Vaccine 37 (2019) 435–443

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Safety and immunogenicity of influenza A(H5N1) vaccine stored up to twelve years in the National Pre-Pandemic Influenza Vaccine Stockpile (NPIVS)

Christine M. Oshansky [a], James Zhou [a], Yonghong Gao [a], Jo Ellen Schweinle [a], Karen Biscardi [a], Jennifer DeBeauchamp [b], Corrina Pavetto [a], Amy Wollish [c], BRITE Study Coordination Team[1], Richard J. Webby [b], Vittoria Cioce [a,*], Ruben O. Donis [a,*], Rick A. Bright [a]

[a] Biomedical Advanced Research and Development Authority (BARDA), Office of the Assistant Secretary for Preparedness and Response (ASPR), Department of Health and Human Services (HHS), Washington, DC, USA
[b] St. Jude Children's Research Hospital, Memphis, TN, USA
[c] PPD, Wilmington, NC, USA

ARTICLE INFO

Article history:
Received 11 May 2018
Received in revised form 23 November 2018
Accepted 26 November 2018
Available online 12 December 2018

Keywords:
NPIVS
Vaccine
Avian influenza
MF59
Stockpile

ABSTRACT

Background: As part of the U.S. Department of Health and Human Services (HHS) Pandemic Influenza Plan preparedness and response strategy, the National Pre-Pandemic Influenza Vaccine Stockpile (NPIVS) program was established by the Biomedical Advanced Research and Development Authority (BARDA) in 2005 with the goal of building and maintaining a stockpile of vaccines for influenza viruses with pandemic potential to vaccinate 20 million people in the critical workforce in the event of a pandemic. The NPIVS program continuously monitors the integrity of influenza vaccine antigens and adjuvants stored within the stockpile. In addition to monitoring physical and chemical properties in stability studies, it is important to regularly assess the safety and immunogenicity of stockpiled vaccines and adjuvants to maintain preparedness for use in the event of an influenza pandemic.
Methods: BARDA conducted a randomized, double-blinded Phase 2 clinical study with the oldest stockpiled influenza A(H5N1) antigen, stored over the previous 10–12 years administered with or without MF59® adjuvant, stored over the previous 2–7 years at the time of vaccination.
Results: Stockpiled vaccines were well-tolerated, adverse events were generally mild, and there was no drop in immunogenicity to the oldest stockpiled A(H5N1) vaccine. Compared to unadjuvanted vaccine, greater peak antibody responses were observed in subjects who were vaccinated with MF59-adjuvanted vaccines, regardless of antigen dose. Vaccination with the A(H5N1) vaccine antigen also results in cross-reactive antibody responses to contemporary circulating strains of A(H5) influenza viruses.
Conclusions: The frequency, type, and severity of AEs observed during this study are similar to historical clinical study data with A(H5N1) vaccines and MF59 adjuvant indicating that a stockpiled A(H5N1) vaccine appears to remain safe and tolerable. The vaccines were immunogenic when administered as a two-dose vaccine regimen in healthy adults, despite extended storage of HA antigen or MF59 adjuvant within the NPIVS. *Trial registration:* ClinicalTrials.gov: NCT02680002.

1. Introduction

To prepare and respond effectively to future influenza pandemic threats, the Biomedical Advanced Research and Development Authority (BARDA) within the Office of the Assistant Secretary for Preparedness and Response (ASPR) in the United States (US) Department of Health and Human Services (HHS) established and maintains the National Pre–Pandemic Influenza

* Corresponding authors at: 330 Independence Avenue, S.W. Room 640 G, Washington, DC 20201, USA.
E-mail addresses: vittoria.cioce@hhs.gov (V. Cioce), ruben.donis@hhs.gov (R.O. Donis).
[1] Members of the BARDA Ready In Times of Emergency (BRITE) Study Coordination Team are listed at the end of the text.

https://doi.org/10.1016/j.vaccine.2018.11.069
0264-410X/

Vaccine Stockpile (NPIVS). Because of the ever-present threat of sustained human-to-human transmission of novel and virulent influenza viruses, global surveillance studies continuously monitor influenza viruses circulating in animals and humans. As they are identified, the associated potential risk of a particular strain to cause a pandemic is characterized and ultimately informs decisions regarding development, manufacturing, use, stockpiling, and distribution of pre-pandemic vaccines (i.e., vaccines to viruses considered to have elevated pandemic potential) [1]. Formally initiated in 2005, the NPIVS program includes not only influenza virus seed lots for ready-manufacturing, but significant manufacturing and reagent development expertise and experience. The NPIVS is currently composed of adjuvants (AS03, MF59) and pre-pandemic influenza virus bulk antigen and final containers of vaccine manufactured from candidate vaccine viruses representing virus subtypes regarded to have the greatest potential to cause a pandemic. Vaccination remains an effective mitigation strategy for reducing the potentially devastating effects of a pandemic. As outlined in the HHS Pandemic Influenza Plan 2017 Update, stockpiled pre-pandemic influenza vaccines and adjuvants could be used for early vaccination efforts among individuals at increased risk of exposure while a vaccine well-matched to the circulating pandemic strain is produced for wider distribution [2]. Several studies conducted or supported by HHS, BARDA, and the National Institute of Allergy and Infectious Diseases (NIAID) have shown the safety and immunogenicity of various pre-pandemic vaccines, irrespective of the manufacturer, combined with AS03 or MF59, supporting emergency use of these vaccine/adjuvant combinations in the event of a pandemic [3–10].

In 2003, avian A(H5N1) influenza viruses of the A/goose/Guangdong/1/1996 lineage were detected in multiple countries and have subsequently been detected in Asia, Europe, Africa, and North America [11,12]. In response, vaccine derived from A/Vietnam/1203/2004 (H5N1; hereafter VN1203) was manufactured and stockpiled as both bulk antigen and multi-dose final container vaccine. This vaccine was clinically evaluated and found to be both safe and immunogenic at 90 μg HA per 1.0 mL dose [3,13,14]. This A(H5N1) vaccine was licensed by the US Food and Drug Administration (FDA) in 2007 for use in adults aged 18 to 64 years at an increased risk of A(H5N1) influenza virus exposure. Avian A (H5N1) influenza viruses are now endemic in poultry in some countries in Asia and Africa [12], and sporadic reports of transmission of avian influenza viruses from birds to humans continue [15]. The BARDA-sponsored study described here was performed as part of an overarching strategy designed to assess whether pre-pandemic influenza antigen and adjuvant that have been stockpiled for an extended period of time not only remain stable in terms of physicochemical properties, but also retain its safety profile and capacity to generate immune responses that are considered protective in the event of an A(H5) influenza pandemic.

2. Methods

2.1. Study design

This randomized, double-blinded, Phase 2 clinical study (Clinicaltrials.gov number: NCT02680002) assessed safety and immunogenicity after each of two doses of Influenza A/Vietnam/1203/2004 (H5N1) monovalent vaccine (Sanofi Pasteur, Swiftwater, PA) administered 21 days apart with or without stored MF59 adjuvant (Seqirus, Holly Springs, NC) (Table 1, Supplemental Table 1). It was conducted in healthy adults, 18–49 years of age, at 6 clinical sites within the US between March 2016 and April 2017 in accordance with Good Clinical Practice guidelines, Declaration of Helsinki, and all applicable regulations. All study-related documents were approved by BARDA, FDA, and an institutional review board. Written informed consent was obtained from all enrolled participants.

2.2. Study vaccine

Influenza A/Vietnam/1203/2004 (H5N1) subvirion inactivated monovalent HA antigen (Sanofi Pasteur) and MF59 adjuvant (Seqirus) were provided by the BARDA-managed NPIVS. All investigational products used in this study passed all release tests and met all specifications for both drug substance and final formulated drug product, and data were submitted, reviewed, and are on file as part of the Investigational New Drug (IND) application to FDA for this study. Vaccinations were administered on day (D) 0 and D21 at dosages of 7.5 μg or 15 μg HA with MF59 adjuvant (Groups A, B, C, D) or 90 μg HA unadjuvanted (Groups E, F).

Specifically, the vaccine administered to Groups A-E was produced in 2005, stored as bulk antigen, then formulated and filled in 2015 as 30, 60, and 90 μg HA/mL. The vaccine administered to Group F was bulk antigen produced in 2004, formulated and filled in 2006 at 90 μg HA/mL then stored within the NPIVS. MF59 adjuvant was either from bulk material manufactured in 2009 (Groups A and B), or recently manufactured in 2013 (Groups C and D). These two bulk lots of adjuvant were then filled in 2014.

The clinical study site mixed equal volumes of antigen and MF59 to yield 0.5 mL doses containing either 7.5 μg or 15 μg HA (Groups A, B, C, and D) with MF59 or administered as a 1.0 mL dose of 90 μg HA without MF59 (Groups E and F). See Table 1 for group descriptions. Vaccines were administered by intramuscular injection.

2.3. Safety

Safety assessments included vital sign measurements, physical examination, clinical safety laboratory tests, solicited local and systemic reactogenicity symptoms, and occurrence of adverse events (AEs), AEs of special interest (AESIs), and serious AEs (SAEs). All

Table 1
Study group definitions.

Study group	Target No. of subjects	Influenza A(H5N1) vaccine (manufacture year/fill year)	MF59 adjuvant (manufacture year/fill year)	Injection volume
A	70	**7.5 μg HA** (2005/2015)	2009/2014	0.5 mL
B	70	**15 μg HA** (2005/2015)	2009/2014	0.5 mL
C	70	**7.5 μg HA** (2005/2015)	2013/2014	0.5 mL
D	70	**15 μg HA** (2005/2015)	2013/2014	0.5 mL
E	70	**90 μg HA** (2005/2015)	Unadjuvanted	1.0 mL
F	70	**90 μg HA** (2004/2006)	Unadjuvanted	1.0 mL
TOTAL	**420**			

unsolicited AEs were assessed from the time the subject received the first vaccination through 21 days following the second vaccination (i.e., D42). AESIs and SAEs were assessed from D0 until exit from the study.

2.4. Immunogenicity assessments

Serum was collected before vaccination at baseline (D0), and on D21 (prior to second vaccination), D28, D42, and D201. Hemagglutination inhibition (HAI) and microneutralization (MN) assays using homologous antigen were performed by Southern Research (Birmingham, AL) against A/Vietnam/1203/2004xPR8 (CDC, Atlanta, GA). Cross-reactive immune responses were evaluated using 6 + 2 A/Puerto Rico/8/1934 (H1N1) reassortant containing the HA and NA of viruses representing divergent influenza clades: rg-A/Vietnam/1203/2004 (H5N1, clade 1), rg-A/Indonesia/05/2005 (H5N1, clade 2.1.3.2), rg-A/bar-headed goose/Qinghai Lake/1A/2005 (H5N1, clade 2.2), rg-A/Egypt/NO3072/2010 (H5N1, clade 2.2.1), rg-A/duck/Bangladesh/19097/2013 (H5N1, clade 2.3.2.1a), rg-A/duck/Vietnam/NCVD-1584/2012 (H5N1, clade 2.3.2.1c), and rg-A/gyrfalcon/Washington/41088-6/2014 (H5N8, clade 2.3.4.4).

2.5. Statistical analysis

Subjects were randomized to 1 of 6 study groups (Table 1). Medidata Rave® electronic data capture system (Medidata, New York City, NY) was used for data collection.

For each group, geometric mean titers (GMTs) of HAI and MN antibodies against VN1203 at baseline (D0), D21, D28, D42, and D201 were calculated, and asymptotic 95% confidence intervals (CIs) and associated p-values using t-distribution were determined for each individual or pooled group. If HAI or MN titer was undetectable, it was assigned a value of half the lower limit of detection, and titers $\geq$1280 were assigned a titer of 1280. Seroprotection rates (SPRs) were defined as the proportion of subjects achieving a serum HAI antibody titer $\geq$1:40 against the VN1203 antigen, and seroconversion rates (SCRs) were defined as the proportion of subjects achieving either a pre-vaccination HAI or MN titer <1:10 and a post-vaccination titer of $\geq$1:40 or a pre-vaccination HAI or MN titer of $\geq$1:10 and a 4-fold or greater increase of HAI or MN post-vaccination antibody titers against the VN1203 antigen at days 21, 28, 42, and 201.

Further details are provided as supplement.

3. Results

The objective of this study was to examine the safety, tolerability, and immunogenicity of two doses of VN1203 vaccine, stored over the previous 10–12 years in the NPIVS, when administered with or without MF59 adjuvant, stored over the previous 2–7 years in the NPIVS.

3.1. Study population

A total of 422 subjects were enrolled and randomly assigned to each treatment group (Fig. 1, Table 1). The mean subject age was 36.4 years (range: 18–49 years), with majority female (59.4%), white (69.0%), and not Hispanic or Latino (95.2%) subjects (Table 2). No notable differences were observed in the demographic characteristics across groups. The safety population included 419 subjects (99.3%) who received at least one dose, and 393 subjects (93.1%) who received both doses. There were 371 subjects (87.9%) who completed the study through the last immunogenicity time point (D201).

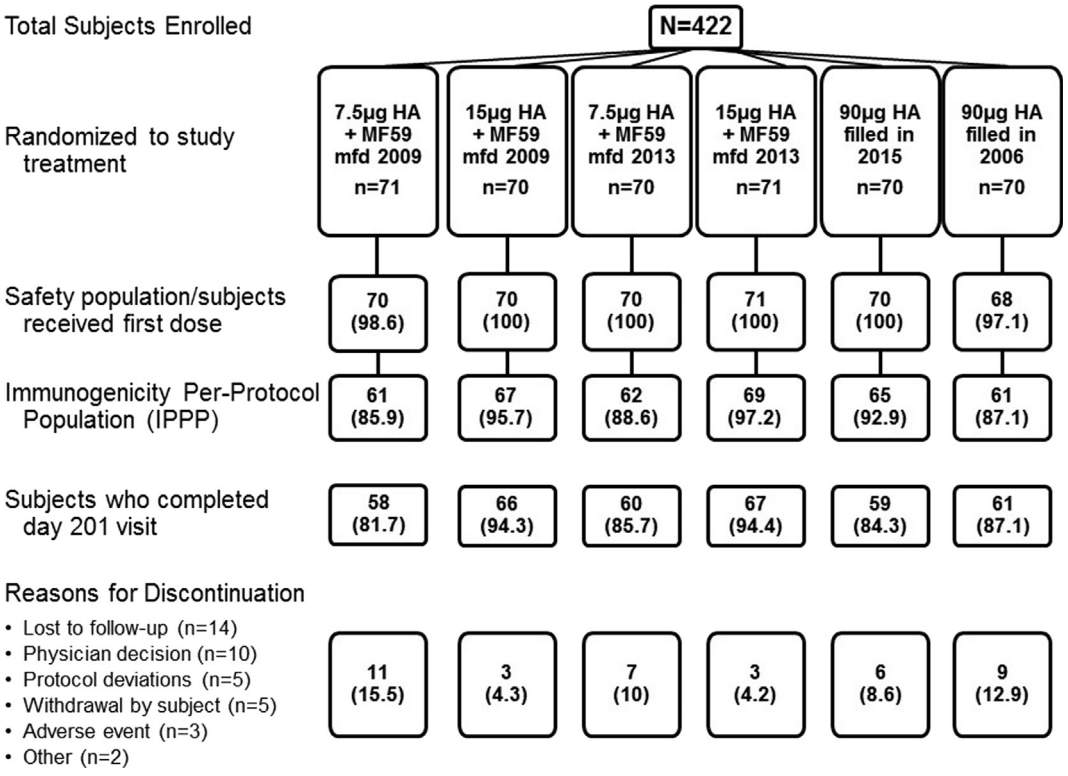

Fig. 1. Subject enrollment and randomization by treatment group. The Safety Population consisted of all subjects who were randomly assigned and received at least one dose of vaccine. The immunogenicity per protocol population (IPPP) included subjects who received two doses of randomized vaccine, had valid HAI results at the D42 visit, and had no major protocol deviations that might impact the assessment of immunogenicity.

Table 2
Safety population demographics.

	Study Group[a]						
	A n = 70	B n = 70	C n = 70	D n = 71	E n = 70	F n = 68	Total N = 419
Age (years)							
n	70	70	70	71	70	68	419
Mean (SD)	36.7 (8.02)	36.8 (8.47)	38.1 (8.37)	35.7 (8.25)	34.9 (8.62)	36.0 (7.60)	36.4 (8.24)
Median	38.0	37.5	38.5	37.0	37.0	35.0	37.0
Min, Max	21, 49	20, 48	18, 49	18, 48	18, 49	18, 49	18, 49
Age Categories (years), n (%)							
18 to <30	16 (22.9)	18 (25.7)	11 (15.7)	17 (23.9)	21 (30.0)	15 (22.1)	98 (23.4)
30 to <40	22 (31.4)	21 (30.0)	26 (37.1)	28 (39.4)	22 (31.4)	31 (45.6)	150 (35.8)
40 to 49	32 (45.7)	31 (44.3)	33 (47.1)	26 (36.6)	27 (38.6)	22 (32.4)	171 (40.8)
Sex, n (%)							
Male	27 (38.6)	20 (28.6)	37 (52.9)	28 (39.4)	29 (41.4)	29 (42.6)	170 (40.6)
Female	43 (61.4)	50 (71.4)	33 (47.1)	43 (60.6)	41 (58.6)	39 (57.4)	249 (59.4)
Race[b], n (%)							
White	43. (61.4)	52 (74.3)	50 (71.4)	47 (66.2)	51 (72.9)	46 (67.6)	289 (69.0)
Black or African American	27 (38.6)	14 (20.0)	18 (25.7)	22 (31.0)	17 (24.3)	18 (26.5)	116 (27.7)
Asian	0	1 (1.4)	1 (1.4)	0	0	1 (1.5)	3 (0.7)
American Indian or Alaska Native	0	0	0	1 (1.4)	2 (2.9)	0	3 (0.7)
Multiracial	0	2 (2.9)	1 (1.4)	0	0	1 (1.5)	4 (1.0)
Other	0	1 (1.4)	0	1 (1.4)	0	2 (2.9)	4 (1.0)
Ethnicity, n (%)							
Hispanic or Latino	4 (5.7)	5 (7.1)	3 (4.3)	4 (5.6)	2 (2.9)	2 (2.9)	20 (4.8)
Not Hispanic or Latino	66 (94.3)	65 (92.9)	67 (95.7)	67 (94.4)	68 (97.1)	66 (97.1)	399 (95.2)
Body mass index (kg/m^2)[c]							
Mean (SD)	32.79 (9.29)	30.76 (8.59)	31.75 (8.35)	30.50 (8.15)	31.76 (8.13)	31.57 (9.27)	31.52 (8.62)
Median	30.93	28.95	29.79	28.08	30.21	30.84	29.70
Min, Max	20.23, 63.66	17.76, 61.42	17.50, 56.96	18.89, 53.45	20.33, 54.13	17.89, 71.43	17.50, 71.43
Substance Type[d], n (%)							
Alcohol	38 (54.3)	40 (57.1)	45 (64.3)	42 (59.2)	40 (57.1)	42 (61.8)	247 (58.9)
Tobacco							
Cigarettes	16 (22.9)	15 (21.4)	22 (31.4)	16 (22.5)	12 (17.1)	12 (17.6)	93 (22.2)
Cigars	1 (1.4)	1 (1.4)	1 (1.4)	3 (4.2)	0	0	6 (1.4)
Pipes	0	0	0	0	0	0	0
Chewing tobacco	2 (2.9)	0	0	1 (1.4)	0	0	3 (0.7)
Subjects with at least one concomitant medication[e], n (%)	56 (80.0)	51 (72.9)	61 (87.1)	53 (74.6)	59 (84.3)	53 (77.9)	333 (79.5)

Abbreviations: min, minimum; max, maximum; SD, standard deviation.
[a] Study Groups were defined by the first vaccinations received by subjects.
[b] Subjects with more than one race category recorded on the case report form appear in the multiracial category.
[c] Body mass index = (body weight in pounds × 703)/(height in inches)^2.
[d] Substance usage at the time of enrollment.
[e] The most common concomitant medications taken by subjects were propionic acid derivatives (124 subjects [29.6%]), anilides (61 subjects [14.6%]), and plain multivitamins (44 subjects [10.5%]).

3.2. Safety and tolerability

The frequency and severity of symptoms reported are shown in Table 3 and Fig. 2. Overall, the vaccines were well-tolerated, regardless of manufacturing date, antigen dose, or inclusion of MF59. Most subjects (67.5%) reported generally mild symptoms in the first seven days following vaccination, with 3.3% of subjects reporting symptoms 8 to 21 days after vaccination. The most commonly reported symptoms considered related to vaccination were injection site pain (42.7%), headache (20.5%), fatigue (17.2%), and myalgia (12.4%) (Fig. 2b). There were no AEs leading to death or AESIs during the study, and there were no severe reactions at the injection site or allergic reactions reported. The total number of mild and moderate symptoms was similar across groups, and no significant differences in the number of subjects with at least one mild, moderate, or severe AE were observed across groups (data not shown). Finally, there were no significant differences observed in changes from baseline in clinical laboratory results or vital sign measurements in any group (data not shown).

3.3. Immunogenicity

Peak HAI and MN antibody titers were observed by D42 and decreased by D201 (Fig. 3a, b and Supplemental Table 2). Overall HAI antibody GMTs at D0, D21, D28, D42, and D201 were 5.2, 11.2, 19.2, 30.6, and 5.8, respectively, and overall MN antibody GMTs at D0, D21, D28, D42, and D201 were 5.7, 11.6, 23.8, 43.5, and 10.0, respectively.

To determine the effect of antigen dose, i.e., 7.5 μg HA versus 15 μg HA, and adjuvant on the antibody response, pooled study group comparisons (Groups A + C [7.5 μg HA + MF59], Groups B + D [15 μg HA + MF59], and Groups E + F [90 μg HA]) of HAI and MN antibody GMTs were conducted. HAI antibody GMT responses were similar (i.e., overlapping 95% CIs) between pooled groups at D0, D21, D28, and D201; however, on D42, subjects vaccinated with 7.5 μg HA + MF59 (Groups A + C) and 15 μg HA + MF59 (Groups B + D) showed greater HAI antibody GMTs (37.7 and 38.4, respectively) and MN antibody GMTs (55.5 and 53.6, respectively) compared to subjects vaccinated with 90 μg HA alone (Groups E + F) (HAI GMT = 19.6; MN GMT = 27.4) (Fig. 3a, b and

Table 3
Summary of all adverse events (safety population).

No. of Subjects with	Group A		Group B		Group C		Group D		Group E		Group F	
	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
	N = 70 n (%)	N = 63 n (%)	N = 70 n (%)	N = 68 n (%)	N = 70 n (%)	N = 65 n (%)	N = 71 n (%)	N = 69 n (%)	N = 70 n (%)	N = 65 n (%)	N = 68 n (%)	N = 63 n (%)
Any AE[a]	39 (55.7)	28 (44.4)	46 (65.7)	31 (45.6)	41 (58.6)	31 (47.7)	50 (70.4)	32 (46.4)	42 (60.0)	26 (40.0)	39 (57.4)	31 (49.2)
Any solicited local AE	25 (35.7)	20 (31.7)	31 (44.3)	20 (29.4)	27 (38.6)	17 (26.2)	27 (38.0)	21 (30.4)	22 (31.4)	17 (26.2)	25 (36.8)	21 (33.3)
Any solicited systemic AE	20 (28.6)	11 (17.5)	17 (24.3)	10 (14.7)	23 (32.9)	11 (16.9)	24 (33.8)	17 (24.6)	17 (24.3)	9 (13.8)	18 (26.5)	9 (14.3)
Any unsolicited AE	22 (31.4)	14 (22.2)	25 (35.7)	18 (26.5)	27 (38.6)	18 (27.7)	29 (40.8)	17 (24.6)	25 (35.7)	9 (13.8)	18 (26.5)	13 (20.6)
Any severe AE	1 (1.4)	0	1 (1.4)	0	3 (4.3)	0	3 (4.2)	0	1 (1.4)	1 (1.5)	2 (2.9)	0
Any SAE	0	0	0	0	2 (2.9)	1 (1.5)	0	0	0	0	1 (1.5)	0
Any AE related to investigational product	35 (50.0)	24 (38.1)	38 (54.3)	26 (38.2)	36 (51.4)	23 (35.4)	42 (59.2)	26 (37.7)	37 (52.9)	22 (33.8)	35 (51.5)	27 (42.9)
Any AE leading to study withdrawal	0	0	0	0	0	0	0	0	1 (1.4)	0	2 (2.9)	0

Abbreviations: AE, adverse event; SAE, serious adverse event.
[a] All AEs included all solicited AEs, unsolicited AEs, SAEs within 21 days of vaccination.

Supplemental Table 2). In addition, D42 GMT ratio estimates for HAI antibody responses were 1.92 ($p < 0.001$) and 1.96 ($p < 0.001$) for the 7.5 μg HA + MF59 (A + C) or 15 μg HA + MF59 (B + D) versus 90 μg HA alone (E + F) comparisons, respectively. Similar results were observed for pooled group comparisons at D42 for MN antibody responses as GMT ratio estimates were 2.03 ($p < 0.001$) and 1.96 ($p < 0.001$) for the 7.5 μg HA + MF59 (A + C) or 15 μg HA + MF59 (B + D) versus 90 μg HA alone (E + F) comparisons, respectively. Overall, groups receiving adjuvanted vaccine (Groups A to D) had greater HAI antibody titers compared to groups receiving unadjuvanted vaccine at a higher dose (Groups E and F).

The peak proportion of subjects achieving seroprotection against VN1203 was observed at D42, with an overall SPR of 54.8%. There was a trend of higher SPR on D42 in groups receiving adjuvanted vaccine (Groups A to D; range 56.7–71.0%) compared to the groups receiving unadjuvanted vaccine at a higher dose (Groups E and F; range 32.3–36.1%) (Supplemental Table 2). While no individual or pooled group met the FDA Center for Biologics Evaluation and Research (CBER) accelerated approval license criterion [16] for post-immunization titer ≥40 (i.e., lower bound of the two-sided 95% CI for the percent of subjects achieving an HAI antibody titer ≥40 should be ≥70%), pooled group comparisons indicated that subjects receiving 7.5 μg HA + MF59 (Groups A + C) and 15 μg HA + MF59 (Groups B + D) showed significantly greater SPRs of HAI antibodies on D42 (65.9% and 64.0%, respectively) compared to subjects vaccinated with 90 μg HA alone (Groups E + F) (34.1%) (Fig. 3c, Supplemental Table 2). The difference in SPR for HAI antibodies was 31.7% greater ($p < 0.001$) and 29.8% greater ($p < 0.001$) for the 7.5 μg HA + MF59 (A + C) versus 90 μg HA alone (E + F) and 15 μg HA + MF59 (B + D) versus 90 μg HA alone (E + F) comparisons, respectively. Therefore, MF59-adjuvanted VN1203 vaccine elicits greater peak antibody titers and SPRs, even if six-to-twelve fold less vaccine is administered as compared to unadjuvanted groups receiving 90 μg HA.

3.4. Heterologous antibody responses

To assess the magnitude and breadth of cross-clade antibody responses, exploratory analyses were conducted by HAI assay to various BPL-inactivated A(H5Nx) influenza viruses derived by reverse genetics on a PR8 backbone representing circulating viruses of diverse A(H5) HA antigenicity (Fig. 4). In general, the highest SPRs were observed using antigen homologous to the vaccine strain, however, cross-reactive HAI antibody responses were elicited to all heterologous antigens tested, including the most divergent clade 2.3.4.4 avian influenza virus tested, A/gyrfalcon/Washington/41088-6/2014 (H5N8). Similar to pooled SPRs to homologous VN1203 antigen (Fig. 3c), no group met the CBER criterion for post-immunization titer ≥40 against any of the other A(H5) influenza antigens tested, and no differences were observed between groups receiving vaccine with or without MF59. It should be noted that the heterologous antigens tested in these unqualified assays (Fig. 4) were BPL-inactivated as opposed to the qualified assays using homologous reverse genetics-derived live virus shown in Fig. 3.

4. Discussion

Because of continuous antigenic evolution of avian influenza viruses and the serious threat for significant morbidity and mortality in humans, there is concern that pre-pandemic influenza vaccines stockpiled for extended periods will not elicit sufficient antibody responses to an emerging A(H5) influenza virus. Confirming previous studies [4–7,17–19], we show that addition of MF59 adjuvant to an A(H5N1) vaccine has a positive role in generating enhanced immune responses, and that a pre-pandemic A(H5N1) vaccine, which has been stockpiled within the NPIVS for over 10 years, appears to remain safe and well-tolerated, eliciting a neutralizing antibody response when administered with MF59 adjuvant stored for over 5 years. The frequency, type, and severity of AEs observed during this study are aligned with historical clinical study data with A(H5N1) vaccines and MF59 adjuvant [20–22]. This study underscores the current NPIVS-managed stability testing plans which measure the potency of antigens over time, including their physical and chemical properties, are clinically relevant and support use of stockpiled pre-pandemic influenza vaccines under Emergency Use Authorization. Moreover, vaccination with the VN1203 vaccine results in modest cross-reactive antibody responses to contemporary circulating strains of A(H5) influenza viruses.

It is important to note that due to short protocol duration, our study was not powered to draw conclusions about safety in terms of unsolicited AEs, nor was it powered to test exploratory comparisons of immunogenicity endpoints between different treatment groups. No adjustments were made to control for multiple compar-

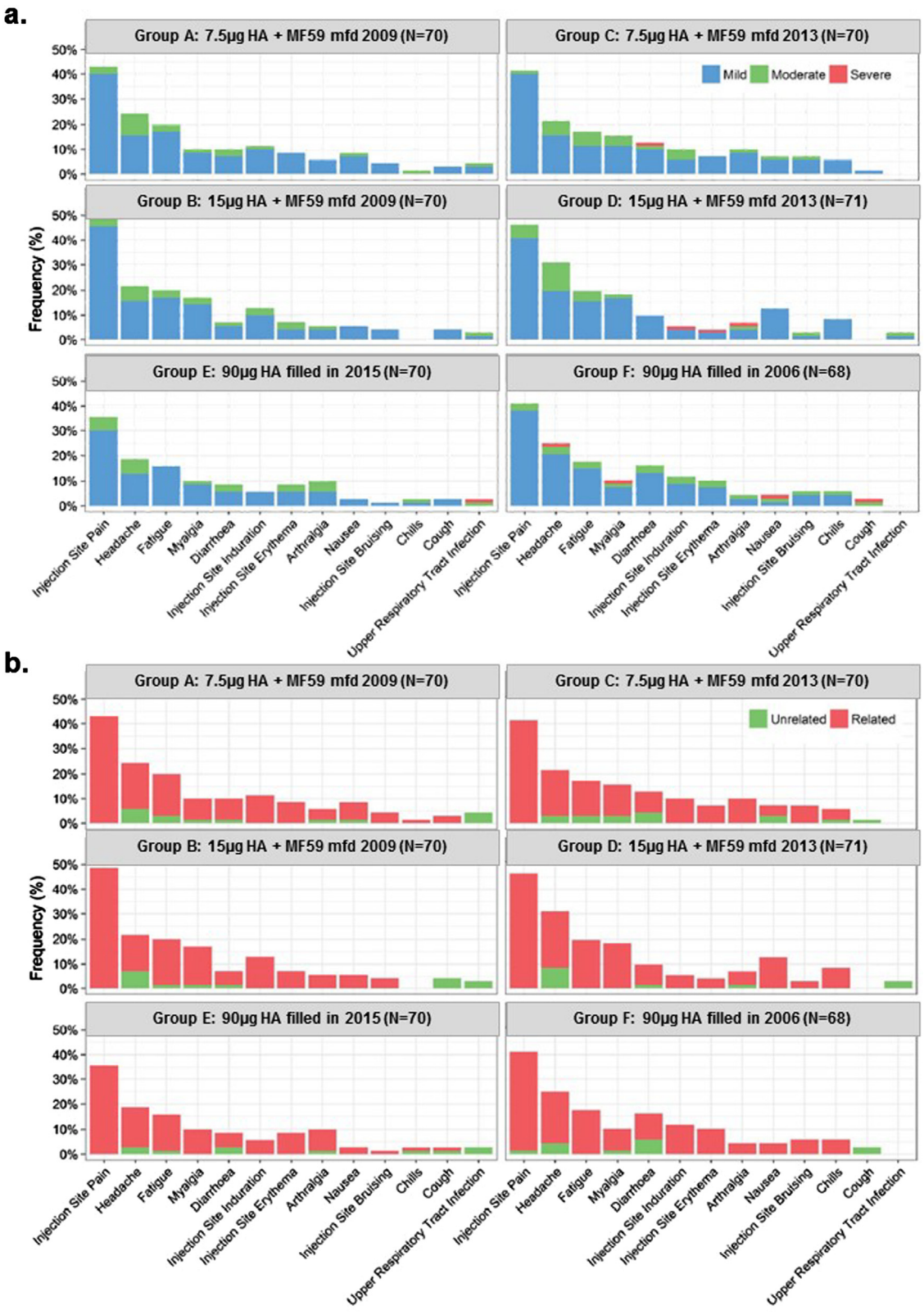

Fig. 2. Frequency and relatedness of adverse events. Solicited local reactions at the injection site included erythema/redness, induration/swelling, and pain. Solicited systemic reactions included fever, myalgia, arthralgia, fatigue, headache, nausea, vomiting, diarrhea, and chills. A vaccination injection site examination was performed to assess for pain, redness, swelling, bruising, and other local symptoms at the site of injection. (a) Subjects' reported symptoms were determined to be mild (blue), moderate (green), or severe (red). Symptoms were considered mild if they were transient in nature and generally not interfering with normal activities, moderate if they were sufficiently discomforting to interfere with normal activities, and severe if they were incapacitating and/or prevented normal activities. (b) Symptoms were considered related if there was a reasonable possibility that the symptom was related to vaccination, and all solicited local and systemic symptoms were considered as related to vaccination by the clinical site investigators. Only those AEs occurring in $\geq$2% of vaccinated subjects are plotted.

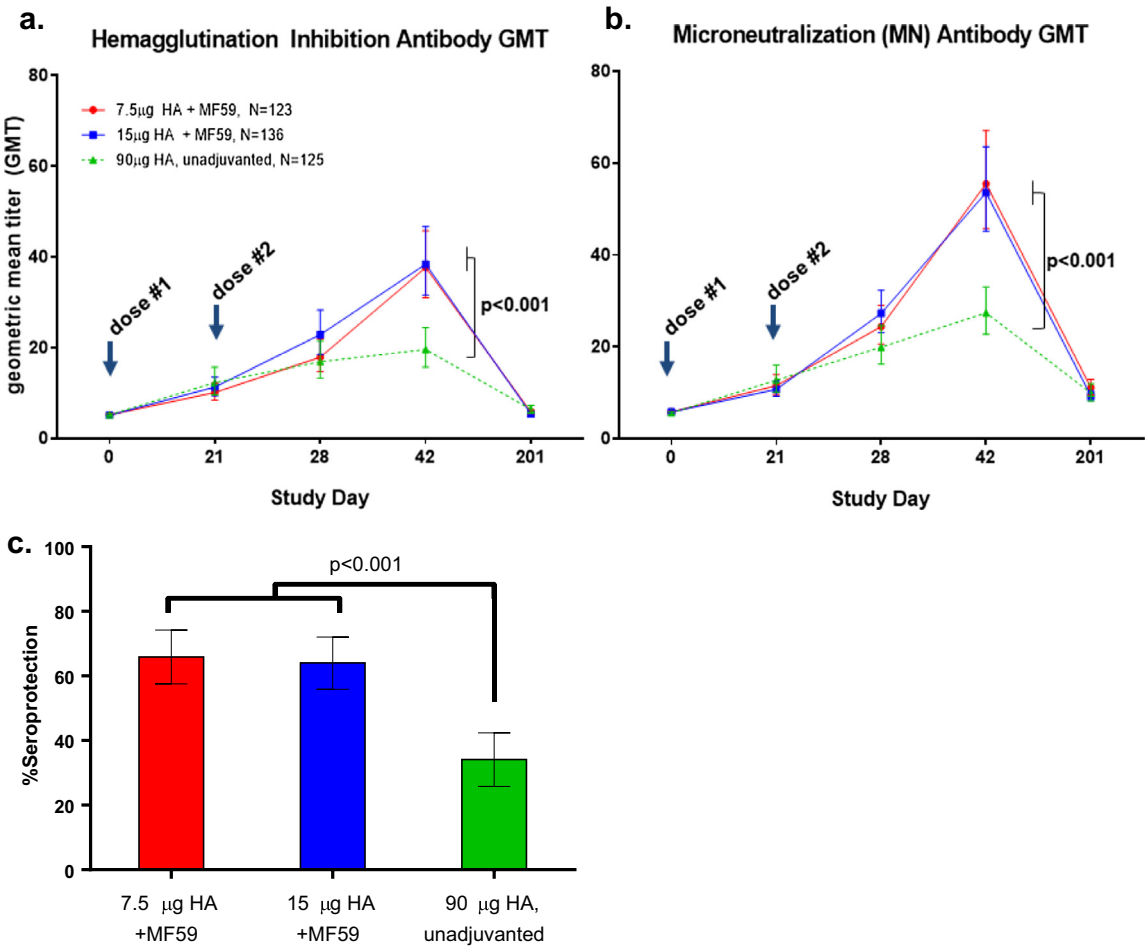

Fig. 3. MF59-adjuvanted VN1203 vaccination elicits higher antibody responses, regardless of vaccine dose. Hemagglutination inhibition (HAI) antibody responses (a), microneutralization (MN) antibody responses (b), and seroprotection rates on D42 (c) to homologous VN1203 (vaccine strain) by pooled group, i.e., 7.5 μg HA + MF59 (red), 15 μg HA + MF59 (blue), 90 μg HA (green). Results are shown as geometric mean titer (GMT); 95% confidence interval (CI; vertical bars), and days of vaccination (arrows) are shown. Seroprotection rates are shown as the proportion of subjects achieving a serum HAI antibody titer of at least 1:40 against the VN1203 antigen (seroprotection rate, SPR and 95% CI).

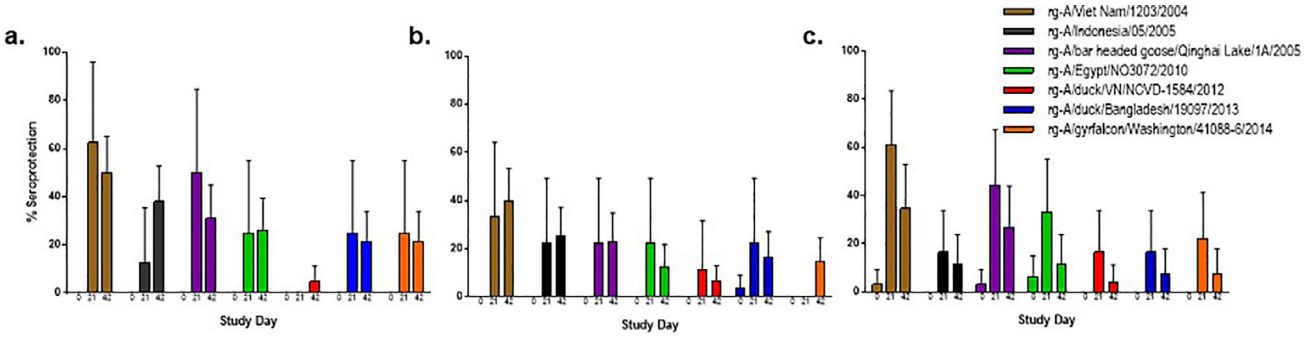

Fig. 4. VN1203 vaccination elicits cross-reactive hemagglutination inhibition (HAI) antibody responses to antigenically diverse HAs. Seroprotection rates by HAI to six heterologous A(H5) influenza viruses by study day and pooled group, (a) 7.5 μg HA + MF59, (b) 15 μg HA + MF59, and (c) 90 μg HA. Results are shown as the proportion of subjects achieving a serum HAI antibody titer of at least 1:40 against the designated antigen (SPR and 95% CI; vertical bars).

isons. Nonsignificant findings generated from these analyses may be explained by small sample size, and significant results could be spurious due to the absence of multiplicity adjustment. Nonetheless, pooled group comparisons were performed, combining groups by dose, i.e., 7.5 μg HA with MF59, 15 μg HA with MF59, and 90 μg HA unadjuvanted. Compared to subjects receiving two unadjuvanted 90 μg HA doses, both MF59-adjuvanted groups (7.5 μg HA and 15 μg HA) elicited significantly higher magnitude antibody responses and SPR, suggesting that inclusion of MF59, regardless of when it was manufactured, with a pre-pandemic A (H5) antigen is important for eliciting a potent immune response. In 2005, Sanofi Pasteur sponsored a clinical study in healthy adults to evaluate the safety and immunogenicity of an A/Vietnam/1203/2004 (H5N1) vaccine, and results showed that a higher vaccine dose (HA content ranging from 7.5 to 90 μg) was associated with a greater antibody response [3,13,14]. While none of the

study groups met the CBER-specified success criterion of 70% SPR for HAI antibodies [16], the Sanofi Pasteur A/Vietnam/1203/2004 (clade 1), 90 μg/mL vaccine was subsequently licensed with a 46% SPR [14]. Of note, this study (Group F) used the same 90 μg HA vaccine as these earlier studies, and despite storage in vials for 10 years in refrigeration and no measurable drop in potency over time, the vaccine elicited similar antibody titers and SPRs as previously reported [3,13,14].

A key challenge for the NPIVS program is management of stored material over time, with ongoing assessments of older stockpiled materials and prioritizing patient safety while balancing antigen stability and immunogenicity to maintain pandemic readiness. Influenza vaccines in particular have variable stability profiles influenced by the virus strain and specific manufacturing process complicating long-term storage predictions. The NPIVS program continuously monitors its stockpiled pre-pandemic influenza vaccines, and accumulated stability data indicates significant potency retention for long-term stored pre-pandemic influenza vaccine. This study was performed as part of an overarching strategy to assess whether pre-pandemic influenza vaccine and adjuvant can be stockpiled long-term and maintain its capacity to generate immune responses that are considered safe and protective. The data obtained from this study provide essential information regarding the safety and immunogenicity of stockpiled vaccines and adjuvants after extended storage. It remains to be determined whether stored vaccine can elicit improved antibody responses to emerging heterologous strains, but there is evidence suggesting superiority of a heterologous prime-boost vaccination regimen including adjuvant as compared to a homologous prime-boost vaccination regimen, and may result in an enhanced and broadened cross-reactive HAI antibody responses to antigenically divergent A(H5) influenza viruses [6,23,24]. Individuals primed 6 years earlier with an A/duck/Singapore/1997 (H5N3)-based vaccine elicits improved recall responses against VN1203 following a VN1203-based vaccine boost (Seqirus) [18,25]. Future NPIVS strategic goals will build on these results and include a heterologous antigen prime and boost vaccine trial (ClinicalTrials.gov Identifier: NCT03497845), whereby the priming potential of clade 1, 2.1.3.2, 2.3.2.1, or 2.3.4.4 stockpiled antigens can be determined. These studies are critical to inform decisions on the strategic value of the NPIVS and provide direct evidence to inform pandemic preparedness strategies.

The results of this study show for the first time that influenza antigen and adjuvant maintain their functional integrity and that it is possible to generate antibody responses from material stored for an extended period. Importantly, our data suggest that a two-dose regimen of 7.5 μg HA + MF59 would likely be well-tolerated in terms of safety, could be effective in preventing severe A (H5N1) influenza disease in healthy adults, and may provide some level of immune protection from divergent A(H5) influenza viruses. These results add to the growing body of evidence that antigen alone may not be sufficient in generating protective immune responses to influenza subtypes not currently circulating in humans and that use of adjuvant is critical to enhance the immune response as well as to provide dose-sparing capacity.

Financial disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

BRITE study coordination team members

Members of the BARDA Ready In Times of Emergency (BRITE) study coordination team are as follows: Kimberly Armstrong, Laura Gibson, Penny Hylton, Angela Jackson, James King, James Little, Michael O'Hara, Katina Robinson-Wright, Silvija Tresnjak-Smith, Robert Walker, Bai Yeh, and Debra Yeskey.

Acknowledgements

The authors thank the volunteers who participated in the trial and acknowledge the program managers, particularly Susan Reddel, Karen Hufham, Joy Wu, and Junli Zhang of PPD, clinical site investigators, study nurses and technicians, and Landon Westfall (Southern Research) who contributed to the study. Thank you is extended to Matthew McCord, Michele Riggs, Jasur Ishmukhamedov, Armen Donabedian, Li-Mei Chen, Jason Asher, Flora Castellino, and Robin Robinson (HHS) for their support and helpful discussion and to Kristina Bradford and Jonathan Edelman (Seqirus) for critical review of the manuscript. This work was funded by the Biomedical Advanced Research and Development Authority (BARDA) under contract HHSO100201400003I, order HHSO10033002T. Cross-reactivity antibody response work was supported by ALSAC. Opinions, findings and conclusions reported by the authors are strictly their own and are in no way meant to represent the opinion, views, or policies of ASPR/BARDA or HHS.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2018.11.069.

References

[1] Centers for Disease Control and Prevention. Influenza Risk Assessment Tool (IRAT). Internet.
[2] U.S. department of health and human services pandemic influenza plan 2017 Update. In: Services HaH, editor. Centers for Disease Control and Prevention; 2017.
[3] Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. New England J Med 2006;354:1343–51.
[4] Bernstein DI, Edwards KM, Dekker CL, Belshe R, Talbot HK, Graham IL, et al. Effects of adjuvants on the safety and immunogenicity of an avian influenza H5N1 vaccine in adults. J Infect Dis 2008;197:667–75.
[5] Jackson LA, Campbell JD, Frey SE, Edwards KM, Keitel WA, Kotloff KL, et al. Effect of varying doses of a monovalent H7N9 influenza vaccine with and without AS03 and MF59 adjuvants on immune response: a randomized clinical trial. JAMA 2015;314:237–46.
[6] Belshe RB, Frey SE, Graham IL, Anderson EL, Jackson LA, Spearman P, et al. Immunogenicity of avian influenza A/Anhui/01/2005(H5N1) vaccine with MF59 adjuvant: a randomized clinical trial. JAMA 2014;312:1420–8.
[7] Mulligan MJ, Bernstein DI, Frey S, Winokur P, Rouphael N, Dickey M, et al. Point-of-use mixing of influenza H5N1 vaccine and MF59 adjuvant for pandemic vaccination preparedness: antibody responses and safety. A Phase 1 Clinical Trial. Open For Infect Dis 2014;1:ofu102.
[8] Banzhoff A, Gasparini R, Laghi-Pasini F, Staniscia T, Durando P, Montomoli E, et al. MF59-adjuvanted H5N1 vaccine induces immunologic memory and heterotypic antibody responses in non-elderly and elderly adults. PLoS One 2009;4:e4384.
[9] Chen WH, Jackson LA, Edwards KM, Keitel WA, Hill H, Noah DL, et al. Safety, reactogenicity, and immunogenicity of inactivated monovalent influenza A (H5N1) virus vaccine administered with or without AS03 adjuvant. Open For Infect Dis 2014;1:ofu091.
[10] Jackson LA, Chen WH, Stapleton JT, Dekker CL, Wald A, Brady RC, et al. Immunogenicity and safety of varying dosages of a monovalent 2009 H1N1 influenza vaccine given with and without AS03 adjuvant system in healthy adults and older persons. J Infect Dis 2012;206:811–20.
[11] Lee DH, Bertran K, Kwon JH, Evolution Swayne DE. global spread, and pathogenicity of highly pathogenic avian influenza H5Nx clade 2.3.4.4. J Vet Sci 2017;18:269–80.
[12] Claes F, Morzaria SP, Donis RO. Emergence and dissemination of clade 2.3.4.4 H5Nx influenza viruses-how is the Asian HPAI H5 lineage maintained. Curr Opin Virol 2016;16:158–63.
[13] Sanofi Pasteur VRBPAC Briefing Document: H5N1 Influenza Virus Vaccine, A/Vietnam/1203/2004 (Clade 1) 90 mcg/ml. 2007.
[14] Memorandum: February 27, 2007b Meeting Topic 1: Safety and Immunogenicity of H5N1 Influenza Virus Vaccine, A/Vietnam/1203/2004 (clade 1), 90μg/mL, Manufactured by Sanofi Pasteur. In: Health and Human Services FaDA, Center for Biologics Evaluation and Research (CBER), Office of

Vaccine Research and Review, Division of Vaccines and Review and the Division of Vaccines and Related Product Applications, editor.
[15] (WHO) WHO. Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO; 2018.
[16] Research FaDACfBEa. Guidance for Industry: Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines. In: Services USDoHaH, editor; 2007.
[17] Black S. Safety and effectiveness of MF-59 adjuvanted influenza vaccines in children and adults. Vaccine 2015;33(Suppl 2):B3–5.
[18] Stephenson I, Nicholson KG, Hoschler K, Zambon MC, Hancock K, DeVos J, et al. Antigenically distinct MF59-adjuvanted vaccine to boost immunity to H5N1. New England J Med 2008;359:1631–3.
[19] Del Giudice G, Rappuoli R. Inactivated and adjuvanted influenza vaccines. Cham: Springer; 2014.
[20] Package Insert: Sanofi Pasteur Influenza Virus Vaccine, H5N1; 2007.
[21] Package Insert: Seqirus Inc. FLUAD; 2017.
[22] Pellegrini M, Nicolay U, Lindert K, Groth N, Della Cioppa G. MF59-adjuvanted versus non-adjuvanted influenza vaccines: integrated analysis from a large safety database. Vaccine 2009;27:6959–65.
[23] Levine MZ, Holiday C, Liu F, Jefferson S, Gillis E, Bellamy AR, et al. Cross-reactive antibody responses to novel H5Nx influenza viruses following homologous and heterologous prime-boost vaccination with a prepandemic stockpiled A(H5N1) vaccine in humans. J Infect Dis 2017;216:S555–9.
[24] Langley JM, Frenette L, Jeanfreau R, Halperin SA, Kyle M, Chu L, et al. Immunogenicity of heterologous H5N1 influenza booster vaccination 6 or 18 months after primary vaccination in adults: a randomized controlled clinical trial. Vaccine 2015;33:559–67.
[25] Khurana S, Coyle EM, Dimitrova M, Castellino F, Nicholson K, Del Giudice G, et al. Heterologous prime-boost vaccination with MF59-adjuvanted H5 vaccines promotes antibody affinity maturation towards the hemagglutinin HA1 domain and broad H5N1 cross-clade neutralization. PLoS One 2014;9:e95496.