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Phase I clinical evaluation of seasonal influenza hemagglutinin (HA) DNA vaccine prime followed by trivalent influenza inactivated vaccine (IIV3) boost

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Abstract

Annual influenza vaccination reduces the risks of influenza when the vaccines are well matched to circulating strains, but development of an approach that induces broader and more durable immune responses would be beneficial. We conducted two companion Phase 1 studies, VRC 307 and VRC 309, over sequential seasons (2008–2009 and 2009–2010) in which only the influenza B strain component of the vaccines differed. Objectives were safety and immunogenicity of prime–boost vaccination schedules. A schedule of DNA vaccine encoding for seasonal influenza hemagglutinins (HA) prime followed by seasonal trivalent influenza inactivated vaccine (IIV3) boost (HA DNA–IIV3) was compared to placebo (PBS)–IIV3 or IIV3–IIV3. Cumulatively, 111 adults were randomized to HA DNA–IIV3 (n = 66), PBS–IIV3 (n = 25) or IIV3–IIV3 (n = 20). Safety was assessed by clinical observations, laboratory parameters and 7-day solicited reactogenicity. The seasonal HA DNA prime–IIV3 boost regimen was evaluated as safe and well tolerated. There were no serious adverse events. The local and systemic reactogenicity for HA DNA, IIV and placebo were reported predominantly as none or mild within the first 5 days post-vaccination. There was no significant difference in immunogenicity detected between the treatment groups as evaluated by hemagglutination inhibition (HAI) assay. The studies demonstrated the safety and immunogenicity of seasonal HA DNA–IIV3 regimen, but the 3–4 week prime–boost interval was suboptimal for improving influenza-specific immune responses. This is consistent with observations in avian H5 DNA vaccine prime–boost studies in which a long

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interval, but not a short interval, was associated with improved immunogenicity. Trial Registration: NCT00858611 for VRC 307 and NCT00995982 for VRC 309.

Keywords

DNA vaccine; Seasonal influenza; Immune response

1. Introduction

The efficacy of seasonal influenza vaccines varies from year to year and is affected by type of vaccine, age group, and match to circulating strains [1,2]. Impaired immune responses in vulnerable populations of older adults and young children require two seasonal vaccine administrations for optimal results [3–5]. In addition, events surrounding the 2009 H1N1 pandemic influenza presented a clear demonstration of the long recognized need to develop influenza vaccines with broader and more durable immune responses [6]. The conduct of influenza vaccine clinical trials presents operational challenges due to the seasonality and unpredictable nature of influenza outbreaks. Widely varying pre-existing immunity of participants is a confounding variable when assessing immune responses to seasonal strains. Over two sequential seasons, the Vaccine Research Center (VRC), NIAID, NIH conducted two companion Phase 1 studies to evaluate a prime–boost approach for seasonal influenza. We report here the outcome of these studies and offer some observations about operational issues related to the conduct of vaccine studies complicated by constraints unique to seasonal influenza.

Compared to other vaccine technologies, rapid production of DNA vaccines that encode for particular antigens is relatively easy [7–12]. Priming with DNA vaccines is an approach studied by VRC/NIAID/NIH as a preventive vaccination strategy against multiple pathogens [9,13] as well as by others for different pathogens and cancer [14–17]. The DNA vaccine priming appears to expand the antibody epitope repertoire and increase affinity maturation, and to direct development of T cells with effector memory phenotype [18–20].

VRC has conducted a series of Phase 1 studies to evaluate HA DNA prime–inactivated influenza vaccine boost regimens. The clinical trials directed towards vaccine development for seasonal influenza strains that began in 2009 are similar in concept to those directed towards H5N1 influenza that began in 2008. Important differences in conducting these studies are that, while the U.S. study population has essentially no pre-existing immunity to H5N1 influenza, the widely variable immunity to seasonal influenza strains and annual outbreak cycles affect the conduct and interpretation of seasonal influenza study results. The studies of H5 DNA vaccine prime followed by inactivated H5N1 vaccine boost showed that 12–24 week prime–boost interval improves the frequency and magnitude of immune responses in comparison to inactivated H5N1 vaccine prime–boost regimens. There was also evidence of greater breadth of response, including epitopes conserved between influenza subtypes [9,18,21].

This finding was not yet known to us in 2009 when we embarked on the clinical trials reported here, in which the 3–4 week HA DNA prime–IIV3 boost intervals were used. This

shorter interval was evaluated as it facilitates completing a two vaccine regimen within the constraints of annual seasonal influenza circulation patterns. The control groups were placebo (PBS)–IIV3 and IIV3–IIV3 administered with the same interval. Both controls are of interest because a single injection of IIV3 is the standard for annual influenza vaccinations and because it is known that two injections are needed to confer better immune responses in populations without preexisting immune responses to novel HA antigens [22,23].

2. Methods

2.1. Study sites and participants

VRC 307 (NCT 00858611) and VRC 309 (NCT 00995982), respectively, included 51 healthy adults ages 18–70 years and 60 healthy adults ages 45–70 years. VRC 307 was conducted at the NIH Clinical Center (Bethesda, MD) while VRC 309 was conducted at the NIH Clinical Center and the Clinical Alliance for Research and Education — Infectious Diseases (CARE-ID) site in Annandale, VA.

IRB oversight for the NIH Clinical Center site was through the NIAID Institutional Review Board (IRB) for both studies. For VRC 309 at the CARE-ID site, IRB oversight was through the Chesapeake IRB (Chesapeake Research Review, Inc., Columbia, MD). All subjects gave written informed consent. As part of their consent process, VRC 307 participants also participated in a consent sub-study with randomization to standard and concise consent forms [24]. The studies followed the applicable regulatory requirements and the U.S. Department of Health and Human Services human experimental guidelines for conducting clinical research.

2.2. Vaccines

The HA DNA vaccines, VRC-FLUDNA047-00-VP and VRC-FLUDNA056-00-VP for VRC 307 and VRC 309, respectively, were manufactured at the VRC/NIAID/Vaccine Pilot Plant operated by SAIC (Frederick, MD). The HA DNA vaccine administered in VRC 307 included three plasmids expressing HA proteins matching the 2008/09 seasonal influenza strains, A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), and B/Florida/4/2006, while the HA DNA vaccine administered in VRC 309 was matched to the 2009/10 seasonal influenza strains which differed only for the influenza B component (B/Brisbane/60/2008). The plasmids contained a CMV/R promoter as previously described [25]. The HA DNA vaccines were prepared under cGMP at 4 mg/mL in phosphate buffered saline (PBS). PBS was used as placebo. The IIV3 vaccines were subunit inactivated vaccines, using AFLURIA® (CSL Limited) for 2008/09 season and FLUVIRIN® (Novartis Vaccines and Diagnostics Inc.) for 2009/10 season in the VRC 307 and VRC 309 studies, respectively. The A/Brisbane/10/2007 (H3N2)-like influenza strain, A/Uruguay/716/2007, was included in both seasonal IIV3s. Each IIV3 dose was composed of 45 mcg hemagglutinin (HA) in 0.5 mL, with 15 mcg HA for each of the incorporated strains.

2.3. Study objectives, design and randomization

The primary objective for both studies was evaluation of the safety and tolerability for the HA DNA prime–IIV3 boost regimen for seasonal influenza. The secondary objectives were related to immunogenicity of this investigational regimen compared to control groups. In both studies, the HA DNA dose was 4 mg and all IIV3 doses were 45 mcg, consistent with the relevant package insert. The randomization sequences for both studies were obtained by computer-generated random numbers and were provided to the study pharmacist(s) by the protocol statistician; all subjects were randomized to a prime–boost schedule. In VRC 307, there was a 1:1 randomization to HA DNA–IIV3 or to PBS–IIV3 schedules with stratification by age groups (18–50 years and 51–70 years), while in VRC 309 there was a 2:1 randomization to HA DNA–IIV3 or to IIV3–IIV3. Prime injections in the VRC 307 were administered by a double-blinded method using the Biojector® 2000 Needle-Free Injection Management System (Biojector, Bioject Medical Technologies Inc., Tigard, OR) for both HA DNA and PBS injections. In VRC 309, prime injections were open-label because Biojector was used for HA DNA while standard needle injection was used for the IIV3. All booster injections of IIV3 were open-label in both studies.

2.4. Safety evaluation

Assessment of safety included clinical observation and monitoring of laboratory parameters. Solicited reactogenicity was collected using a 7-day Diary Card. All adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) with a graded severity scale. All AEs were reported for each subject from enrollment through 28 days after each study injection, and after this, only SAEs, new chronic medical conditions, and influenza or influenza-like illness were recorded through the last study visit.

2.5. Immunogenicity evaluation

The primary timepoint for immunogenicity evaluation was at 3–4 weeks after the IIV3 boost. The NIAID Vaccine Immune T Cell and Antibody Laboratory (NVITAL) in Gaithersburg, MD processed the research blood samples. The BioQual, Inc., Rockville, MD tested antibody responses by hemagglutination inhibition (HAI) assay. The positivity was defined as the strain-specific antibodies that are as either a 1:40 titer or, if positive at baseline, a 4-fold increase at 3–4 weeks after last vaccination. HAI assays were performed in V-bottom 96-well plates using four hemagglutinating units of influenza virus and 0.5% turkey red blood cells (RBC) similar to previously described [26]. Exploratory immunogenicity evaluations by ELISA, viral neutralization, and T cell assays were performed by NVITAL as previously described [9,21]. T cell responses were evaluated by ICS and ELISpot assays.

2.6. Statistical methods

Intention-to-treat analyses were applied for all endpoints. Positive immunogenicity response rates with exact 95% confidence intervals were computed by the method of Pearson–Clopper. Magnitude of response is reported as the geometric mean titer and 95% confidence intervals. All statistical analyses were performed using Statistical Analysis System (SAS)

and R statistical software. No formal multiple comparison adjustments were employed for safety endpoints or secondary endpoints.

3. Results

3.1. Participant flow and operational issues

Consort diagram is shown on Fig. 1. In VRC 307, 24 subjects received HA DNA prime–IIV3 boost injections, 2 subjects were lost to follow-up after receiving only the HA DNA injection and 1 subject after completion of all study injections. In the PBS prime–IIV3 boost group, 24 subjects received all study injections and 1 subject was lost to follow-up after a single PBS injection. Due to vaccine expiration, of the 48 booster injections administered in the study, 21 were the CSL Northern Hemisphere (NH) IIV3 and 27 were the CSL Southern Hemisphere (SH) IIV3 that had the same components. A total of 6 booster injections were beyond the preferred 28 ± 7 day window due to a delay in obtaining the SH IIV3; the range for these delayed boosters was 41 to 85 days after the prime injection. These were recorded as minor protocol violations for clarity of the record.

In VRC 309, 1 subject was randomized to HA DNA–IIV3 group, but was never vaccinated due to study ineligibility; 39 subjects received the HA DNA–IIV3 injections and completed the study. In the IIV3–IIV3 group, 19 subjects received both injections, 1 subject had discontinued vaccination schedule after one IIV3 injection due to a treatment with systemic glucocorticoids, and 3 subjects were lost to follow-up after completing both IIV3 injections.

VRC 307 study was opened for accrual 3/18/2009; last subject was enrolled 8/25/2009, last study vaccination was completed on 9/22/2009, and the last study visit occurred 3/24/2010. VRC 309 was opened for enrollment 10/15/2009; last subject was enrolled 12/23/2009, last study vaccination was completed 1/13/2010, and the last study visit occurred 6/29/2010.

3.2. Subject demographics

Baseline subject characteristics are summarized in Table 1. VRC 307 study was designed for healthy adults ages 18–70 years, VRC 309 for healthy adults ages 45–70 years.

3.3. Safety

In total, 111 subjects were enrolled into the studies with 66 randomized to the HA DNA prime–IIV3 boost, 25 to the PBS prime–IIV3 boost, and 20 to the IIV3 prime–IIV3 boost schedule. Overall, 65/66 (99%) of the expected HA DNA injections, 25/25 (100%) of the expected PBS injections and 126/131 (96%) of the expected IIV3 injections were administered to 110 subjects.

The worst severity per subject for the solicited local and systemic reactogenicity parameters is summarized in Table 2. There was no severe local or systemic reactogenicity. In summary, 13 (20%) of HA DNA vaccine recipients reported no local reactogenicity, 51 (78%) reported mild, and 1 (2%) reported moderate systemic symptoms. In the placebo group, 12 (48%) of placebo recipients reported no local reactogenicity, and 13 (52%) reported mild symptoms. Similar outcomes were detected for systemic reactogenicity with no symptoms reported by 43 (66%), mild by 20 (31%) and moderate symptoms by 2 (3%) of the HA DNA recipients.

The respective frequencies were 15 (60%), 8 (32%) and 2 (8%) for none, mild and moderate systemic symptoms in placebo group. HA DNA vaccine had higher local reactogenicity than PBS or IIV3, and no difference in systemic reactogenicity was noted (Table 2). Overall, HA DNA was safe and well tolerated.

The frequency of any local or systemic reactogenicity by day for the VRC 307 and VRC 309 studies combined is shown in Fig. 2.

There were no serious adverse events reported. Following HA DNA injections there were three grade 3 (severe) unsolicited adverse events: urticaria, influenza, and increased alanine aminotransferase (ALT), of which only the urticaria was assessed as related to HA DNA administration. Specifically, the occurrence of transient (resolved on the same day), singular, pruritic papules on knee, forearms and abdomen that the subject reported occurred the day after HA DNA vaccine were presumed to be urticaria by description. This subject remained

eligible for the seasonal IIV3 vaccine booster, as there was no contraindication to receiving the licensed IIV3 vaccine. Following IIV3 injections, there was one grade 3 adverse event, gastroenteritis, which was assessed as unrelated to vaccination. Following PBS prime injections, there was one grade 3 adverse event, neutropenia. Other unsolicited adverse events assessed as related to injections were grade 1 (mild); these were superficial injection site erosion (n = 4), pruritus (n = 2), flushing (n = 1) and upper respiratory infection (n = 1) following HA DNA and

3.4. Immunogenicity of the HA DNA vaccine prime–IIV3 boost regimen

The recognized standard for immunogenicity evaluation of an influenza vaccine is the HAI assay. The HAI responses in VRC 307 and VRC 309 studies are summarized in Table 3. The frequency of positive response is defined as either a 1:40 titer or, if positive at baseline, a 4-fold increase at 3–4 weeks after last vaccination. Magnitude of response is reported as geometric mean titer (GMT), calculated across all participants regardless of response. No statistically significant differences in frequency or magnitude of HAI responses (Table 3 and Supplementary Fig.3) or T cell responses (Supplementary Fig. 4) were detected between treatment groups. Similar results were obtained in ELISA, intracellular cytokine staining (ICS), and viral neutralization assays (not shown).

4. Discussion

VRC 307 and VRC 309 studies were designed to evaluate safety and immunogenicity of priming with HA DNA vaccines and boosting with inactivated vaccines against seasonal influenza. There are significant logistical and operational challenges for clinical studies conducted for evaluation of novel seasonal influenza vaccine candidates because of time constraints of the influenza season and changes in seasonal influenza strains. Compared to vaccines produced by other technologies, DNA vaccines are relatively easy to manufacture, constructs that encode for novel antigens can be produced rapidly, and these plasmid-based vaccines are stable for a long time.

As we demonstrated in our studies with H1N1 pandemic influenza, HA DNA vaccines can be produced faster than inactivated vaccine, and there is a real potential to vaccinate with the HA DNA prime before inactivated vaccine is available in the season [12].

The VRC 307 study began enrolling in March 2009, which was very late in the northern hemisphere (NH) 2008–2009 influenza season. The IIV3 used for booster injections administered through June 30, 2009 was the NH product AFLURIA® manufactured by CSL Limited. After expiration, the IIV3 administered was the comparable product manufactured by CSL for the southern hemisphere (SH). Six booster injections in the study were administered out of window due to an unexpected delay in obtaining the SH product. Although this was a logistical constraint in this first Phase 1 study of seasonal influenza HA DNA vaccine, this could be avoided by planning the protocol in advance of the influenza season and initiating manufacture of the HA DNA vaccine as soon as the seasonal strains needed that year become known.

As tested in VRC 307 and VRC 309 studies, the prime–boost regimen of seasonal trivalent HA DNA vaccine followed by IIV3 boost was safe and well tolerated. No severe local or systemic reactogenicity was observed (Table 2). The majority (51, 78%) of seasonal HA DNA vaccine recipients reported mild local reactogenicity, while moderate local reactogenicity was infrequent (1, 2%). The majority (43, 66%) of HA DNA vaccine recipients reported no systemic reactogenicity, 20 (31%) reported mild and 2 (3%) reported moderate systemic symptoms. The reactogenicity was self-limited with majority of symptoms persisting in the first 1–5 days after vaccination (Fig. 2). Overall, HA DNA vaccine injections were safe and well tolerated.

While some DNA vaccines have been shown to induce robust immune responses administered alone [8,10,11], some require additional steps to improve immunogenicity such as using electroporation or adjuvants [27,28]. In comparison with the previous season, all 3 seasonal strains were new in the 2008–2009 influenza season, and in addition to the short prime–boost interval, this may explain an absence of robust immune responses in these studies. In preclinical studies, the HA DNA vaccine prime–inactivated vaccine boost strategy has shown evidence of improved immune response, including to epitopes conserved between influenza subtypes [29,30]. We confirmed that HA DNA prime–IIV3 boost is immunogenic in healthy adults (Table 3, Supplementary Figs 3 and 4).

In human Phase 1 studies with avian influenza vaccines, a single H5 DNA vaccine prime with a single monovalent inactivated vaccine boost at short intervals (4–8 weeks) does not significantly improve HAI titers over that achieved with inactivated vaccine alone, and boost intervals at 12 or more weeks significantly increase the immune response [9,21]. In the VRC 307 and VRC 309 studies, these findings were confirmed such that immune responses to IIV3 were not significantly improved when the HA DNA prime was administered in a 3 to 4-week interval prior to IIV3 administration. However, data from the H5 DNA vaccine prime–boost trials indicate that a single H5 DNA 4 mg IM vaccination prime significantly improves HAI responses when the inactivated vaccine boost interval is 16–24 weeks as compared to two vaccinations with the inactivated vaccine [9,18,21]. In conclusion, seasonal trivalent HA DNA vaccines alone are not highly immunogenic nor markedly improve

responses to inactivated vaccine boost administered at the 3–4 week interval. Based on experience with the H5 DNA vaccine, evaluation of an inactivated boost at 16–24 weeks after the prime is warranted as a potential method of reliably improving immune response to seasonal influenza vaccine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.cct.2015.08.006>.

Fig. 1. CONSORT flow diagram. Study enrollment, allocation and follow-up are shown for all screened and enrolled subjects.

Fig. 2.
Severity of solicited reactogenicity by day as reported by subjects using a 7-day diary card.

Table 1

Baseline characteristics of subjects by group.

Category	Sub-category	VRC 307			VRC 309		
		HA DNA–HIV3 (N = 26)	PBS–HIV3 (N = 25)	HA DNA–HIV3 (N = 20)	HA DNA–HIV3 (N = 40)	IIV3–HIV3 (N = 20)	
Gender	Male — no. (%)	14 (54%)	13 (52%)	22 (55%)	9 (45%)		
	Female — no. (%)	12 (46%)	12 (48%)	18 (45%)	11 (55%)		
Age	18–20	1 (4%)	3 (12%)	n/a	n/a		
	21–30	12 (46%)	8 (32%)	n/a	n/a		
	31–40	3 (12%)	7 (28%)	n/a	n/a		
	41–50	4 (15%)	2 (8%)	18 (45%)	8 (40%)		
	51–60	3 (12%)	3 (12%)	15 (38%)	6 (30%)		
	61–70	3 (12%)	2 (8%)	7 (18%)	6 (30%)		
Race	Mean (S.D.)	37.5 (15)	35.0 (14)	53.4 (7)	55.6 (8)		
	Range	[20, 68]	[19, 64]	[45, 68]	[45, 69]		
	Asian	4 (15%)	3 (12%)	2 (5%)	1 (5%)		
	Black or African American	9 (35%)	6 (24%)	9 (23%)	5 (25%)		
	White	13 (50%)	15 (60%)	28 (70%)	12 (60%)		
	All other races combined	0 (0%)	0 (0%)	1 (2%)	2 (10%)		
Ethnicity	Non-Hispanic/Latino	26 (100%)	23 (92%)	38 (95.0%)	19 (95.0%)		
	Hispanic/Latino	0 (0%)	2 (8%)	2 (5.0%)	1 (5.0%)		
BMI	Mean (S.D.)	26.1 (4.5)	25.0 (4.8)	28.1 (5.5)	27.0 (3.8)		
	Range	[19.6, 36.8]	[19.0, 36.8]	[18.7, 42.1]	[21.9, 34.8]		

Table 2

Solicited reactogenicity following study injections.

Parameter	Intensity	HA DNA (N = 65)	Number (%) of subjects			P values for HA DNA–PBS comparison ³	P values for HA DNA–IVV comparison ³
			HA DNA (N = 65)	IVV3 (N = 107)	PBS (N = 25)		
Local reactogenicity							
Pain/Tenderness	None	15 (23%)	67 (63%)	14 (56%)	0.005	<0.001	
	Mild	49 (75%)	40 (37%)	11 (44%)			
	Moderate	1 (2%)	0	0			
Swelling	None	48 (74%)	101 (94%)	23 (92%)	0.083	<0.001	
	Mild	17 (26%)	6 (6%)	2 (8%)			
	Moderate	0	0	0			
Redness	None	44 (68%)	99 (93%)	24 (96%)	0.005	<0.001	
	Mild	21 (32%)	8 (7%)	1 (4%)			
	Moderate	0	0	0			
Any local	None	13 (20%)	60 (56%)	12 (48%)	0.016	<0.001	
	Mild	51 (78%)	47 (44%)	13 (52%)			
	Moderate	1 (2%)	0	0			
Systemic reactogenicity							
Malaise	None	52 (80%)	85 (79%)	19 (76%)	0.774	1.0	
	Mild	12 (18%)	21 (20%)	6 (24%)			
	Moderate	1 (2%)	1 (1%)	0			
Myalgia	None	56 (86%)	94 (88%)	21 (84%)	0.749	0.638	
	Mild	9 (14%)	12 (11%)	4 (16%)			
	Moderate	0	1 (1%)	0			
Headache	None	53 (81%)	88 (82%)	20 (80%)	1.0	1.0	
	Mild	11 (17%)	17 (16%)	4 (16%)			
	Moderate	1 (2%)	2 (2%)	1 (4%)			
Chills	None	64 (98%)	103 (96%)	24 (96%)	0.481	0.651	
	Mild	1 (2%)	3 (3%)	1 (4%)			
	Moderate	0	1 (1%)	0			
Nausea	None	65 (100%)	106 (99%)	24 (96%)	0.278	1.0	

Parameter	Intensity	HA DNA (N = 65)	IIV3 (N = 107)	PBS (N = 25)	P values for HA DNA–PBS comparison ³	P values for HA DNA–IIV comparison ³
Number (%) of subjects						
Fever	Mild	0	1 (1%)	0		
	Moderate	0	0	1 (4%)		
	None	64 (98%)	105 (98%)	24 (96%)	1.0	1.0
	Mild	1 (2%)	2 (2%)	0		
Any systemic	Moderate	0	0	0		
	Missing	0	0	1 (4%)		
	None	43 (66%)	74 (69%)	15 (60%)	0.628	0.737
	Mild	20 (31%)	30 (28%)	8 (32%)		
	Moderate	2 (3%)	3 (3%)	2 (8%)		

* Fisher's exact test was used for calculation of p-values.

Table 3

HAI responses.

Study	VRC 307		VRC 309	
Treatment group	HA DNA–IIV3 (n = 24)	PBS–IIV3 (n = 24)	HA DNA–IIV3 (n = 39)	IIV3–IIV3 (n = 19)
Boost interval	4 weeks	4 weeks	3 weeks	3 weeks
<i>A/Brisbane/59/2007 (H1N1)</i>				
HAI positive response frequency, n (% 95% CI)	15 (63%; 41–81)	17 (71%; 49–87)	25 (64%; 47–79)	15 (79%; 54–94)
HAI before vaccination (GMT, 95% CI)	14 (10–19)	13 (10–19)	20 (13–33)	16 (10–24)
HAI after vaccination (GMT, 95% CI)	57 (38–86)	92 (51–167)	118 (82–171)	192 (100–371)
<i>A/Uruguay/716/2007 (H3N2)</i>				
HAI positive response frequency, n (% 95% CI)	15 (63%; 41–81)	15 (63%; 41–81)	29 (74%; 58–87)	11 (58%; 33–80)
HAI before vaccination (GMT, 95% CI)	13 (10–16)	12 (10–14)	27 (16–46)	19 (9–41)
HAI after vaccination (GMT, 95% CI)	82 (48–141)	92 (44–194)	344 (215–549)	185 (76–453)
<i>B/Florida/04/2006 for VRC 307; B/Brisbane/60/2008 for VRC 309</i>				
HAI positive response frequency, n (% 95% CI)	15 (63%; 41–81)	13 (54%; 33–74)	18 (46%; 30–63)	7 (37%; 16–62)
HAI before vaccination (GMT, 95% CI)	16 (12–21)	22 (14–35)	11 (8–15)	7 (5–10)
HAI after vaccination (GMT, 95% CI)	60 (39–93)	117 (63–216)	49 (33–71)	26 (14–50)

injection site papules (n = 1) and lymphadenopathy (n = 1) following IIV3. There was also a superficial injection site erosion following a PBS injection.