Clinical Infectious Diseases

MAJOR ARTICLE

A Phase 2 Clinical Trial to Evaluate the Safety, Reactogenicity, and Immunogenicity of Different Prime-Boost Vaccination Schedules of 2013 and 2017 A(H7N9) Inactivated Influenza Virus Vaccines Administered With and Without AS03 Adjuvant in Healthy US Adults

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Introduction. A surge of human influenza A(H7N9) cases began in 2016 in China from an antigenically distinct lineage. Data are needed about the safety and immunogenicity of 2013 and 2017 A(H7N9) inactivated influenza vaccines (IIVs) and the effects of AS03 adjuvant, prime-boost interval, and priming effects of 2013 and 2017 A(H7N9) IIVs.

Methods. Healthy adults (n = 180), ages 19–50 years, were enrolled into this partially blinded, randomized, multicenter phase 2 clinical trial. Participants were randomly assigned to 1 of 6 vaccination groups evaluating homologous versus heterologous prime-boost strategies with 2 different boost intervals (21 vs 120 days) and 2 dosages (3.75 or 15 μg of hemagglutinin) administered with or without AS03 adjuvant. Reactogenicity, safety, and immunogenicity measured by hemagglutination inhibition and neutralizing antibody titers were assessed.

Results. Two doses of A(H7N9) IIV were well tolerated, and no safety issues were identified. Although most participants had injection site and systemic reactogenicity, these symptoms were mostly mild to moderate in severity; injection site reactogenicity was greater in vaccination groups receiving adjuvant. Immune responses were greater after an adjuvanted second dose, and with a longer interval between prime and boost. The highest hemagglutination inhibition geometric mean titer (95% confidence interval) observed against the 2017 A(H7N9) strain was 133.4 (83.6–212.6) among participants who received homologous, adjuvanted 3.75 μg + AS03/2017 doses with delayed boost interval.

Conclusions. Administering AS03 adjuvant with the second H7N9 IIV dose and extending the boost interval to 4 months resulted in higher peak antibody responses. These observations can broadly inform strategic approaches for pandemic preparedness. Clinical Trials Registration. NCT03589807.

Keywords. avian influenza; boost; antibody; 2013 H7N9; 2017 H7N9.

Received 23 January 2024; editorial decision 20 March 2024; published online 27 March 2024

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Clinical Infectious Diseases® 2024;78(6):1757–68

https://doi.org/10.1093/cid/ciae173

Emerging and reemerging infectious pathogens can rapidly spread through the progressively interconnected world to threaten human health [1]. Since the 1918 influenza pandemic, the emergence of influenza A strains from avian or porcine reservoirs highlights the need to prepare for the next influenza pandemic [2–8]. Influenza A(H7N9) viruses emerged to cause infections in people in 2013 [9], prompting studies of an inactivated 2013 H7N9 influenza vaccine [10–12]. A subsequent surge of human influenza A(H7N9) cases began in the fall of 2016 in China with emergence of the antigenically distinct Yangtze River Delta lineage [13, 14]. As a result, the US Department of Health and Human Services determined that influenza A(H7N9) virus has significant pandemic potential and supported the production of A/Hong Kong/125/2017 (H7N9) inactivated influenza vaccine (IIVs) for the US strategic national stockpile and for assessments of safety and immunogenicity. Additional drift within highly pathogenic A(H7N9) was subsequently observed, which rendered it capable of infecting ferrets via respiratory droplets without adaptation, supporting the potential for sustained human-to-human transmission (A/Guangdong/17SF003/2016) [15].

A critical question is how to optimize vaccine-induced immune responses against novel pathogens like A(H7N9). In general, vaccines made from novel avian influenza viruses are poorly immunogenic even at high hemagglutinin doses [10, 16, 17]. Several immunization strategies have shown potential to enhance immunogenicity. First, oil-in-water emulsion adjuvants, (eg, AS03 [GlaxoSmithKline Biologicals) are well tolerated and dose sparing resulting in increased antibody responses to IIVs containing novel hemagglutinins 10–12, 18–20]. Second, evidence with antigens of other pathogens (eg, influenza A(H5N1), modified vaccinia Ankara, anthrax, and coronavirus disease 2019 [COVID-19]) suggests that antibody responses are impaired by shortening the interval between prime and boost [21–25]. Extending the prime–boost interval may improve antibody responses [26–28] because of ongoing immune response maturation [29]. Finally, the use of heterologous prime–boost vaccination regimens could expand the breadth and durability of cross-clade antibody responses [26–28].

The goal of this clinical trial was to assess the safety, reactogenicity, and immunogenicity of 2013 and 2017 A(H7N9) IIVs in healthy adults to better understand the impact of dose, adjuvant, prime–boost interval (21 vs 120 days), and homologous versus heterologous priming effects of 2013 or 2017 A(H7N9) IIVs.

METHODS

Trial Design and Participants

After institutional review board approval at the participating institutions, adults aged 19–50 years who provided written informed consent were enrolled in this partially blinded (blinded to treatment assignment and unblinded to treatment interval), randomized, multicenter phase 2 clinical trial from 21 August to 13 November 2018.

Eligibility criteria included participants in good health or with stable chronic medical conditions without recent changes in prescription medication (Supplementary Methods). Eligible participants were randomly assigned to 1 of 6 vaccination groups (Supplementary Table 1) stratified by site and prior receipt of licensed, seasonal influenza vaccine in at least 1 of the 2017–2018 and/or 2018–2019 seasons. Subjects in study groups 1 and 4 received vaccination on days 1 and 22, whereas study groups 2, 3, 5, and 6 received vaccination on days 1 and 121. Subjects were followed through 12 months after their last study vaccination.

Study Product

The first dose was 3.75 mcg of either A/Shanghai/2/2013 (H7N9) or A/Hong Kong/125/2017 (H7N9) administered with AS03 adjuvant via 0.5 mL intramuscular injection into the deltoid muscle. The second dose was either 3.75 mcg of 2017 A(H7N9) administered with AS03 or 15 mcg of 2017 A(H7N9) administered without AS03 (Supplementary Methods).

Assessment of Subject Reactogenicity and Safety

The occurrence of solicited injection site and systemic reactions was assessed from the time of study vaccination through 7 days after each study vaccination. Unsolicited nonserious adverse events (AEs) were collected from the time of each study vaccination through 21 days after each study vaccination. Serious AEs (SAEs) and medically attended adverse events, including new-onset chronic medical conditions (NOCMCs) and potentially immune-mediated medical conditions (PIMMCs), were collected from first study vaccination throughout the study. Clinical safety laboratory evaluations were obtained before and 7 days after each study vaccination.

Assessment of Antibody Response

Antibody titers were assessed on day 1 before the first vaccination, 21 days after vaccination 1 (groups 1 and 4 only), 120 days after vaccination 1 (groups 2, 3, 5, and 6 only), and 21 days and 180 days after vaccination 2. The primary analysis included assessment of hemagglutinin inhibition (HAI) and neutralizing (Neut) antibodies against the A/Shanghai/2/2013 H7N9 strain and the A/Hong Kong/125/2017 H7N9 strain. The geometric mean titer (GMT), geometric mean fold rise from baseline, the percentage of subjects with a titer ≥40, and the percentage of subjects with seroconversion (defined as either a prevaccination titer <10 and a postvaccination titer ≥40 or a prevaccination titer ≥10 and a minimum 4-fold rise in postvaccination titer) were determined. In addition, HAI and Neut antibodies were assessed against the antigenically drifted A(H7N9) strain A/Guangdong/17SF003/2016. Plasmablasts were also analyzed for a subset of participants (Supplementary Methods).

Statistical Analysis

The sample size for this study was selected to obtain preliminary estimates of immune responses and common safety issues. The study was not designed to test a specific null hypothesis. The Safety Analysis Population consisted of all participants who received at least 1 study vaccination and for whom any safety data were available. The number and percentage of subjects reporting at least 1 solicited event following any vaccination were summarized for each solicited symptom, any systemic symptom, any injection site symptom, and any symptom. The associated 95% confidence intervals (CIs) were calculated using Clopper-Pearson methodology based on the binomial distribution. Logistic regression was performed to evaluate the effect of vaccine regimens on the probability of reporting any injection site solicited event or reporting any systemic solicited event. The incidence, frequency, timing, severity, and relatedness of unsolicited AEs were summarized.

Immunogenicity data summaries and analyses were performed for the modified intent-to-treat (mITT) population (Supplementary Methods). Immune responses measured by strain-specific HAI and Neut titers were summarized by study group at each time point and against each A(H7N9) vaccine strain. Exact Clopper-Pearson 95% CIs were calculated for proportional endpoints; 95% CIs for continuous endpoints were calculated using the Student *t*-distribution. The primary immunogenicity analysis was an assessment of these endpoints at 21 days after the second study vaccination. Secondary analyses included assessment of the previously described HAI and Neut against the A(H7N9) vaccine study strains at 21 days after the first study vaccination, immediately before the second study vaccination, and 180 days after the second study vaccination. As an exploratory analysis, multiple linear regression was performed to examine the relationship between log-transformed HAI and Neut titers and prime–boost (homologous [reference group] versus heterologous), boost dose (15 mcg [reference group] versus 3.75 mcg + AS03), and vaccination interval (21 days [reference group] versus 120 days), against the study vaccination strains at 21 days after the second vaccination.

RESULTS

Study Participants

A total of 254 healthy adults were screened and 180 were randomized between 21 August and 13 November 2018 (Figure 1). Overall, the median age was 29 years and the majority of enrolled subjects were female (59%), non-Hispanic (89%), and White (67%) (Table 1). Most subjects (82%) had not received the 2018–2019 seasonal vaccine before study participation. Enrolled subjects were randomized to 1 of 6 groups (Figure 1). Small numerical imbalances were observed between the groups because of the randomization by site and by prior receipt of licensed, seasonal influenza vaccine. Age, ethnicity, race, and body mass index were similarly distributed across study groups with the exception that group 1 had a higher proportion of males (59%).

Vaccine Reactogenicity & Safety

Injection site and systemic reactogenicity were observed in most participants. After both doses of vaccine, solicited injection site symptoms occurred in nearly all participants (94%), with tenderness (87%), pain (56%), and erythema (34%) occurring most commonly. Most injection site reactions were mild to moderate (or small to medium in size). Grade 3 injection site reactions occurred in 4% of participants after the first dose and 1% of participants after the second dose and were mostly erythema or induration/swelling. Solicited systemic symptoms occurred in most participants (63%), with fatigue (39%), myalgia (35%), and headache (27%) occurring most commonly. Most systemic symptoms were mild to moderate (Figure 2). A single individual had grade 3 arthralgia after dose 1 (group 6), and another had grade 3 fatigue after dose 2 (group 5). The logistic regression model after the second vaccine dose identified that receipt of 3.75 μg with AS03 was associated with increased odds (odds ratio, 37.87; 95% CI, 8.28–173.25) of injection site AEs (particularly pain, tenderness, and induration) when compared with 15 μg unadjuvanted vaccine. The longer boost interval was also associated with increased odds of injection site AEs (odds ratio, 7.61; 95% CI, 1.63–35.51) (Supplementary Table 2). The logistic regression model did not identify any factors associated with systemic symptoms after the boost. Vital sign data, laboratory findings, and unsolicited AEs are detailed in the Supplementary Results.

Of the 9 (5%) subjects who had unsolicited AEs that were considered by investigators to be related to vaccination, 6 were mild, 3 were moderate (episcleritis, upper respiratory infection, tendonitis), and none was severe (Supplementary Table 3, Supplementary Figure 1). The episcleritis also was a medically attended AEs and an NOCMC. No PIMMCs were reported in the study. An ectopic pregnancy followed by miscarriage occurred at an estimated gestational age of 16 days (146 days after the second vaccination) in 1 subject who became pregnant during the study. The 4 SAEs reported (1 in group 3, 2 in group 4, and 1 in group 6) were not considered related to vaccine, and no deaths occurred.

Immunogenicity Outcomes

HAI responses were detected against A/Hong Kong/125/2017 (A/H7N9) at ≥40 at baseline in ≤6% of participants in each group, with the exception of group 1, in which 11% of subjects had a baseline HAI titer ≥40 (Table 2, Figure 3). Overall, minimal increases in HAI and Neut titers were observed following the first study vaccination (Supplementary Table 4). HAI and Neut titers were highest and most highly correlated (Supplementary Figure 2) at 21 days after vaccination 2, at which time HAI

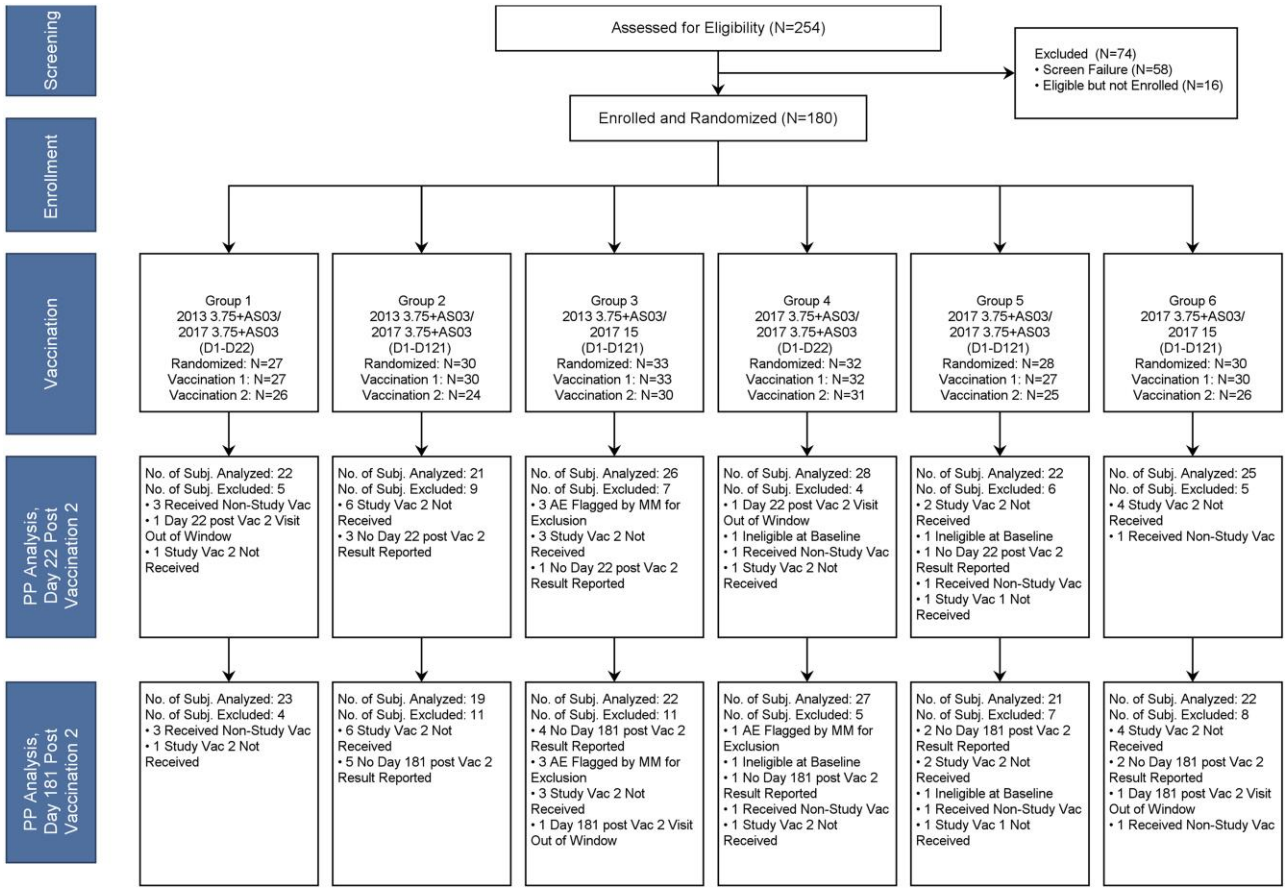

Figure 1. CONSORT flow diagram of subject participation. See Supplementary Methods and Results for additional details about the Per Protocol Analysis (PP) and reasons for exclusion. Abbreviations: AE, adverse events; CONSORT, Consolidated Standards of Reporting Trials; MM, medical monitor; Subj, subject; Vac, vaccine.

GMTs (95% CI) against A/Hong Kong/125/2017 were 75.7 (48.0–119.4) for group 1, 97.2 (55.9–169.0) for group 2, 43.1 (26.6–69.6) for group 3, 65.0 (42.4–99.5) for group 4, 133.4 (83.6–212.6) for group 5, and 33.2 (20.3–54.5) for group 6. The 15-μg unadjuvanted boost dose groups (groups 3 and 6) displayed modest increases in both HAI and Neut titers compared with the other study groups. At 21 days after vaccination 2, study groups with a 120-day vaccination interval and 3.75-μg + AS03 boost dose (groups 2 and 5) displayed higher GMTs, geometric mean fold rises, and rates of seroconversion for both study vaccine strains compared with other study groups (Table 2, Figure 3). By 180 days after vaccination 2, HAI and Neut titers decreased across study groups but remained higher than before receipt of the second vaccination. At 180 days, the HAI GMTs (95% CI) against A/Hong Kong/125/2017 were 24.7 (15.2–40.1) for group 1, 47.3 (24.7–90.7) for group 2, 13.8 (8.6–22.0) for group 3, 20.8 (13.5–32.1) for group 4, 38.8 (22.6–66.4) for group 5, and 8.5 (6.4–11.1) for group 6. Overall, HAI titers against the A/Shanghai/2/2013 H7N9 strain were lower compared with responses to the A/Hong Kong/125/2017 H7N9 strain across timepoints, whereas Neut titers were similar.

Linear regression models of log-transformed HAI and Neut antibody titers at 21 days after the second vaccination indicated that subjects who received the AS03-adjuvanted 3.75-μg boost dose had significantly higher mean log titers against both strains (Table 3) and against the antigenically drifted variant A/Guangdong/17SF003/2016 strain (Supplementary Table 5) compared with subjects who received the unadjuvanted boost dose. Subjects who had a 120-day interval between study vaccinations had significantly higher mean log titers against both vaccine strains and against the A/Guangdong/17SF003/2016 strain compared with subjects who had a 21-day interval between study vaccinations (Supplementary Table 6). Subjects who received a heterologous prime–boost had significantly higher mean log Neut titers against the A/Shanghai/2/2013 H7N9 strain compared with subjects who received a homologous prime–boost; no significant effects of heterologous boosting were observed for prime–boost on mean HAI log titer for either study strain (Table 3) or A/Guangdong/17SF003/2016 strain HAI (Supplementary Table 5a) or for mean Neut log titer against A/Hong Kong/125/2017 (Table 3) or A/Guangdong/17SF003/2016 (Supplementary Table 5b).

Table 1. Summary of Categorical and Continuous Demographic and Baseline Characteristics by Study Group, All Enrolled Subjects

Variable	Statistic	Characteristic	Group 1 2013 3.75 + AS03/ 2017 3.75 + AS03 (D1-D22) (N = 27)	Group 2 2013 3.75 + AS03/ 2017 3.75 + AS03 (D1-D121) (N = 30)	Group 3 2013 3.75 + AS03/ 2017 15 (D1-D121) (N = 33)	Group 4 2017 3.75 + AS03/ 2017 3.75 + AS03 (D1-D22) (N = 32)	Group 5 2017 3.75 + AS03/ 2017 3.75 + AS03 (D1-D121) (N = 28)	Group 6 2017 3.75 + AS03/ 2017 15 (D1-D121) (N = 30)	All Subjects (N = 180)
Sex	n (%)	Male	16 (59)	11 (37)	10 (30)	13 (41)	9 (32)	15 (50)	74 (41)
	n (%)	Female	11 (41)	19 (63)	23 (70)	19 (59)	19 (68)	15 (50)	106 (59)
BMI, kg/m^2	n (%)	<30	21 (78)	27 (90)	23 (70)	24 (75)	18 (64)	20 (67)	133 (74)
	n (%)	≥30	6 (22)	3 (10)	10 (30)	8 (25)	9 (32)	10 (33)	46 (26)
	n (%)	Unknown	-	-	-	-	1 (4)	-	1 (<1)
	Mean (SD)	-	27.1 (3.3)	25.0 (4.3)	28.3 (5.9)	26.3 (5.0)	27.2 (6.2)	28.2 (6.8)	27.0 (5.4)
	Median (Min, Max)	-	27.6 (20.1,32.8)	24.1 (18.3,38.6)	26.6 (18.2,45.2)	26.4 (19.2,38.5)	25.8 (18.7,40.1)	25.5 (17.0,41.5)	25.7 (17.0,45.2)
Age, y	n (%)	19–34	16 (59)	24 (80)	25 (76)	19 (59)	19 (68)	19 (63)	122 (68)
	n (%)	35–50	11 (41)	6 (20)	8 (24)	13 (41)	9 (32)	11 (37)	58 (32)
	Mean (SD)	-	32.3 (8.2)	29.7 (7.5)	29.6 (7.7)	32.8 (9.2)	31.2 (7.7)	30.6 (8.4)	31.0 (8.1)
	Median (Min, Max)	-	32.0 (21, 50)	29.0 (20, 48)	27.0 (19, 49)	32.5 (19, 50)	29.0 (21, 48)	27.5 (19, 48)	29.0 (19, 50)
Ethnicity	n (%)	Not Hispanic or Latino	25 (93)	26 (87)	29 (88)	28 (88)	25 (89)	28 (93)	161 (89)
	n (%)	Hispanic or Latino	2 (7)	4 (13)	4 (12)	4 (13)	3 (11)	2 (7)	19 (11)
Race	n (%)	Asian	1 (4)	2 (7)	-	1 (3)	-	2 (7)	6 (3)
	n (%)	Black or African American	5 (19)	5 (17)	11 (33)	6 (19)	9 (32)	4 (13)	40 (22)
	n (%)	White	18 (67)	22 (73)	20 (61)	23 (72)	17 (61)	21 (70)	121 (67)
	n (%)	Multiracial	3 (11)	-	2 (6)	2 (6)	2 (7)	2 (7)	11 (6)
	n (%)	Unknown	-	1 (3)	-	-	-	1 (3)	2 (1)
Prior seasonal influenza vaccination (prior seasons)	n (%)	2016–2017 received	15 (56)	16 (53)	14 (42)	20 (63)	19 (68)	17 (57)	101 (56)
	n (%)	2016–2017 not received	12 (44)	14 (47)	19 (58)	12 (38)	9 (32)	13 (43)	79 (44)
	n (%)	2017–2018 received	17 (63)	18 (60)	22 (67)	20 (63)	20 (71)	18 (60)	115 (64)
	n (%)	2017–2018 not received	10 (37)	12 (40)	11 (33)	12 (38)	8 (29)	12 (40)	65 (36)
Seasonal influenza vaccination (current season)	n (%)	2018–2019 received	6 (22)	3 (10)	6 (18)	7 (22)	6 (21)	5 (17)	33 (18)
	n (%)	2018–2019 not received	21 (78)	27 (90)	27 (82)	25 (78)	22 (79)	25 (83)	147 (82)
Prior receipt of H5 influenza vaccine	n (%)	No prior H5 influenza vaccination(s) received	24 (89)	29 (97)	30 (91)	31 (97)	27 (96)	28 (93)	169 (94)
	n (%)	Received prior H5 influenza vaccination(s)	3 (11)	1 (3)	3 (9)	1 (3)	1 (4)	2 (7)	11 (6)

N = Number of subjects enrolled. One subject with missing weight data is excluded from BMI summaries.

Abbreviations: BMI, body mass index; Max, maximum; Min, minimum; SD, standard deviation.

Logistic regression models of HAI and Neut seroconversion at 21 days after the second vaccination yielded generally similar results to the linear regression models. At 180 days, the AS03-adjuvanted boost and 120-day dosing interval remained statistically predictive of Neut seroconversion against both A/Hong Kong/125/2017 and A/Shanghai/2/2013 H7N9 strains (Supplementary Table 7).

HAI and Neut antibody responses against the antigenically drifted A/Guangdong/17SF003/2016 strain were generally lower in magnitude compared with A/Hong Kong/125/2017 but similar when compared with A/Shanghai/2/2013 and had similar kinetics across study time points (Supplementary Table 6). Plasmablast responses ascertained from a subset of participants are shown in Supplementary Figures 3 and 4. Broadly, hemagglutinin-specific responses were qualitatively greater than neuraminidase-specific responses across study groups, and the greatest plasmablast responses were hemagglutinin-specific IgG following the second vaccination.

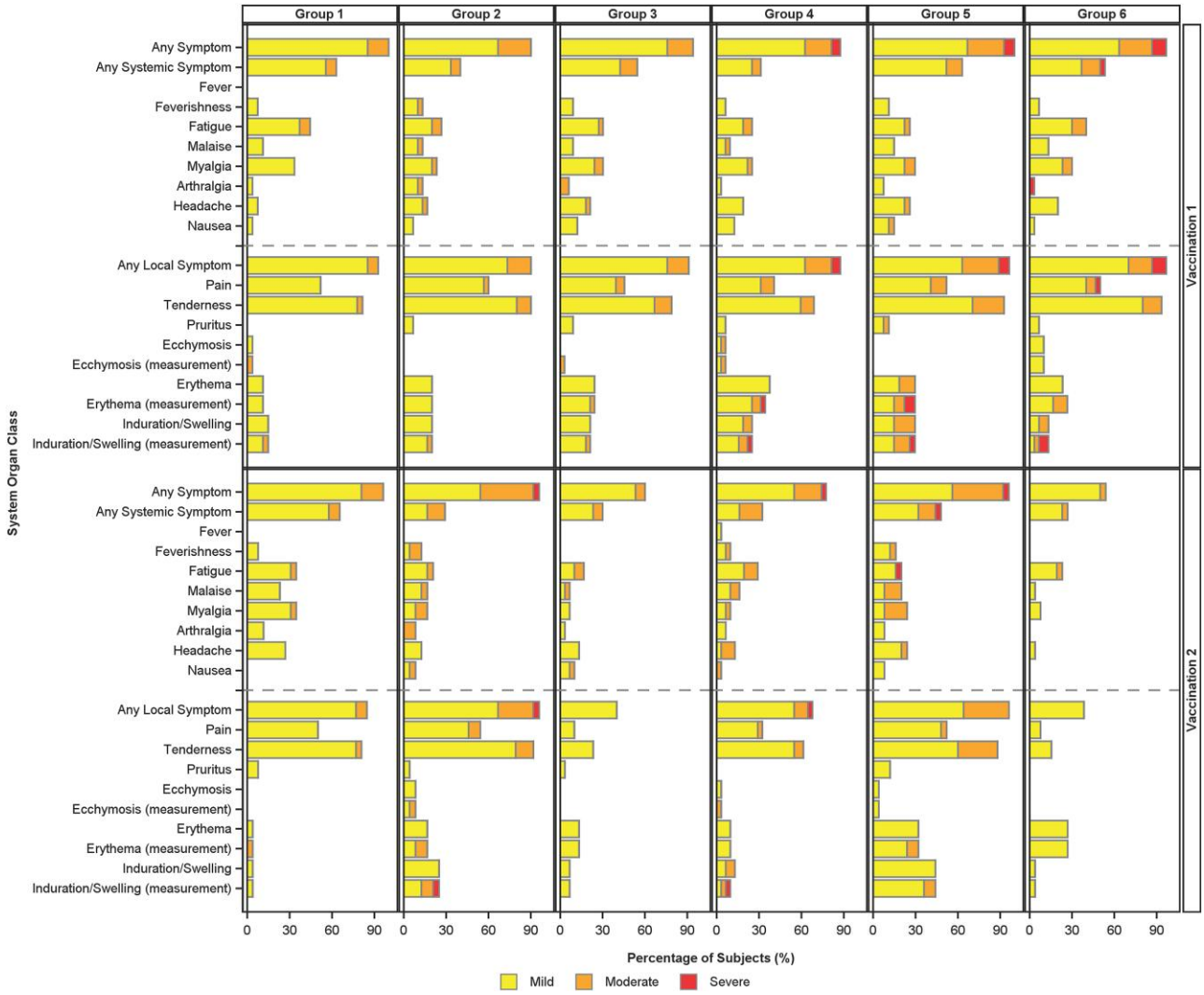

Figure 2. Systemic and injection site* reactogenicity of H7N9 influenza vaccination by dose and study group. *Grading for measurements of erythema and induration were small, medium, and large.

DISCUSSION

This phase 2 clinical trial assessed the safety, reactogenicity, and immunogenicity of various prime–boost regimens using 2013 and 2017 A(H7N9) IIVs. Immune responses were most robust with an AS03-containing boost, and with extending the prime–boost interval from 3 weeks to 4 months. Cross-reactive immune responses (HAI and Neut) observed against the antigenically drifted strain A/Guangdong/17SF003/2016 were generally similar to those observed against the A/Shanghai/2/2013 strain, but lower than those observed against the A/Hong Kong/125/2017 strain.

Overall, the vaccine formulations were safe and well tolerated. As has been observed in other H5 and H7 studies, injection site reactogenicity was greater with addition of adjuvant [2, 10–12, 30]. Although systemic symptoms have sometimes been more significant with AS03 adjuvant [11, 20], this was not observed in this study, perhaps because only a subset of the booster doses (groups 3 and 6) were administered without adjuvant. No PIMMCs occurred. The only pregnancy that occurred in this study was ectopic and ended in miscarriage at a gestational age of 16 days, which was considered unrelated to vaccine because miscarriages occur with a background incidence of 11%–21% of all recognized first trimester pregnancies [31]. A single participant had a NOCMC of episcleritis in group 5, which was considered related to vaccination by the investigator. Importantly, no vaccine-related SAEs occurred and no subjects died.

Baseline HAI and Neut titers were undetectable in nearly all participants, and a single dose resulted in minimal HAI and Neut responses [10–12]. The adjuvant-sparing approach to the boost dose (15 mcg of 2017 H7N9 without AS03) resulted in lower antibody responses than those observed with the antigen-sparing approach (3.75 mcg of 2017H7N9 with

Table 2. Summaries of Hemagglutination Inhibition Antibody Against A(H7N9) Strains by Study Day and Study Group, mITT Population

Time Point	Statistic	Group 1 2013 3.75 + AS03/ 2017 3.75 + AS03 (D1-D22) (N = 27)	Group 2 2013 3.75 + AS03/ 2017 3.75 + AS03 (D1-D121) (N = 28)	Group 3 2013 3.75 + AS03/ 2017 15 (D1-D121) (N = 33)	Group 4 2017 3.75 + AS03/ 2017 3.75 + AS03 (D1-D22) (N = 31)	Group 5 2017 3.75 + AS03/ 2017 3.75 + AS03 (D1-D121) (N = 27)	Group 6 2017 3.75 + AS03/ 2017 15 (D1-D121) (N = 30)
Hemagglutination inhibition antibody against A/Shanghai/2/2013 (A/H7N9)							
Day 1 (pre-vaccination 1)	n	27	28	33	31	27	30
	GMT (95% CI)[c]	7.1 (NC)	7.1 (NC)	7.1 (NC)	7.1 (NC)	7.3 (7.0–7.6)	7.1 (NC)
	Titer ≥ 40—% (95% CI)[d]	0 (0–13)	0 (0–12)	0 (0–11)	0 (0–11)	0 (0–13)	0 (0–12)
Day 22 (post-vaccination 1)	n	27	28	33	31	27	30
	GMT (95% CI)[c]	8.8 (6.8–11.4)	7.9 (6.9–9.0)	7.6 (6.8–8.5)	7.5 (7.0–8.1)	7.1 (NC)	7.1 (NC)
	Titer ≥ 40—% (95% CI)[d]	4 (0–19)	4 (0–18)	3 (0–16)	0 (0–11)	0 (0–13)	0 (0–12)
	GMFR (95% CI)[c]	1.2 (1.0–1.6)	1.1 (1.0–1.3)	1.1 (1.0–1.2)	1.1 (1.0–1.1)	1.0 (0.9–1.0)	1.0 (NC)
	Seroconversion—% (95% CI)[d]	4 (0–19)	4 (0–18)	3 (0–16)	0 (0–11)	0 (0–13)	0 (0–12)
Day 121 (post-vaccination 1)	n	NA	25	32	NA	27	28
	GMT (95% CI)[c]	NA	7.7 (6.6–8.8)	7.1 (NC)	NA	7.1 (NC)	7.1 (NC)
	Titer ≥ 40—% (95% CI)[d]	NA	4 (0–20)	0 (0–11)	NA	0 (0–13)	0 (0–12)
	GMFR (95% CI)[c]	NA	1.1 (.9–1.2)	1.0 (NC)	NA	1.0 (.9–1.0)	1.0 (NC)
	Seroconversion—% (95% CI)[d]	NA	4 (0–20)	0 (0–11)	NA	0 (0–13)	0 (0–12)
Day 22 (post-vaccination 2)[a]	n	27	22	31	31	26	27
	GMT (95% CI)[c]	35.9 (22.5–57.5)	53.8 (31.7–91.4)	22.6 (14.8–34.5)	19.5 (14.0–27.1)	50.6 (30.8–83.2)	17.0 (11.2–25.9)
	Titer ≥ 40—% (95% CI)[d]	52 (32–71)	77 (55–92)	32 (17–51)	35 (19–55)	69 (48–86)	19 (6–38)
	GMFR (95% CI)[c]	5.0 (3.1–8.0)	7.5 (4.4–12.8)	3.2 (2.1–4.8)	2.7 (2.0–3.8)	6.9 (4.2–11.4)	2.4 (1.6–3.6)
	Seroconversion—% (95% CI)[d]	52 (32–71)	77 (55–92)	32 (17–51)	35 (19–55)	69 (48–86)	19 (6–38)
Day 181 (post-vaccination 2)[b]	n	27	20	27	30	25	25
	GMT (95% CI)[c]	8.1 (6.9–9.4)	13.3 (9.5–18.6)	8.8 (7.3–10.7)	7.4 (7.0–7.8)	11.9 (8.8–16.0)	7.1 (NC)
	Titer ≥ 40—% (95% CI)[d]	4 (0–19)	10 (1–32)	4 (0–19)	0 (0–12)	12 (3–31)	0 (0–14)
	GMFR (95% CI)[c]	1.1 (1.0–1.3)	1.9 (1.3–2.6)	1.2 (1.0–1.5)	1.0 (1.0–1.1)	1.6 (1.2–2.2)	1.0 (NC)
	Seroconversion—% (95% CI)[d]	4 (0–19)	10 (1–32)	4 (0–19)	0 (0–12)	12 (3–31)	0 (0–14)
Hemagglutination inhibition antibody against A/Shanghai/2/2017 (A/H7N9)							
Day 1 (pre-vaccination 1)	n	27	28	33	31	27	30
	GMT (95% CI)[c]	7.4 (4.9–11.3)	5.6 (4.7–6.8)	6.8 (5.1–9.1)	6.5 (5.0–8.4)	6.0 (4.8–7.6)	5.5 (4.5–6.9)
	Titer ≥ 40—% (95% CI)[d]	11 (2–29)	4 (0–18)	6 (1–20)	6 (1–21)	4 (0–19)	3 (0–17)

Table 2. Continued

Time Point	Statistic	Group 1 2013 3.75 + AS03/ 2017 3.75 + AS03 (D1-D22) (N = 27)	Group 2 2013 3.75 + AS03/ 2017 3.75 + AS03 (D1-D121) (N = 28)	Group 3 2013 3.75 + AS03/ 2017 15 (D1-D121) (N = 33)	Group 4 2017 3.75 + AS03/ 2017 3.75 + AS03 (D1-D22) (N = 31)	Group 5 2017 3.75 + AS03/ 2017 3.75 + AS03 (D1-D121) (N = 27)	Group 6 2017 3.75 + AS03/ 2017 15 (D1-D121) (N = 30)
Day 22 (post-vaccination 1)	n	27	28	33	31	27	30
	GMT (95% CI)[c]	9.7 (6.7–14.0)	8.8 (5.9–12.9)	8.2 (5.9–11.4)	14.6 (8.8–24.2)	8.9 (6.5–12.2)	10.1 (6.5–15.5)
	Titer ≥ 10—% (95% CI)[d]	15 (4–34)	14 (4–33)	12 (3–28)	29 (14–48)	11 (2–29)	17 (6–35)
	GMFR (95% CI)[c]	1.3 (.8–2.2)	1.6 (1.1–2.2)	1.2 (.8–1.7)	2.3 (1.5–3.5)	1.5 (1.0–2.2)	1.8 (1.1–3.0)
	Seroconversion—% (95% CI)[d]	11 (2–29)	11 (2–28)	9 (2–24)	26 (12–45)	11 (2–29)	17 (6–35)
Day 121 (post-vaccination 1)	n	NA	25	32	NA	27	28
	GMT (95% CI)[c]	NA	9.3 (6.0–14.5)	8.1 (6.0–10.9)	NA	9.8 (5.9–16.2)	6.4 (4.8–8.5)
	Titer ≥ 40—% (95% CI)[d]	NA	20 (7–41)	6 (1–21)	NA	11 (2–29)	7 (1–24)
	GMFR (95% CI)[c]	NA	1.6 (1.1–2.4)	1.3 (.9–1.9)	NA	1.6 (.9–2.9)	1.1 (.8–1.7)
	Seroconversion—% (95% CI)[d]	NA	16 (5–36)	6 (1–21)	NA	11 (2–29)	7 (1–24)
Day 22 (post-vaccination 2)[a]	n	27	22	31	31	26	27
	GMT (95% CI)[c]	75.7 (48.0–119.4)	97.2 (55.9–169.0)	43.1 (26.6–69.6)	65.0 (42.4–99.5)	133.4 (83.6–212.6)	33.2 (20.3–54.5)
	Titer ≥ 40—% (95% CI)[d]	81 (62–94)	86 (65–97)	61 (42–78)	81 (63–93)	92 (75–99)	59 (39–78)
	GMFR (95% CI)[c]	10.2 (5.3–19.5)	18.6 (10.7–32.3)	7.0 (4.2–11.8)	10.0 (6.5–15.5)	21.9 (12.8–37.4)	5.9 (3.6–9.7)
	Seroconversion—% (95% CI)[d]	78 (58–91)	86 (65–97)	55 (36–73)	81 (63–93)	85 (65–96)	56 (35–75)
Day 181 (post-vaccination 2)[b]	n	27	20	27	30	25	25
	GMT (95% CI)[c]	24.7 (15.2–40.1)	47.3 (24.7–90.7)	13.8 (8.6, 22.0)	20.8 (13.5–32.1)	38.8 (22.6–66.4)	8.5 (6.4–11.1)
	Titer ≥ 40—% (95% CI)[d]	48 (29–68)	75 (51–91)	30 (14–50)	37 (20–56)	64 (43–82)	4 (0–20)
	GMFR (95% CI)[c]	3.3 (1.8–6.1)	9.0 (4.7–17.4)	2.2 (1.3–3.6)	3.2 (2.1–4.8)	6.3 (3.6–11.0)	1.5 (1.0–2.2)
	Seroconversion—% (95% CI)[d]	41 (22–61)	75 (51–91)	26 (11–46)	33 (17–53)	56 (35–76)	4 (0–20)

N = Number of subjects in the Modified Intent-to-Treat Population; n = Number of subjects with available results.

Abbreviations: CI, confidence interval; GMFR, geometric mean fold rise (relative to baseline); GMT, geometric mean titer; mITT, modified intention-to-treat; NA, not applicable; NC, not calculable.

[a]Day 22 postvaccination 2 varies by group (day 43 for groups 1 and 4; day 142 for groups 2, 3,5, and 6).

[b]Day 181 postvaccination 2 varies by group (day 202 for groups 1 and 4; day 301 for groups 2, 3,5, and 6).

[c]CI calculated based on the Student *t*-distribution.

[d]Exact binomial CI calculated using the Clopper-Pearson methodology.

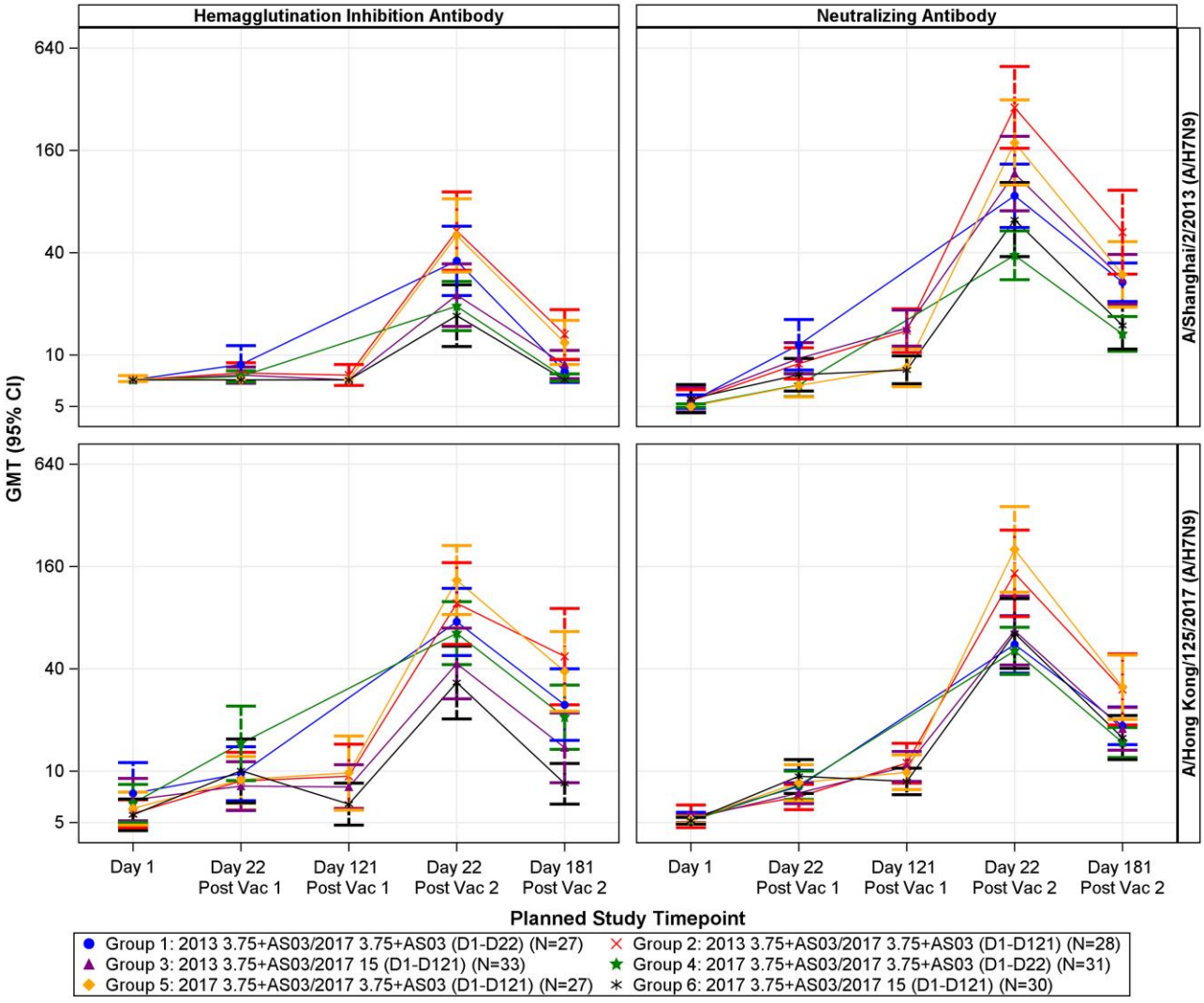

Figure 3. Geometric mean titers of hemagglutination inhibition and neutralizing antibodies by study day and study group, modified intent-to-treat population. Shown are GMTs with 95% CIs from participants before vaccination and up to 181 d after vaccination 2. Each line represents a study cohort over time. Note that groups 1 and 4 received the second dose at day 22 after vaccination 1 (and so do not have a day 121 postvaccination 1), whereas groups 2, 3, 5, and 6 received the second dose at day 121. Abbreviations: CI, confidence interval; GMT, geometric mean titer; Vac, vaccination.

AS03), similar to prior H7N9 studies [10, 11]. Unsurprisingly, individuals who received 2013 H7N9 for the first dose had better 2013 A(H7N9) HAI GMTs, while maintaining serological responses to 2017 H7N9, similar to other studies that have assessed a heterologous prime–boost approach [26–28, 32]. Use of such a heterologous prime–boost strategy could allow priming of a population in a pandemic before boosting with a homologous strain to provide strain-specific responses. Similar to the findings of others, significant declines in H7N9 antibody titers were observed by 6 months after the second dose [12, 33], although titers remained above baseline. This raises the question about whether a late boost (third dose) might be needed to provide protection in a persistent pandemic.

The timing of boost administration is recognized as having an impact on immunogenicity, as shortening the interval between the first and second doses to <3–4 weeks impairs the anamnestic serological responses [21–24, 26]. Here, we observed that extending the boost dose from 21 days to 4 months resulted in substantially higher HAI and Neut GMTs. These, and other trial and real-world data derived from the COVID-19 pandemic, suggest there is potential to improve immune responses across multiple vaccine platforms by delaying boost administration [25, 34, 35], but this approach must be weighed against the need to quickly generate immunological protection. Our data suggest that in a pandemic, vaccinating with a stockpiled related but distant H7N9 strain could provide some priming effect until a more closely matched booster vaccine could be manufactured. In a recent study, baseline HAI titers waned to <40 in >90% of participants at 5 years after a second dose of a 2013H7N9 IIV, but a single AS03-adjuvanted 2017H7N9 IIV dose resulted in ≥88%

Table 3. Multiple Linear Regression Model to Evaluate the Relationship of Administration Schedule With Log-Adjusted HAI Titer and Log-Adjusted Neutralization Titer to A(H7N9) Strains 21 Days After Second Study Vaccination, mITT Population

		A/Shanghai/2/2013 (A/H7N9) (N* = 164)				A/Hong Kong/125/2017 (A/H7N9) (N* = 164)			
Model Parameter	Parameter Category	Parameter Estimate	SE	95% CI	*P*	Parameter Estimate	SE	95% CI	*P*
Log-Adjusted HAI Titer									
Intercept	N/A	2.11	0.28	1.55–2.66	<.001	3.11	0.30	2.51–3.71	<.001
Prime–boost	Homologous (reference)	-	-	-	-	-	-	-	-
	Heterologous	0.33	0.18	−.01 to .68	.058	0.05	0.19	−.32 to .43	.779
Boost dose	15 mcg (reference)	-	-	-	-	-	-	-	-
	3.75 mcg + AS03	0.99	0.22	.56–1.42	<.001	1.11	0.24	.64–1.58	<.001
Interval	21 d (reference)	-	-	-	-	-	-	-	-
	120 d	0.70	0.22	.27–1.13	.002	0.50	0.24	.04–.97	.035
Log-adjusted neutralization titer									
Intercept	N/A	2.75	0.31	2.15–3.35	<.001	3.03	0.30	2.45–3.62	<.001
Prime–boost	Homologous (reference)	-	-	-	-	-	-	-	-
	Heterologous	0.64	0.19	.27–1.02	<.001	−0.05	0.19	−.42 to .31	.783
Boost dose	15 mcg (reference)	-	-	-	-	-	-	-	-
	3.75 mcg + AS03	0.98	0.24	.51–1.45	<.001	0.97	0.23	.51–1.42	<.001
Interval	21 d (reference)	-	-	-	-	-	-	-	-
	120 d	1.38	0.24	.90–1.85	<.001	1.19	0.23	.73–1.64	<.001

N = 176 (Number of subjects in the mITT population); N* = Number of subjects with uncensored results available 21 days after second study vaccination in the mITT population.

0 subjects missing covariate data were excluded from this analysis.

Abbreviations: CI, confidence interval; HAI, hemagglutination inhibition; mITT, modified intention-to-treat; N/A, not available; SE, standard error.

of participants seroconverting at 3 weeks after this late boost [36]. These results suggest long-lasting memory B cells are capable of rapidly responding to a heterologous HA.

Limitations of this study include the small number of participants in each group, which decreased our ability to assess for rare safety events and to identify small differences in immunogenicity. The study did not include children or adults >50 years of age, or all potential iterations of vaccine, dose, adjuvant, and interval. Other functional antibody, T-cell, and memory B-cell responses were not assessed. Because correlates of protection for avian influenza strains are poorly understood, additional data are needed to inform potential efficacy. Although our study evaluated only the IIV platform, it is likely that other more adaptable vaccine platforms may be implemented in a future pandemic. Nevertheless, the data generated from our study corroborate the immunologic benefits of heterologous and delayed interval boosting as observed with COVID-19 vaccines across multiple vaccine platforms.

In conclusion, we found the administration of 2013 and 2017 H7N9 influenza vaccines with AS03 using variable dosing intervals to be safe and immunogenic. Utilization of AS03 adjuvant with both the first and the second dose allowed for H7N9 antigen sparing. Extending the interval between prime and boost doses from 21 days to 4 months resulted in higher peak antibody titers. HAI and Neut antibodies declined by 6 months after boost, which suggests the potential need for another dose in a prolonged pandemic setting. These data are informative for facilitating pandemic preparedness and for optimally designing future pandemic vaccine clinical trials.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. R. L. A., E. B. W., S. F., J. L. M., P. C. R., M. J. M., and E. J. A. conceived and designed the study. C. A. R., R. L. A., E. B. W., S. F., J. L. M., A. S., L. L., C. M. K., V. R., H. M. E., W. A. K., J. A. W., M. J. S., K. E. S., G. K. W., G. A., P. W., W. B., Y. X., I. Y., S. K., N. R., M. J. M., and E. J. A. collected data. L. L. and Y. X. performed laboratory experiments and analyzed data. R. T., K. C., and A. W. performed primary statistical analysis. C. A. R., N. R., and E. J. A. drafted the manuscript. All authors provided intellectual contribution, reviewed and approved of the final manuscript.

Acknowledgments. The authors thank the volunteers who consented to participate in this study. The Emory Children's Center-Vaccine Research Clinic and Hope Clinic of the Emory Vaccine Center teams, including Larry Anderson, Teresa Ball, Theda Gibson, Laila Hussaini, Joanne Altieri-Rivera, Sreelatha Aramgam, Dilshad Rafi Ahmed, Alexis Ahonen, Alicarmen Alvarez, Kristy Bachman, Dawn Battle, Mary Bower, Laura Clegg, Sharon Curate-Ingram, Matthew Collins, Rijalda Deovic, Alexandra Dretler, Ana-Maria Drobeniuc, Francine Dyer, Srilatha Edupuganti, Tamera Franks, Tigisty Girmay, Felicia Glover, Rebecca Feng Gonzalez, Lisa Harewood, Mary Hart, Christopher Huerta, Brandi Johnson, Sara Jo Johnson, Taylor Johnson, Ariel Kay, Colleen Kelley, Peggy Kettle, Lilin Lai, Pamela Lankford-Turner, Tiraje Lester, Hollie Macenczak, Lisa Macoy, Michele Mccullough, William Millians, Regina Mosley, Amy Muchinsky, Tuong-Vy N Ngo, G Eileen Osinski, Ester Sue Park, Etza Peters, Varun Phadke, Divyanshu Rehaja, Brittany Robinson, Susan Rogers, April Ross, Nadine Rouphael, Brittany Robinson, Robert William Shepard, Kathleen Stephens, Ashley Tippett, Jessica Traenkner, Cynthia Whitney, Dongli Wang, Juton Winston, Terra Winter, Jinguo

Xu, Yongxian Xu, Kathryn Zaks, Jingru Zhao, Angelle Ijeoma, Inara Jooma, Dean Kleinhenz, and Wensheng Li. The BCM study team, including Caroline Doughty-Skierski, Coni Cheesman, PA-C, Barbara Annette Nagel, RN, Connie Rangel, RN, Janet Brown, PharmD, Lisreina Toro, MD, Kathy Bosworth, and Yvette Rugeley. The University of Iowa study team, including Jack Stapleton, Dilek Ince, Deb Pfab, Laura Stulken, Michelle Rodenburg, and A. J. Carr. The Saint Louis University Center for Vaccine Development study team, including Joan Siegner, Janice Tennant Kate Leifer, Missy Loyet, Sabrina Dipiazza, Linda Eggemeyer-Sharpe, Karla Mosby, Carol Duane, Kathleen Chirco, Irene Graham, Daniel Hoft, Donald Kennedy, Geoffrey Gorse, Sarah George, Soumya Chatterjee, Kiana Wilder, Nicole Purcell, Susan Stewart, Helay Amini, Michelle Harris, Richard Nickel, Fred Gattas, Tamara Blevins, Yinyi Yu, Azra Blazevic, Huan Ning, Chase Colbert, Kristy White, Fahreta Hamzabegovic, Amanda Nethington, Krystal Meza, Camerra Miller, and Keith Meyer. The Duke Vaccine and Trials Unit study team, including Lynn Harrington RN, BSN, Lori Hendrickson RN, BSN, Stephanie Smith, Luis Ballon, Joyce Gandee, Elizabeth Schmidt, Christopher Todd MPH, and Thad Gurley MS. From Emmes, the authors thank Shu Yang and the rest of the study team. The authors also thank the Division of Microbiology and Infectious Diseases (DMID) for their support of this study.

Financial support. This work was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health under Contract Number HHSN272201300018I (N.R.) to Emory University, HHSN272201300015I (H. M. E. S.) to Baylor College of Medicine, HHSN272201300020I to the University of Iowa, National Institutes of Health grant UL1 TR002537 to the University of Iowa, HHSN272201300021I to the Saint Louis University, and HHSN272201300017I (E. B. W.) to Duke University. The content is solely the responsibility of the authors and does not necessarily reflect the official views of the National Institutes of Health. Additional support was provided by the Georgia Research Alliance, the Emory University School of Medicine, and Children's Healthcare of Atlanta, and New York University institutional funds. The H7N9 vaccine and AS03 adjuvant were manufactured by Sanofi and GSK, respectively. The work reported here was also supported in whole or in part with federal funds from the U.S. Department of Health and Human Services Administration for Strategic Preparedness and Response, and BARDA under contracts HHSO100201600004I and HHSO100201600006I. The findings and conclusions in this work are those of the authors and do not necessarily represent the views of the U.S. Department of Health and Human Services or its components. AS03 is a trademark owned by or licensed to the GSK group of companies.

Potential conflicts of interest. E. J. A. has consulted for Pfizer, Sanofi Pasteur, GSK, Janssen, Moderna, and Medscape, and his institution has received funds to conduct clinical research unrelated to this manuscript from MedImmune, Regeneron, PaxVax, Pfizer, GSK, Merck, Sanofi-Pasteur, Janssen, and Micron. He has served on a safety monitoring board for Kentucky BioProcessing, Inc. and Sanofi Pasteur. He has served on a data adjudication board for WCG and ACI Clinical. His institution has also received funding from the National Institutes of Health (NIH) to conduct clinical trials of COVID-19 vaccines. He is currently employed by Moderna, Inc. N. R. has consulted for EMMES, Moderna, GSK, Sanofi, Seqirus, and ICON, and her institution receives funds to conduct clinical research unrelated to this manuscript from Pfizer, Sanofi, Quidel, Lilly, Immorna, Vaccine Company, and Merck as well as NIH to conduct translational clinical studies and interventional clinical trials. P. W. has consulted for EMMES and Pfizer and her institution has received funds to conduct clinical research unrelated to this manuscript from Pfizer, Glaxo Smith Kline, Sanofi-Pasteur, and Gilead as well as NIH. C. A. R.'s institution has received funds to conduct clinical research unrelated to this manuscript from the Centers for Disease Control and Prevention, BioFire Inc, GSK, MedImmune, Janssen, Merck, Moderna, Novavax, PaxVax, Pfizer, Regeneron, and Sanofi-Pasteur. She is co-inventor of patented respiratory syncytial virus vaccine technology, which has been licensed to Meissa Vaccines, Inc. C. M. K.'s institution has received funds to conduct clinical research unrelated to this manuscript from the CDC, Pfizer, Merck, and the NIH to conduct clinical trials of the Moderna COVID-19 vaccine. V. R. is currently employed by Pfizer, Inc., and works in vaccine clinical research unrelated to the current manuscript. This material reflects the personal views of the author and may not reflect the views of her current employer. M. J. M. reported these potential competing interests: laboratory research and clinical trials contracts for vaccines or MAB with Lilly, Pfizer, and Sanofi; contract funding from USG/HHS/BARDA for research specimen characterization and repository; research grant funding from USG/HHS/NIH for vaccine and MAB clinical trials; personal fees for Scientific Advisory Board service from Merck, Meissa Vaccines, Inc., and Pfizer. EBW has received funding support from Pfizer, Moderna, Sequiris, Najit Technologies Inc, and Clinetic for the conduct of clinical trials and clinical research. E. B. W. has served as an advisor to Vaxcyte and consultant to ILiAD biotechnologies. A. C. S.'s institution has received funds to conduct clinical research unrelated to this manuscript from NIH, HIV Vaccine Trials Network, COVID Vaccine Prevention Network, International AIDS Vaccine Initiative, Crucell/Janssen, Moderna, Pfizer, and Sanofi-Pasteur. S. K.'s institution has received funds to conduct clinical research and vaccine trials unrelated to this manuscript from the CDC, Pfizer, Meissa, Emergent BioSolutions, and the NIH. I. Y. reported being a member of the NIAID/COVPN mRNA-1273 Study Group and her institution received funding to conduct clinical research from NIH, Gates Foundation, Centers for Disease Control and Prevention, Pfizer, and Moderna outside the submitted work. She has consulted for Merck, Sanofi-Pasteur, and UptoDate. S. E. F. has received funding to conduct research from Novartis, Moderna, Janssen, Pfizer, and Bavarian Nordic and is a member of the HIV Vaccine Trials Network safety monitoring board. G. A. has received funding to conduct research from Bavarian Nordic. All other authors report no potential conflicts.

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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