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MAJOR ARTICLE

Immune responses to twice-annual influenza vaccination in older adults in Hong Kong

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Key points: Vaccination with the southern hemisphere influenza vaccine improved protection for older adults in the summer and autumn, but led to slightly blunted immune responses to the northern hemisphere vaccination with implications for protection in the following winter.

ABSTRACT

Background: Many health authorities recommend influenza vaccination of older adults to reduce disease burden. We hypothesized that in tropical and subtropical areas with more prolonged influenza seasons, twice-annual influenza vaccination might provide older adults with improved immunity against influenza.

Methods: In 2014/15, Hong Kong experienced a substantial A(H3N2) winter epidemic with a mismatched vaccine. Local authorities procured and administered to older adults the 2015 southern hemisphere influenza vaccine which included an updated and matching A/Switzerland/9715293/2013(H3N2) strain. We compared immune parameters in pre- and post-vaccination sera from older adults ≥75 years of age who received one versus two influenza vaccines per year.

Results: We enrolled 978 older adults with 470 vaccinations for summer 2015 and 827 vaccinations for winter 2015/16. Recipients of southern hemisphere vaccination had higher geometric mean titers (GMTs) by the hemagglutination inhibition assay against all three vaccine strains. When receiving influenza vaccination for the subsequent winter, the southern hemisphere vaccine recipients had higher pre-vaccination GMTs but lower post-vaccination GMTs, compared to those who had not received the southern hemisphere vaccine. Furthermore, cellular immunity was impacted by bi-annual vaccination, with reduced influenza-specific CD4 T cell responses in the second season of vaccination.

Conclusions: We observed some reductions in immune responses in the twice-annual vaccination group compared to once-annual vaccination group, in the context of unchanging vaccine strains, while protection was likely to have been improved during the summer and autumn for the twice-annual vaccination group due to the continued circulation of the A/Switzerland/9715293/2013(H3N2) virus.

INTRODUCTION

Influenza vaccination is the cornerstone of influenza prevention programmes around the world, and inactivated influenza vaccines are the most commonly used in these programmes. The effectiveness of inactivated influenza vaccines depends, in part, on the degree of matching between vaccine and circulating strains of influenza virus [1]. As the prevailing strains change over time, regular updates of the vaccine strains are necessary to maintain moderate to high vaccine effectiveness [2]. Twice a year the World Health Organization (WHO) issues recommendations on the vaccine strains to be used in the northern hemisphere (NH) and southern hemisphere (SH) [3].

In subtropical and tropical locations, influenza viruses circulate for prolonged periods each year [4], but national authorities usually choose to use either NH or SH vaccine in their annual campaigns [5-7]. Since the immune responses to influenza vaccination may be weaker in older adults [8], and may wane within a year [9-11], older adults who receive once-annual vaccination may not be as well protected during the period 6-12 months after vaccination, and a booster vaccination halfway through the year could sustain protection [12,13]. On the other hand, there is growing evidence that immune responses to repeated vaccination can be blunted, particularly when antigens are unchanged in successive vaccines [14-20], and cellular immunity could also be impaired [21,22]. If blunted immune responses to influenza vaccination led to poorer clinical protection over time [23], that could disadvantage recipients of twice-annual vaccination, in addition to the increased cost of such a strategy [13].

In Hong Kong, a city in southern China with a subtropical climate, influenza epidemics often occur twice per year in the winter and summer, and in some years there can be elevated influenza activity for >9 months [4,24,25]. Older adults are included as a priority group for

influenza vaccination, and the government subsidises the cost in public and private outpatient clinics, with annual campaigns using NH inactivated influenza vaccines each autumn. In the winter of 2014/15, a large influenza A(H3N2) epidemic occurred in Hong Kong with the A/Switzerland/9715293/2013-like strain, which was antigenically mismatched with the A/Texas/50/2012-like virus included in the 2014/15 NH vaccine. Local authorities consequently imported and administered the 2015 SH trivalent inactivated influenza vaccine (TIV), which included an updated and matching A/Switzerland/9715293/2013-like strain [26], primarily targeting older adults ≥75 years old with initial priority given to those ≥85 years of age. We therefore had an opportunity to evaluate the potential impact of twice-annual vaccination on immunity against influenza in older adults. The aim of our study was to assess the immunogenicity of the SH TIV among older adults in Hong Kong and its effect on the immunogenicity of subsequent NH quadrivalent inactivated influenza vaccine (QIV).

METHODS

Study design

In the summer of 2015 we enrolled older adults who presented at public-sector outpatient clinics to receive the SH TIV. Older adults were eligible to participate in the study if they were ≥75 years of age attending public-sector outpatient clinics for routine appointments. We excluded older adults who were not competent to give their consent. Participants received vaccines procured and administered in the government vaccination campaign recommended by the WHO, including the trivalent formulation of the 2015 SH TIV (Vaxigrip, Sanofi Pasteur), and the quadrivalent formulation of the 2015/16 NH QIV (Fluarix Tetra, GlaxoSmithKline) (Supplementary Table 1).

We collected serum samples immediately before vaccination (day 0) and 30 days after vaccination (median 29 days, range: 28-35 days) (Group A1). The same blood collection schedule was repeated for those who were re-enrolled in the subsequent 2015/16 winter for receiving the NH QIV (Group A2), and additional new enrolees who received the 2015/16 NH QIV but had not received the 2015 SH TIV as single-dose comparison group (Group B2). Group A2 included additional enrolees who had received the SH TIV but who had not been enrolled in Group A1. In a subset (20%) of these participants, we collected additionally heparinized whole blood samples immediately before vaccination, and 7 and 30 days after vaccination.

Ethical approval

The study received ethical approval from the Institutional Review Board of the Hospital Authority/Hong Kong West Cluster. Written informed consent was obtained from participants.

Influenza-specific antibody titers by hemagglutination inhibition (HAI), neuraminidase inhibition (NAI) and antibody dependent cellular cytotoxicity (ADCC) assays

Upon collection, blood samples were stored at 2-8°C immediately and delivered to a central laboratory within 2 days for serum extraction, and stored at -80°C prior to testing. Sera were tested in parallel for humoral immune responses against the vaccine strains by the HAI assay [27], and by a newly standardised lectin-based NAI assay [28]. Egg-based virus antigens were prepared following the standard WHO reagent preparation protocols. The HAI assay was carried out using turkey red blood cells with the relevant in-house serum controls in place. Sera were tested in serial doubling dilutions starting at 1:10, and the antibody titer was taken as the reciprocal of the greatest dilution that gave a positive result.

The ability of plasma to bind influenza proteins and activate antibody-dependent cell-mediated cytotoxicity (ADCC) function of NK cells was assessed in a previously described plate-style flow cytometry assay [29,30]. Plate-bound recombinant HA proteins representing the vaccine was used for A/California/07/2009(H1N1) and A/Switzerland/9715293/2013(H3N2) (Sinobiological).

Intracellular cytokine staining (ICS) for influenza-specific T cells

Peripheral blood mononuclear cell (PBMC) fractionation was done on heparinized blood samples by density gradient centrifugation within 12 hours of collection and stored in liquid nitrogen for batch analysis. We determined CD4 and CD8 T cell responses against whole A(H1N1) and A(H3N2) viruses by IFNγ production by ICS at day 0, 7 and 30, representing baseline, acute peak, and memory responses respectively. PBMCs were thawed in cRPMI (with Benzoase (Merck), 5000U), $1x10^6$ PBMCs were stimulated with MOI 4 of live virus representing the vaccine strains A/California/07/2009(H1N1) and A/Switzerland/9715293/2013(H3N2) for 6 hours in the presence of rhIL-2 (Roche), LEAF CD49d and CD28 (BioLegend). Then Brefeldin A, Monensin and anti-human CD107a-Pacific blue were added for a further 16 hours. Following stimulation, cells were stained for live/dead (Zombie-NIR), then in FACS buffer (PBS 10% FBS, 0.1% NaN_3) for a panel of anti-human surface markers: CD3-PETexasDazzle, CD4-BV605, CD8-AF700, dump (CD19/CD16/CD56- BV510), CD45RA-APC, CCR7-PerCPCy5.5 and CCR5-PE (BioLegend). Cells were then fixed with BD Cytofix/Cytoperm, and intracellular staining in BD PermWash buffer for anti-human IFNγ-FITC and IL-2-PECy7. Samples were acquired by flow cytometry on an LSR Fortessa and analysed with FlowJo software. Background cytokine production was determined from no virus stimulation of PBMCs.

Statistical analysis

The outcome measures were assessed in each group to estimate the immunogenicity of the 2015 SH TIV and 2015/16 NH QIV. We estimated the geometric mean titre (GMT) of HAI titers pre- and post-vaccination (i.e. pre-vaccination and 1 month post-vaccination), and the proportion of participants with post-vaccination HAI titre ≥40, a threshold that is considered to be associated with at least 50% protection from influenza virus infection [31]. For NAI titers, we examined pre- and post-vaccination GMTs. When estimating GMTs, we imputed 5 for titers <10, and 2560 for titers ≥1280.

We then compared GMTs following receipt of 2015/16 NH QIV between participants who had received the 2015 SH TIV compared to those who had not. We estimated *a priori* that 400 older adults in each group in the final analysis would be sufficient to detect a ≥10% difference in the prevalence of titers ≥40 between these two groups with 5% significance level and 80% power. We performed stratified analyses to account for baseline differences between groups and used log-linear regression models to examine correlates of post-vaccination GMTs. The magnitude of $IFN\gamma^+$ influenza-specific T cells within each group at day 7 and 30 were compared with the respective day 0 response using Kruskal-Wallis rank sum tests, with analyses restricted to individuals who provided all three samples. The EC_{50} of serum concentration for 50% of maximum NK cell activation versus 1:20 serum dilution was determined by best fit of non-linear log regression analysis.

RESULTS

From May through July 2015, we approached 1684 older adults and finally enrolled 470 who received SH TIV (Group A1) in four public outpatient clinics. The most frequent reasons for

refusal to participate were inability to provide consent, unwillingness to provide blood samples, or lack of interest in the study. Between October and December 2015, we approached 5224 older adults and enrolled 827 who received the 2015/16 NH QIV (group A2), and 408 participants who received NH QIV only (Group B2) (Figure 1). We included 1297 vaccination events in 978 participants in this study (Figure 1). Participants in group A2 were 3 years older on average and more likely to have received influenza vaccination in the prior two years than those in group B2 (Table 1). Because responses varied by age, we stratified the serologic analyses into three age groups: 75-79y, 80-84y, and 85+y.

HAI titers were determined against both influenza A vaccine strains before and after receiving 2015 SH TIV in group A1 and 2015/16 NH QIV in groups A2 and B2 (Figure 2). In group A1, GMTs rose by factors of 1.8-3.4 with the largest fold rise against the updated A/Switzerland/9715293/2013 (H3N2)-like strain (Figure 2). In groups A2 and B2 within each age group, the GMTs against A(H1N1) and A(H3N2) rose after vaccination, with group B2 starting from a lower baseline and rising to a larger post-vaccination GMT than group A2 (Figure 2). Similar patterns were observed for influenza B virus within each age group, where post-vaccination GMTs after receipt of the 2015/16 NH QIV were mostly lower in the group that had received the 2015 SH TIV (Figure 3).

The differences between groups A2 and B2 were maintained after adjustment for age, sex, and prior vaccination with the 2014/15 NH vaccine (Table 2), with group A2 having significantly lower post-vaccination GMTs against A(H3N2), B/Yamagata and B/Victoria strains compared to group B2. A pattern consistent with the GMT changes was also noted in the increase of the proportion of participants reaching titers ≥40 post-vaccination (Table 3), with more participants in group B2 compared to group A2 reaching this threshold against

A(H1N1), A(H3N2) and B/Yamagata, which were included in both the 2015 SH TIV and the 2015/16 NH QIV. The proportion of participants in group A1 with titers ≥40 against A/Switzerland/9715293/2013(H3N2) increased from 58.8% to 86.1% after receiving 2015 SH TIV (Table 3).

NAI antibody titres were determined against influenza A(H1N1) and A(H3N2) before and after receiving 2015 SH TIV in group A1, and 2015/16 NH QIV in groups A2 and B2 (Appendix Figure 1). There was no significant difference in pre-vaccination or post-vaccination GMTs by NAI for all three groups. Furthermore, ADCC antibodies were not boosted by vaccination and there was no measurable difference to the magnitude of the response to the ADCC antibodies generated by the novel H3N2-Switzerland vaccine component (Figure 5B) or in their avidity (Appendix Figure 2).

Analysis of cellular immunity was performed to determine the immunological effect of twice-annual vaccination. In group A1, there was no significant differences post vaccination for H1N1-specific CD4 or CD8 T cells, whilst memory H3N2-specific CD4 T cells at day 30 were significantly increased by vaccination (Figure 4). However, the winter NH vaccination showed significantly reduced T cell responses across groups A2 and B2. Acute day 7 H1N1 and H3N2-specific CD4 and CD8 T cell responses were significantly reduced in group A2 (Figure 4). Furthermore, in the subset of participants who provided longitudinal samples for analysis in groups A1 and A2, there was a significantly lower H3N2-specific CD4 T effector memory ($CCR7^-$ $CD45RA^-$) response at day 30 ($p=0.002$) (Figure 4E).

DISCUSSION

We found that older adults who received the 2015 SH TIV had improved protection against the prevalent A/Switzerland/9715293/2013(H3N2) strain in the summer of 2015, as indicated by the significantly higher post-vaccination HAI titers in group A1 (Figure 2), and over 25% increase of participants with antibody titers ≥40 after vaccination (Table 3). Consistently, within each age stratum, group A2 tended to have higher GMTs by HAI than group B2 prior to receipt of the 2015/16 NH QIV (Figures 2 and 3), and a higher proportion with antibody titers ≥40 prior to receipt of the 2015/16 NH QIV against A(H1N1), A(H3N2) and B/Victoria (Table 3).

However, in the subsequent winter, we found evidence of reduced responses to the 2015/16 NH QIV in those in group A2 who had received the 2015 SH TIV, compared to those in group B2 who had not (Figures 2 and 3). Specifically, despite starting with higher pre-vaccination GMTs by HAI, group A2 had weaker rises in HAI titers and significantly lower post-vaccination GMTs by HAI than group B2 for A(H3N2), B/Yamagata and B/Victoria (Table 3). A lower proportion of participants in group A2 had titers ≥40 to B/Yamagata after receipt of the 2015/16 NH QIV (Table 3). A blunted response to repeated vaccination with the same vaccine components was predicted by the antigenic distance hypothesis, that some of the antigen in the most recent vaccine will be partially eliminated by pre-existing cross-reactive antibody from the prior vaccination leading to reduced responses [15,23]. This phenomenon has also been shown in healthcare workers who received repeated annual influenza vaccination [19].

Nevertheless, vaccination for 2015/16 winter did improve GMTs in participants in our study in the group that received the 2015 SH TIV, with increases in the proportion that had titers

≥40 by 6% to 21% from day 0 to 30 (Table 3). This implies that, despite a reduced response, the NH QIV still improved overall protection levels in older adults who had received SH TIV.

Whereas influenza vaccination led to significant increases in GMTs measured by the HAI assay (Figures 2 and 3, Table 3), there were no significant increases in GMTs measured by the NAI assay (Appendix Figure 1). The subunit inactivated influenza vaccine is purified and includes a defined amount of hemagglutinin protein and a very small amount of neuraminidase protein. NA content was not determined in our study, however no differences in NAI GMTs were observed between A1 (Vaxigrip TIV) and B2 (Fluarix Tetra QIV). Since NAI titers have been associated with protection [32] we infer no increased NAI protective benefit from twice-annual vaccination.

Overall, we found that ADCC antibodies had higher magnitude towards the pandemic A/California/07/2009(H1N1) virus than the A/Switzerland/9715293/2013(H3N2) virus. It is most likely group A1 participants experienced a primary exposure to A/Switzerland/9715293/2013 (H3N2) virus, however no significant boosting effects were seen (Appendix Figure 2), which may be attributed to cross-reactivity with H3N2 viruses that circulated previously and/or an immune ceiling threshold. There was no boosting effects on response magnitude or affinity attributed to biannual vaccination against the A/Switzerland/9715293/2013(H3N2) virus.

This is the first report of direct measurements of vaccine effects on T cell immunity for twice-annual vaccination. Overall, we showed small but statistically significantly reduced T cell responses post-vaccination in a subset of participants in groups A2 and B2 against some

strains (Figure 4). Participants who received both SH TIV and NH QIV (group A2) had reduced CD4 and CD8 T cell responses against both the H1N1 and H3N2 viruses at day 7 and 30 post-vaccination. This could reflect a recruitment of a robust response to the lymph nodes or tissues [33], or diminished T cell response overall, and this unknown is a limitation of human studies on peripheral blood. However, our results indicate twice-annual vaccination may reduce the magnitude of T cell memory responses, particularly CD4 effector memory responses. Considering the half-life of T cells, estimated at 1-5 years [34] and can be detected for the lifetime of an individual [35] and their recognition of conserved influenza epitopes [36], twice-annual vaccination does not appear to have a likely benefit to T cell responses.

There are some limitations to our study. First, this was an observational study, and there may have been other systematic differences between groups in addition to the difference in age that we identified. There was no comparator for group A1 although we did collect sera to assess pre-vaccination titers in this group that serve as a comparison. Second, participants in this study were very old adults, with majority in group A1 and A2 older than 80 years of age. Our results might not generalize to adults between 65-74 years of age, or younger adults. Third, this study was designed to assess the immunogenicity of influenza vaccination over only one year, and the vaccine strains did not change (Appendix Table 1). As the circulating strains of seasonal influenza change over time, a longer-term study would be valuable to investigate how changes in antigens and circulating strains contribute to the boosting and blunting of immune responses to influenza vaccination [15]. We did not have detailed information on prior vaccination history and participants in our study could have received multiple prior vaccinations. Finally, we were not able to study vaccine effectiveness for protection from infection in this study due to a relatively smaller sample size. While HAI

titres are correlated with clinical protection [31,37], other mechanisms may also play a role in the immunity conferred by influenza vaccination.

In conclusion, in this study of immune responses following twice-annual vaccination with SH and NH formulations compared to once-annual vaccination with the NH formulation in older adults, we found that NH QIV improved GMTs regardless of prior receipt of SH TIV, although there was some reduction in immune responses to subsequent NH QIV in the twice-annual vaccination group. Based on HAI titers, protection during the summer did appear to be higher for the twice-annual vaccination group that received SH TIV. Deciding whether to adopt twice-annual vaccination for older adults in the future may depend on predicted patterns in influenza circulation as well as appropriate vaccine availability [13], and our study highlights both the potential advantages and disadvantages of such an approach.

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POTENTIAL CONFLICTS OF INTEREST

BJC has received research funding from Sanofi Pasteur for a study of influenza vaccine effectiveness. The authors report no other potential conflicts of interest.

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FIGURE LEGENDS

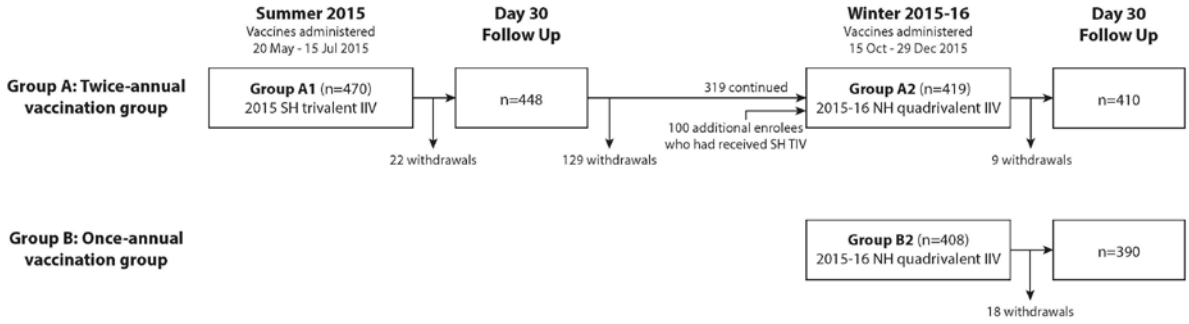

Figure 1. Flow chart of participants in the study. Among all participants enrolled, 49 withdrew after enrolment and vaccination and prior to the post-vaccination blood draw. The reasons for withdrawal included being unwell to return for follow-up (n=14), afraid of blood taking (n=12), hospitalised (n=9), traveling overseas (n=6), unwilling to participate further (n=5) or failed to be reached despite repeated attempts (n=3).

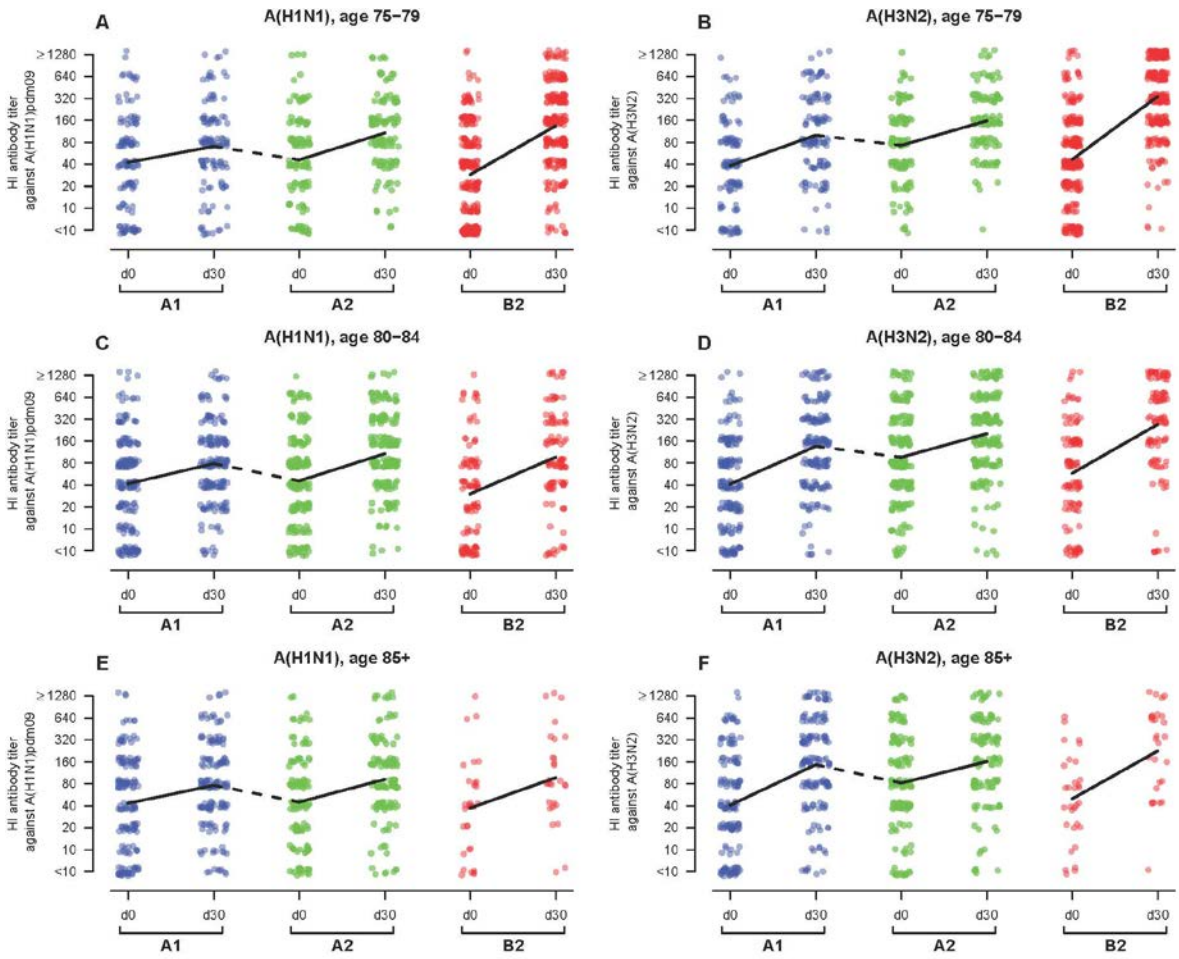

Figure 2. Antibody titres against influenza A(H1N1) and A(H3N2) measured by hemagglutination inhibition assays immediately before and 30 days after vaccination in groups A1, A2 and B2, stratified by age group and plotted by day of specimen collection. Group A1 received the 2015 SH TIV, group A2 received the 2015/16 NH QIV (and had previously received 2015 SH TIV), group B2 received the 2015/16 NH QIV (and had not previously received 2015 SH TIV). The geometric mean pre-vaccination and post-vaccination titers in each group are also shown, connected by straight lines, and a dotted line connects group A1 post-vaccination with group A2 pre-vaccination to represent the decline in GMTs during the fall.

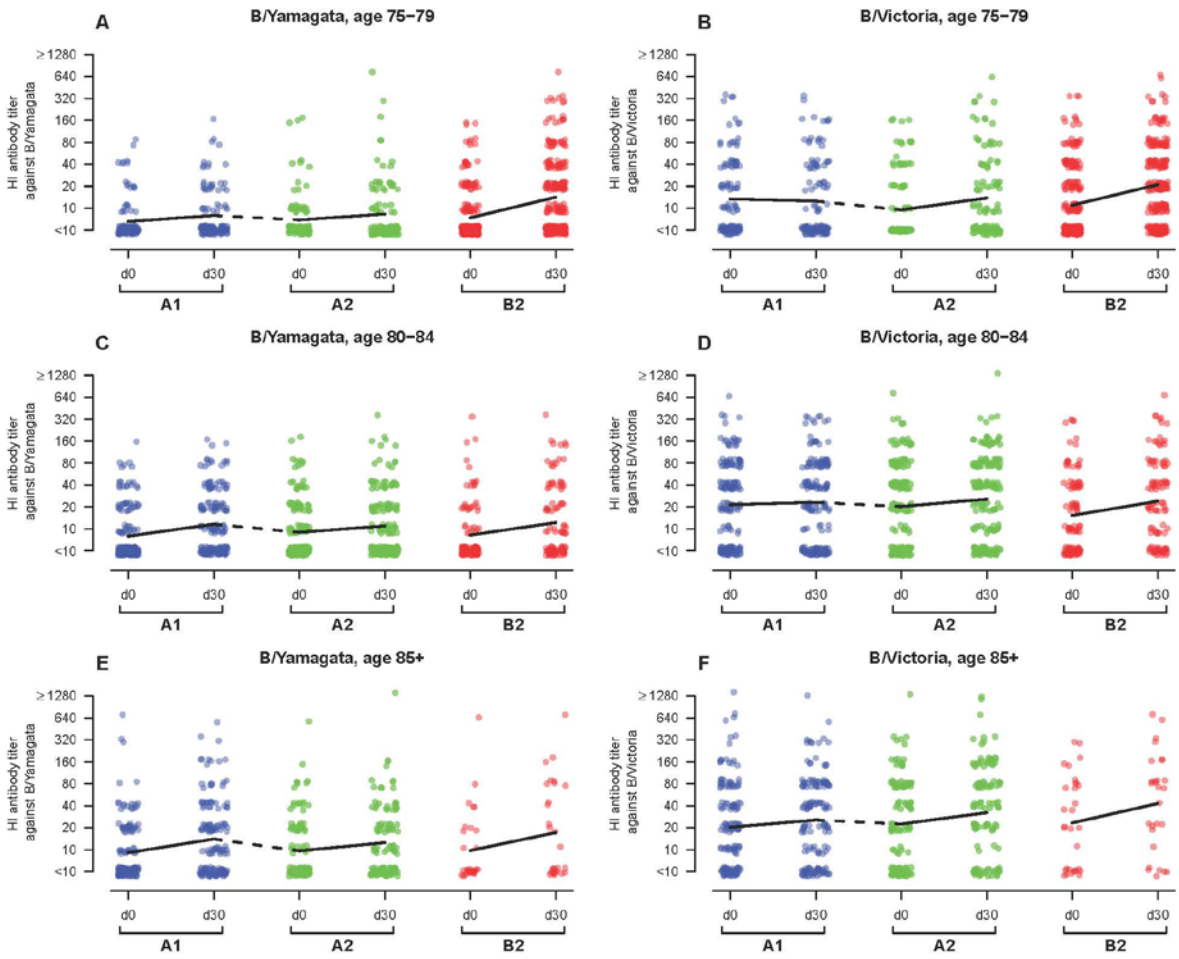

Figure 3. Antibody titres against influenza B/Yamagata and B/Victoria measured by hemagglutination inhibition assays immediately before and 30 days after vaccination in groups A1, A2 and B2, stratified by age group and plotted by day of specimen collection. Group A1 received the 2015 SH TIV, group A2 received the 2015/16 NH QIV (and had previously received 2015 SH TIV), group B2 received the 2015/16 NH QIV (and had not previously received 2015 SH TIV). The geometric mean titers in each group are also shown, connected by straight lines, and a dotted line connects group A1 post-vaccination with group A2 pre-vaccination to represent the decline in GMTs during the fall. The 2015 SH TIV administered to group A1 did not contain a B/Victoria component.

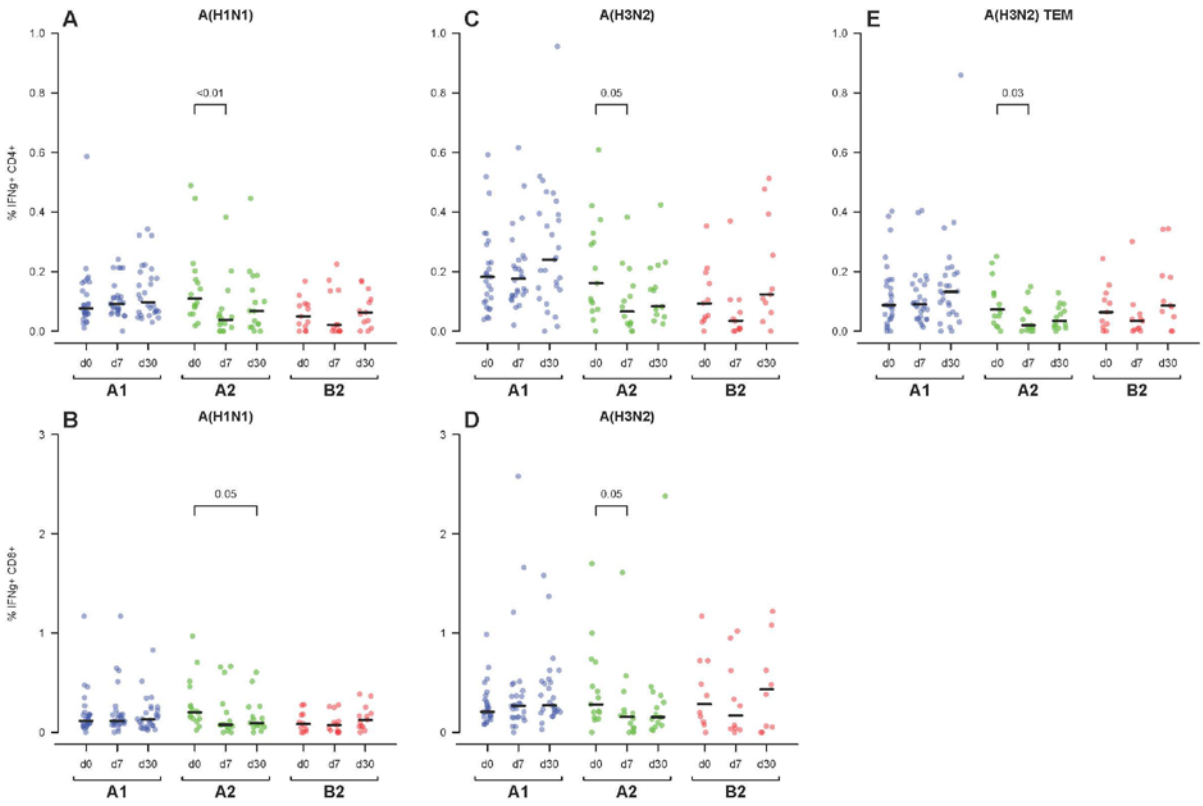

Figure 4. Kinetics of influenza-specific T cell responses following SH and NH vaccination. CD4 T cell and CD8 T cell responses 7 days and 30 days following receipt of SH TIV in 2015 (group A1, n=12), NH QIV in 2015-16 in participants who had previously received SH TIV in 2015 (group A2, n=16), and NH IIV in 2015-16 in participants who had not received SH TIV in 2015 (group B2, n=16). PBMCs were stimulated with A(H1N1) (A, B) and A(H3N2) vaccine strains (C-E), and the frequency of influenza-specific CD4 and CD8 T cells identified by IFNγ production by flow cytometry. A subset of IFNγ+ CD4 T cells that were $CCR7^{-}$ $CD45RA^{-}$, denoted as T effector memory (TEM), showed significant changes post vaccination (panel E), while other subsets and CD8 T cells had no differences. Data are presented as percentage change from day 0 to day 7 and day 30, and mean differences from day 0 that were statistically significant by paired t-tests are indicated. The experiment was repeated twice, using 2 different sets of participants.

Appendix Figure 1. Antibody titres against the neuraminidase of the A(H1N1) and A(H3N2) components of the 2015 SH TIV (the same as the 2015-16 NH QIV) measured by neuraminidase inhibition assays immediately before and 30 days after vaccination in groups A1, A2 and B2. The median and inter-quartile range is shown alongside each group.

Appendix Figure 2. Affinity maturation of the ADCC antibody response was determined by plasma serial titration to determine the EC_{50} of NK cell activation for the H1-HA and H3Switz-HA proteins. In a subset of 12 participants who provided 6 longitudinal samples in groups A1 and A2, serial titration (1:20-1280) was used to determine the EC_{50} serum dilution by non-linear regression analysis. Mean differences between corresponding time points in groups A1 and A2 that were statistically significant by paired t-tests are indicated with an asterisk above the x-axis.

Table 1. Characteristics of participants in the three groups

Characteristics	**Group A1** (n = 470)	**Group A2** (n = 419)	**Group B2** (n = 408)	**Group A2 vs Group B2** p-value*
Age group				
75-79 years	120 (25.5%)	114 (27.2%)	264 (64.7%)	<0.01 [a]
80-84 years	186 (39.6%)	178 (42.5%)	113 (27.7%)	
≥85 years	164 (34.9%)	127 (30.3%)	31 (7.6%)	
Male	284 (60.4%)	253 (60.4%)	241 (59.1%)	0.75 [b]
Received 2014/15 NH influenza vaccine	380 (80.9%)	355 (84.7%)	302 (74.0%)	<0.01 [b]
Received 2013/14 NH influenza vaccine	378 (80.4%)	330 (78.8%)	275 (67.4%)	<0.01 [b]
Presence of one or more underlying medical condition	459 (97.7%)	415 (99.0%)	406 (99.5%)	0.69 [a]
Hypertension	363 (77.2%)	344 (82.1%)	349 (85.5%)	0.21 [b]
Hyperlipidemia	204 (43.4%)	219 (52.3%)	231 (56.6%)	0.24 [b]
Diabetes	139 (29.6%)	159 (37.9%)	165 (40.4%)	0.51 [b]
Cardiac diseases	64 (13.6%)	72 (17.2%)	67 (16.4%)	0.84 [b]

NH: northern hemisphere formulation; SH: southern hemisphere formulation; Group A1: received 2015 SH TIV; Group A2: received 2015/16 NH QIV and had previously received 2015 SH TIV; Group B2: received 2015/16 NH IIV and had not previously received 2015 SH TIV.

[a] Using Fisher's exact test

[b] Using Chi-squared test

Table 2. The association of receipt of SH IIV with the geometric mean HAI titers following receipt of 2015/16 NH IIV, against each vaccine component*.

Comparisons	**Ratio of post-vaccination HAI titers after receipt of 2015/16 NH QIV, comparing participants who did versus did not receive the 2015 SH TIV†**							
	A(H1N1)		**A(H3N2)**		**B/Yamagata**		**B/Victoria**	
	Ratio	(95% CI)	Ratio	(95% CI)	Ratio	(95% CI)	Ratio	(95% CI)
Univariate comparison	0.84	(0.69, 1.02)	0.51	(0.42, 0.63)	0.77	(0.66, 0.90)	1.01	(0.84, 1.22)
Adjusted for age and sex	0.91	(0.74, 1.12)	0.55	(0.45, 0.69)	0.71	(0.60, 0.84)	0.79	(0.65, 0.96)
Adjusted for age, sex and prior receipt of 2014/15 NH IIV	0.91	(0.74, 1.12)	0.55	(0.45, 0.69)	0.71	(0.60, 0.84)	0.79	(0.65, 0.97)

NH: northern hemisphere formulation; SH: southern hemisphere formulation; HAI: hemagglutinin inhibition.

*HAI assays were performed against A/California/7/2009 (H1N1)pdm09-like virus, A/Switzerland/9715293/2013 (H3N2)-like virus, B/Phuket/3073/2013-like virus (Yamagata lineage), and B/Brisbane/60/2008-like virus (Victoria lineage).

†The values shown are the ratios of post-vaccination geometric mean titers. A ratio <1 indicates that the group who received 2015 SH IIV had a lower geometric mean post-vaccination titer after receipt of 2015/16 NH IIV than the group who did not receive 2015 SH IIV.

Table 3. Proportions of participants with antibody titers measured by HAI ≥40 before and after receipt of influenza vaccination in each group

Vaccine strains	**Group A1** (n=470)	**Group A2** (n=419)	**Group B2** (n=408)	**Group A2 vs B2*** **p-value**
A(H1N1)				
Day 0	61.1%	64.7%	52.0%	<0.01
Day 30	78.7%	86.0%	86.6%	0.80
A(H3N2)				
Day 0	58.8%	79.8%	67.2%	<0.01
Day 30	86.1%	93.1%	95.9%	0.08
B/Yamagata				
Day 0	9.6%	10.8%	9.1%	0.42
Day 30	18.4%	17.0%	25.6%	<0.01
B/Victoria				
Day 0	38.3%†	36.2%	26.1%	<0.01
Day 30	43.3%†	44.5%	41.5%	0.40

NH: northern hemisphere formulation; SH: southern hemisphere formulation; Group A1: received 2015 SH TIV; Group A2: received 2015/16 NH QIV and had previously received 2015 SH TIV; Group B2: received 2015/16 NH QIV and had not previously received 2015 SH TIV.

* Wilcoxon Signed-Rank Test, p-value

† A B/Victoria strain was not included in the trivalent SH TIV used in Hong Kong in 2015 (group A1) but was included in the NH QIV used in Hong Kong in 2015/16 (groups A2 and B2).

Appendix Table 1. Vaccine strains recommended by the World Health Organization for NH and SH inactivated influenza vaccines, and the vaccines selected for use in public outpatient clinics in Hong Kong, in 2014/15, 2015, and 2015/16. Changes in WHO recommended vaccine strains from the preceding vaccine are highlighted in bold font. Our study included data before and after receipt of 2015 southern hemisphere and 2015/16 northern hemisphere inactivated influenza vaccines.

Northern hemisphere formulation, 2014/15

Influenza strain	WHO recommended vaccine strains	Fluarix Tetra (GlaxoSmithKline)
A(H1N1)	A/California/7/2009 (H1N1)pdm09-like virus	A/California/7/2009 NIB-74xp
A(H3N2)	A/Texas/50/2012 (H3N2)-like strain	A/Texas/50/2012 NYMC X-223A
B/Yamagata	B/Massachusetts/2/2012-like strain	B/Massachusetts/2/2012
B/Victoria	B/Brisbane/60/2008-like virus	B/Brisbane/60/2008

Southern hemisphere formulation, 2015

Influenza strain	WHO recommended vaccine strains	Vaxigrip (Sanofi Pasteur)
A(H1N1)	A/California/7/2009 (H1N1)pdm09-like virus	A/California/7/2009 NYMC X-179A
A(H3N2)	**A/Switzerland/9715293/2013 (H3N2)-like virus (A/South Australia/55/2014, A/Norway/466/2014, and A/Stockholm/6/2014)**	A/South Australia/55/2014 IVR-175
B/Yamagata	**B/Phuket/3073/2013-like virus**	B/Phuket/3073/2013
B/Victoria	B/Brisbane/60/2008-like virus	Not included (trivalent vaccine)

Northern hemisphere formulation, 2015/16

Influenza strain	WHO recommended vaccine strains	Fluarix Tetra (GlaxoSmithKline)
A(H1N1)	A/California/7/2009 (H1N1)pdm09-like virus	A/Christchurch/16/2010 NIB 74XP
A(H3N2)	A/Switzerland/9715293/2013 (H3N2)-like virus	A/Switzerland/9715293/2013 NIB-88
B/Yamagata	B/Phuket/3073/2013-like virus	B/Phuket/3073/2013
B/Victoria	B/Brisbane/60/2008-like virus	B/Brisbane/60/2008