days after second vaccination) for HAI and microneutralization (MN) testing. The HAI assay was conducted on all Part A samples by Focus Diagnostics, Inc. using a validated assay with National Institute for Biological Standards and Control (NIBSC) procured cell culture grown A/California/07/2009 (H1N1) virus. The MN assay was conducted on samples derived from a subset of subjects by Southern Research Institute using A/California/07/2009 (H1N1) virus from the CDC.

2.6. Statistical methods

All subjects in the study were assigned to treatment according to a blinded randomization scheme generated by a statistician at ICON Clinical Research. For Part A, subjects were allocated to one of four treatment groups in a 1:1:1:1 ratio: 5 μg, 15 μg, or 45 μg novel A (H1N1) 2009 influenza VLP vaccine, or placebo. In Part B, subjects were allocated in a 2:5 ratio (placebo:active) to receive placebo or a 15 μg dose of the VLP vaccine, for a minimum inclusion of 3000 subjects overall (Parts A and B combined) in the H1N1 VLP vaccine safety cohort. Part A had a power of >80% to achieve lower bound criteria of 40% for seroconversion and 70% for seroprotection with a sample size of 250 subjects per group. For the entire VLP safety cohort of 3000 subjects minimum, and given an event rate of 0.33%, the probability of observing one AE was >99.99%.

For immunogenicity analyses, geometric mean titers (GMT), geometric mean ratio (GMR), and rates of seroconversion and seroprotection, were measured and described for each of the randomized treatment groups. An exploratory analysis to evaluate HAI immune responses in subjects categorized as seronegative (HAI titer < 1:10) and seropositive (HAI titer $\geq$ 1:10) based on pre-vaccination titers was also performed. GMTs with two-sided 95% confidence intervals (CI) for HAI and MN were calculated at each time point (Days 1, 14 and 36) using the back-transformed mean of the $\log_{10}$ transformed HAI or MN titer for all treatment groups. The GMR, defined as the back-calculated mean difference (post-vaccination–pre-vaccination) of $\log_{10}$ transformed HAI titers, was calculated at Day 14 and Day 36 for all treatment groups along with its two-sided 95% CI using a *t*-statistic.

Seroconversion rates at Days 14 and 36 and the percentage of subjects who achieved an HAI (seroprotection) or MN titer of $\geq$1:40 at Days 1, 14, and 36 with corresponding two-sided 95% CIs were calculated. Confidence intervals for the point estimates were obtained using the Clopper–Pearson's exact method. Seroconversion was defined as a pre-vaccination titer <1:10 and a post-vaccination titer $\geq$1:40 or a pre-vaccination titer $\geq$1:10 and a minimum of four-fold rise in post-vaccination HAI antibody titer.

For Parts A and B, analysis of all AEs experienced overall were summarized for each treatment group by incidence of subjects with one or more vaccine related AE, severe or life-threatening AE, SAE, unsolicited AE, and SE. The two-sided *p*-value was based on the Cochran–Armitage trend test in incidence with dose (as applicable). SEs were summarized by treatment group, vaccination,

Table 2
Overall summary of adverse events.

	Part A					Part B	
	Placebo ($N=253$)	5 μg dose ($N=251$)	15 μg dose ($N=255$)	45 μg dose ($N=254$)	All VLP ($N=760$)	Placebo ($N=1011$)	15 μg dose ($N=2537$)
Serious AE	0 (0.0)	4 (1.6) $p=0.139$	3 (1.2)	4 (1.6)	11 (1.4)	15 (1.5)	25 (1.0) $p=0.204$
Solicited AE	185 (73.1)	177 (70.5) $p=0.012$	205 (80.4)	203 (79.9)	585 (77.0)	N/A	N/A N/A
Unsolicited AE	142 (56.1)	139 (55.4) $p=0.649$	144 (56.5)	147 (57.9)	430 (56.6)	537 (53.1)	1415 (55.8) $p=0.151$
Vaccine related AE	46 (18.2)	50 (19.9) $p=0.848$	46 (18.0)	46 (18.1)	142 (18.7)	150 (14.8)	401 (15.8) $p=0.472$
Severe or life-threatening AE	12 (4.7)	9 (3.6) $p=0.567$	16 (6.3)	7 (2.8)	32 (4.2)	13 (1.3)	52 (2.0) $p=0.126$
Life-threatening AE	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	1 (0.1)	1 (0.1)	4 (0.2)

A subject is counted only once per category using the event with the highest severity.

incidence and severity in Part A using the total vaccinated cohort; the Cochran–Armitage trend test was used to evaluate differences across groups. A subject was counted only once per adverse event category; the event with the highest severity was used for subjects that experienced multiple events.

3. Results

3.1. Study subjects

A total of 1013 subjects were enrolled in Part A and 3547 in Part B at a single site in Mexico. Of the 1013 subjects randomized to treatment in Part A, 253 (100%) subjects were vaccinated with placebo, and 251(99%), 255 (<100%), and 254 (100%) subjects were vaccinated with the 5 μg, 15 μg, and 45 μg influenza VLP vaccine (respectively) on Day 1. Two subjects randomized to receive the 5 μg VLP dose were administered the 15 μg VLP dose in error; these subjects received the same treatment dose for second vaccination and were analyzed according to the treatment received (15 μg VLP). The second treatment dose was administered on Day 22 to 237 (94%), 232 (92%), 243 (96%), and 237 (93%) subjects in the placebo and the 5 μg, 15 μg, and 45 μg influenza VLP vaccine groups, respectively. There were 873 (86%) subjects who completed the study on Day 202. Of the 3547 subjects randomized to treatment in Part B, 1011 subjects received placebo (this total includes one subject who received two doses of placebo and is counted twice and another subject who received a dose of placebo and VLP vaccine) and 2537 subjects received the influenza VLP vaccine at a dose of 15 μg HA protein on Day 1. There were 3315 (94%) subjects who completed the study on Day 181 (Fig. 1).

The mean age of all subjects in Part A was 35.0 years, with 41.5% and 58.5% of subjects being male and female, respectively. In part B, the mean age of all subjects was 35.1 years, and 37.2% were male and 62.8% were female. Greater than 99% of all subjects in both parts were of Hispanic origin. Demographic characteristics of the VLP vaccine groups and the placebo groups were generally comparable and are provided in Table 1.

3.2. Safety

In Part A of the study, SEs (i.e., select injection site and systemic reactions) were recorded by subjects in a symptom diary for a 7-day period following each vaccination. As sufficient information on the SEs experienced by the 1013 subjects was available during Part A of the study, and it was assumed that the safety results would be comparable in Part B, a symptom diary was not used in Part B. Solicited events were reported by 185 (73.1%) subjects in the placebo group and 177 (70.5%), 205 (80.4%), and 203 (79.9%) subjects in the 5 μg, 15 μg, and 45 μg VLP vaccine dosage groups, respectively (Table 2). Results of the Cochran–Armitage trend test suggested a dose-related increasing incidence in SEs ($p=0.012$) as might be expected. This was primarily attributable to injection site events of local pain, swelling, tenderness, and redness, which were reported by more subjects in the higher VLP dose groups (15 μg and 45 μg) compared to the placebo and 5 μg VLP groups following both vaccinations (Fig. 2a). Overall, the majority of local AEs were mild in severity; however, moderate or greater local injection site reactions of pain, tenderness, redness and swelling also showed a dose-trend by the Cochran–Armitage test after both vaccinations (data not shown). There were no differences between placebo and vaccine recipients in systemic SE incidences (Fig. 2b); however a dose-trend in incidence of moderate to severe intensity was observed for cough (after vaccination one only) and muscle pain (after vaccination two only) (data not shown). Like the injection site events, systemic SEs overall were generally mild in severity.

The incidence of AEs other than SEs was comparable across treatment groups, with 142 (56.1%) subjects in the placebo group and 139 (55.4%), 144 (56.5%), and 147 (57.9%) subjects in the 5 μg, 15 μg and 45 μg VLP dosage groups (respectively) experiencing at least one AE through 202 days following the first vaccination (Table 2). AEs considered to be related to vaccine were reported in 46 (18.2%) subjects in the placebo group and 50 (19.9%), 46 (18.0%), and 46 (18.1%) subjects in the 5 μg, 15 μg and 45 μg VLP dosage groups, respectively. Across all groups, the majority of the events were of mild or moderate severity. A total of 31 (4.1%) VLP-treated subjects and 12 (4.7%) placebo-treated subjects reported at least one severe or life threatening AE (Table 2). For the VLP groups, the majority were severe events associated with gastrointestinal and nervous system disorders (incidence by system organ class [SOC] was 1.1% for both disorders) and were unrelated to the study vaccine. Eleven subjects experienced SAEs; none were considered related to the vaccine.

The incidence of AEs in Part B of the study was consistent with Part A and comparable between the VLP and placebo groups. A total of 537 (53.1%) subjects in the placebo group and 1415 (55.8%) subjects in VLP group experienced at least one AE. Vaccine-related AEs were reported in 150 (14.8%) subjects in the placebo group and 401 (15.8%) subjects in the VLP group (Table 2). The majority of the events in both treatment groups were of mild or moderate severity. A total of 52 (2.0%) VLP-treated subjects and 13 (1.3%) placebo-treated subjects reported at least one severe or life threatening AE. These events were primarily severe in intensity (i.e., only six were life-threatening), unrelated to the vaccine, and infrequently reported by both VLP and placebo recipients across the SOCs. Forty subjects experienced SAEs, none were considered related to vaccine.

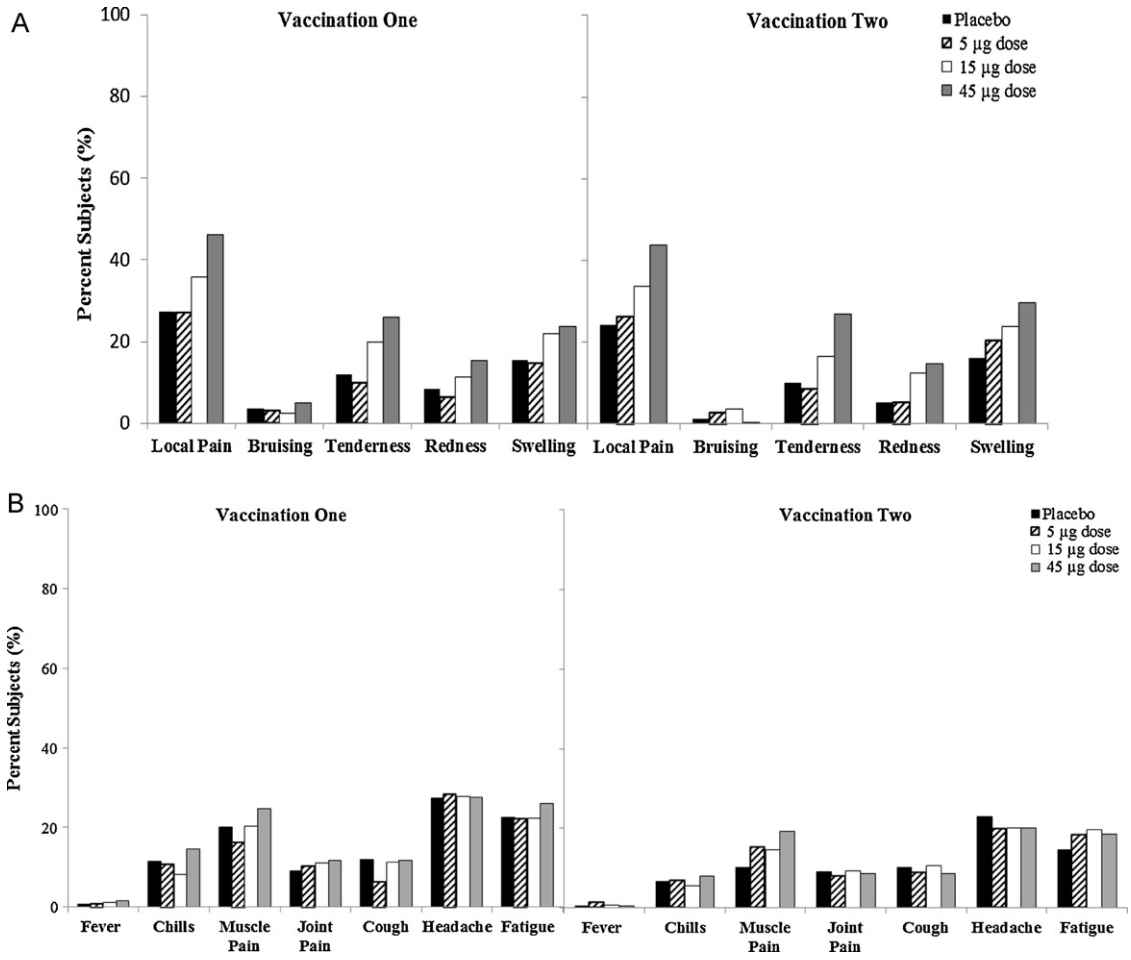

Fig. 2. Solicited adverse events. A total of 1013 subjects were enrolled and vaccinated in Part A and solicited AEs were collected through the first 7 days following each immunization. (A) Solicited local AE incidence after the first and second vaccinations. (B) Solicited systemic AE incidence after the first and second vaccinations. No significant differences in local or systemic AEs were seen between placebo and 5 μg, 15 μg or 45 μg at either the first or second immunization.

3.3. Immunogenicity

HAI responses were measured in 967 subjects (using the modified intent-to treat population [mITT] which included all subjects who received a full or partial dose of the study treatment, had pre- and post-vaccination immunogenicity data, and had protocol violations) enrolled in Part A of the study. Immunogenicity analysis results in the per-protocol (which included all mITT subjects with no protocol violations) and the mITT populations very similar.

Baseline (Day 1) HAI titers were similar across all groups (HAI GMT: 20–23). Over the course of the study where subjects were potentially exposed to the H1N1 virus, a slight increase in HAI GMTs in the placebo group was observed (HAI GMT of 20 at baseline compared to 24 and 25, at Day 14 and Day 36), but these changes were not statistically significant (95% CI responses overlapped). HAI responses in all VLP groups were significantly higher than placebo at all post baseline time points. A dose-dependent rise in Day 14 HAI responses was evident in comparisons between the 5 μg and 15 μg or 45 μg VLP vaccine groups, but the increase in HAI GMT between the 45 μg VLP group and the 15 μg VLP group was not statistically significant (Table 3). The second immunization showed no effect on HAI titers in the 5 μg and 15 μg VLP vaccine groups and resulted in only a slight increase in the 45 μg VLP group. Similarly, the HAI GMR indicated a dose–response relationship, with a 4.0, 6.6, and 8.7 mean increase at Day 14 in the 5 μg, 15 μg and 45 μg vaccine groups (respectively), and no additional increase (relative to the Day 14 result) at Day 36 (2 weeks after the second injection) except in the 45 μg VLP group (HAI GMR: 10.0).

Seroconversion rates at Day 14 ranged from 6% in the placebo group, to 48%, 65% and 75% (respectively) in the 5 μg, 15 μg and 45 μg vaccine groups (Table 3). The percentage of subjects with an HAI titer of ≥40 (seroprotection) ranged from 36% to 41% across all groups at baseline and reached 82%, 91% and 92% respectively for the 5 μg, 15 μg and 45 μg VLP vaccine groups at Day 14 (Table 3). No added benefit was seen for either seroconversion or seroprotection after a second dose.

As the trial was conducted after the second wave of the H1N1 pandemic, approximately 40% of the subjects had preexisting, measurable immunity by HAI, a finding in concert with other H1N1 pandemic influenza vaccine trials [18]. Thus, an exploratory analysis was performed on subjects who had no measurable HAI titers (i.e., HAI titer <1:10) at baseline (seronegative). Day 14 HAI seroprotection and seroconversion rates, which were identical in seronegative subjects, were 64%, 79% and 85% for the 5 μg, 15 μg and 45 μg vaccine dose groups, respectively. Unlike seropositive subjects, seronegative subjects had a trend towards higher GMTs at all dosage levels and following both vaccinations, suggesting that the seronegative population as a whole was primed with the first vaccine dose and boosted by the second (Table 3). The high rates of seroconversion and seroprotection achieved in the seronegative population after one injection further suggest that the VLP vaccine was highly immunogenic. The microneutralization assay, which demonstrated higher GMTs overall in the subset of sub-

Table 3
Immune responses as measured by the hemagglutination inhibition assay (HAI).

Parameter	Placebo (N = 242)			5 μg dose (N = 237)			15 μg dose (N = 244)			45 μg dose (n = 244)		
Population	D1	D14	D36	D1	D14	D36	D1	D14	D36	D1	D14	D36
GMT (95% CI)												
Total	20 (17–23)	24 (20–28)	25 (21–29)	23 (19–26)	88 (75–103)	85 (73–99)	21 (18–25)	141 (121–164)	138 (119–160)	20 (18–24)	174 (147–204)	209 (180–243)
Seronegative	5 (5–5)	8 (7–10)	12 (10–15)	5 (5–5)	48 (34–68)	53 (40–72)	5 (5–5)	88 (66–116)	98 (77–126)	5 (5–5)	85 (63–114)	120 (91–159)
Seropositive	32 (28–38)	34 (29–40)	33 (27–39)	37 (32–42)	107 (90–127)	98 (82–117)	37 (32–43)	168 (142–200)	158 (131–189)	37 (32–42)	235 (195–282)	264 (223–312)
Seroprotection (≥1:40) (95% CI)												
Total	36 (30–42)	40 (34–47)	43 (36–50)	41 (35–48)	82 (76–86)	81 (75–86)	39 (33–45)	91 (86–94)	93 (89–96)	40 (34–46)	92 (87–95)	96 (93–98)
Seronegative	0 (0–6)	13 (6–25)	15 (7–27)	0 (0–6)	64 (50–76)	70 (56–82)	0 (0–5)	79 (67–88)	92 (83–98)	0 (0–5)	85 (74–92)	94 (86–98)
Seropositive	48 (41–56)	49 (42–57)	53 (45–60)	55 (47–62)	88 (82–92)	85 (78–90)	54 (47–62)	95 (90–98)	93 (88–97)	56 (49–64)	95 (90–98)	97 (93–99)
Seroconversion (95% CI)												
Total	NA	6 (3–10)	7 (4–11)	NA	48 (42–55)	48 (42–55)	NA	65 (59–71)	64 (58–71)	NA	75 (69–80)	80 (74–85)
Seronegative	NA	12 (5–23)	7 (2–16)	NA	64 (50–76)	59 (44–72)	NA	79 (67–88)	85 (74–92)	NA	85 (74–92)	87 (76–94)
Seropositive	NA	4 (2–8)	7 (3–11)	NA	43 (36–51)	45 (38–53)	NA	60 (52–67)	56 (48–64)	NA	71 (63–78)	77 (69–83)
GMR (95% CI)												
Total	NA	1.2 (1–1.3)	1.1 (1–1.2)	NA	4.0 (3.4–4.7)	3.7 (3.12–4.4)	NA	6.6 (5.6–7.8)	6.2 (5.2–7.4)	NA	8.7 (7.2–10.4)	10.0 (8.5–11.9)
Seronegative	NA	1.6 (1.3–1.9)	1.4 (1.1–1.8)	NA	9.6 (6.8–13.6)	5.7 (4.2–7.8)	NA	17.5 (13.2–23.3)	12.2 (9.1–16.3)	NA	16.9 (12.6–22.7)	15.5 (11.4–21.1)
Seropositive	NA	1.0 (0.9–1.2)	1.0 (0.9–1.2)	NA	3.0 (2.5–3.5)	3.2 (2.6–3.9)	NA	4.5 (3.9–5.5)	4.8 (3.9–5.8)	NA	6.5 (5.3–8.1)	8.4 (6.9–10.2)

Fig. 3. Reverse cumulative distribution of HAI responses by treatment group percent of part A subjects with HAI responses on Day 14 following a single immunization with placebo (●), or a 5 μg (○), 15 μg (△), or 45 μg (□) dose of the VLP vaccine.

jects evaluated, essentially confirmed HAI findings, although the dose–response differences were not significant (Table 4).

A reverse cumulative distribution of HAI responses in part A was performed to display the range of individual responses after a single dose (Fig. 3). The dose–response relationship between the VLP doses is evident, with the greatest differences seen between the low (5 μg) and the two higher (15 μg and 45 μg) VLP doses. Additionally, it is also clear that a significant number of subjects in the placebo group had HAI antibodies.

4. Discussion

In the current clinical study, an insect cell-derived H1N1 (A/California/04/2009) pandemic influenza vaccine candidate proved to be both immunogenic and well-tolerated in healthy adults in the midst of the pandemic in Mexico. Comparable safety and immunogenicity results in adults administered a single injection have been published for this strain with inactivated subvirion vaccines [19,20]. The current study population had a relatively high rate of preexisting immunity (HAI titer ≥40 rate: 36–41%) for a novel pandemic strain. Baseline H1N1 pandemic strain seroprotection rates of 30–33% was reported in the Australian population [19]. Consistent with other studies, we found no measurable cross reactivity with other pandemic strains in our study (data not shown) suggesting strain specific immunity. As the sera sampling occurred at the end of the second wave in Mexico, the data suggests that this novel H1N1 pandemic strain exposure established a relatively high rate of subjects with high HAI titers in the general population in a short period of time. The strain specificity provided an opportunity to evaluate the VLP vaccine immunogenicity in a relatively naïve population. A sub-analysis was performed to evaluate the immunogenicity of the vaccine in the seronegative population (no measurable HAI titers to H1N1 at baseline), as an indicator of the VLP vaccine's immunogenicity. As shown in Table 3, responses in this population were robust as indicated by a 79% seroprotection rate achieved after a single 15 μg dose.

Extensive deployment of licensed vaccines during the 2009 H1N1 pandemic occurred and the immunogenicity results were also robust [21–27]. The immunogenicity appeared to translate into clinically significant levels of vaccine efficacy, especially in the young. In a very large study, Chinese school children immunized with a single 15 μg dose of a monovalent split-virion vaccine resulted in an estimated vaccine effectiveness of 87.3% (95% confidence interval, 75.4–93.4) [20]. A large multicenter case–control study, based on medical practice-based active surveillance for medically attended culture-confirmed H1N1 pandemic influenza-like illness (ILI) in multiple European countries, in all ages using multi-

Table 4
Immune response as measured by the microneutralization assay (MN).

Parameter	Placebo (n = 29)			5 μg dose (n = 31)			15 μg dose (n = 31)			45 μg dose (n = 29)		
	D1	D14	D36	D1	D14	D36	D1	D14	D36	D1	D14	D36
GMT (95% CI)	15 (9–25)	14 (9–24)	22 (11–44)	15 (9–26)	104 (62–173)	142 (87–233)	18 (10–30)	293 (164–522)	309 (193–496)	12 (8–18)	261 (141–483)	388 (221–682)
%Seroconversion (95% CI)	NA	0 (0–12)	4 (0–19)	NA	58 (39–76)	72 (51–88)	NA	74 (55–88)	88 (69–96)	NA	83 (64–94)	100 (87–100)
%Subjects ≥1:40 (95% CI)	23 (10–42)	24 (10–44)	30 (14–50)	32 (17–51)	84 (66–95)	88 (69–98)	29 (14–48)	90 (74–98)	97 (83–100)	24 (10–44)	90 (73–98)	100 (87–100)

ple vaccines, estimated vaccine efficacy at 71.9% (95% CI 45.6–85.5) [28]. Adjuvanted vaccines achieved up to 100% vaccine efficacy in children [29], but were accompanied by high rates of fever and local side effects. Additionally, in some studies the data indicated that HAI titers correlated with protection [19,30].

The announcement of the pandemic in Mexico created a public health crisis and initiated widespread efforts to manufacture appropriate vaccine. The licensed egg-based vaccines were effective, but deficiencies in vaccine availability due to limitations of this manufacturing technique hampered efforts to have a major impact on the disease incidence [1]. Although the insect cell produced VLP vaccine is in development, a manufactured lot of insect cell recombinant VLP influenza H1N1 vaccine was complete 12 weeks after release of the relevant sequence by the CDC. The data presented in this study indicate that the vaccine is highly immunogenic, even in the seronegative population, and was well-tolerated in a large and placebo-controlled study. The expectation by public health authorities that a transmissible, highly pathogenic pandemic virus will emerge remains a threat, and delivery of effective and rapidly available vaccines continue to be an important development priority. Results of this study suggests that the insect cell derived VLP should be further developed as a candidate influenza vaccine [21]. In support of this objective, additional clinical trials are in development to evaluate influenza vaccine candidate antigens manufactured using Sf9/baculovirus VLP technology.

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References

[1] (PCAST) PsCoAoSaT. Plan to improve Nation's vaccine response against pandemic influenza and other outbreaks. In: Policy OoSaT, Washington, DC, 2010.
[2] Pushko P, Tumpey TM, Bu F, Knell J, Robinson R, Smith G. Influenza virus-like particles comprised of the HA NA, and M1 proteins of H9N2 influenza virus induce protective immune responses in BALB/c mice. Vaccine 2005;23(50):5751–9.
[3] Safdar A, Cox MM. Baculovirus-expressed influenza vaccine. A novel technology for safe and expeditious vaccine production for human use. Expert Opin Invest Drugs 2007;16(July (7)):927–34.
[4] Pushko P, Tumpey TM, Van Hoeven N, Belser JA, Robinson R, Nathan M, et al. Evaluation of influenza virus-like particles and Novasome adjuvant as candidate vaccine for avian influenza. Vaccine 2007;25(21):4283–90.
[5] Bright RA, Carter DM, Crevar CJ, Toapanta FR, Steckbeck JD, Cole KS, et al. Cross-clade protective immune responses to influenza viruses with H5N1 HA and NA elicited by an influenza virus-like particle. PLoS One 2008;3(1):e1501.
[6] Mahmood K, Bright RA, Mytle N, Carter DM, Crevar CJ, Achenbach JE, et al. H5N1 VLP vaccine induced protection in ferrets against lethal challenge with highly pathogenic H5N1 influenza viruses. Vaccine 2008;26(42):5393–9.
[7] Perrone LA, Ahmad A, Veguilla V, Lu X, Smith G, Katz JM, et al. Intranasal vaccination with 1918 influenza virus-like particles protects mice and ferrets from lethal 1918 and H5N1 influenza virus challenge. J Virol 2009;83(11):5726–34.
[8] Pushko P, Kort T, Nathan M, Pearce MB, Smith G, Tumpey TM. Recombinant H1N1 virus-like particle vaccine elicits protective immunity in ferrets against the 2009 pandemic H1N1 influenza virus. Vaccine 2010;28(30):4771–6.
[9] Liu YV, Massare MJ, Barnard DL, Kort T, Nathan M, Wang L, Smith G. Chimeric severe acute respiratory syndrome coronavirus (SARS-CoV) S glyoprotein and influenza matrix 1 effciently form virus-like particles (VLPs) that protect mice against challenge with SARS-CoV. Vaccine 2011, doi:10.1016/j.vaccine.2011.06.111. Jul 14 [Epub ahead of print].
[10] Raghunandan R, Massare M, Smith G, Connolly K. Safety and efficacy of a novel RSV F-particle vaccine candidate in two preclinical RSV challenge models.

In: 50th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). 2010.
[11] Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet 2004;364(9447):1757–65.
[12] Cohen J, Enserink M. Swine flu. After delays WHO agrees: the 2009 pandemic has begun. Science 2009;324(5934):1496–7.
[13] Use of influenza A (H1N1) 2009 monovalent vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. MMWR Recomm Rep 2009; 58(RR-10):1–8.
[14] Girard MP, Katz J, Pervikov Y, Palkonyay L, Kieny MP. Report of the 6th meeting on the evaluation of pandemic influenza vaccines in clinical trials World Health Organization, Geneva Switzerland, 17–18 February 2010. Vaccine 2010;28(42):6811–20.
[15] Wu J, Xu F, Lu L, Lu M, Miao L, Gao T, et al. Safety and effectiveness of a 2009 H1N1 vaccine in Beijing. N Engl J Med 2010;363(25):2416–23.
[16] Boletín Epidemiología; 2010; Available from: http://www.dgepi.salud.gob.mx/boletin/boletin.htm. [cited 2011 19-04-11].
[17] MedDRA® Term Selection, Version 12.1. Application to adverse drugs reactions/adverse events & medical and social history & indications. Geneva: ICH; 2009.
[18] Carrat F, Pelat C, Levy-Bruhl D, Bonmarin I, Lapidus N. Planning for the next influenza H1N1 season: a modelling study. BMC Infect Dis 2010;10:301.
[19] Plennevaux E, Sheldon E, Blatter M, Reeves-Hoche MK, Denis M. Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomised controlled phase 2 trials. Lancet 2010;375(9708):41–8.
[20] Girard MP, Tam JS, Assossou OM, Kieny MP. The 2009 A (H1N1) influenza virus pandemic: a review. Vaccine 2010;28(31):4895–902.
[21] Greenberg ME, Lai MH, Hartel GF, Wichems CH, Gittleson C, Bennet J, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. N Engl J Med 2009;361(25):2405–13.
[22] Liang XF, Wang HQ, Wang JZ, Fang HH, Wu J, Zhu FC, et al. Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines in China: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet 2010;375(9708):56–66.
[23] Zhu FC, Wang H, Fang HH, Yang JG, Lin XJ, Liang XF, et al. A novel influenza A (H1N1) vaccine in various age groups. N Engl J Med 2009;361(25):2414–23.
[24] Nolan T, McVernon J, Skeljo M, Richmond P, Wadia U, Lambert S, et al. Immunogenicity of a monovalent 2009 influenza A(H1N1) vaccine in infants and children: a randomized trial. JAMA 2010;303(1):37–46.
[25] Waddington CS, Walker WT, Oeser C, Reiner A, John T, Wilkins S, et al. Safety and immunogenicity of AS03B adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. BMJ 2010;340:c2649.
[26] Lu CY, Shao PL, Chang LY, Huang YC, Chiu CH, Hsieh YC, et al. Immunogenicity and safety of a monovalent vaccine for the 2009 pandemic influenza virus A (H1N1) in children and adolescents. Vaccine 2010;28(36):5864–70.
[27] Kao TM, Hsieh SM, Kung HC, Lee YC, Huang KC, Huang LM, et al. Immune response of single dose vaccination against 2009 pandemic influenza A (H1N1) in the Taiwanese elderly. Vaccine 2010;28(38):6159–63.
[28] Valenciano M, Kissling E, Cohen JM, Oroszi B, Barret AS, Rizzo C, et al. Estimates of pandemic influenza vaccine effectiveness in Europe, 2009–2010: results of influenza monitoring vaccine effectiveness in Europe (I-MOVE) multicentre case–control study. PLoS Med 2011;8(1):e1000388.
[29] Van Buynder PG, Carcione D, Rettura V, Daly A, Woods E. Marketing paediatric influenza vaccination: results of a major metropolitan trial. Influenza Other Respir Viruses 2011;5(1):33–8.
[30] Andrews N, Waight P, Yung CF, Miller E. Age-specific effectiveness of an oil-in-water adjuvanted pandemic (H1N1) 2009 vaccine against confirmed infection in high risk groups in England. J Infect Dis 2011;203(1):32–9.