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MAJOR ARTICLE

Preliminary findings from the Dynamics of the Immune Responses to Repeat Influenza Vaccination Exposures (DRIVE I) Study: a Randomized Controlled Trial

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Background: Studies have reported that repeated annual vaccination may influence influenza vaccination effectiveness in the current season.

Methods: We established a 5-year randomized placebo-controlled trial of repeated influenza vaccination (Flublok, Sanofi Pasteur) in adults 18-45 years of age. In the first two years, participants received vaccination (V) or saline placebo (P) as follows: P-P, P-V, or V-V. Serum samples were collected each year just before vaccination and after 30 and 182 days. A subset of sera collected at 5 timepoints from 95 participants were tested for antibodies against vaccine strains.

Results: From 23 October 2020 through 11 March 2021 we enrolled and randomized 447 adults. Among vaccinated individuals, antibody titers increased between days 0 and 30 against each of the vaccine strains, with smaller increases for repeat vaccinees who on average had higher pre-vaccination titers in year 2. There were statistically significant differences in the proportion of participants achieving >=four-fold rises in antibody titer for the repeat vaccinees for influenza A(H1N1), B/Victoria and B/Yamagata, but not for A(H3N2). Among participants who received vaccination in year 2, there were no statistically significant differences between the P-V and V-V groups in geometric mean titers at day 30 or the proportions of participants with antibody titers ≥40 at day 30 for any of the vaccine strains.

Conclusions: In the first two years, during which influenza did not circulate, repeat vaccinees and first-time vaccinees had similar post-vaccination geometric mean titers to all four vaccine strains, indicative of similar levels of clinical protection.

Key words: influenza; vaccination; immunogenicity; antibody

INTRODUCTION

A number of studies have reported that repeated annual vaccination may influence the effectiveness of the influenza vaccination in the current season [1-5]. The effect of repeated vaccination on immunogenicity has been less frequently assessed in multi-year randomized trials, but repeat vaccination effects are also observed [6-10]. The mechanisms underlying these differences are unclear, but might include "focusing" of the adaptive immune response to older strains [11]. Under this model, repeated exposures boost responses to conserved and potentially less protective epitopes, which might in some years reduce protection against circulating strains [12,13].

Enhanced influenza vaccines, including the recombinant hemagglutinin vaccine Flublok (Sanofi Pasteur), stimulate stronger immune responses and may be able to overcome repeat vaccination

effects [14]. The Flublok vaccine has two major differences with the standard egg-grown inactivated influenza vaccine [8,15,16]. First, because eggs are not used in the production process for Flublok, the antigens included in the vaccine should be more similar to circulating viruses [8,17]. Second, it includes three times more HA antigen than standard-dose vaccines and can therefore generate a stronger, more HA-specific humoral immune response [8,9]. Evidence from randomized trials have shown improved immunogenicity and efficacy and a slightly lower local reactogenicity compared with standard inactivated influenza vaccine in adults ≥18 years [18].

We designed a randomized controlled trial to explore immune responses to first-time or repeated influenza vaccination with the Flublok vaccine, with a particular interest in the possible occurrence of repeat vaccination effects.

METHODS

Study design

Participants in this randomized controlled trial (Clinicaltrials.gov: NCT04576377) were enrolled from the general community in Hong Kong through various approaches (see Appendix). Individuals were eligible to participate if they were between 18 and 45 years of age, capable of providing informed consent, and intending to reside in Hong Kong for at least the next two years. Potential participants were excluded if they had been vaccinated against influenza in the preceding 24 months, if they were included in a priority group for influenza vaccination, if they had a diagnosed immunosuppressive condition or were taking immunosuppressive medication, or if they had severe allergies or bleeding conditions that contraindicated intramuscular influenza vaccination.

We collected a baseline 9ml clotted blood sample from enrolled participants, and we used a standardized questionnaire to collect baseline information on demographics, current health status, medical history and medication use. Participants were randomized equally among five equal groups using a permuted block approach with block sizes of 5 and 10, using R. The five groups involved annual receipt of Flublok vaccination (V) or saline placebo (P) over five years (Appendix Figure 1). Allocation to these groups was concealed using REDCap [19]. In the first two years the participants were randomized to P-P, P-V and V-V.

The influenza vaccine used in our trial is recombinant HA quadrivalent influenza vaccine (0.5ml Flublok®, Sanofi Pasteur) for the northern hemisphere (Appendix Table 1). To maintain blinding of participants, we used 0.5ml saline placebo in syringes prepared in advance and packed the vaccines and placebo doses in numbered boxes according to the randomization scheme. Other than the nurse who conducted the injections, all other study staff were blinded to the study intervention.

Following vaccination, we invited participants to return for scheduled follow-up visits at 30 days and 182 days after vaccination for further blood draws. In a subset of participants we arranged for

collection of peripheral blood mononuclear cells at baseline, day 7 and day 30, and additional clotted blood samples on days 91 and 273. All participants were invited to report any acute respiratory illnesses. During periods of influenza activity, we planned to implement weekly active illness surveillance, collecting nasal swabs from ill participants for testing by PCR and an additional blood sample 30 days after illness onset.

Ethics

Written informed consent was obtained from all participants. Participants were compensated with a gift voucher worth HK$100 (US$13) at each blood draw. The study protocol was approved by the Institutional Review Boards of the University of Hong Kong (ref: UW19-551) and of the University of Chicago Biological Sciences Division (ref: IRB20-0217).

Primary and secondary outcomes

The primary outcome measure in this trial is the vaccine immunogenicity, measured in terms of antibody titers in hemagglutination-inhibition (HAI) assays or foci reduction neutralization tests (FRNTs) for each vaccine strain. We compared the proportion of participants who achieved the targeted rise in antibody titre against each of the vaccine strains at 30 days. The targeted rise in antibody titre is defined as a four-fold or greater rise in titer, including either a pre-vaccination HAI titer <10 and a post-vaccination HAI titre ≥20 or a pre-vaccination HAI titer ≥10 and at least a four-fold rise in post-vaccination HAI antibody titer. As an alternate outcome we defined a targeted rise as a four-fold or greater rise in titer to a post-vaccination titer ≥40. We also assessed the geometric mean titer (GMT) ratios between the various randomized groups against each of the vaccine strains at 30 days and 182 days. HAI assays were completed with influenza A(H1N1) and B viruses; however; we used FRNTs to quantify antibodies against A(H3N2) strains, since some contemporary A(H3N2) strains inefficiently agglutinate red blood cells [8,20].

Other secondary outcomes specified in the protocol include additional comparisons of antibody titers; analyses of cellular immunity, including transcriptional activity of immune cells; comparisons of adverse reactions after vaccination; and the occurrence of influenza or other acute respiratory illnesses. These secondary outcomes will be addressed in subsequent reports. Of particular note, there was no influenza circulation in Hong Kong between March 2020 and February 2023 [21-23], which included the first two years of the trial.

Laboratory analysis

Blood samples were collected in tubes for clotted blood, stored at 2-8°C immediately and delivered to the laboratory within two days for storage at -80°C. Sera were treated with receptor destroying enzyme and tested by HAI against the influenza A(H1N1) and B vaccine strains using a standard protocol [24]. For influenza A(H1N1), the vaccine strains were A/Hawaii/70/2019 in 2020/21 (GISAID Accession #EPI397028) and A/Wisconsin/588/2019 (GISAID Accession #EPI404460) in 2021/22. Due to the inability to obtain sufficient volume of cell-grown virus stock,

A/Wisconsin/588/2019 was propagated once in eggs. A single mutation D204V was found in the egg-grown stock. A comparison of antigenicity using 22 serum samples from five participants showed that the egg-grown stock had slightly higher sensitivity that yielded 2-fold higher titers in 50% of the samples but otherwise showed good correlation with cell-grown stock (Appendix Figure 2). For influenza B, ether-treated egg-grown antigens were used, and the vaccine strains were B/Washington/02/2019 (GISAID Accession #EPI347829) and B/Phuket/3073/2014 (GISAID Accession #EPI168822) in both years.

We used a FRNT assay as previously described [25] for the two influenza A(H3N2) vaccine strains A/Hong Kong/45/2019 in 2020/21 and A/Cambodia/e0826360/2020 in 2021/22. A/Hong Kong/45/2019 HA (GISAID Accession #EPI1397376) is identical at the amino acid level to A/Minnesota/41/2019 HA (GISAID Accession #EPI1487157) included in the 2020/21 Flublok. However, A/Cambodia/e0826360/2020 HA (GISAID Accession #EPI1837753) differs by a single amino acid substitution (N171K at antigenic site D) from A/Tasmania/503/2020 HA (GISAID Accession #EPI1759269) included in the 2021/22 Flublok.

To estimate total binding antibody of specific IgG responses, Enzyme Linked Immunosorbent Assays (ELISAs) were performed as previously described [26] with recombinant HA of the vaccine strains A/Hawaii/70/2019(H1N1), A/Wisconsin/588/2019(H1N1), A/Minnesota/41/2019(H3N2) and A/Tasmania/503/2020(H3N2) (Appendix Table 1).

Sample size justification

We aimed to enrol 820 participants into our study. This would permit us to have a sample size of at least 100 participants in each of the five groups at the fourth year of follow-up, allowing for an anticipated drop-out rate of 15% per year without replacement. Most of our outcome measures, including both of our primary outcome measures, are based on geometric mean antibody titers. In year 2, with a target sample size of 139 in each group, we expected to have 80% power to identify 1.6-fold differences in GMT between groups, assuming a standard deviation of $\log_2$(GMT) of 1.8. However, due to disruption in study activities during the COVID-19 pandemic we were unable to reach our target sample size, and as a consequence we updated the protocol to include enrolment of an additional cohort of 530 participants starting in 2021/22 with a similar trial design, with participants randomized across four groups for four years, named "DRIVE II". For consistency, the present cohort with participant enrolment in 2020/21 is named "DRIVE I".

Statistical analysis

We assessed the proportion of participants who achieved a targeted rise in antibody titre against each of the vaccine strains at 30 days, and compared these proportions between the V-V, P-V and P-P groups using Fisher exact tests. We estimated GMT ratios versus the P-P group at day 30 of year 2 and compared them between first-time vaccines and repeat vaccinees using t-tests on the log-transformed GMT ratios. Confidence intervals were estimated using t distributions. All

analyses were conducted in R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

From 23 October 2020 through 11 March 2021, 447 individuals were enrolled and received influenza vaccination or placebo according to the randomization scheme, with 88 to 91 individuals in each arm (Figure 1, Appendix Table 2). The median age of participants was 31 years, and 54% were male, with similar characteristics across the three groups (Table 1). None of the participants reported receiving influenza vaccination in the two years prior to the start of the trial, and only 12% reported ever previously receiving influenza vaccination. We randomly selected for laboratory analysis a subset of 15 participants from the P-P group, 40 participants from the P-V group, and 40 participants from the V-V group, with similar characteristics to the overall participants (Appendix Table 3).

Antibody titers increased between days 0 and 30 against each of the vaccine strains, with greater fold increases for first-time vaccinees compared to repeat vaccinees. The repeat vaccinees had higher antibody titers prior to vaccination in year 2 despite some waning, especially 30 to 182 days after their first vaccination (Figure 2). At day 30 of year 2, the GMTs were similar in the P-V and V-V groups for each strain (Appendix Table 4). While there were statistically significant differences in the proportion achieving a targeted rise in antibody titer for the repeat vaccinees in year 2 for A(H1N1), B/Victoria and B/Yamagata, there were no statistically significant differences in GMTs at day 30 or the proportions with antibody titers ≥40 at day 30 for any of the strains (Table 2). These observations were repeated with the alternate definition of a targeted rise (Table 2).

The A(H1N1) and A(H3N2) components of the northern hemisphere vaccine were updated between 2020/21 and 2021/22. For each subtype, we examined whether vaccination with one strain increased antibody titers to the other, which is a measure of cross-reactivity or antigenic distance. We focused on the responses in first-time vaccinees in each of the two years (i.e. the V-V group in year 1 and the P-V group in year 2). For A(H1N1), the year 1 GMT at day 30 was approximately 3.2-fold higher to the 2020/21 vaccine strain (A/Hawaii/70/2019) than to the vaccine strain used the next year (A/Wisconsin/588/2019), consistent with a modest antigenic distance between the two strains (Table 3). For A(H3N2), the fold-difference between the day 30 GMT to the 2020/21 strain (A/Hong Kong/45/2019) and the 2021/22 strain (A/Cambodia/e0826360/2020) was approximately 3.1, also indicating modest antigenic distance.

In year 2, first-time vaccinees mounted slightly stronger responses to the prior-season vaccine strains than to the current-season vaccine strains. First-time vaccinees in year 2 had approximately threefold higher titers to the previous year's strain, A/Hawaii/70/2019, than to the strain with which they were vaccinated, A/Wisconsin/588/2019 (180.6 vs. 60.6, respectively) (Table 3).

Similarly, first-time vaccinees in year 2 mounted approximately 1.5-fold higher titers to the previous year's A/Hong Kong/45/2019(H3N2) strain than they did to the A/Cambodia/e0826360/2020(H3N2) strain in the vaccine. For both H1N1 and H3N2, the absolute post-vaccination titers to the year 2 vaccine strains in year 2 was higher than after vaccination in year 1 (geometric mean titers of 60.6 vs. 40 for the H1N1 vaccine strains and 45.6 vs. 34.7 for the H3N2 vaccine strains), showing the strain updates still led to increased titers to the intended strain.

That first-time vaccinees in year 2 mounted higher titers to the prior year's influenza A vaccine strains than to the current vaccine strains might reflect a strong influence of prior immunity, but it could also reflect differences in receptor avidity between the strains. Viruses that bind to cells with lower avidity are more easily neutralized by antibodies compared to viruses that bind with higher avidity, irrespective of antigenic differences [27]. To address this, we measured total HA-specific IgG antibody responses by ELISAs, which are not affected by differences in viral receptor binding avidities, to measure antibody binding to the influenza A(H1N1) and A(H3N2) vaccine strains, with results shown in Figure 3 and Table 3. We found that the year 2, geometric mean post-vaccination antibody levels to the current vaccine strain were similar to or slightly higher than those to the previous vaccine strain in first-time vaccinees, suggesting no disproportionate boosting of prior immunity targeting past strains. That there were no statistically significant differences in the geometric mean post-vaccination antibody levels measured by ELISA between first-time vaccinees in years 1 and 2 suggests the previously reported low HAI and FRNT titers to A/Wisconsin/588/2019 and A/Cambodia/e0826360/2020 likely arise from differences in the receptor avidity. The antigenic distances implied by the ELISA data are also smaller and do not show the same asymmetry. For instance, the amount of antibody reactive to A/Wisconsin/588/2019 after vaccination with A/Hawaii/70/2019 (V-V group, year 1, day 30) was similar to the amount of antibody reactive to A/Hawaii/70/2019 after vaccination with A/Wisconsin/588/2019 (P-V group, year 2, day 30) (Table 3). Finally, consistent with the data from the HAI and FRNT assays, there were no statistically significant differences in absolute post-vaccination antibody levels measured by ELISA between first-time and repeat vaccinees in year 2.

DISCUSSION

In the second year of this five-year trial we identified reduced fold-rises in HAI titers after repeat vaccination for influenza A(H1N1), B/Victoria and B/Yamagata. However, post-vaccination GMTs were similar in repeat vaccinees and first-time vaccinees, indicating that these reduced responses likely would not hinder overall protection [28]. During the study period, public health measures used to contain COVID-19 also prevented the community circulation of influenza [21], and our analysis of antibody titers is therefore unaffected by any potential differences in incidence of influenza virus infections in vaccine versus placebo recipients in the first year of the study that could have occurred if influenza had been circulating.

We found that repeat and first-time vaccinees also had similar post-vaccination GMTs to A(H3N2) measured by FRNT, indicative of similar levels of clinical protection. However, there was no substantial blunting of the fold rises to A(H3N2) in repeat vaccinees. Both groups started with low GMTs to A/Cambodia/e0826360/2020(H3N2) in year 2 and increased those GMTs approximately 4-fold by 30 days post-vaccination (Figure 2, Appendix Table 4). Our ELISA analyses measuring direct antibody binding suggest that the observed asymmetric cross-reactivities implied by HAI and FRNT titers between groups could be due to differences in receptor binding avidities of the viral strains used in our studies, and that first vaccination in either year 1 or year 2 induces comparable amounts of cross-reactive antibodies to the other year's vaccine strain.

Both the A(H1N1) and A(H3N2) vaccine strains were updated between years 1 and 2 of the study, raising opportunities to investigate the cross-reactivity of antibody titers induced by vaccination. The 2020/21 vaccine induced HAI titers to A(H1N1) and FRNT titers to A(H3N2) that were approximately 3-fold lower to the strains used in the 2021/22 vaccine. The suggested benefits of a vaccine update were evidenced by slightly higher titers to those strains among first-time vaccinees the following season compared to titers of vaccinees from the 2020/21 season. However, first-time vaccinees in the 2021/22 season did not have titers that were three-fold lower to strains from the 2020/21 season, as might be expected after vaccination in naïve animals or associated cartographic methods. Instead, individuals vaccinated in 2021/22 had approximately 3-fold and approximately 1.5-fold higher post-vaccination GMTs to prior season's A(H1N1) and A(H3N2) vaccine strains. Measuring anti-HA antibody responses with ELISA implied even higher cross-reactivity and limited antigenic distances between viral strains used in successive years.

Our study has a number of limitations. To preserve specimens for later longitudinal analyses, we have analyzed a subset of participants and thus are limited in the effect sizes we can detect in the present analysis. While the strains used in our serologic assays did not have 100% sequence identity with the vaccine strains, the minor genetic differences likely did not affect our conclusions. Additionally, although the ELISA data suggest that antibody responses were comparable in magnitude between year 1 and 2 influenza A vaccine strains, at present we cannot conclude whether the contrasting HAI and FRNT results reflect strain-specific differences in receptor binding avidity, neutralization potential, and/or the relative immunogenicity of the stalk and other epitopes that are poorly measured by HAI. Understanding the roles of these factors is an important area for further work [29]. In contrast to many vaccinees in other countries, our repeat vaccinees had mostly not been previously vaccinated to influenza, and they were relatively healthy young adults. Due to lack of influenza circulation in the first two years of our study, our results might not reflect typical differences in titers or vaccine responsiveness between repeat and non-repeat vaccinees. Additionally, our study does not investigate egg-grown vaccines, whose immunogenicity can be influenced by egg-associated substitutions and glycans [30].

In conclusion, our randomized trial of repeat influenza vaccination has found that repeat vaccination can be associated with reduced fold changes in antibody titers, but our preliminary results show no evidence of reduced post-vaccination titers, consistent with similar levels of

protection after vaccination regardless of vaccination history. The apparently low immunogenicity of influenza A vaccine strains in 2021/22 relative to the prior year may reflect increases in receptor avidity of updated vaccine strains and deserves further study.

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Potential Conflicts of Interest: B.J.C. consults for AstraZeneca, Fosun Pharma, GlaxoSmithKline, Haleon, Moderna, Novavax, Pfizer, Roche and Sanofi Pasteur. S.C. has consulted for Seqirus. S.E.H. is a co-inventor on patents that describe the use of nucleoside-modified mRNA as a vaccine platform. S.E.H reports receiving consulting fees from Sanofi, Pfizer, Lumen, Novavax, and Merck. The authors report no other potential conflicts of interest.

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Figure 1. Study flow chart showing participant enrolment into the study, randomization into the P-P, P-V and V-V groups, interventions received, and follow up.

Footnote: *The random samples were selected from participants who provided serum samples at these five timepoints: year 1 day 0, day 30 and day 182, and year 2 day 0 and day 30.

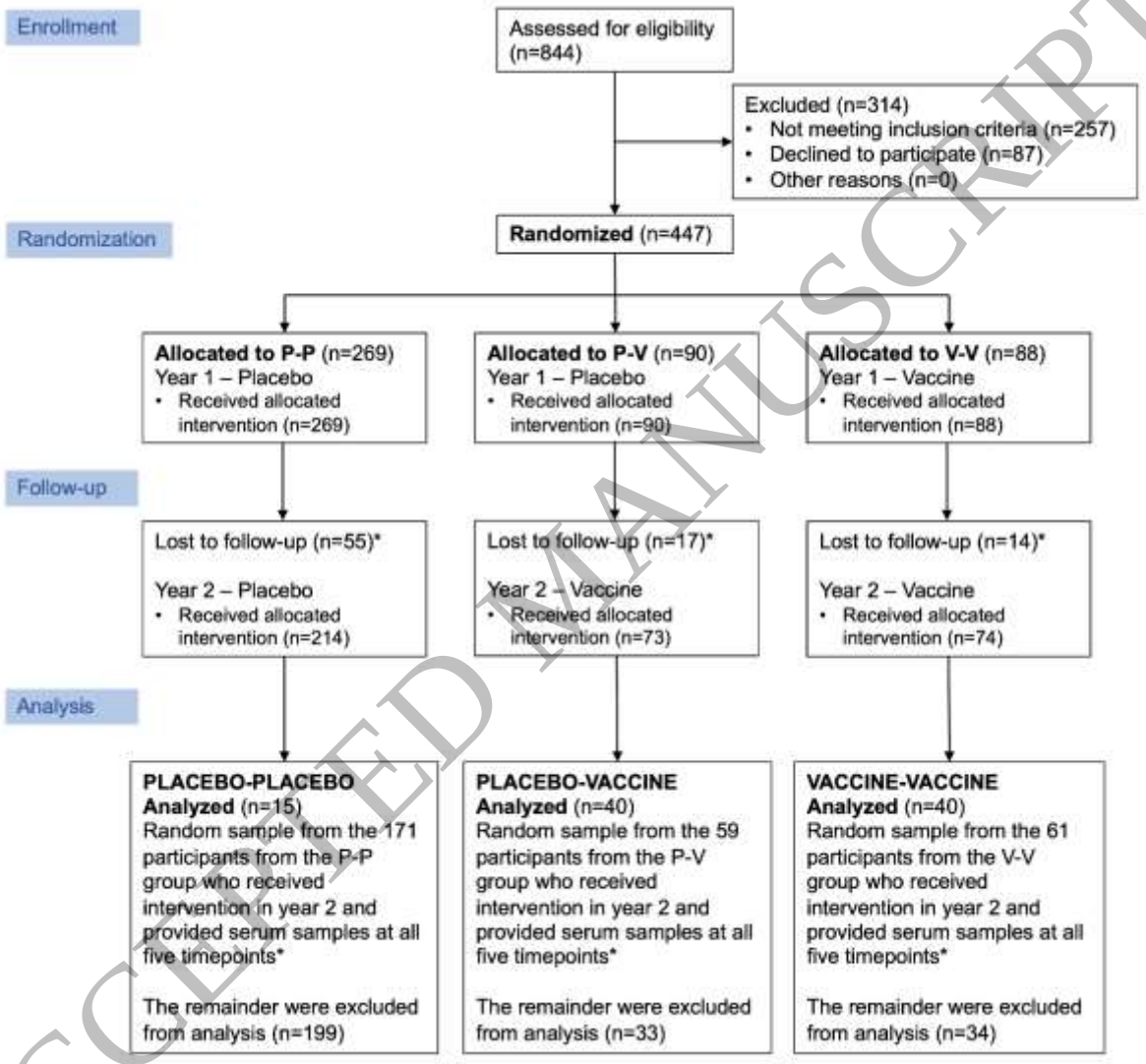

Figure 2. Antibody titers at various timepoints measured by hemagglutination inhibition assay for influenza A(H1N1) and B, and by focus reduction neutralization test for influenza A(H3N2). Measured titers are plotted for each group at 0, 30 and 182 days post-vaccination of year 1 and at 0 and 30 days post-vaccination of year 2, and lines represent the geometric mean titers at each timepoint. Data from the V-V group are shown in red. Data from the P-V group are shown in blue. Data from the P-P group are shown in gray.

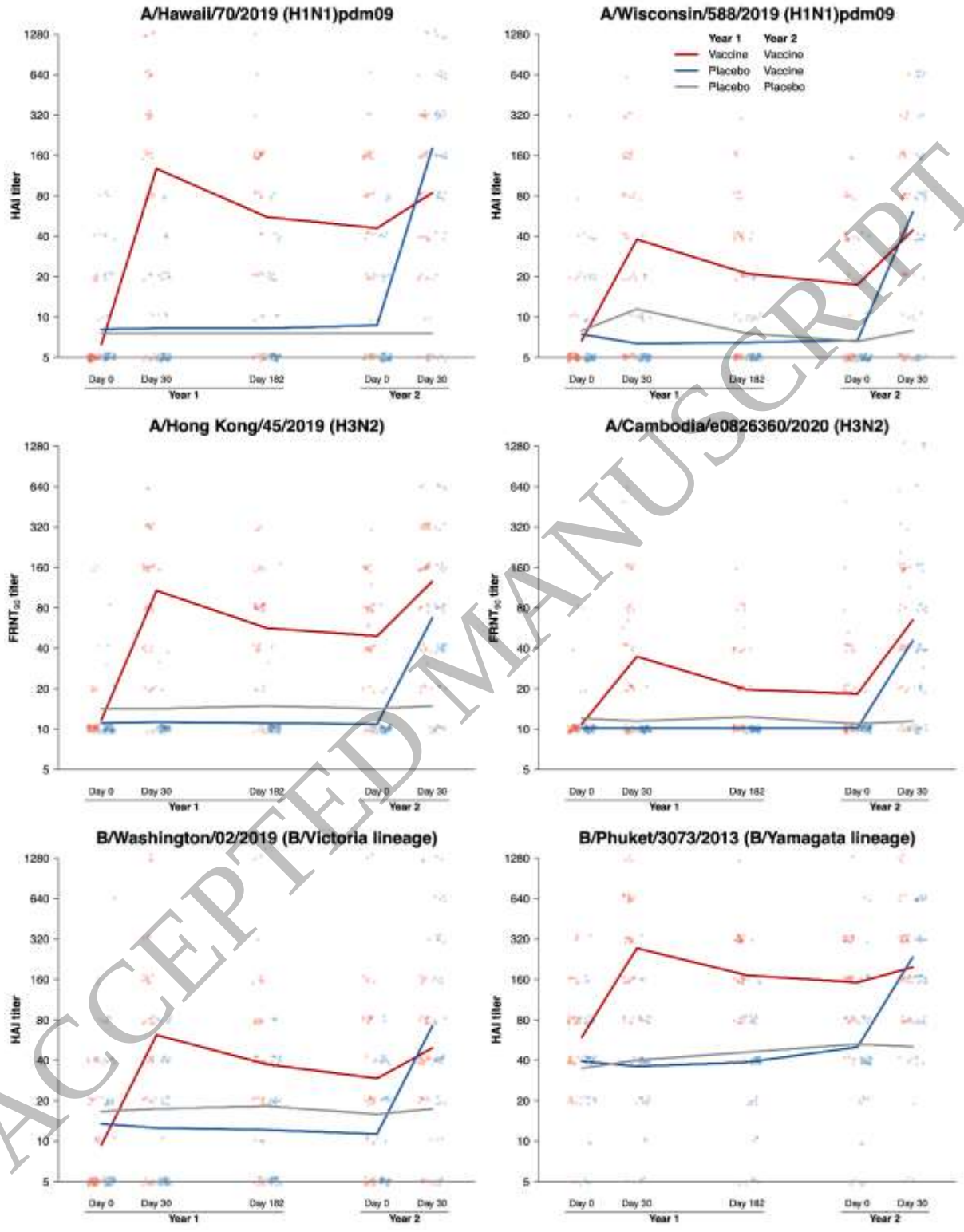

Figure 3. Antibody levels at various timepoints measured by enzyme-linked immunosorbent assay for influenza A(H1N1) and A(H3N2). Measured titers are plotted at 0 and 30 days post-vaccination in years 1 and 2, and lines represent the geometric mean antibody levels at each timepoint. Data from the V-V group are shown in red. Data from the P-V group are shown in blue. Data from the P-P group are shown in gray.

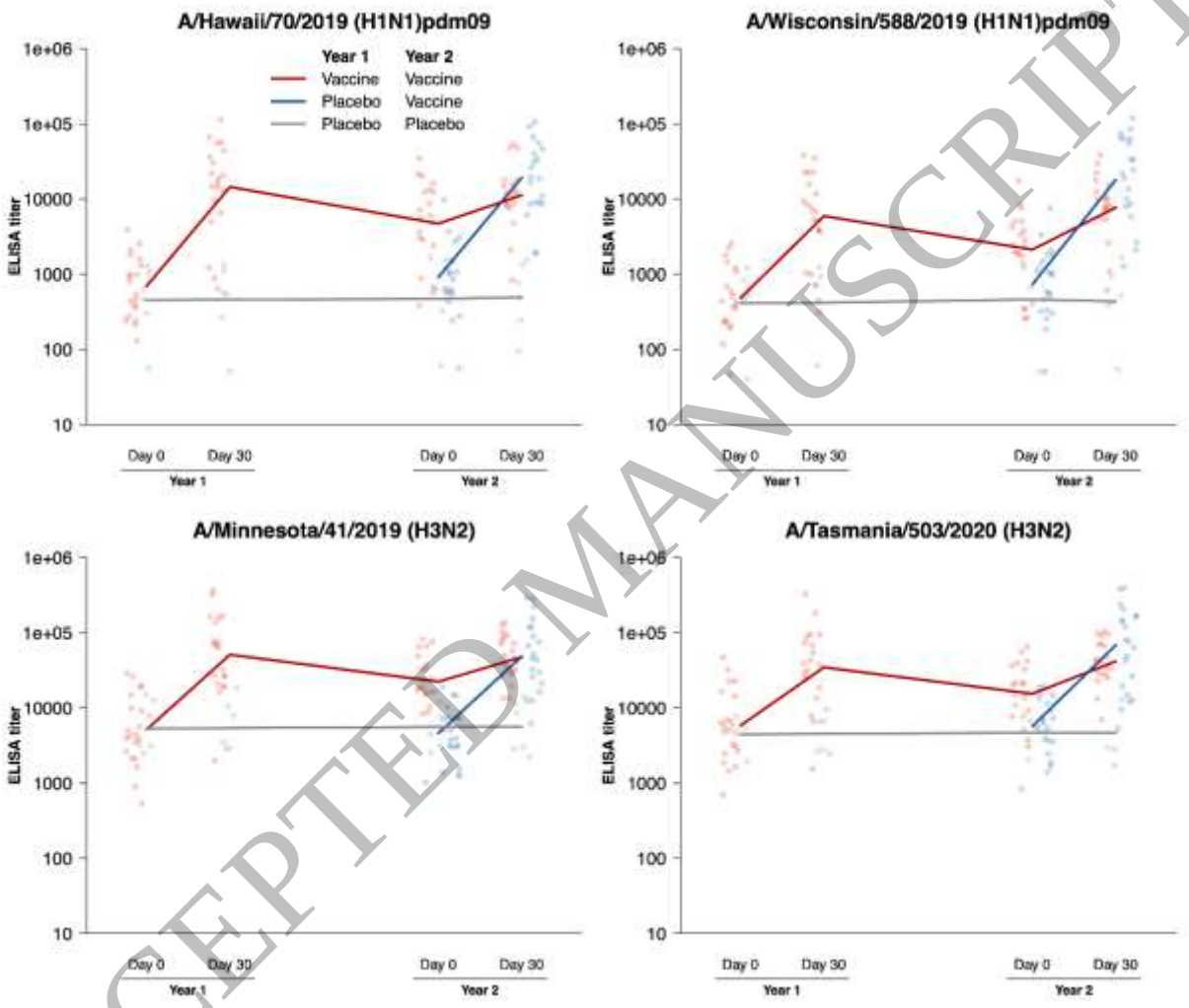

Table 1. Characteristics of the subset of 95 participants selected for serological analysis

Characteristic	Randomized allocation to receipt of placebo (P) or influenza vaccination (V) in the first two years			p-value*
	P-P (n=15)	P-V (n=40)	V-V (n=40)	
Age at randomization				
18-25 years	3 (20%)	10 (25%)	8 (20%)	
26-35 years	5 (33%)	15 (38%)	14 (35%)	
36-45 years	7 (47%)	15 (38%)	18 (45%)	0.96
Male sex	8 (53%)	23 (58%)	17 (43%)	0.44
Ever received influenza vaccination	3 (20%)	4 (10%)	4 (10%)	0.52

*p-value using Fisher's exact test

Table 2. Antibody responses to influenza vaccination and placebo in year 2 of the trial, with 95% confidence intervals in the three groups Placebo-Placebo, Placebo-Vaccine and Vaccine-Vaccine. Antibody responses were measured by HAI for influenza A(H1N1) and B, and by FRNT for influenza A(H3N2).

Outcome and Virus	P-P (n=15)	P-V (n=40)	V-V (n=40)	p-value comparing V-V with P-V
*Proportion with ≥4-fold rise in antibody titer from day 0 to day 30 post-vaccination**				
A/Wisconsin/588/2019 (H1N1)	13% (1.6%, 40%)	78% (62%, 89%)	45% (29%, 62%)	0.005
A/Cambodia/e0826360/2020 (H3N2)	0% (0%, 21%)	60% (43%, 75%)	53% (36%, 68%)	0.65
B/Washington/02/2019	6.7% (0.2%, 32%)	70% (53%, 83%)	25% (13%, 41%)	<0.001

B/Phuket/3073/2013	0% (0%, 22%)	68% (51%, 81%)	10% (0.3%, 24%)	<0.001
Proportion with ≥4-fold rise in antibody titer from day 0 to day 30 post-vaccination[†]				
A/Wisconsin/588/2019 (H1N1)	6.7% (0.2%, 32%)	75% (59%, 83%)	35% (21%, 52%)	<0.001
A/Cambodia/e0826360/2020 (H3N2)	0% (0%, 22%)	60% (43%, 75%)	53% (36%, 68%)	0.65
B/Washington/02/2019	6.7% (0.2%, 32%)	63% (46%, 77%)	20% (9.1%, 36%)	<0.001
B/Phuket/3073/2013	0% (0%, 22%)	68% (51%, 81%)	10% (2.8%, 24%)	<0.001
Antibody GMT ratio at day 30 post-vaccination compared to P-P reference group				
A/Wisconsin/588/2019 (H1N1)	-	7.64 (4.0, 15)	5.59 (3.1, 10)	-
A/Cambodia/e0826360/2020 (H3N2)	-	3.97 (2.4, 6.4)	5.65 (3.6, 8.8)	-
B/Washington/02/2019	-	4.14 (1.9, 9.2)	2.83 (1.3, 6.0)	-
B/Phuket/3073/2013	-	4.65 (2.4, 9.1)	3.91 (2.0, 7.8)	-
Antibody GMT ratio at day 30 post-vaccination compared to P-V reference group				
A/Wisconsin/588/2019 (H1N1)	-	-	0.73 (0.37, 1.43)	0.36
A/Cambodia/e0826360/2020 (H3N2)	-	-	1.42 (0.79, 2.55)	0.23
B/Washington/02/2019	-	-	0.68 (0.36, 1.29)	0.23
B/Phuket/3073/2013	-	-	0.84 (0.55, 1.29)	0.42

Proportion with antibody titer ≥40 at day 30 post-vaccination				
A/Wisconsin/588/2019	20%	78%	78%	1.00
(H1N1)	(4.3%, 48%)	(62%, 89%)	(62%, 89%)	
A/Cambodia/e0826360/2020	13%	75%	85%	0.40
(H3N2)	(1.7%, 40%)	(59%. 87%)	(70%, 94%)	
B/Washington/02/2019	53%	88%	88%	1.00
	(26%, 79%)	(73%, 96%)	(73%, 96%)	
B/Phuket/3073/2013	87%	100%	98%	1.00
	(60%, 98%)	(91%, 100%)	(87%, 100%)	

*For influenza A(H1N1) and B this was defined as either a pre-vaccination antibody titer <10 and a post-vaccination antibody titre ≥20, or a pre-vaccination antibody titer ≥10 and at least a four-fold rise in post-vaccination antibody titer. For influenza A(H3N2) this was defined as either a pre-vaccination antibody titer <20 and a post-vaccination antibody titre ≥40, or a pre-vaccination antibody titer ≥20 and at least a four-fold rise in post-vaccination antibody titer.

†Alternate definition of target rise in titer where there must be at least a four-fold rise in titer to a post-vaccination titer ≥40.

Table 3. Geometric mean antibody levels 30 days post-vaccination for participants receiving vaccination for the first time in this study

Group/year	A(H1N1)		A(H3N2)		B/Vic	B/Yam
	A/Hawaii/70/2019 (year 1 vaccine strain)	A/Wisconsin/588/2019 (year 2 vaccine strain)	A/Minnesota/41/2019 (year 1 vaccine strain)	A/Tasmania/503/2020 (year 2 vaccine strain)	B/Washington/02/2019 (vaccine strain in both years)	B/Phuket/3073/2013 (vaccine strain in both years)
Assay	*HAI*	*HAI*‡	*FRNT**	*FRNT*†	*HAI*	*HAI*
V-V group, Year 1	**127.7**	40.0	**107.4**	34.7	**61.7**	**273.8**
P-V group, Year 2	180.6	**60.6**	67.2	**45.6**	**72.1**	**234.3**
Assay	*ELISA*	*ELISA*	*ELISA*	*ELISA*		
V-V group, Year 1	**14400**	5920	**51100**	34600	-	-
P-V group, Year 2	19200	**18000**	48600	**68500**	-	-

Cells in bold indicate homologous responses to vaccine strains. Values for all three assays are antibody titers.

‡ This virus contained an egg adaptation, D204V.

* The similar virus A/Hong Kong/45/2019 was used in place of A/Minnesota/41/2019 for the FRNT assay

† The similar virus A/Cambodia/e0826360/2020 was used in place of A/Tasmania/503/2020 for the FRNT assay