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Phase 1 Safety and Immunogenicity Study of a Quadrivalent Seasonal Flu Vaccine Comprising Recombinant Hemagglutinin-Flagellin Fusion Proteins

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Background. We evaluated the safety and immunogenicity of VAX2012Q, a quadrivalent influenza vaccine comprising 4 hemagglutinin subunits fused to flagellin.

Methods. In this dose-ranging, open-label study, healthy adults (18–40 years) were divided into 7 cohorts for evaluation of 5 dose levels and 3 component ratios. Dose levels were as follows: (1) 1 mcg per component of VAX128C (H1N1), VAX181 (H3N2), VAX173 (B-YAM), and VAX172 (B-VIC), respectively; (2) 2 mcg per component, respectively; (3) 2, 4, 4, and 4 mcg of each component, respectively; (4) 2, 4, 6, and 6 mcg of each component, respectively; and (5) 3 mcg per component, respectively. Tolerability and immunogenicity data were analyzed.

Results. Three hundred sixteen subjects received VAX2012Q (309 per protocol). At all dose levels, 54% to 65% of subjects reported mild injection site pain, the most common local reaction. Moderate injection site pain increased at dose levels 2 through 5 (22%–42%, compared with 20% at dose level 1). Systemic symptoms were mostly mild to moderate with moderate symptoms increasing in dose levels 3 and 4. Three dose level 3 subjects (6%) reported severe, transient chills and or fever. Mean fold rises in hemagglutination inhibition titers ranged from 2.5 to 6.9 despite high baseline titers. Mean seroprotection rates were ≥90% and mean seroconversion rates were ≥40% for all strains in all groups postvaccination.

Conclusions. VAX2012Q elicited immune responses at all dose levels with no significant safety concerns. Doses of 2 or 3 mcg per component provided a favorable balance of tolerability and immunogenicity.

Keywords. flagellin adjuvant; influenza vaccine; recombinant vaccine; seasonal influenza; vaccine.

Control of seasonal and pandemic influenza represents a global public health challenge due to the virus's ability to circumvent protective immune responses through frequent mutation and subunit recombination. These characteristics, coupled with influenza's ability to spread rapidly during outbreaks, require continuous global surveillance, frequent vaccine reformulation, and tightly scheduled manufacturing. Egg- and cell-culture–based vaccine production methods are resource- and time-intensive, leaving little room for error in selection of vaccine components or timing of production. For example, in 2014–2015, long manufacturing lead times prevented the industry from addressing a discrepancy between the H3 virus predominantly circulating in the northern hemisphere and the H3 included in the licensed vaccine [1]. Furthermore, low vaccine effectiveness in the 2012–2013 season was attributed to mutations introduced as part of egg-based manufacturing [2]. Rapid response, high-yield methods that remain faithful to the circulating virus sequences are therefore needed to address the shortcomings of current approaches.

Recombinant technologies have the potential to address such current shortcomings and include production of recombinant HA [3], virus-like particles consisting of hemagglutinin (HA), neuraminidase (NA), and matrix (M1) proteins [4, 5], vaccinia virus-based expression of HA and NA [6], the HA1 fragment of HA [7], or the HA2 stalk of HA [8], as well as DNA vector-based expression of multiple antigens [9]. To date, none has achieved a combination of rapid, high-yield production, potency, and ability to remain faithful to the circulating sequence that is sufficient to address the deficiencies associated with egg- and cell-based approaches.

We have developed a recombinant influenza vaccine platform whereby the globular head domain of the major protective antigen, HA, is fused to the Toll-like receptor (TLR)5 agonist, flagellin (*Salmonella typhimurium* flagellin type 2 [STF2]) [10–12]. The binding of STF2 to TLR5 on the surface of sentinel immune cells activates the innate immune system and, in turn,

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enhances the adaptive immune response [10]. The flagellin moiety of the vaccine therefore serves as a built-in adjuvant. These relatively simple recombinant fusion proteins can be rapidly and inexpensively produced in standard *Escherichia coli* production systems at high yield. Specifically, current production yields range from 0.3 to 1 mg of purified bulk drug substance per liter of fermentation, with a cycle time of under 2 weeks (unpublished observations).

In animal models and in humans, the individual HA-STF2 fusion proteins elicit HA-specific neutralizing antibodies at low microgram doses of vaccine [13]. We have reproduced these results using HA from a variety of influenza type A and B strains, and we have also determined the optimal, subtype or type-specific placement of each HA subunit within the STF2 protein sequence [12–17]. The low dose of antigen required, coupled with the rapidity and efficiency of production, further demonstrate that the platform has the capacity to address the shortcomings associated with current licensed vaccines.

Extending on this work, we are developing a prototypic quadrivalent seasonal flu vaccine, VAX2012Q. We report results from a Phase I dose-ranging study designed to identify the total antigen dose and component ratio that provides optimal tolerability and immunogenicity profiles.

MATERIALS AND METHODS

Study Design

This was a Phase I, first-in-man, open-label, dose-ranging study with adaptive dose selection, in up to 320 healthy adults, performed at 4 United States clinical sites (Clinicaltrials.gov/ct2/show/NCT02015494). The primary objective was to evaluate the safety and tolerability of the quadrivalent seasonal influenza vaccine VAX2012Q. The secondary objective was to evaluate the immunogenicity of VAX2012Q. This study was conducted in compliance with the International Conference on Harmonization guidelines and all US Food and Drug Administration regulations for Good Clinical Practice. A central Institutional Review Board (Schulman Associates IRB, Cincinnati, OH) reviewed and approved the protocol. All study participants provided written informed consent.

Study Participants

Healthy adult volunteers (aged 18 to 40 years inclusive) with an intact immunological status were eligible. Comprehensive eligibility criteria are listed at https://www.clinicaltrials.gov/ct2/show/NCT02015494. Key exclusion criteria included the following: clinical diagnosis of influenza within the last 6 months; recent or planned receipt of any live or inactivated vaccine product; history of severe allergic reaction or hypersensitivity to previous vaccinations or seasonal influenza vaccine components; and recent history of immunosuppressive or immunomodulatory therapeutic modality.

Prototypic Vaccine

The 4 antigenic components in VAX2012Q, based on recently or currently circulating strains, were manufactured under good manufacturing practice conditions in *E coli* (Lonza, Hopkinton, MA) as described [11, 12]. Proteins were ≥90% pure by reverse-phase chromatography and contained <50 endotoxin units/mg protein. For each component, the HA globular head subunit was expressed as a recombinant fusion protein with STF2 [15, 17] (Supplementary Figure 1). VAX128C comprises 1 copy of the HA1–2 subunit of A/California/07/2009 (H1N1) fused to the STF2 C-terminus and a second copy in place of the D3 domain [17]; VAX181 comprises a 57-amino acid longer portion of the HA1 globular head (HA1-1L), from A/Perth/16/2009 (H3N2) fused in place of the STF2 D3 domain; VAX173 comprises the HA1-2 subunit from B/Wisconsin/01/2010 (B-YAM) fused at a site within the D3 domain [15]; and VAX172 comprises the HA1-2 subunit from the B/Bangladesh/5945/2009 (B-VIC), also fused at a site within the D3 domain [15].

The diluent for all mixes contained 10 mM Tris, 150 mM NaCl, 10 mM l-histidine, ethanol 0.5% (v/v), trehalose 5% (w/v), 0.1 mM EDTA, polysorbate-80 0.02% (w/v), pH 7.0, and contained no added adjuvants [17].

Vaccine doses were formulated by protein mass from single lots of each component (Table 1). For each dose level, the "aggregate" dose was the total protein mass (microgram) of the 4 antigens, and the "component ratio" represented the relative mass of each component in the mix. Lot numbers were 128C913237 (VAX128C), 181913238 (VAX181), 173913239 (VAX173), 172913240 (VAX172), and 147913236 (vaccine diluent).

Treatment

The starting dose and escalation plan for dose levels 1–4 (Table 1) were prespecified based on experience with the quadrivalent vaccine in rabbits and with monovalent vaccines in prior preclinical and clinical studies [12–17]. The first dose level was expected to be suboptimal and effectively serve as a comparator for higher doses. Participants were assigned to cohorts sequentially, based on the timing of enrollment. Before administration, the quadrivalent VAX2012Q vaccine was formulated at the clinical site according to the dose escalation plan and administered within 12 hours of formulation. Vaccinations were delivered by single intramuscular injection (deltoid, nondominant arm) on study day 0. Subjects remained at the clinic for 2 to 2.5 hours after vaccination and were evaluated at clinic visits on days 0, 1, 7, and 21.

Data Collection, Safety Monitoring, and Escalation Plan Amendments

Demographic and immunogenicity data were collected at baseline. Safety and immunogenicity data were collected and assessed after each dose level. During a scheduled study pause after dose group 4, safety and immunogenicity results were reviewed by the Safety Monitoring Committee and US Food and Drug Administration Center for Biologics Evaluation and

Table 1. Dose Escalation Schedule for VAX2012Q Quadrivalent Vaccine Phase I Study (Final Schedule)

		Component Dose (mcg)[b]						
Cohort[a]	N	VAX128C (H1N1)	VAX181 (H3N2)	VAX173 (B-YAM)	VAX172 (B-VIC)	Aggregate Dose (mcg)[c]	Component Ratio[d]	Dose Level
1	20	1	1	1	1	4	1:1:1:1	1
2	48	2	2	2	2	8	1:1:1:1	2
3	51	2	4	4	4	14	1:2:2:2	3
4	48	2	4	6	6	18	1:2:3:3	4
5	49	3	3	3	3	12	1:1:1:1	5
6	51	2	2	2	2	8	1:1:1:1	2
7	49	3	3	3	3	12	1:1:1:1	5
Total	316							

Abbreviations: B-YAM, B Yamagata lineage; B-VIC, B Victoria lineage; HAI, hemagglutination.

[a] Dose levels for groups 1–4 were prespecified at the beginning of the study. Study was paused after dose group 4 for interim safety and immunogenicity analyses, the results of which were used to inform dose levels for groups 5–7.

[b] VAX128C: HA1-2 subunit of A/California/07/2009 (H1N1; licensed vaccine year 2010 to present); VAX181: HA1-1L subunit from A/Perth/16/2009 (H3N2; licensed vaccine year 2010 and 2011); VAX173: HA1-2 subunit from B/Wisconsin/01/2010 (B-YAM; licensed vaccine year 2012); VAX172: HA1-2 subunit from B/Bangladesh/5945/2009 (B-VIC; licensed vaccine year 2009 to present). Refer to Materials and Methods for complete description of antigen constructs.

[c] Aggregate dose: total protein mass (mcg) of all 4 vaccine HAI antigens (components).

[d] Component ratio: relative amount of each vaccine component, by protein mass.

Research (CBER). Dose levels for groups 5 to 7 were selected based on these analyses.

Safety and Tolerability Measures

Subjects used a memory aid to record oral digital temperature and adverse events (AEs) daily on study days 0 through 6. These records were reviewed at the day 7 clinic visit. Additional AEs and changes to concomitant medications were recorded during days 7 through 21. Follow-up to track AEs of special interest, including those considered to be vaccine-related, all serious AEs (SAEs), and new-onset chronic illness continued through day 360. Serum samples were assessed (1) for C-reactive protein (CRP) before vaccination and on days 1 and 7 by enzyme-linked immunosorbent assay (ELISA) and (2) for cytokines (interleukin [IL]-12p70, tumor necrosis factor [TNF]-α, IL-10, IL-6, IL-1β, and/or IL-8) before vaccination and 2 hours after vaccination by Cytokine Bead Array (BD Biosciences, San Jose, CA) as previously described [17]. Timepoints for cytokine and CRP analysis were based on prior clinical and preclinical experience demonstrating that proinflammatory cytokines peak in the first several hours after immunization, and CRP peaks at approximately 24 hours (unpublished observations).

Immune Response Measures

Serum samples for HA inhibition (HAI) assays were collected before vaccine administration and on day 21 (±3 days). The HAI assay was performed with turkey red blood cells by a validated method at Focus Diagnostics, Inc. (Cypress, CA). Live virus was used for A/California/07/2009-X179A and A/Perth/16/2009; ether-extracted virus was used for B/Wisconsin/1/2010 and B/Bangladesh/5945/2009.

The geometric mean titer (GMT) ± the 95% confidence interval (CI) at day 21 was calculated by taking the exponent ($\log_{10}$) of the mean and of the limits of the 95% CIs of the $\log_{10}$-transformed titers [18].

Seroprotection rate (SPR) was defined as the proportion of subjects with HAI titer ≥1:40. Seroconversion rate (SCR) was defined as the proportion of subjects with a postvaccination titer ≥1:40 and a prevaccination titer <1:10, or a minimum 4-fold increase in HAI titers and a prevaccination titer ≥1:10 [18]. Mean rates were calculated and presented along with 95% CIs. The immunogenicity target was a dose that elicited HAI antibody titers >1:40 in the largest proportion of subjects.

Testing for flagellin-specific immunoglobulin (Ig)G antibody was by ELISA as previously described [16].

Sample Size Determination

Planned enrollment was 320 subjects, with 20 subjects to be treated in cohort 1 (dose level 1) and 50 subjects each in cohorts 2 through 7. The sample size was selected to obtain an initial safety assessment and to evaluate the dose-related immune response to the candidate vaccine. The first-dose cohort was smaller because the dose level was expected to be suboptimal for immunogenicity, and because the group was to serve as baseline for assessing safety and immunogenicity.

Statistical Analysis

Statistical analyses were performed using SAS version 9.1.3 or higher (SAS Institute, Inc.). The intent-to-treat (ITT) population included all subjects who were administered vaccine. The per protocol (PP) population received the full assigned dose of vaccine and provided pre- and postvaccination serum samples for testing, and they did not otherwise deviate from the study protocol.

Categorical data were summarized with frequencies and percentages. Hypothesis testing was performed with a prespecified

Table 2. Baseline Characteristics by Dose Level (ITT Population; N = 316)[a]

Characteristic	Dose Level[b] 1 (n = 20)	2 (n = 99)	3 (n = 51)	4 (n = 48)	5 (n = 98)	Total (N = 316)
Age, year	30.6 ± 5.80	28.3 ± 5.31	27.0 ± 5.42	30.2 ± 5.44	28.6 ± 6.12	28.6 ± 5.70
Body mass index	26.2 ± 2.3	24.3 ± 3.4	23.8 ± 3.6	24.8 ± 2.7	24.4 ± 2.9	24.5 ± 3.2
Sex						
Female	10 (50)	51 (51.5)	24 (47.1)	26 (54.2)	64 (65.3)	175 (55.4)
Male	10 (50)	48 (48.5)	27 (52.9)	22 (45.8)	34 (34.7)	141 (44.6)
Race						
American Indian or Alaska Native	0	0	0	0	0	0
Asian	1 (5.0)	1 (1.0)	0	0	1 (1.0)	3 (0.9)
Black or African American	6 (30.0)	15 (15.2)	14 (27.5)	9 (18.8)	10 (10.2)	54 (17.1)
Native Hawaiian/Pacific Islander	0	0	1 (2.0)	0	0	1 (0.3)
White	12 (60.0)	83 (83.8)	36 (70.6)	39 (81.3)	84 (85.7)	254 (80.4)
Other	1 (5.0)	0	0	0	3 (3.1)	4 (1.3)
Ethnicity						
Hispanic or Latino	1 (5.0)	10 (10.1)	7 (13.7)	4 (8.3)	16 (16.3)	38 (12.0)
Not Hispanic or Latino	19 (95.0)	89 (89.9)	44 (86.3)	44 (91.7)	82 (83.7)	278 (88.0)
Dominant arm, right	20 (100)	87 (87.9)	46 (90.2)	43 (89.6)	94 (95.9)	290 (91.8)
Received influenza vaccine 2013	2 (10.0)	19 (19.2)	7 (13.7)	6 (12.5)	26 (26.5)	60 (19.0)

Abbreviations: ITT, intent-to-treat; SD, standard deviation.

[a] Data are presented as mean ± SD or n (%) except where otherwise noted.

[b] Dose level 2 included subjects from cohorts 2 and 6; dose level 5 included subjects from cohorts 5 and 7.

alpha of 0.05. Where noted, data from cohorts 2 and 6 were pooled because the dose levels were identical (8 mcg); data from cohorts 5 and 7 (12 mcg) were similarly pooled.

RESULTS

Participants and Demographics

Between March 2014 and September 2014, 316 subjects were randomized to treatment (ITT population). Seven subjects exited the study early (2 at each dose level other than dose level 1); 4 of these 7 withdrew consent. An eighth subject exited the study early but after serum samples were collected; this subject's data were analyzed with the PP/safety set (total N = 309). Baseline demographics were similar between cohorts (Table 2).

Safety and Tolerability

More than 85% of subjects at each dose level experienced at least 1 treatment-emergent AE (TEAE) that was at least possibly related to the vaccine. The most frequently reported TEAEs were injection site pain, headache, fatigue, and myalgia. The frequency and severity of the most common TEAEs were roughly dose-dependent (Tables 3 and 4), with the largest proportions of subjects affected at the highest dose level (dose level 4; 18 mcg total antigen).

Local AE data are presented by dose level and symptom in Table 3. At dose level 1, the most common symptom was mild injection site pain (13 of 20 subjects; 65%). Mild injection site pain was common at higher dose levels (54%–63%), and incidence of moderate injection site pain increased (22%–42%, compared with 20% at dose level 1). Injection site erythema and swelling were reported at mostly mild intensities in ≤5 subjects at the higher dose levels. Injection site bruising was reported sporadically, and injection site hematoma was not reported at all. One subject reported severe injection site pain at dose level 2 (8 mcg). The difference in incidence of injection site (local) symptoms relative to dose level 1 was not statistically significant for any of the higher dose levels. Incidence and intensities of events did not differ appreciably between dose levels 2 and 5.

Table 3. Local Adverse Events by Dose Level and Symptom

Cohort	Grade	Arm Pain	Redness	Swelling	Bruising
Dose level 1 (4 mcg dose) N = 20	None	3 (15%)	18 (90%)	20 (100%)	19 (95%)
	Mild	13 (65%)	2 (10%)	0	1 (5%)
	Mod	4 (20%)	0	0	0
	Severe	0	0	0	0
Dose level 2 (8 mcg dose) N = 99	None	9 (9%)	95 (96%)	94 (95%)	99 (100%)
	Mild	58 (59%)	4 (4%)	4 (4%)	0
	Mod	31 (31%)	0	1 (1%)	0
	Severe	1 (1%)	0	0	0
Dose level 3 (14 mcg dose) N = 51	None	8 (16%)	50 (99%)	46 (90%)	51 (100%)
	Mild	32 (63%)	1 (1%)	3 (6%)	0
	Mod	11 (22%)	0	2 (4%)	0
	Severe	0	0	0	0
Dose level 4 (18 mcg dose) N = 48	None	2 (4%)	43 (90%)	42 (88%)	48 (100%)
	Mild	26 (54%)	4 (8%)	5 (10%)	0
	Mod	20 (42%)	1 (2%)	1 (2%)	0
	Severe	0	0	0	0
Dose level 5 (12 mcg dose) N = 98	None	5 (5%)	94 (96%)	96 (98%)	98 (100%
	Mild	55 (56%)	3 (3%)	2 (2%)	0
	Mod	38 (39%)	1 (1%)	0	0
	Severe	0	0	0	0

Table 4. Systemic AEs by Dose Level and Symptom

Cohort	Grade	Fever	Headache	Fatigue	Joint Pain	Muscle Ache	Chills	Sweats	Any Sys
Dose level 1 (4 mcg) N = 20	None	20 (100%)	17 (85%)	17 (85%)	19 (95%)	18 (90%)	20 (100%)	20 (100%)	14 (70%)
	Mild	0	3 (15%)	3 (15%)	1 (5%)	2 (10%)	0	0	6 (30%)
	Mod	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0
Dose level 2 (8 mcg) N = 99	None	98 (99%)	72 (73%)	76 (77%)	90 (91%)	75 (76%)	88 (89%)	93 (94%)	55 (55%)
	Mild	1 (1%)	21 (21%)	18 (18%)	5 (5%)	17 (17%)	7 (7%)	1 1%)	32 (32%)
	Mod	0	5 (5%)	5 (5%)	4 (4%)	7 (7%)	4 (4%)	1 (1%)	13 (13%)
	Severe	0	0	0	0	0	0	0	0
Dose level 3 (14 mcg) N = 51	None	46 (90%)	34 (67%)	30 (59%)	44 (86%)	36 (71%)	39 (76%)	48 (94%)	22 (43%)
	Mild	2 (4%)	12 (23%)	14 (28%)	6 (12%)	12 (23%)	6 (12%)	3 (6%)	16 (31%)
	Mod	1 (2%)	5 (10%)	7 (14%)	1 (2%)	3 (6%)	4 (8%)	0	10 (20%)
	Severe	2 (4%)	0	0	0	0	2 (4%)	0	3 (6%)
Dose level 4 (18 mcg) N = 48	None	47 (98%)	30 (62%)	25 (52%)	41 (85%)	32 (67%)	34 (71%)	45 (94%)	16 (33%)
	Mild	1 (2%)	11 (23%)	15 (31%)	6 (13%)	10 (21%)	10 (21%)	3 (6%)	19 (40%)
	Mod	0	7 (15%)	8 (17%)	1 (2%)	6 (12%)	4 (8%)	0	13 (27%)
	Severe	0	0	0	0	0	0	0	0
Dose level 5 (12 mcg) N = 98	None	95 (97%)	76 (78%)	71 (73%)	93 (95%)	86 (88%)	91 (93%)	91 (93%)	57 (58%)
	Mild	2 (2%)	17 (17%)	20 (20%)	2 (2%)	8 (8%)	6 (6%)	2 (2%)	25 (26%)
	Mod	1 (1%)	5 (5.1%)	7 (7%)	3 (3%)	4 (4%)	1 (1%)	0	16 (16%)
	Severe	0	0	0	0	0	0	0	0

Abbreviations: AE, adverse event; Mod, moderate; Sys, systemic.

Systemic AE data are presented by dose level and symptom in Table 4. The majority of systemic AEs occurred and resolved within the first 24 hours. At dose level 1, only mild systemic symptoms were reported among 6 subjects (30.0%). Mild symptoms were reported in similar proportions at higher dose levels (26%–40%), but moderate systemic symptoms were reported for 16% (dose level 5) to 27% (dose level 4; $P = .006$ vs dose level 1) of subjects. Fatigue and headache were the most frequently reported symptoms at mild and moderate severities at all dose levels. At the highest dose levels (3 and 4), mild myalgia was as common or nearly as common as headache and fatigue. Chills, arthralgia, and pyrexia were reported primarily at the higher dose levels. Severe systemic symptoms of chills (2 subjects, 4%) and pyrexia (2 subjects, 4%) were reported for 3 subjects treated at dose level 3 (14 mcg). Incidence and intensities of events did not differ appreciably between dose levels 2 and 5.

Small median increases in IL-6 and IL-8 from baseline to 2 hours after vaccination were loosely dose-dependent (Supplementary Table 1). For IL-1β, IL-10, IL-12p70, and TNF-α, there was essentially no change from baseline to 2 hours postvaccination at all dose levels. For IL-10 and in particular TNF-α, some subjects treated at higher dose levels had large increases, which affected mean change results, but median change was 0 at all dose levels (Supplementary Table 2).

Median increases in CRP from day 0 to day 1 (postvaccination) followed a dose-dependent pattern and ranged from 3.7 mg/L at dose level 1 to 10.8 mg/L at dose level 4 (Supplementary Table 3). By day 7, CRP values had nearly dropped to baseline.

A weak but significant correlation was observed both between postvaccination IL-6 values and number of general systemic symptoms (coefficient = 0.192 [$P = .0006$]) and a moderate but significant correlation between day 1 CRP results and the number of general systemic symptoms (coefficient = 0.425 [$P < .0001$]). There were no clinically relevant clinical laboratory result abnormalities judged related to vaccination.

Immunogenicity

Table 5 provides the GMTs, SCR, and SPR for each component, by dose level. The H1 (VAX128C) component was given at 1 mcg in dose level 1, 2 mcg in dose levels 2 to 4, and 3 mcg in dose level 5. Results did not follow a clearly dose-dependent pattern; mean GMTs at day 21 ranged from 274.7 at dose level 4 to 505.5 at dose level 5 (median GMTs were highest, 640.0, at dose levels 3 and 5). The mean fold response at day 21 ranged from 3.4 at dose level 3 to 6.2 at dose level 1.

The H3 (VAX181) component was given at 1 mcg in dose level 1, 2 mcg in dose level 2, 4 mcg in dose levels 3 and 4, and 3 mcg in dose level 5. Results followed an approximately dose-dependent pattern with respect to GMTs at day 21, which were lowest (102.0) at dose level 1 and highest (208.5 and 191.9, respectively) at dose levels 3 and 4. Mean-fold response at day 21 were lowest (4.0 and 4.3, respectively) at dose levels 1 and 5 and highest (6.7 and 6.9, respectively) at dose levels 3 and 4.

The B/Yamagata (VAX173) component was given at 1 mcg in dose level 1, 2 mcg in dose level 2, 4 mcg in dose level 3, 6 mcg in dose level 4, and 3 mcg in dose level 5. Results at day 21 followed a dose-dependent pattern only for dose levels 1 to 3, with

Table 5. Geometric Mean Titer, Fold Rise, Seroconversion, and Seroprotection by Dose Level (PP Population)[a]

	Dose Level (Aggregate Dose)				
Component-Specific Response Data	1 (4 mcg) N = 20	2 (8 mcg) N = 98	3 (14 mcg) N = 51	4 (18 mcg) N = 48	5 (12 mcg) N = 96
VAX128C (A/California/07/2009; H1N1)					
Day 0 GMT (95% CI)	47.6 (25.8–87.8)	92.2 (65.9–128.9)	113.7 (68.3–189.1)	71.8 (43.8–117.6)	127.6 (94.0–173.2)
Day 21 GMT (95% CI)	298.6 (186.7–477.5)	395.6 (312.9–500.2)	388.0 (267.7–562.5)	274.7 (199.3–378.7)	505.5 (402.8–634.4)
GMT fold (mean)	6.2	4.3	3.4	3.8	4.0
Day 21 SCR (%, 95% CI)	55.0 (33.2–76.8)	55.1 (45.3–64.9)	39.6 (25.7–53.4)	46.8 (32.5–61.1)	46.9 (36.9–56.9)
Day 0 SPR (%, 95% CI)	70.0 (45.7–88.1)	78.6 (69.1–86.2)	79.2 (65.0–89.5)	68.1 (52.9–80.9)	82.3 (73.2–89.3)
Day 21 SPR (%, 95% CI)	95.0 (75.1–99.9)	96.9 (91.3–99.4)	93.8 (82.8–98.7)	95.7 (85.5–99.5)	97.9 (92.7–99.7)
VAX181 (A/Perth/16/2009; H3N2)					
Day 0 GMT (95% CI)	25.5 (12.2–53.4)	29.7 (21.9–40.4)	31.3 (20.3–48.1)	27.8 (18.0–42.8)	36.2 (26.4–49.8)
Day 21 GMT (95% CI)	102.0 (55.1–188.8)	183.9 (145.3–232.7)	208.5 (154.9–280.7)	191.9 (138.6–265.7)	156.9 (124.0–198.6)
GMT fold (mean)	4.0	6.2	6.7	6.9	4.3
Day 21 SCR (%, 95% CI)	50.0 (28.1–71.9)	58.2 (48.4–67.9)	62.5 (48.8–76.2)	57.4 (43.3–71.6)	45.8 (35.9–55.8)
Day 0 SPR (%, 95% CI)	45.0 (23.1–68.5)	52.0 (41.7–62.2)	45.8 (31.4–60.8)	44.7 (30.2–59.9)	53.1 (42.7–63.4)
Day 21 SPR (%, 95% CI)	85.0 (62.1–96.8)	94.9 (88.5–98.3)	95.8 (85.7–99.5)	95.7 (85.5–99.5)	90.6 (82.9–95.6)
VAX173 (B/Wisconsin/01/2010; B-YAM)					
Day 0 GMT (95% CI)	106.7 (58.8–193.6)	127.3 (98.3–164.8)	109.9 (77.1–156.8)	128.9 (91.5–181.5)	183.0 (146.4–228.8)
Day 21 GMT (95% CI)	327.5 (188.3–569.6)	488.0 (397.1–599.8)	510.5 (378.2–689.0)	451.5 (326.4–624.4)	757.4 (639.3–897.4)
GMT fold (mean)	3.1	3.8	4.6	3.5	4.1
Day 21 SCR (%, 95% CI)	35.0 (14.1–55.9)	49.0 (39.1–58.9)	58.3 (44.4–72.3)	42.6 (28.4–56.7)	56.3 (46.3–66.2)
Day 0 SPR (%, 95% CI)	90.0 (68.3–98.8)	86.7 (78.4–92.7)	81.3 (67.4–91.1)	93.6 (82.5–98.7)	96.9 (91.1–99.4)
Day 21 SPR (%, 95% CI)	95 (75.1–99.9)	99.0 (94.4–100)	100 (92.6–100)	97.9 (88.7–99.9)	100 (96.2–100)
VAX172 (B/Bangladesh/5945/2009; B-VIC)					
Day 0 GMT (95% CI)	47.6 (24.9–90.9)	54.6 (41.4–72.1)	48.5 (35.0–67.3)	61.1 (46.5–80.2)	95.4 (73.7–123.4)
Day 21 GMT (95% CI)	118.4 (64.0–219.0)	216.4 (171.8–272.6)	200.6 (151.0–266.6)	229.1 (174.9–300.0)	323.9 (264.0–397.3)
GMT fold (mean)	2.5	4.0	4.1	3.7	3.4
Day 21 SCR (%, 95% CI)	30.0 (9.9–50.1)	54.1 (44.2–63.9)	50.0 (35.9–64.1)	51.1 (36.8–65.4)	47.9 (37.9–57.9)
Day 0 SPR (%, 95% CI)	65.0 (40.8–84.6)	65.3 (55.0–74.6)	64.6 (49.5–77.8)	83.0 (69.2–92.4)	81.3 (72.0–88.5)
Day 21 SPR (%, 95% CI)	90.0 (68.3–98.8)	95.9 (89.8–98.9)	97.9 (88.9–99.9)	97.9 (88.7–99.9)	97.9 (92.7–99.7)

Abbreviations: B-YAM, B Yamagata lineage; B-VIC, B Victoria lineage; CI, confidence interval; GMT, geometric mean titer; PP, per protocol; SCR, seroconversion rate; SPR, seroprotection rate.

[a] Aggregate dose: total protein mass of all vaccine components; refer to Table 1.

linear increases in GMTs and mean-fold responses. The highest GMT was observed at dose level 5 (3 mcg), whereas the GMT and mean-fold response at dose level 4 (6 mcg) were similar to those observed for dose level 2 (2 mcg).

The B/Victoria (VAX172) component was given at the same doses as the B/Yamagata component. Responses were lowest at dose level 1 but otherwise did not follow a dose-dependent pattern (highest by GMT at dose level 5 and highest by mean fold response at dose level 3). Thus, the highest response based on GMT was observed at dose level 5 for 3 of the 4 components (H1, B/Yamagata, and B/Victoria).

Day 0 mean SPRs were high and ranged from 44.7% to 96.9% (Table 5). At all dose levels and for all components, mean SPRs were >90% at day 21 (with the exception of H3 [VAX181] at dose level 1; Table 5 and Supplementary Figure 2A). Seroprotection rates at day 21 were not statistically significantly different compared with rates at dose level 1 for any component.

Mean SCRs at day 21 (Table 5 and Supplementary Figure 2B) for H1 (VAX128C) were higher at dose levels 1 and 2 (1 and 2 mcg, respectively; 55.0% and 55.1%) than at dose levels 3, 4, and 5 (2, 2, and 3 mcg, respectively; 39.6%–46.8%). The lower limit of the 95% CI was >40%, as recommended by CBER, for dose level 2.

Mean SCRs for H3 (VAX181) at day 21 were 50.0%, 58.2%, and 62.5% for dose levels 1, 2, and 3, respectively (1, 2, and 4 mcg). The lower limit of the 95% CI was >40% for dose levels 2 to 4.

For B/Yamagata (VAX173), mean SCRs at day 21 followed a dose-dependent pattern for dose levels 1, 2, 3, and 5. The lower limit of the 95% CI was >40% at dose levels 3 and 5 (the value was 39.1% for dose level 2).

For B/Victoria (VAX172), mean SCRs at day 21 did not follow a dose-dependent pattern, and the highest rate (54.1%) was observed for dose level 2. At this dose level, the lower limit of the 95% CI was >40%.

Taken together, the results suggest that dose levels 2 and 5 had very favorable immune responses. Ad hoc analyses evaluating titers showed a benefit for dose level 5 relative to dose level 2 for the B Yamagata (VAX173) component ($P = .024$). For SCRs, it is noteworthy that baseline titers and SPRs were high and, importantly, differed among the dose levels (Table 5). When SCRs for subjects at dose levels 2 and 5 were stratified by baseline titer

levels, they were 35% to 56% lower in subjects with starting titers >40 (Supplementary Figure 3). Ad hoc logistic regression analyses, using baseline titers as a covariate to correct for the confounding effect of the high baseline titers, showed that the SCRs were comparable at both dose levels for H1, H3, and B-VIC, and were positively impacted by dose level for the B-YAM strain (odds ratio = 2.38; $P = .014$).

Anti-flagellin titers were assessed on day 0 for dose levels 2 and 5. No relationship between individual flagellin titers versus HAI titers on day 21 (linear correlations $r^2 \leq 0.13$) or fold rises in flagellin IgG versus HAI titers on day 21 was observed ($r^2 \leq 0.33$). No relationship between fold rises in flagellin IgG and CRP was observed ($r^2 \leq 0.03$).

DISCUSSION

We have described the first-in-human trial of a novel, quadrivalent influenza vaccine comprised solely of recombinant HA-STF2 fusion proteins. We have shown that these fusion proteins elicit functional immune responses to the HA moiety in the absence of exogenous adjuvant, at dose levels that exhibit an acceptable tolerability profile.

The study was designed as a Phase I multicenter, open-label, dose-ranging study aimed at establishing a target dose and component ratio for future clinical studies of VAX2012Q. Interim findings for cohorts 1 through 5 did not suggest any serious safety concerns at any dose level, although the frequency and severity of systemic symptoms at dose level 4 (18 mcg total antigenic components) was higher than our target safety profile. At dose levels 2 and 5 (8 mcg and 12 mcg total antigen, respectively, 1:1 component ratio), incidence and intensities of local and systemic events and CRP and cytokine results were within the acceptable safety window and did not differ appreciably between the groups. In general, observations related to safety were unremarkable and consistent with the safety profiles of current licensed and investigational influenza vaccines [19, 20].

Dose levels 3 and 4 were designed to explore whether altered component levels and ratios could boost the immune response against the type B strains. However, assessment of GMT, SPRs, and SCRs suggested that the 1:1 ratios in dose levels 2 and 5 elicited the most favorable response. Furthermore, ad hoc analyses showed a benefit for dose level 5 relative to dose level 2 for the B-YAM (VAX173) component. Accordingly, the dose escalation plans for cohorts 6 and 7 were modified during the study to provide additional data in subjects receiving dose levels 2 and 5.

High pre-existing titers were observed in the study, particularly for dose level 5, at which subjects had significantly higher baseline titers against the 2 B strains compared with dose level 2 (Table 5). This may have affected SCRs.

Because of the timing and capacity constraints associated with seasonal influenza vaccine manufacturing along with the pandemic potential of influenza, efficiency, yield and vaccine dose are important considerations. The manufacturing advantages of VaxInnate's vaccine platform coupled with the low dose of antigen required demonstrate the potential of VaxInnate's platform.

CONCLUSIONS

All 4 components of VAX2012Q elicited seroprotective immune responses against their respective influenza type A and B viruses. At doses of 2 to 3 mcg per component, formulated at a 1:1 component ratio, VAX2012Q exhibited a tolerability and immunogenicity profile that favorably compares with licensed vaccines.

Supplementary Data

Supplementary material is available online at Open Forum Infectious Diseases online (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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Potential conflicts of interest. L. T., S. U., L. S. and G. L. are employees of VaxInnate Corporation. K. A. is a former employee and C. J. W. is a former employee and shareholder of VaxInnate Corporation. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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