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## Safety and immunogenicity of a plant-produced recombinant monomer hemagglutinin-based influenza vaccine derived from influenza A (H1N1)pdm09 virus: A Phase 1 dose-escalation study in healthy adults,★★

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### Abstract

**Background:** Novel influenza viruses continue to pose a potential pandemic threat worldwide.

In recent years, plants have been used to produce recombinant proteins, including subunit vaccines. A subunit influenza vaccine, HAC1, based on recombinant hemagglutinin from the 2009 pandemic A/California/04/2009 (H1N1) strain of influenza virus, has been manufactured using a plant virus-based transient expression technology in *Nicotiana benthamiana* plants and demonstrated to be immunogenic and safe in pre-clinical studies (Shoji et al., 2011).

**Methods:** A first-in-human, Phase 1, single-center, randomized, placebo-controlled, single-blind, dose escalation study was conducted to investigate safety, reactogenicity and immunogenicity of an HAC1 formulation at three escalating dose levels (15µg, 45µg and 90µg) with and without Alhydrogel®, in healthy adults 18–50 years of age (inclusive). Eighty participants were randomized into six study vaccine groups, a saline placebo group and an approved monovalent H1N1 vaccine group. Recipients received two doses of vaccine or placebo (except for the monovalent H1N1 vaccine cohort, which received a single dose of vaccine, later followed by a dose of placebo).

**Results:** The experimental vaccine was safe and well tolerated, and comparable to placebo and the approved monovalent H1N1 vaccine. Pain and tenderness at the injection site were the only local solicited reactions reported following vaccinations. Nearly all adverse events were mild to moderate in severity. The HAC1 vaccine was also immunogenic, with the highest seroconversion

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rates, based on serum hemagglutination-inhibition and virus microneutralization antibody titers, in the 90 g non-adjuvanted HAC1 vaccine group after the second vaccine dose (78% and 100%, respectively).

**Conclusions:** This is the first study demonstrating the safety and immunogenicity of a plant-produced subunit H1N1 influenza vaccine in healthy adults. The results support further clinical investigation of the HAC1 vaccine as well as demonstrate the feasibility of the plant-based technology for vaccine antigen production.

## Keywords

Influenza A; H1N1; Recombinant vaccine; Hemagglutinin; Plant-produced

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## 1. Introduction

Influenza is a serious respiratory disease caused by influenza viruses. During annual epidemics, three to five million cases of severe influenza illness are recorded worldwide, resulting in 250,000–500,000 deaths [1]. In addition to seasonal influenza outbreaks, occasional influenza pandemics can arise at any time when influenza A virus containing a novel hemagglutinin (HA) subtype is introduced and spreads efficiently among humans. In 2009, a novel, swine-origin influenza A H1N1 virus [A(H1N1)pdm09] emerged and infected humans. The virus, a triple reassortant with genes acquired from swine, avian and human influenza viruses [2], was first detected in people in the U.S. in April 2009 [3] and spread rapidly across the globe, primarily in children and younger adult populations with little pre-existing serologic immunity to the novel HA [4], causing the World Health Organization to declare a pandemic on June 11, 2009 [5].

Currently licensed influenza vaccines are made in embryonated eggs [6]. For the past four decades, these vaccines have been successfully used and proven to be safe and effective [7–9]. Recently, safety and immunogenicity of several 2009 H1N1 influenza A vaccines in healthy volunteers have been demonstrated [10–13]. However, the 2009 H1N1 pandemic demonstrated that egg-based technologies fall short of satisfying the global need for an emerging pandemic influenza vaccine in a timely manner. Therefore, a number of countries, including the U.S., are developing alternative economic and scalable platforms for production of large amounts of safe and effective recombinant, or subunit, vaccines in a short time frame. Some of these alternative manufacturing approaches are based on mammalian and insect cell expression systems [14–17]. Over the past ten years, plants have emerged as a highly promising approach to economically manufacture subunit vaccines and therapeutic proteins [18–22]. The immunogenicity and protective efficacy of plant-produced vaccine candidates against a variety of pathogens has been demonstrated in numerous pre-clinical studies [21,23–28]. More recently, safety and biological relevance of plant-produced therapeutic proteins and subunit vaccines, including H1N1 and H5N1 influenza vaccines, have been demonstrated in humans in Phase 1–3 clinical trials [29–33].

Fraunhofer USA Center for Molecular Biotechnology (FhCMB) has developed a transient expression system using *Nicotiana benthamiana* plants agroinfiltrated with a plant virus-based ‘launch’ vector encoding target sequence [34] and demonstrated its utility for

producing vaccine antigens [23–27]. A recombinant HA influenza vaccine, HAC1, based on the A/California/04/2009 (H1N1) strain, for the prevention of disease caused by a novel A(H1N1)pdm09 virus, was developed and produced in *N. benthamiana* at pilot plant scale under current Good Manufacturing Practice (cGMP) guidelines [35]. Pre-clinical studies demonstrated safety and protective immunogenicity of HAC1 in animals [35] and prompted further investigation of this vaccine candidate in humans. A Phase 1 study was conducted to determine the safety, reactogenicity and immunogenicity of HAC1 delivered intramuscularly at three escalating dose levels in healthy adults.

## 2. Materials and methods

### 2.1 Study design

This study was a first-in-human, Phase 1, single-center, randomized, placebo-controlled, single-blind, dose-escalation clinical study conducted at the Walter Reed Army Institute of Research (WRAIR) in Silver Spring, Maryland. The protocol was approved by the Human Use Review Committee of the WRAIR and by the U.S. Army Medical Research and Materiel Command's Human Subjects Research Board, Fort Detrick, Maryland. The study was conducted in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice (as defined by the International Conference on Harmonization) and federal regulations. All participants provided written informed consent prior to screening and enrollment into the study.

The primary objective was to evaluate the safety, reactogenicity and tolerability of an HAC1 vaccine formulation delivered unadjuvanted or adjuvanted with Alhydrogel® intramuscularly at doses of 15 µg, 45 µg or 90 µg in a two-dose regimen delivered 21 days apart. The secondary objective was to evaluate and compare immunogenicity of two injections of this formulation with a single dose of a licensed monovalent H1N1 vaccine by measuring hemagglutination-inhibition (HAI) and virus microneutralization (MN) antibody titers.

This study was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) under reference identifier NCT01177202.

### 2.2 Vaccine

The HAC1 vaccine, developed by FhCMB, is a formulated recombinant HA monomer based on A/California/04/2009 (H1N1) influenza virus containing a poly-histidine (6 × His) affinity purification tag and the endoplasmic reticulum retention signal, KDEL, at the C-terminus. The HA antigen was cloned, expressed in *N. benthamiana*, purified and characterized as reported previously [35]. The concentration of HAC1 was 360 µg/mL in an aqueous formulation of normal saline with trace amounts of PBS (45 mM NaCl, 0.9 mM KCl, 3 mM Na<sub>2</sub>HPO<sub>4</sub> and 0.6 mM KH<sub>2</sub>PO<sub>4</sub>) for intramuscular administration.

The active control was a licensed Influenza A (H1N1) 2009 monovalent inactivated influenza virus vaccine that was administered per prescribing information as a single 0.5 mL intramuscular dose. No adjuvant was used with this licensed vaccine.

### 2.3 Study population and treatment

Healthy, non-pregnant adults 18–50 years of age (inclusive) were eligible for enrollment if they had a screening H1N1 HAI titer  $\geq 40$ , no prior vaccination with 2010–2011 seasonal influenza vaccine containing A/California/04/2009-like virus, no medical condition that may be associated with impaired immune responsiveness, including diabetes mellitus, and no cancer or treatment for cancer within the previous three years, excluding basal cell carcinoma or squamous cell carcinoma. Subjects were excluded if they did not meet any of these criteria or had a history of anaphylactic type reaction to injected vaccines, positive serology for HIV-1, HIV-2, HBsAg or HCV antibodies or any acute or chronic pulmonary, cardiovascular, hepatic, neurologic or renal disease that might confound evaluation of the vaccine, or had recently taken or planned to take any other experimental vaccine within 30 days prior to vaccination.

A total of 80 subjects were enrolled and randomized into 8 groups of 10 subjects, to receive two doses of the vaccine formulation, placebo, or active control (an FDA-approved monovalent H1N1 vaccine). Subjects in the study vaccine groups received two doses of HAC1 at 15 $\mu$ g, 45 $\mu$ g or 90  $\mu$ g, administered either nonadjuvanted (Group A [15  $\mu$ g], Group C [45  $\mu$ g] and Group E [90  $\mu$ g]) or with 0.3% aluminum hydroxide (Alhydrogel®) adjuvant (Brenntag Biosector, Denmark) (Group B [15  $\mu$ g], Group D [45  $\mu$ g] and Group F [90  $\mu$ g]). The placebo control group received two doses of saline (0.9% Sodium Chloride, USP, Hospira, Lake Forrest, IL). The subjects in the active control group received an approved monovalent H1N1 vaccine on Day 0 and saline on Day 21 (the approved vaccine had a single-dose vaccine regimen for study subjects). All doses were administered in a volume of 0.5 mL. Subjects were blinded as to the group to which they were assigned. Vaccinations were administered in the deltoid muscle of the same non-dominant arm at Day 0 and Day 21. Dose escalation was staggered by at least 7 days to assess adverse events (AEs) before proceeding to higher doses.

### 2.4 Safety assessments

Safety was assessed by recording medical history, monitoring AEs and vital signs, and performing physical examinations and laboratory tests for chemistry and hematology. The safety endpoint was the proportion of subjects in each treatment group reporting one or more local or systemic solicited and unsolicited reactions within 7 days after vaccination (Days 0–6 and 21–27), and the occurrence of any serious AEs (SAEs), defined as any untoward events resulting in hospitalization or death, during the study. Clinical safety laboratory assessments were performed at baseline, day of vaccination, and 7 days after each vaccination. The presence of solicited local and general signs and symptoms, including measurement of oral temperature, were assessed after each vaccination and at 1, 2, 3, 7 and 14 days post-vaccination. The solicited injection site AEs were pain, tenderness, erythema, and induration. Solicited general AEs were fever, fatigue, headache, malaise, myalgia, nausea, and vomiting. Also, the investigators recorded any other AEs occurring on the first administration of the vaccine through Day 56 as unsolicited AEs. AEs were assessed for intensity. Injection site pain was graded as 1 = mild pain not interfering with function, 2 = moderate pain; repeated use of non-narcotic pain reliever  $>24$  h or interferes with activity, 3 = severe pain; any use of narcotic pain reliever or prevents normal activity,

and 4 = hospitalization. Solicited systemic AEs were graded as 1 = no interference with normal activity, 2 = some interference with normal activity, 3 = significant; prevents daily activity, and 4 = hospitalization. Additional grading scales were applied to visible swelling or redness at the injection site: 1 = 2.5–5 cm, 2 = 5.1–10 cm, 3 = >10 cm, and 4 = necrosis or exfoliative dermatitis and to oral temperature: 1 = 37–38.4°C, 2 = 38.5–38.9°C, 3 = 39–40°C, and 4 = >40°C. Unsolicited AEs were graded as 1 = easily tolerated with no interference with everyday activities, 2 = sufficiently discomforting to interfere with everyday activities, 3 = preventing everyday activities, and 4 = ER visit or hospitalization required. Vaccine-related AEs were those that the investigator judged as having a reasonable possibility that the vaccine contributed to the AE.

## 2.5 Immunogenicity assessments

Sera for immunogenicity assessments were collected on Day 0 (pre-vaccination), Day 21 (pre-vaccination) and Day 56, and sent to the Influenza Division, CDC for analysis by HAI and MN assays using previously described methods and an A/California/04/2009-like virus [36]. Immunogenicity assessments included determination of (1) geometric mean titer (GMT) of HAI and MN antibody titers; (2) the proportion of subjects in each group who seroconverted on Days 21 and 56; and (3) the proportion of subjects in each group who achieved an HAI titer of  $\geq 40$  or an MN titer of  $\geq 80$  or  $\geq 160$  on Days 21 and 56. Seroconversion was defined for HAI and MN titers according to the FDA guidance as the percentage of subjects with either a pre-vaccination titer of  $<10$  and a post-vaccination titer of  $\geq 40$  or a pre-vaccination titer of  $>10$  and a minimum 4-fold rise in a post-vaccination antibody titer.

## 2.6 Statistical analysis

Statistical analyses were performed by Statistics Collaborative, Inc. (SCI). The safety population included all subjects who received at least one dose of HAC1. Only subjects who received both doses of HAC1 were included in the immunogenicity analyses.

For clinical laboratory tests, shift and grade summaries were based on the FDA's guidelines [37]. All AEs were coded for intensity and vaccine-relatedness and summarized by MedDRA System Organ Class. Incidence of any AE, any solicited AE (overall, local and systemic) and any unsolicited AE in each HAC1 vaccine group were compared against the recipients of the approved H1N1 vaccine, as well as against the recipients of placebo control. For analyses of AEs, Fisher's exact test was used to compare each investigational vaccine group to the placebo control group and to the active control group.

Standard statistical analyses were used to distinguish significant changes in antibody titers. For each post-vaccination assessment, natural log-transformed HAI and MN titers between dose groups and between adjuvanted and non-adjuvanted groups were compared using the analysis of variance (ANOVA) method. The proportion of subjects who seroconverted and/or had an HAI titer achievement of  $\geq 40$  was determined using Fisher's exact test. The relationship between the percentages of subjects with each endpoint and dose group was determined using the Cochran–Armitage trend test. No adjustment of *P*-values was made for multiple analyses.

### 3. Results

#### 3.1 Study population

Eighty subjects were enrolled, randomized and received the first scheduled vaccination; 78 subjects received both vaccinations (two doses of the vaccine formulation, two doses of placebo, or one dose of the FDA-approved monovalent H1N1 vaccine and one dose of saline) and were included in the immunogenicity analysis (Fig. 1). The patient demographic characteristics are summarized in Table 1.

#### 3.2 Safety and reactogenicity

Vaccinations were safe and well tolerated in all groups. No deaths, SAEs, clinically significant laboratory abnormalities, or AEs of special interest were reported during the trial. No subject withdrew due to an adverse event.

Pain and/or tenderness at the injection site were the only local solicited AEs noted, being reported in 37 subjects (46%) (Tables 2 and 3). All such AEs were considered vaccine-related, and all but one were Grade 1 intensity, with a single subject in Group D (45 µg + adjuvant) reporting a Grade 2 reaction (Table 2). The occurrence of local solicited AEs increased with increasing dose levels, in both adjuvanted and non-adjuvanted groups, as well as with the addition of Alhydrogel® (Table 2).

Systemic solicited AEs occurred in 33 subjects (41%), evenly distributed across study groups (Table 2). The most common systemic solicited AE was headache (23 subjects; 29%), which occurred in all study groups with similar incidence ranging from 30 to 50%, except for Group E (90 µg) where no headaches were reported (Table 3). Only four of these headache AEs (5%) were considered vaccine related.

Unsolicited AEs were more common in all study groups, with 66 subjects (83%) experiencing at least one unsolicited AE (Table 2). Unsolicited AEs had a larger range of intensity, with 15 subjects (19%) experiencing at least one Grade 2 AE, and one subject (1%) experiencing a Grade 3 AE (Table 2). Four of the unsolicited AEs (5%) were considered vaccine related and all four of these were Grade 1 intensity. The occurrence of unsolicited AEs was similar across all groups following each vaccination.

#### 3.3. Immunogenicity

All vaccine cohorts demonstrated and maintained HAI and MN titers above the placebo cohort throughout Day 56 (Tables 4 and 5). The HAI titers were highest in Group H (H1N1 active control group) on Day 21 (GMT: 101.5; 95% confidence interval [CI]: 26.2, 393.1) and Day 56 (GMT: 75.4; 95% CI: 19.4, 292.9), followed by Group E (90 µg; GMT: 73.9; 95% CI: 36.5, 149.5), Group D (45 µg + adjuvant; GMT: 53.4; 95% CI: 182, 156.3) and Group F (90 µg + adjuvant; GMT: 36.4; 95% CI: 11.1, 119.2) on Day 56 (Table 4). The MN titers were highest in Group E (90 µg) on Day 56 (GMT: 436.6; 95% CI: 196.4, 970.7), followed by Group H (H1N1 active control group) on Day 21 (GMT: 342.5; 95% CI: 82.9, 1414.2), Group D (45 µg + adjuvant; GMT: 307.2; 95% CI: 123.3, 765.2) on Day 56, Group

H (H1N1 active control group; GMT: 186.5; 95% CI: 44.9, 775.1) on Day 56, and Group F (90 µg + adjuvant; GMT: 124.5; 95% CI: 36.5, 424.9) on Day 56 (Table 5).

HAI seroconversion rates (and the proportions of subjects achieving HAI titers of  $\geq 40$ ) among the non-adjuvanted HAC1 vaccinated cohorts increased with dose escalation, ranging from 20% in Group A (15 µg) to 50% in Group E (90 µg) on Day 21 and from 30% in Group A (15 µg) to 78% in Group E (90 µg) on Day 56, indicative of a dose response (Table 4). Seroconversion rates for MN titers in non-adjuvanted vaccine cohorts also increased with dose escalation ranging from 50% in Group A (15 µg) to 80% in Group E (90 µg) on Day 21 and from 60% in Group A (15 µg) to 100% in Group E (90 µg) on Day 56 (Table 5). A dose response was also observed with the proportions of subjects achieving MN antibody titers of  $\geq 80$  and  $\geq 160$  (Table 5). Analysis of both HAI and MN seroconversion rates in the groups receiving adjuvanted HAC1 demonstrated variable results (Tables 4 and 5). No subject in the placebo control group seroconverted; in contrast, 7 (70%) subjects seroconverted on Day 21 in the H1N1 active control group (Tables 4 and 5).

In all study vaccine groups, MN seroconversion rates were significantly different compared to the placebo control group on both Days 21 ( $p < 0.05$  for Groups A–C,  $p = 0.003$  for Groups D–F) and 56 ( $p = 0.011$  for Groups A and B,  $p = 0.003$  for Groups C–F). Seroconversion rates for HAI were statistically different ( $p = 0.033$ ) compared to placebo control in Group D (45 µg + adjuvant), Group E (90 µg) and Group F (90 µg + adjuvant) on Day 21 and in all groups except Group A (15 µg) on Day 56.

#### 4. Discussion

This first-in-human, Phase 1 clinical study assessed safety and immunogenicity of HAC1, a plant-produced recombinant HA influenza vaccine candidate developed by FhCMB for the prevention of disease caused by a novel A (H1N1)pdm09 virus. FhCMB engineered and produced HAC1 in less than one month and subsequently scaled it up for cGMP manufacturing in FhCMB's pilot facility [35].

At the doses tested in this Phase 1 study, the vaccine was generally shown to be safe in healthy volunteers, with no reported SAEs and no evidence of any dose-limiting or dose-related toxicity. As expected, the most frequent AE was local injection site reaction after either dose, which was generally mild and self-limited. In addition, the vaccine was shown to be highly immunogenic. HAI and MN GMTs were enhanced with increasing doses of non-adjuvanted HAC1 vaccine. Responses detected post-1st dose of HAC1, showed seroconversion rates between 20 and 56% by HAI and 50–80% by MN. These responses were enhanced following a second immunization with the highest seroconversion rates seen in the 90 µg non-adjuvanted HAC1 group at 78% and 100% for HAI and MN, respectively. Additionally, responses elicited by the second 90 µg dose of non-adjuvanted HAC1 were comparable (by HAI) to or greater (by MN) than immune responses elicited by a single dose of the licensed, control H1N1 vaccine. The extent of the impact the second dose of HAC1 had on the immune responses should be evaluated further in the next stage of product development. Inclusion of a single dose group as a comparator control that enables the

kinetics of the antibody responses to be followed post first dose could provide valuable insight on the utility of a booster dose.

It has been shown in extensive preclinical testing that the development of anti-HA antibody responses were not affected by the presence of the 6 × His tag and the KDEL sequence present at the Cterminus of HAC1 and that this C-terminal tag did not itself induce any detectable antibody titers [35]. In addition, we have previously shown that the plant-produced HAC1 is a glycosylated protein with six of the seven potential N-linked glycosylation sites being glycosylated [35]. No apparent safety concerns were noted as a result of the presence of the C-terminal tags or plant glycans in a repeat dose toxicology study in New Zealand White rabbits [35]. Data from this clinical trial confirms the safety profile of this vaccine in humans.

It was previously demonstrated in pre-clinical studies that Alhydrogel® adjuvant enhanced antibody responses elicited by HAC1 and provided a dose-sparing effect in animal models [35]. In addition, in a randomized, Phase 1 clinical trial, alum-adjuvanted whole-virus avian A/Hong Kong/1073/99 (H9N2) vaccine was shown to be more immunogenic than the unadjuvanted vaccine [38]. Therefore, we chose to evaluate the HAC1 vaccine in the clinic with and without Alhydrogel®. In contrast to the effects of Alhydrogel® in the indicated pre-clinical and clinical studies, the present clinical study demonstrated variable results with regard to enhancement of antibody responses to different doses of HAC1 when the vaccine was adsorbed on Alhydrogel®. These results, however, are consistent with the findings from other randomized, controlled influenza vaccine clinical studies in different populations of human volunteers. Indeed, several groups evaluating split-virion 2009 pandemic influenza A H1N1 vaccines [11,39,40] as well as split-virion or whole-virus influenza A H5N1 vaccines have reported that alum either failed to enhance or even decreased antibody production [41–47]. The reason for Alhydrogel®'s inefficiency is not known, since potency of this adjuvant in stimulating both T helper 1 and 2 type responses and antibody production has been well documented [48]. As suggested by Liang et al. [24], the absence of benefit may be due to a delayed antigen release from alum-adjuvanted vaccine formulations.

In conclusion, the plant-produced experimental, recombinant HAC1 vaccine demonstrated an acceptable safety profile with a dose-dependent immune response in healthy adults. These results validate FhCMB's plant-based transient expression system as an alternative platform for manufacturing influenza vaccines and support further clinical development of HAC1.

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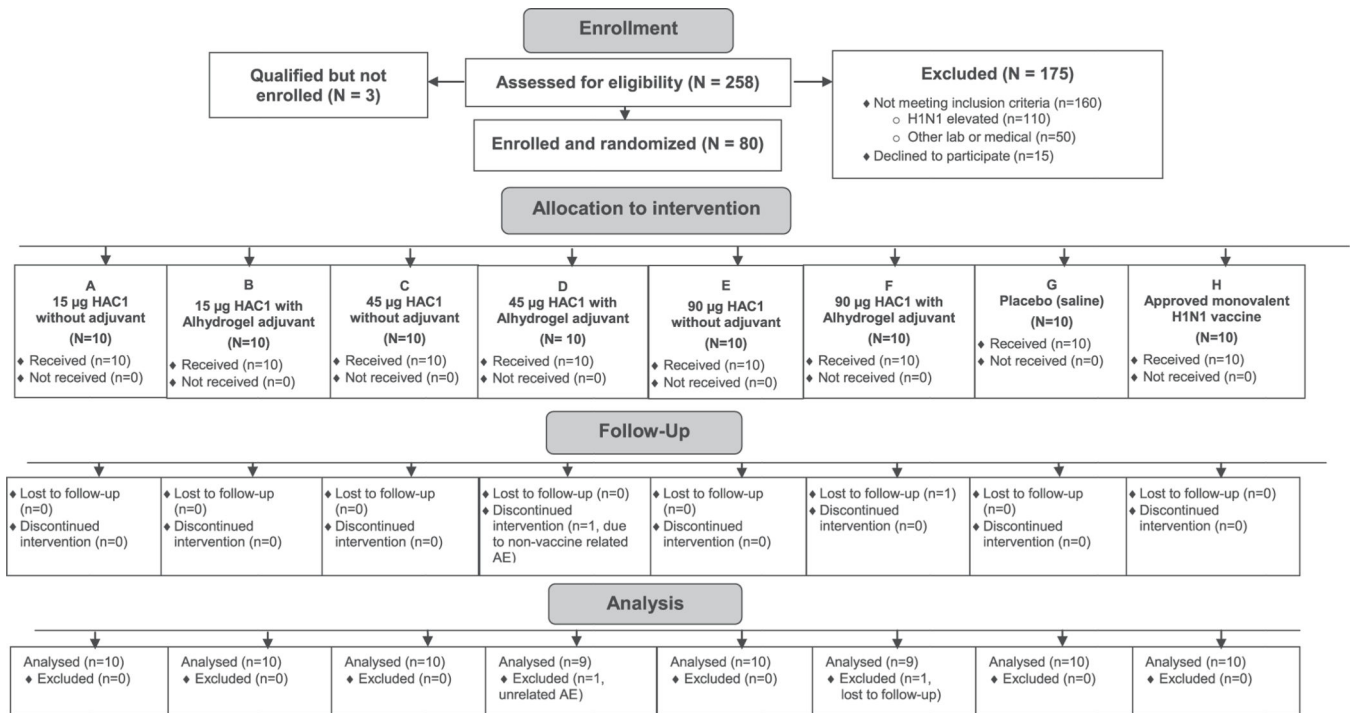
The findings, conclusions and opinions expressed herein are those of the authors and do not represent official positions of the Departments of the Army or Defense or of the Centers for Disease Control and Prevention.

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**Fig. 1.**  
Subject disposition.

Table 1

Demographic characteristics.

	Group A (15 µg) N = 10 <sup>a</sup>	Group B (15 µg + A) <sup>b</sup> N = 10	Group C (45 µg) N = 10	Group D (45 µg + A) N = 10	Group E (90 µg) N = 10	Group F (90 µg + A) N = 10	Group G (saline) N = 10	Group H (H1N1) <sup>c</sup> N = 10	Overall N = 80
Female, n (%)	6 (60)	7 (70)	8 (80)	9 (90)	5 (50)	2 (20)	6 (60)	2 (20)	45 (56)
Age (years)									
Mean (SD)	32.3 (8.2)	33.2 (7.5)	35.1 (8.7)	31.9 (7.4)	28.3 (6.4)	32.5 (7.9)	30.8 (7.9)	28.1 (8.7)	31.5 (7.8)
Median	32	33	36	34	27	31	30	27	30
Range (min, max)	20, 48	22, 45	22, 47	22, 44	22, 39	23, 46	23, 45	19, 49	19, 49
Ethnicity, n (%)									
Not Hispanic or Latino	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	9 (90)	10 (100)	9 (90)	78 (98)
Hispanic or Latino	0	0	0	0	0	1 (10)	0	1 (10)	2 (3)
Race, n (%)									
Black or African American	6 (60)	6 (60)	6 (60)	2 (20)	4 (40)	4 (40)	5 (50)	5 (50)	38 (48)
White	4 (40)	4 (40)	4 (40)	6 (60)	4 (40)	4 (40)	3 (30)	4 (40)	33 (41)
Asian	0	0	0	2 (20)	2 (20)	1 (10)	2 (20)	1 (10)	8 (10)
Other	0	0	0	0	0	1 (10)	0	0	1 (1)

<sup>a</sup>N: number of subjects.<sup>b</sup>A: alhydrogel® adjuvant.<sup>c</sup>Approved monovalent vaccine containing an A/California (H1N1)-like strain.

**Table 2**

Summary of all AEs after any vaccination.

AEs, n (%)	Group A (15 µg) N = 10 <sup>a</sup>	Group B (15 µg + A) <sup>b</sup> N = 10	Group C (45 µg) N = 10	Group D (45 µg + A) N = 10	Group E (90 µg) N = 10	Group F (90 µg + A) N = 10	Group G (saline) N = 10	Group H (H1N1) <sup>c</sup> N = 10	Overall N = 80
Any AEs	9/10 (90)	9/10 (90)	10/10 (100)	10/10 (100)	8/10 (80)	10/10 (100)	7/10 (70)	10/10 (100)	73/80 (91)
Solicited AEs	6/10 (60)	7/10 (70)	7/10 (70)	9/10 (90)	7/10 (70)	8/10 (80)	4/10 (40)	5/10 (50)	53/80 (66)
Local	2/10 (20)	6/10 (60)	6/10 (60)	8/10 (80)	6/10 (60)	7/10 (70)	1/10 (10)	1/10 (10)	37/80 (46)
Grade 1	2/10 (20)	6/10 (60)	6/10 (60)	8/10 (80)	6/10 (60)	7/10 (70)	1/10 (10)	1/10 (10)	37/80 (46)
Grade 2	0	0	0	1/10 (10)	0	0	0	0	1/80 (1)
Grade 3	0	0	0	0	0	0	0	0	0
Grade 4	0	0	0	0	0	0	0	0	0
Systemic	5/10 (50)	6/10 (60)	3/10 (30)	5/10 (50)	2/10 (20)	5/10 (50)	3/10 (30)	4/10 (40)	33/80 (41)
Grade 1	5/10 (50)	6/10 (60)	3/10 (30)	5/10 (50)	2/10 (20)	5/10 (50)	3/10 (30)	4/10 (40)	33/80 (41)
Grade 2	0	0	0	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0	0	0	0
Grade 4	0	0	0	0	0	0	0	0	0
Unsolicited AEs	8/10 (80)	9/10 (90)	10/10 (100)	9/10 (90)	6/10 (60)	7/10 (70)	7/10 (70)	10/10 (100)	66/80 (83)
Grade 1	8/10 (80)	9/10 (90)	10/10 (100)	8/10 (80)	6/10 (60)	7/10 (70)	7/10 (70)	10/10 (100)	65/80 (81)
Grade 2	0	2/10 (20)	3/10 (30)	3/10 (30)	1/10 (10)	0	4/10 (40)	2/10 (20)	15/80 (19)
Grade 3	0	0	0	1/10 (10)	0	0	0	0	1/80 (1)
Grade 4	0	0	0	0	0	0	0	0	0
SAEs	0	0	0	0	0	0	0	0	0

<sup>a</sup>N: number of subjects

<sup>b</sup>A: alhydroge® adjuvant.

<sup>c</sup>Approved monovalent vaccine containing an A/California (H1N1)-like strain.

Table 3

Summary of solicited AEs after any vaccination.

	Group A (15 µg) N=10 <sup>e</sup>	Group B (15 µg + A) <sup>b</sup> N=10	Group C (45 µg) N=10	Group D (45 µg + A) N=10	Group E (90 µg) N=10	Group F (90 µg + A) N=10	Group G (saline) N=10	Group H (H1N1) <sup>c</sup> N=10	Overall N=80
Solicited AEs, n (%)	6/10(60)	7/10(70)	7/10(70)	9/10(90)	7/10(70)	8/10(80)	4/10(40)	5/10(50)	53/80 (66)
Local	2/10(20)	6/10(60)	6/10(60)	8/10(80)	6/10(60)	7/10(70)	1/10(10)	1/10(10)	37/80 (46)
Pain	2/10(20)	6/10(60)	3/10(30)	6/10(60)	3/10(30)	3/10(30)	1/10(10)	0	24/80 (30)
Tenderness	0	4/10(40)	4/10(40)	3/10(30)	6/10(60)	7/10(70)	0	1/10(10)	25/80(31)
Erythema	0	0	0	0	0	0	0	0	0
Induration/swelling	0	0	0	0	0	0	0	0	0
Systemic	5/10(50)	6/10(60)	3/10(30)	5/10(50)	2/10(20)	5/10(50)	3/10(30)	4/10(40)	33/80 (41)
Fever	0	0	0	1/10(10)	0	0	0	0	1/80(1)
Gastrointestinal	1/10(10)	0	1/10(10)	1/10(10)	2/10(20)	2/10(20)	0	1/10(10)	8/80(10)
Headache	4/10(40)	5/10(50)	3/10(30)	3/10(30)	0	3/10(30)	2/10(20)	3/10(30)	23/80 (29)
Fatigue	2/10(20)	2/10(20)	2/10(20)	1/10(10)	0	2/10(20)	0	2/10(20)	11/80(14)
Myalgia	0	1/10(10)	0	1/10(10)	0	0	1/10(10)	1/10(10)	4/80 (5)

<sup>a</sup>N: number of subjects.

<sup>b</sup>A: alhydrogel@adjuvant.

<sup>c</sup>Approved monovalent vaccine containing an A/California (H1N1)-like strain.

**Table 4**

HAI antibody titer, seroconversion, and proportion of subjects achieving titer 40.

Treatment Group	N <sup>a</sup>	HAI titer		Seroconverted <sup>c</sup>		Titer 40	
		GMT	95% CI <sup>b</sup>	n (%)	95% CI <sup>d</sup>	n (%)	95% CI
Group A (15 µg)							
Day 0	10	6.3	4.7, 8.5	–	–	–	–
Day 21	10	20.9	6.8, 63.9	2 (20)	3, 56	2 (20)	3, 56
Day 56	10	22.9	7.7, 67.1	3 (30)	7, 65	3 (30)	7, 65
Group B (15 µg + A <sup>e</sup> )							
Day 0	10	6.6	5.2, 8.4	–	–	–	–
Day 21	10	23.4	9.8, 55.9	4(40)	12, 74	4(40)	12, 74
Day 56	10	28.8	12.8, 64.9	5 (50)	19, 81	5 (50)	19, 81
Group C (45 µg)							
Day 0	10	5.9	4.0, 8.8	–	–	–	–
Day 21	10	20.3	6.6, 62.4	3 (30)	7, 65	3 (30)	7, 65
Day 56	10	24.5	8.9, 67.9	5 (50)	19, 81	5 (50)	19, 81
Group D (45 µg + A)							
Day 0	9	5.6	4.4, 7.1	–	–	–	–
Day 21	9	42.5	12.3, 146.5	5 (56)	21, 86	5 (56)	21, 86
Day 56	9	53.4	18.2, 156.3	4 (44)	14, 79	4 (44)	14, 79
Group E (90 µg)							
Day 0	10	5.2	4.8, 5.6	–	–	–	–
Day 21	10	33.6	11.4, 98.9	5(50)	19, 81	5(50)	19, 81
Day 56	9	73.9	36.5, 149.4	7(78)	40, 97	7(78)	40, 97
Group F (90 µg + A)							
Day 0	9	5.0	–	–	–	–	–
Day 21	9	27.2	6.9, 107.2	4(44)	14, 79	4(44)	14, 79
Day 56	9	36.4	11.1, 119.2	5(56)	21, 86	5(56)	21, 86
Group G (saline)							
Day 0	10	7.4	3.3, 16.4	–	–	–	–



Treatment Group	N <sup>a</sup>	HAI titer		Seroconverted <sup>c</sup>		Titer 40	
		GMT	95% CI <sup>b</sup>	n (%)	95% CI <sup>d</sup>	n (%)	95% CI
Day 21	10	7.6	3.5, 16.4	0	0, 31	1	(10) 0, 45
Day 56	10	6.7	3.8, 11.7	0	0, 31	1	(10) 0, 45
Group H (H1N1) <sup>f</sup>							
Day 0	10	5.4	4.6, 6.3	-	-	-	-
Day 21	10	101.5	26.2, 393.1	7	(70) 35, 93	7	(70) 35, 93
Day 56	10	75.4	19.4, 292.9	6	(60) 26, 88	6	(60) 26, 88

<sup>a</sup>N: number of subjects.

<sup>b</sup>95% upper and lower confidence limits of the GMT, based on the t distribution with  $n - 1$  degrees of freedom.

<sup>c</sup>Subjects have achieved seroconversion if they have either a pre-vaccination HAI titer <10 and a post-vaccination HAI titer ≥40 or a pre- vaccination HAI titer ≥10 and a minimum 4-fold rise in a post-vaccination HAI antibody titer.

<sup>d</sup>Exact (Clopper–Pearson) confidence limits were computed for each binomial proportion

<sup>e</sup>A: alhydrogel® adjuvant.

<sup>f</sup>Approved monovalent vaccine containing an A/California (H1N1)-like strain.

**Table 5**  
 MN antibody titer, seroconversion, and proportion of subjects achieving titer 80 and 160.

Treatment Group	N <sup>a</sup>	MN Titer		Seroconverted <sup>c</sup>		Titer 80		Titer 160	
		GMT	95% CI <sup>b</sup>	n (%)	95% CI <sup>d</sup>	n (%)	95% CI <sup>d</sup>	n (%)	95% CI
Group A (15 µg)									
Day 0	10	7.2	4.5, 11.4	–	–	–	–	–	–
Day 21	10	45.9	9.4, 223.4	5 (50)	(19, 81)	2 (20)	(3, 56)	2 (20)	(3, 56)
Day 56	10	65.8	15.8, 273.1	6 (60)	(26, 88)	3 (30)	(7, 65)	2 (20)	(3, 56)
Group B (15 µg + A <sup>e</sup> )									
Day 0	10	13.0	7.4, 22.9	–	–	–	–	–	–
Day 21	10	83.9	28.6, 246.5	5(50)	(19, 81)	5(50)	(19, 81)	5(50)	(19, 81)
Day 56	10	110.1	40.4, 300.0	6(60)	(26, 88)	6(60)	(26, 88)	5(50)	(19, 81)
Group C (45 µg)									
Day 0	10	8.4	4.1, 17.4	–	–	–	–	–	–
Day 21	10	67.8	14.6, 313.8	5(50)	(19, 81)	4(40)	(12, 74)	3(30)	(7, 65)
Day 56	10	93.6	22.7, 387.0	7(70)	(35, 93)	5(50)	(19, 81)	5(50)	(19, 81)
Group D (45 µg + A)									
Day 0	9	9.5	4.3, 20.9	–	–	–	–	–	–
Day 21	9	244.4	64.8, 922.1	7 (78)	(40, 97)	6 (67)	(30, 93)	5 (56)	(21, 86)
Day 56	9	307.2	123.3, 765.2	9 (100)	(66, 100)	7 (78)	(40, 97)	7 (78)	(40, 97)
Group E (90 µg)									
Day 0	10	7.1	5.0, 10.2	–	–	–	–	–	–
Day 21	10	171.2	55.8, 525.2	8 (80)	(44, 97)	7 (70)	(35, 93)	5 (50)	(19, 81)
Day 56	9	436.6	196.4, 970.7	9 (100)	(66, 100)	9 (100)	(66, 100)	8 (89)	(52, 100)
Group F (90 µg + A)									
Day 0	9	6.1	4.8, 7.7	–	–	–	–	–	–
Day 21	9	86.4	14.6, 510.3	6(67)	(30, 93)	5(56)	(21, 86)	5(56)	(21, 86)
Day 56	9	124.5	36.5, 424.9	8(89)	(52, 100)	5(56)	(21, 86)	5(56)	(21, 86)
Group G (saline)									
Day 0	10	20.3	7.5, 55.0	–	–	–	–	–	–

Treatment Group	N <sup>a</sup>	MN Titer	Seroconverted <sup>c</sup>		Titer 80		Titer 160		
			n (%)	95% CI <sup>d</sup>	n (%)	95% CI <sup>d</sup>	n (%)	95% CI	
		GMT	95% CI <sup>b</sup>						
Day 21	10	17.5	7.6, 40.6	0 (0, 31)	1 (10)	(0, 45)	1 (10)	(0, 45)	
Day 56	10	13.3	7.4, 24.0	0 (0, 31)	1 (10)	(0, 45)	0	(0, 31)	
Group H (H1N1) <sup>f</sup>									
Day 0	10	7.3	4.3, 12.4	–	–	–	–	–	
Day 21	10	342.5	82.9, 1414.2	7 (70)	7 (70)	(35, 93)	6 (60)	(26, 88)	
Day 56	10	186.5	44.9, 775.1	7 (70)	5 (50)	(19, 81)	5 (50)	(19, 81)	

<sup>a</sup>N: number of subjects.

<sup>b</sup>95% upper and lower confidence limits of the GMT, based on the t distribution with  $n - 1$  degrees of freedom.

<sup>c</sup>Subjects have achieved seroconversion if they have either a pre-vaccination MN titer <10 and a post-vaccination MN titer 40 or a pre-vaccination MN titer 10 and a minimum 4-fold rise in a post-vaccination MN antibody titer.

<sup>d</sup>Exact (Clopper–Pearson) confidence limits were computed for each binomial proportion.

<sup>e</sup>A: alhydrogel® adjuvant.

<sup>f</sup>Approved monovalent vaccine containing an A/California (H1N1)-like strain.