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Comparative Immunogenicity of Several Enhanced Influenza Vaccine Options for Older Adults: A Randomized, Controlled Trial

Benjamin J. Cowling[1], **Ranawaka A. P. M. Perera**[1], **Sophie A. Valkenburg**[1,2], **Nancy H. L. Leung**[1], **A. Danielle Iuliano**[3], **Yat Hung Tam**[1], **Jennifer H. F. Wong**[1], **Vicky J. Fang**[1], **Athena P. Y. Li**[1,2], **Hau Chi So**[1], **Dennis K. M. Ip**[1], **Eduardo Azziz-Baumgartner**[3], **Alicia M. Fry**[3], **Min Z. Levine**[3], **Shivaprakash Gangappa**[3], **Suryaprakash Sambhara**[3], **Ian G. Barr**[4,5], **Danuta M. Skowronski**[6,7], **J. S. Malik Peiris**[1], **Mark G. Thompson**[3]

[1]World Health Organization Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, The University of Hong Kong, Hong Kong Special Administrative Region, Hong Kong, China,

[2]The University of Hong Kong-Pasteur Research Pole, School of Public Health, The University of Hong Kong, Hong Kong Special Administrative Region, Hong Kong, China,

[3]Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia, USA,

[4]World Health Organization Collaborating Centre for Reference and Research, Melbourne, Victoria, Australia,

[5]Department of Microbiology and Immunology, University of Melbourne, Victoria, Australia,

[6]British Columbia Centre for Disease Control, Vancouver, Canada,

[7]University of British Columbia, Vancouver, Canada

Abstract

Correspondence: B. J. Cowling, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 7 Sassoon Road, Pokfulam, Hong Kong Special Administrative Region, China (bcowling@hku.hk).

Author contributions. B. J. C. and M. G. T. designed the study and drafted the paper. B. J. C. wrote the statistical analysis plan and is the guarantor. R. A. P. M. P., S. A. V., and A. P. Y. L. conducted laboratory testing. B. J. C., M. G. T., N. H. L. L., and A. D. I. designed the data collection tools. Y. H. T. and J. H. F. W. implemented the trial. V. J. F. cleaned and analyzed the data. B. J. C., M. G. T., N. H. L. L., A. D. I., H. C. S. and D. K. M. I. monitored the data collection for the whole trial. B. J. C., R. A. P. M. P., S. A. V., N. H. L. L., A. D. I., A. P. Y. L., E. A.-B., A. M. F., M. Z. L., S. G., S. S., I. G. B., D. M. S., J. S. M. P., and M. G. T. interpreted the data. All authors revised the paper.

Supplementary Data

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

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Background.—Enhanced influenza vaccines may improve protection for older adults, but comparative immunogenicity data are limited. Our objective was to examine immune responses to enhanced influenza vaccines, compared to standard-dose vaccines, in community-dwelling older adults.

Methods.—Community-dwelling older adults aged 65–82 years in Hong Kong were randomly allocated (October 2017–January 2018) to receive 2017–2018 Northern hemisphere formulations of a standard-dose quadrivalent vaccine, MF59-adjuvanted trivalent vaccine, high-dose trivalent vaccine, or recombinant-hemagglutinin (rHA) quadrivalent vaccine. Sera collected from 200 recipients of each vaccine before and at 30-days postvaccination were assessed for antibodies to egg-propagated vaccine strains by hemagglutination inhibition (HAI) and to cell-propagated A/Hong Kong/4801/2014(H3N2) virus by microneutralization (MN). Influenzaspecific $CD4^+$ and $CD8^+$ T cell responses were assessed in 20 participants per group.

Results.—Mean fold rises (MFR) in HAI titers to egg-propagated A(H1N1) and A(H3N2) and the MFR in MN to cell-propagated A(H3N2) were statistically significantly higher in the enhanced vaccine groups, compared to the standard-dose vaccine. The MFR in MN to cell-propagated A(H3N2) was highest among rHA recipients (4.7), followed by high-dose (3.4) and MF59-adjuvanted (2.9) recipients, compared to standard-dose recipients (2.3). Similarly, the ratio of postvaccination MN titers among rHA recipients to cell-propagated A(H3N2) recipients was 2.57-fold higher than the standard-dose vaccine, which was statistically higher than the high-dose (1.33-fold) and MF59-adjuvanted (1.43-fold) recipient ratios. Enhanced vaccines also resulted in the boosting of T-cell responses.

Conclusions.—In this head-to-head comparison, older adults receiving enhanced vaccines showed improved humoral and cell-mediated immune responses, compared to standard-dose vaccine recipients.

Clinical Trials Registration.—NCT03330132.

Keywords

influenza; vaccination; public health

Influenza vaccination is the cornerstone public health intervention to reduce the annual burden of influenza epidemics [1]. The most widely used influenza vaccines are inactivated influenza vaccines, administered intramuscularly [2]. Trivalent inactivated influenza vaccines contain a total of 45 μg of hemagglutinin (HA), with a “standard dose” (SD) of 15 μg each of influenza A(H1N1), A(H3N2), and B strains, while quadrivalent vaccines contain an additional 15 μg HA of a second B strain. While these are the most commonly used vaccines, their effectiveness is variable from year to year [3].

Enhanced influenza vaccines have the potential to overcome issues associated with an aging immune system and reduced vaccine effectiveness [4–7]. The MF59-adjuvanted vaccine (FluAD) includes MF59, a squalene-based emulsion, in addition to 45 μg of HA (trivalent formulation), and produces a stronger immune response than the SD vaccine [8, 9], including increased breadth, diversity, and avidity of HA antibodies [10]. However, there are no available trials comparing the clinical efficacy of the MF59-adjuvanted vaccine to the SD

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vaccine in older adults. The trivalent high-dose (HD) vaccine, FluZone, contains a total of 180 μg of HA (60 μg of each HA): that is, 4 times the antigen of a trivalent SD vaccine. Compared to an SD vaccine, the HD vaccine induced significantly higher antibody responses [11] and provided better efficacy than the SD vaccine against laboratory-confirmed influenza among older adults [12]. A recombinant HA-protein quadrivalent influenza vaccine, FluBlok, includes 180 μg of HA (45 μg of each HA), produced by Baculovirus expression in insect SF9 cell culture [13, 14]. This vaccine was more efficacious than an SD vaccine in preventing polymerase chain reaction–confirmed influenza among adults aged ≥50 years [15].

Although there is some evidence to support improved protection among older adults for these 3 enhanced influenza vaccines, multiple knowledge gaps remain. The immunogenicity of enhanced vaccines has not been directly compared against the same influenza vaccine antigens within the same population. This is especially important for the A(H3N2) vaccine component, given the lower clinical effectiveness against A(H3N2) illness among older adults [16] and the effects of egg adaptations observed in association with A(H3N2) vaccine components [17]. Therefore, we conducted a randomized, controlled trial to examine immune responses to 3 enhanced influenza vaccines, compared to SD vaccines, in community-dwelling older adults. Because antibody responses have limitations as predictors of vaccine efficacy, especially among older adults [18], we also compared T-cell responses to the vaccines [19]. Although large vaccine efficacy trials will ultimately be needed to clarify the relative preventive benefits of enhanced vaccines among older adults, over the course of this immunogenicity trial we aim to directly quantify and compare relative differences in immune responses to inform this substantial future investment.

METHODS

Recruitment and Follow-up of Participants

We enrolled community-dwelling older adults who were: (1) 65–82 years of age; (2) residing in Hong Kong; and (3) had not already received the Northern hemisphere 2017–2018 formulation of the influenza vaccine. Minor exclusion criteria and details on recruitment methods are described in the Supplementary Appendix.

Because we intend to examine alternative combinations of repeated vaccinations with SD and enhanced vaccines in future years of this trial, we randomized participants to 11 different groups (see the Supplementary Appendix). In Year 1, we randomized 3/11 (27%) participants to receive an SD quadrivalent vaccine (0.5 mL FluQuadri), 3/11 (27%) to receive a trivalent MF59-adjuvanted vaccine (0.5 mL FLUAD, Seqirus), 3/11 (27%) to receive a trivalent high-dose vaccine (0.5 mL Fluzone High-Dose, Sanofi Pasteur), and 2/11 (18%) to receive a quadrivalent recombinant-HA vaccine (0.5 mL Flublok, Sanofi Pasteur). All vaccines included the strains recommended for the Northern hemisphere 2017–2018 formulation: namely, the A/Michigan/45/2015(H1N1)-like virus (clade 6B.1), A/Hong Kong/4801/2014(H3N2)-like virus (clade 3C.2a), and B/Brisbane/60/2008-like virus (Victoria lineage; clade 1A), and the quadrivalent vaccines also included the B/Phuket/3073/2013-like virus (Yamagata lineage; clade 3).

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We used R software to generate a block-randomized allocation sequence (see the Supplementary Appendix), which was concealed from the study staff. Study vaccines were repackaged into numbered boxes, based on the allocation sequence, by a research assistant who had no contact with participants. The study was blinded to participants, researchers, and laboratory staff, but was not blinded to the nurse who removed the vaccine from the numbered box and administered it. Randomization was done immediately prior to the administration of the vaccine for each participant.

We collected a 9 ml blood sample from each participant immediately prior to vaccination and 30 days (range, 26–35 days) after vaccination. A nonrandom, voluntary subset of 10% of participants (see the Supplementary Appendix) was invited to provide an additional 10 ml of heparinized whole-blood specimens at baseline, 7 days, and 30 days after vaccination for the extraction of peripheral blood mononuclear cells (PBMCs) and testing for cell-mediated immune responses to vaccination.

After vaccination, participants were observed for 15 minutes for acute reactions, and contacted at 1, 3, 7, and 14 days after vaccination to record any adverse events. During the 30-day postvaccination visit, we collected information on any serious adverse events since vaccination.

Ethics

Signed, informed consent was obtained from all participants. The study protocol was approved by the Institutional Review Board of the University of Hong Kong.

Laboratory Methods

A subset of 200 pairs of Day 0 and Day 30 sera from each of the 4 vaccine groups were selected, including all sera from the participants in the nonrandom 10% subset who provided PBMCs, plus a random sample of the other participants (see the Supplementary Appendix). Sera were tested by hemagglutination inhibition (HAI) assays against egg-propagated vaccine strains A/Singapore/GP1908/2015 (A/Michigan/45/2015[H1N1]-like virus), A/Hong Kong/4801/2014(H3N2), B/Brisbane/60/2008, and B/Phuket/3073/2013. In addition, we tested the sera by virus microneutralization (MN) against cell-propagated A/Hong Kong/4801/2014(H3N2), because the cell-propagated virus, which is more similar to the circulating strains, failed to agglutinate red blood cells (see the Supplementary Appendix).

In a subset of 20 participants in each of the 4 vaccine groups, influenza-specific $CD4^+$ and $CD8^+$ T-cell responses against influenza A(H1N1), A(H3N2), and B vaccine strains were assessed by flow cytometry for interferon (IFN)-γ^+ production after in vitro stimulation (see the Supplementary Appendix) to determine $CD8^+$ and $CD4^+$ T-cell recall responses at Day 7 and memory responses at Day 30, compared to prevaccination (Day 0) [20].

Outcome Measures

Following the approach taken by a recent review of randomized trials comparing the immunogenicity of alternative influenza vaccine types among older adults [5], the primary outcome measures relate to the immune responses, measured by antibody titers against the

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vaccine strains in each enhanced vaccine group, compared to SD, including: (1) the proportion of participants who achieved a 4-fold or greater rise in postvaccination titers from Day 0 to Day 30 with a postvaccination titer ≥40; and (2) the geometric mean titer (GMT) at Day 30. In addition, we examined 3 protocol-specified secondary outcome measures: (1) the proportion of participants who achieved a postvaccination (Day 30) antibody titer ≥40 (and further elevated titers of ≥80 and ≥160); (2) the fold rise of vaccine-specific IFN-γ^+ $CD4^+$ and IFN-γ^+ $CD8^+$ T-cell responses at 7 (acute) and 30 (memory) days postvaccination, compared to prevaccination (Day 0); and (3) the rate of adverse events within 30 days after each vaccination.

Statistical Analysis

We estimated the proportion of participants with a ≥4-fold rise in antibody titers that led to a postvaccination titer ≥40, the GMTs at Day 0 and Day 30, and the mean fold rise (MFR) from Day 0 to Day 30, with 95% confidence intervals. We calculated the ratio of postvaccination GMTs in each enhanced vaccine group, compared to the postvaccination GMT in the SD group, with 95% confidence intervals. We estimated the proportions of participants with postvaccination titers ≥40, ≥80, and ≥160 in each vaccination group, and compared the proportions in the other 3 vaccination groups versus an SD quadrivalent influenza vaccine (QIV), using Chi-squared tests. Although *P* values <.05 were considered statistically significant in order to optimize sensitivity to differences between exposure groups, we considered as noteworthy any patterns of effects that were repeated across outcomes. As previously noted [21], with a *P* value threshold <.05, a statistically significant difference can be observed even when 95% confidence intervals for the estimate overlap. All statistical analyses were conducted using R version 3.4.3.

Sample Size Justification

For the present comparison of responses to 4 different vaccines in Year 1, we sought to detect small to moderate but meaningful relative differences in immunogenicity. A sample size of 200 participants per group would permit 80% power to detect increases of ≥14% in the proportion of enhanced vaccine recipients with ≥4-fold rises in titer, and GMT ratios of 1.4 or higher in the enhanced vaccine groups, compared to a SD vaccine. Overall, we aimed to enroll 2200 participants into our study, anticipating a 10% dropout rate per year and a final sample size of at least 146 participants in each group through to the fourth year of the planned trial (see the Supplementary Appendix).

RESULTS

We screened 3283 older adults, of whom 2695 were eligible and 2039 agreed to participate. We obtained signed informed consent from 2008 persons and were able to randomize and vaccinate 1861 participants from 7 October 2017 through 12 January 2018, of whom 1826 provided Day 30 postvaccination serum samples (Figure 1). About 10% of participants were asked to give additional blood; 168 contributed PBMCs for analyses of cell-mediated immunity.

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Participants were balanced by age, sex, underlying medical conditions, and prior vaccination history between the 4 study groups, with no statistically significant differences in these key variables overall or among those contributing to the primary antibody findings reported here (Table 1). The most common underlying conditions were hypertension, osteoarthritis, and diabetes. Most participants (66%) had received an influenza vaccination in the preceding year (2016–2017), and only 22% had not received any influenza vaccination in the preceding 5 years. All prior influenza vaccinations were with an SD vaccine. We selected 200 participants from each group for a serologic analysis, and the characteristics of this subset were representative of the entire cohort (Table 2). Pre- and post-vaccination GMTs and corresponding MFRs are described in Table 3 for each vaccine group, and Figure 2 presents comparisons of the MFRs between the enhanced vaccine groups and the SD group. Table 4 shows the proportions of participants achieving specific titer thresholds after vaccination.

There were no statistically significant baseline differences between the groups in prevaccination GMTs for each of the antigens tested, except that the baseline GMTs to the B/Victoria antigen were statistically significantly lower in the SD group (Table 3). As shown in Table 3, among SD QIV recipients, the HAI GMT to A(H1N1) increased from 17 to 69. MF59-adjuvanted and HD recipients achieved a significantly higher postvaccination GMT to A(H1N1) than SD recipients; recombinant-HA recipients did not. Nonetheless, MFRs for A(H1N1) were statistically significantly higher for all 3 enhanced vaccines (range, 5.3–6.1) compared to the SD (4.1; Table 3; Figure 2). The proportions with ≥4-fold rises to titers ≥40 were statistically significantly higher for all 3 enhanced vaccines (range, 59–60%) compared to the SD QIV (42%). As shown in Table 4, the proportions achieving elevated titers of ≥40 were higher for the MF59-adjuvanted (82%) and HD (83%) groups, compared to the SD recipients (72%). Of note, the proportions achieving very high titers of ≥160 were significantly higher for all 3 enhanced vaccines (45–55%), compared to the SD vaccine (35%).

Recipients of all 3 enhanced vaccines achieved higher postvaccination GMTs and greater MFRs to both the egg-propagated A(H3N2) by HAI and the cell-propagated A(H3N2) by MN than recipients of SD vaccines (Table 3; Figure 2). The directions of the effects were similar using other indicators. Of note, only 28% of SD recipients achieved ≥4-fold rises in MN responses to cell-propagated A(H3N2), compared to 39% of MF59-adjuvant, 47% of HD QIV, and 57% of recombinant-HA QIV recipients ($P < .01$; Table 3).

On several indicators, antibody responses to cell-propagated A(H3N2) by MN were significantly higher among recombinant-HA QIV recipients, compared to SD vaccine, MF59-adjuvant, and HD recipients (Table 3). Specifically, recipients of recombinant-HA QIV achieved a postvaccination GMT to cell-propagated A(H3N2) that was 2.57-fold higher than that of SD recipients; the magnitude of this difference was significantly higher than the 1.43- and 1.33-fold differences of MF59-adjuvanted and HD recipients, respectively, compared to SD recipients (Figure 2). Recombinant-HA recipients were the only enhanced vaccine group with a significantly larger proportion (74%) of very high titers (≥160) against cell-propagated A(H3N2), compared to SD recipients (48%; Table 4).

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Responses to the B/Victoria component of both the trivalent influenza vaccine (TIV) and QIV in the study year (B/Brisbane/60/2008) were similar across vaccines, with the exception of a significantly higher GMT of HD TIV, compared with SD QIV (Figure 2). Although the MFRs against B/Phuket/3073/2013 were statistically significantly >1 for both SD and enhanced vaccines (Table 3), the GMTs were significantly lower, compared with the SD QIV, for the MF-59-adjuvanted and HD TIVs, which did not include this B/Yamagata component (Figure 2). The response to recombinant-HA QIV that did include B/Yamagata was not significantly different to SD QIV on any indicator.

For T-cell responses at 7 and 30 days postvaccination, we noted a trend whereby enhanced influenza vaccines elicited increased peak acute IFN-γ^+ T-cell responses and maintained higher average memory responses for selected time points and viruses (Figure 3; Supplementary Appendix Table 5). For influenza-specific IFN-γ^+ $CD8^+$ T-cell responses, statistically significant rises in T-cell responses from Day 0 to Day 30 were noted for the recombinant-HA vaccine against A(H1N1) and B/Victoria and for the MF59-adjuvanted vaccine against B/Victoria; no significant rises in the SD group were observed. IFN-γ^+ $CD8^+$ T cells to B/Victoria increased from Day 7 to Day 30 for all 3 enhanced vaccines; this trend was statistically significant when contrasted with a decline in T cells among SD vaccinees.

For influenza-specific IFN-γ^+ $CD4^+$ T-cell responses, statistically significant rises in T-cell responses were noted at 7 and/or 30 days postvaccination for the enhanced vaccines, though none of these were statistically significantly higher than the trend for the SD vaccine. Overall, the recombinant-HA vaccine caused sustained memory IFN-γ^+ $CD4^+$ T-cell responses by significantly increased Day 30 mean fold rises for 3 of 3 viruses tested, whilst the HD vaccine increased $CD4^+$ T-cell Day 30 memory fold rises for 2 of 3 viruses and the MF59-adjuvanted vaccine resulted in early Day 7 rises for 2 of 3 viruses and sustained memory rises for only 1 of 3 viruses. SD group had a significant rise in $CD4^+$ T-cell response to 1 virus at day 7 and no significant rises for all 3 viruses tested at Day 30.

The rate of contraction of T-cell responses from acute Day 7 to memory Day 30 was determined by the fold change from Day 30, divided by Day 7 (Supplementary Appendix Table 5). We did not observe statistically significant contractions (or expansions) for virus-specific $CD4^+$ T cells for any vaccines. For virus-specific $CD8^+$ T cells, a significant difference for the fold change from acute Day 7 to memory Day 30 responses was noted for B/Victoria only. Among those receiving the SD vaccine, B/Victoria-specific $CD8^+$ T cells significantly contracted by 0.65-fold from 7 to 30 days postvaccination. In contrast, among those receiving the enhanced vaccines, B/Victoria-specific $CD8^+$ T cells continued to increase by 1.34- to 1.40-fold from Day 7 to Day 30 (P values all <.05, compared to the standard vaccine group); therefore, enhanced vaccines maintained and expanded a larger pool of circulating B/Victoria-specific $CD8^+$ T cells than the SD vaccine.

The most frequently reported adverse events following vaccination included local reactions, such as tenderness (range across vaccine groups, 12–20%), pain (10–22%) and swelling (4–10%; Supplementary Appendix Figure 4). Compared to the SD group, recipients of the MF59-adjuvanted vaccine and HD vaccine experienced more tenderness and recipients of

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the HD vaccine experienced more swelling, mostly of a mild nature. Hospitalizations were not uncommon, but the rates of postvaccination hospitalizations were not statistically different between the 4 vaccine groups (Supplementary Appendix Table 3). We did not identify any potentially vaccine-related serious adverse events.

DISCUSSION

We found that all 3 of the 2017–2018 Northern hemisphere–enhanced vaccines had improved immunogenicity for influenza A(H1N1) and A(H3N2), compared to the SD vaccine, among older adults, while the recombinant-HA vaccine elicited a particularly high antibody response to the cell-like H3N2 strain, relative to the other enhanced vaccines and the SD vaccine (Figure 2). Improved $CD4^+$ T-cell responses against H3N2 were also seen at Day 7 or Day 30 for each enhanced vaccine (Figure 3). Influenza A(H3N2) virus infection causes the greatest morbidity and mortality effects in older adults [22] and there have been particular concerns in recent years over poorer vaccine effectiveness against influenza A(H3N2) [16, 23], which may be at least partially due to egg adaptations in A(H3N2) vaccine strains and/or poorer immunogenicity in repeated vaccinees [17, 24–27].

Enhanced vaccines resulted in higher antibody responses on almost all indicators, compared to a SD vaccine, to the A/Michigan/45/2015(H1N1)-like virus, which was introduced in the 2017–2018 season. The ratios of postvaccination titers against the A(H1N1) component for the MF59-adjuvanted vaccine and the HD vaccine, compared to the SD vaccine (1.37-fold higher and 1.80-fold higher, respectively), were very similar to pooled estimates in a recent meta-analysis for this ratio across previous immunogenicity trials (1.28 and 1.72, respectively) [5]. The antibody response to A(H1N1) among recombinant-HA recipients in our study was better than the A(H1N1) response among older adults in a previous recombinant-HA trial [15]. Our findings for A(H3N2) were also within the range of postvaccination ratios for the HD vaccine and MF59-adjuvanted vaccine, compared to the SD vaccine, when observed across prior trials [5]. Our findings reinforce other studies suggesting that enhanced vaccines offer improved antibody responses over standard vaccines in older adults. However, our study was not designed to demonstrate efficacy against polymerase chain reaction–confirmed influenza, and comparisons of clinical efficacy and effectiveness are still needed.

However, the findings for influenza B viruses were less clear. Antibody responses to the influenza B/Victoria-lineage component, which was in the TIV and QIV formulations in 2017–2018 and the previous season, were high for all vaccines. There were also significant improvements over the SD vaccine in $CD8^+$ T-cell responses to B/Victoria (Figure 3). Not surprisingly, the quadrivalent SD vaccine resulted in higher antibody responses to the influenza B/Yamagata-lineage strain, compared to the trivalent MF59-adjuvanted and HD vaccines, which did not include this component; nonetheless, both vaccines were associated with significant rises in B/Yamagata titers (Table 3). The QIV recombinant-HA vaccine did not result in a higher antibody response to the influenza B/Yamagata lineage, compared to the QIV SD vaccine, consistent with a previous trial [13].

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Our study had a number of limitations. First, vaccine immunogenicity is not equivalent to vaccine efficacy or effectiveness, nor is there yet a way to directly extrapolate our findings into an epidemiologic measure of differences in actual vaccine protection. Second, we only tested sera from a subset of 800 participants, determining that this would be sufficient to compare vaccines in the first year and allowing us to save more of the sera for future cross-season analyses. Two-thirds of participants were vaccinated during the previous season; examining the association between vaccination histories and immune response will also require additional future testing. Finally, here we only examined the immediate response to vaccination; we did not examine the duration of protection or effects on subsequent vaccinations with enhanced or SD vaccines.

In conclusion, this randomized, controlled trial provides a unique comparison of the immune response to multiple enhanced influenza vaccine options for older adults. Although distinguishing the relative preventive value of alternative vaccine types among older adults will ultimately depend on future efficacy trials and/or well-controlled effectiveness studies, the findings from this ongoing immunogenicity trial can help to establish the conditions for those more definitive evaluations, including what differences in efficacy/effectiveness might plausibly be expected.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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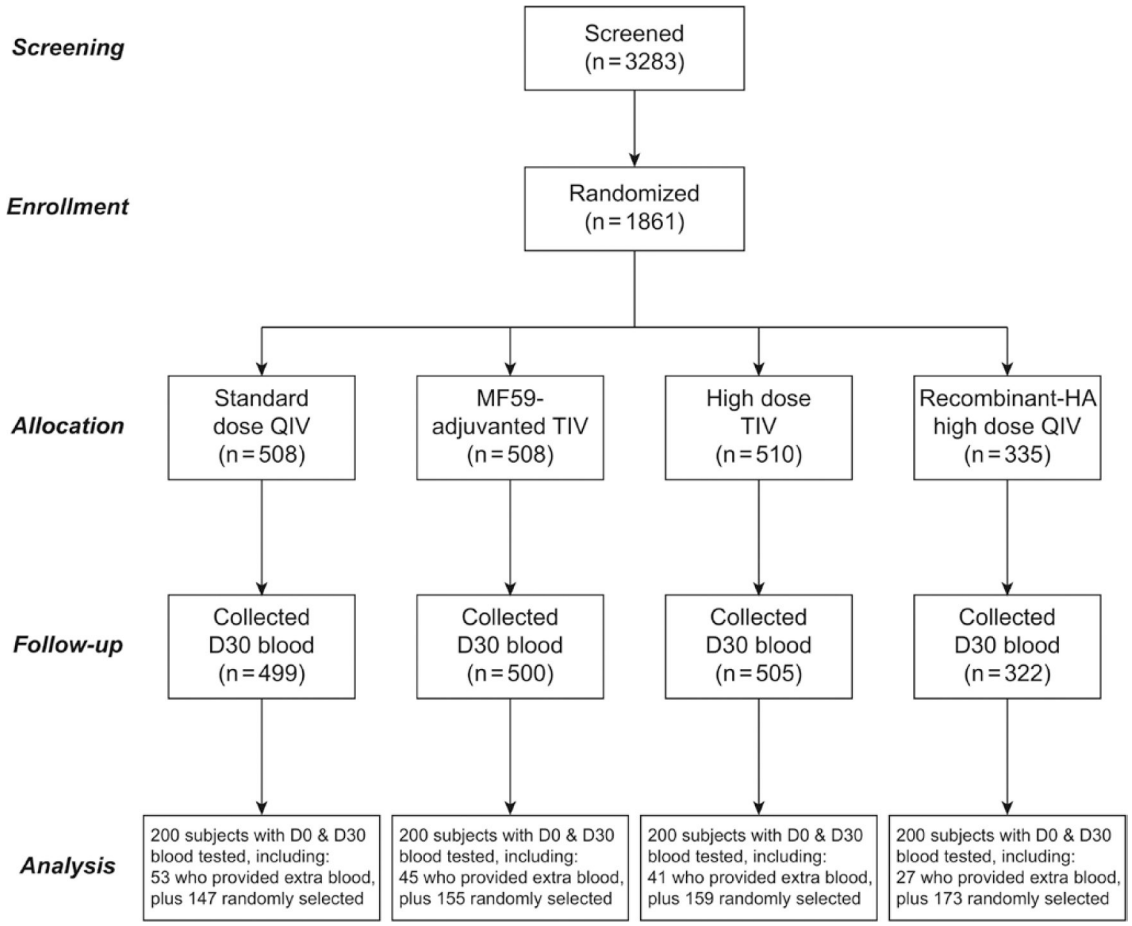

Figure 1.

Flow chart of participants through the study, and selection of 200 participants per group for serologic analysis. Abbreviations: D, day; HA, hemagglutinin; QIV, quadrivalent influenza vaccine; TIV, trivalent influenza vaccine.

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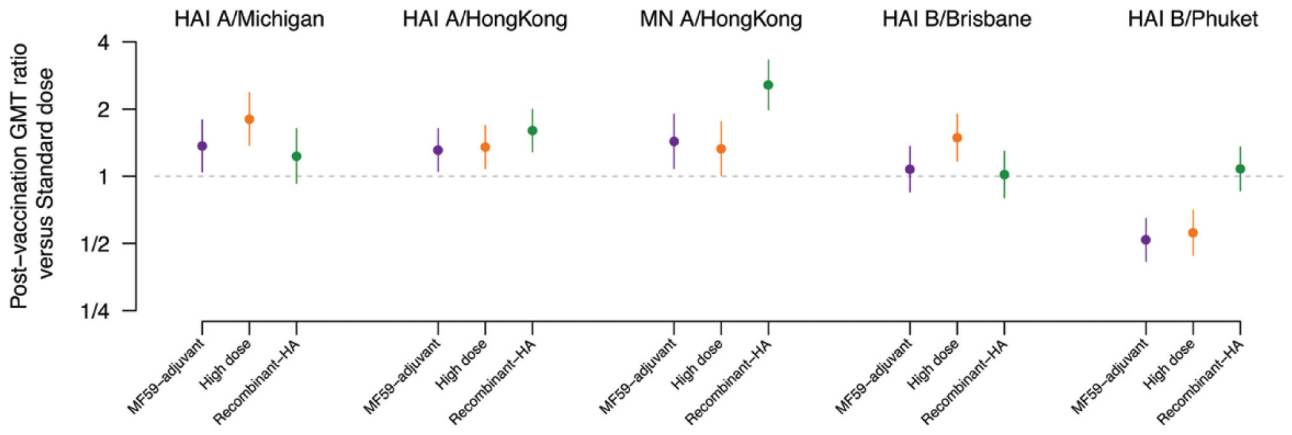

	GMTR	(95% CI)	GMTR	(95% CI)	GMTR	(95% CI)	GMTR	(95% CI)	GMTR	(95% CI)
Standard dose	1.00		1.00		1.00		1.00		1.00	
MF59−adjuvant	**1.37**	**(1.05 – 1.78)**	**1.31**	**(1.05 – 1.63)**	**1.43**	**(1.09 – 1.89)**	1.08	(0.85 – 1.36)	**0.52**	**(0.42 – 0.65)**
High dose	**1.80**	**(1.38 – 2.36)**	**1.35**	**(1.08 – 1.69)**	**1.33**	**(1.01 – 1.75)**	**1.49**	**(1.17 – 1.89)**	**0.56**	**(0.44 – 0.70)**
Recombinant−HA	1.23	(0.93 – 1.63)	**1.60**	**(1.29 – 1.99)**	**2.57**	**(1.99 – 3.31)**	1.02	(0.80 – 1.29)	1.08	(0.86 – 1.35)

Figure 2.

Comparisons of postvaccination geometric mean antibody titers in each of the enhanced vaccine groups, compared to the postvaccination geometric mean titer in the standard-dose QIV group (n = 200 individuals per group). Values significantly different from 1.0 are highlighted in bold. Note that a B/Yamagata lineage virus was not included in the MF59-adjuvanted TIV and the high-dose TIV. Antigens used were A/Singapore/GP1908/2015 (A/Michigan/45/2015[H1N1]-like virus); A/Hong Kong/4801/2014(H3N2), with both egg-propagated and cell-propagated variants; B/Brisbane/60/2008; and B/Phuket/3073/2013. Abbreviations: CI, confidence interval; GMT, geometric mean titer; GMTR, geometric mean titer ratio comparing post-vaccination geometric mean titers between enhanced vaccine groups and the standard dose group; HA, hemagglutinin; HAI, hemagglutination inhibition assay; MN, microneutralization assay; QIV, quadrivalent influenza vaccine; TIV, trivalent influenza vaccine.

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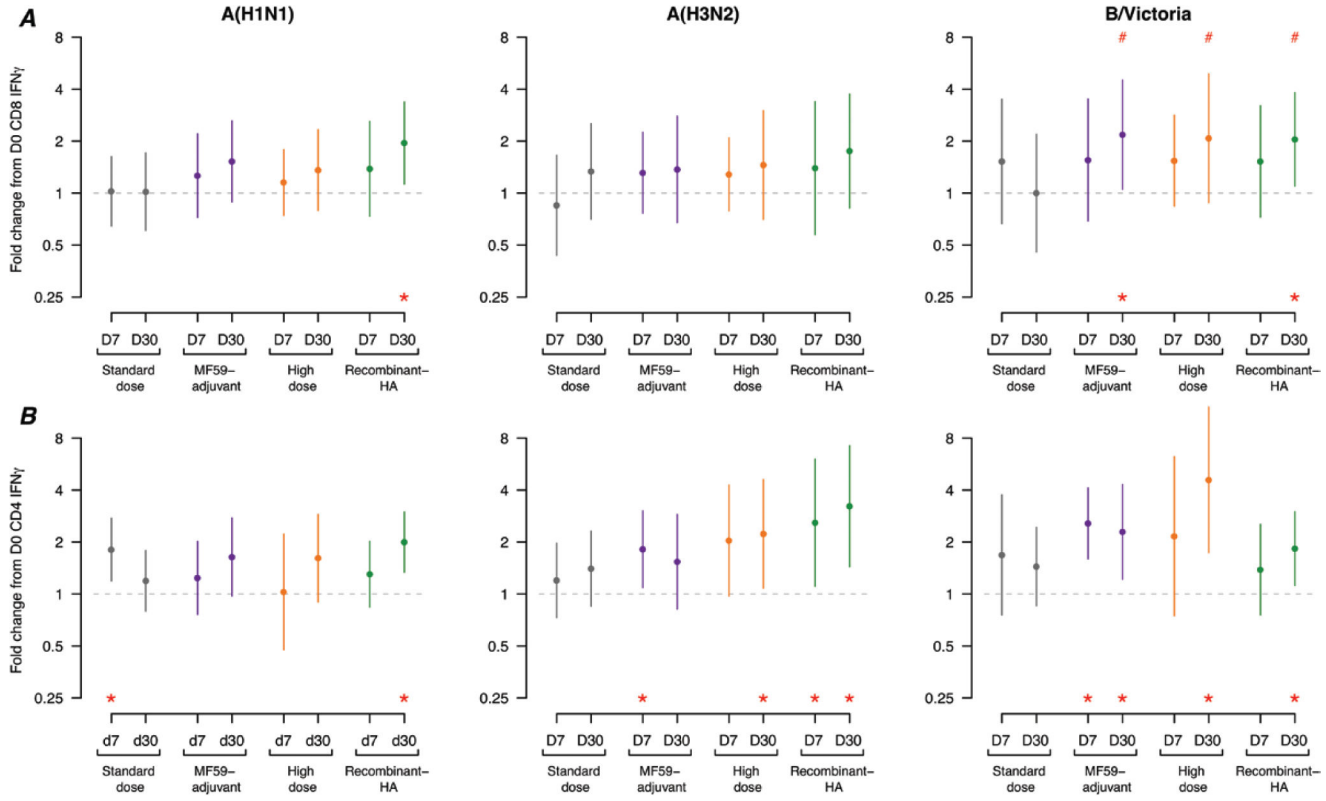

Figure 3.

The mean and standard deviations of the fold changes of Days 7 and 30 IFN-γ^+ T cells, compared to Day 0 responses for A/Michigan/45/2015(H1N1), A/Switzerland/9715293/2013(H3N2), and B/Brisbane/60/2008 viruses after in vitro stimulation for (*A*) $CD8^+$ and (*B*) $CD4^+$ IFN-γ^+ T cells (n = 20 individuals per group). Values marked with a # symbol indicate a statistically significant difference ($P < .05$), compared to the response in the standard-dose vaccine group at the same time point. Values marked with an * indicate a statistically significant difference ($P < .05$), compared to the baseline (Day 0) response within the same vaccine group. Abbreviations: D, day; HA, hemagglutinin; IFN, interferon.

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Table 1.

Baseline Characteristics of the 1861 Participants, by Vaccination Group

Characteristics	All Participants							
	Standard-dose quadrivalent vaccine, n = 508		MF59-adjuvanted trivalent vaccine, n = 508		High-dose trivalent vaccine, n = 510		Recombinant-HA quadrivalent vaccine, n = 335	
	n	(%)	n	(%)	n	(%)	n	(%)
Age, years								
65–70	269	(53%)	248	(49%)	258	(51%)	171	(51%)
71–76	130	(26%)	149	(29%)	143	(28%)	82	(24%)
77–82	109	(21%)	111	(22%)	109	(21%)	82	(24%)
Female sex	301	(59%)	308	(61%)	327	(64%)	195	(58%)
Underlying medical conditions								
Hypertension	230	(45%)	262	(52%)	239	(47%)	161	(48%)
Osteoarthritis	110	(22%)	102	(20%)	109	(21%)	70	(21%)
Diabetes	98	(19%)	104	(20%)	88	(17%)	61	(18%)
Heart diseases	52	(10%)	47	(9%)	52	(10%)	29	(9%)
Cancer	46	(9%)	43	(8%)	40	(8%)	22	(7%)
Others[a]	209	(41%)	221	(44%)	226	(44%)	141	(42%)
Received influenza vaccination in 2016–2017 season	328	(65%)	332	(65%)	351	(69%)	226	(67%)
Number of times received influenza vaccination in the preceding 5 years[b]								
0	126	(25%)	104	(20%)	111	(22%)	71	(21%)
1–2	113	(22%)	120	(24%)	112	(22%)	68	(20%)
3–4	54	(11%)	45	(9%)	61	(12%)	38	(11%)
5–6	195	(38%)	201	(40%)	212	(42%)	144	(43%)

Abbreviation: HA, hemagglutinin.

[a]Other underlying conditions included: stroke, chronic lung disease, kidney disease, liver disease, depression or anxiety disorder, neurologic disorder, autoimmune disease, disease of the digestive system, hypothyroidism, dermatological disease, etc.

[b]There were 6 possible influenza vaccines, including the 5 Northern hemisphere influenza vaccines from 2012–2013 through 2016–2017 plus the Southern hemisphere 2015 vaccine, which was available in Hong Kong for a special vaccination campaign [20]. The Southern hemisphere formulation is not usually available in Hong Kong.

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Table 2.

Baseline Characteristics of the Subset of 800 Participants Selected for Immunogenicity Analyses

	Participants Selected for Immunogenicity Analysis							
	Standard-dose quadrivalent vaccine, n = 200		MF59-adjuvanted trivalent vaccine, n = 200		High-dose trivalent vaccine, n = 200		Recombinant-HA quadrivalent vaccine, n = 200	
	n	(%)	n	(%)	n	(%)	n	(%)
Age, years								
65–70	105	(52%)	96	(48%)	106	(53%)	101	(50%)
71–76	44	(22%)	59	(30%)	52	(26%)	53	(26%)
77–82	51	(26%)	45	(22%)	42	(21%)	46	(23%)
Female sex	119	(60%)	119	(60%)	127	(64%)	120	(60%)
Underlying medical conditions								
Hypertension	95	(48%)	105	(52%)	92	(46%)	101	(50%)
Osteoarthritis	44	(22%)	36	(18%)	41	(20%)	49	(24%)
Diabetes	43	(22%)	41	(20%)	38	(19%)	37	(18%)
Heart diseases	22	(11%)	11	(6%)	20	(10%)	21	(10%)
Cancer	20	(10%)	14	(7%)	15	(8%)	15	(8%)
Others[a]	83	(42%)	81	(40%)	82	(41%)	89	(44%)
Received influenza vaccination in 2016–2017 season	136	(68%)	130	(65%)	139	(70%)	137	(69%)
Number of times received influenza vaccination in the preceding 5 years[b]								
0	46	(23%)	45	(22%)	46	(23%)	47	(24%)
1–2	46	(23%)	43	(22%)	34	(17%)	41	(20%)
3–4	29	(14%)	21	(10%)	26	(13%)	19	(10%)
5–6	74	(37%)	78	(39%)	90	(45%)	87	(44%)

Abbreviation: HA, hemagglutinin.

[a]Other underlying conditions included: stroke, chronic lung disease, kidney disease, liver disease, depression or anxiety disorder, neurologic disorder, autoimmune disease, disease of the digestive system, hypothyroidism, dermatological disease, etc.

[b]There were 6 possible influenza vaccines, including the 5 Northern hemisphere influenza vaccines from 2012–2013 through 2016–2017 plus the Southern hemisphere 2015 vaccine, which was available in Hong Kong for a special vaccination campaign [20]. The Southern hemisphere formulation is not usually available in Hong Kong.

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Table 3.

Summary of Pre- and Post-vaccination Antibody Titers and Fold Rises in Each Vaccination Group

		Vaccination Group							
		Standard-dose QIV, n = 200		MF59-adjuvanted TIV, n = 200		High-dose TIV, n = 200		Recombinant-HA QIV, n = 200	
Assay	**Strain**	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**
HAI	A/Michigan/45/2015 (H1N1)								
	Day 0 GMT	17	(14–20)	17	(15–20)	20	(17–24)	16	(14–19)
	Day 30 GMT	69	(58–83)	94*	(78–114)	125*	(102–152)	85	(69–105)
	Mean fold rise from D0 to D30	4.1	(3.5–4.9)	5.5*	(4.6–6.6)	6.1*	(5.1–7.3)	5.3*	(4.4–6.3)
	% with ≥4-fold rise from D0 to D30[b]	42%	(36–50%)	60%*	(53–67%)	59%*	(52–66%)	60%*	(53–67%)
HAI	A/Hong Kong/4801/2014 (H3N2) egg-like								
	Day 0 GMT	46	(39–56)	49	(41–59)	45	(38–54)	54	(45–64)
	Day 30 GMT	158	(135–186)	207*	(178–241)	214*	(183–250)	254*	(218–295)
	Mean fold rise from D0 to D30	3.4	(2.8–4.1)	4.2*	(3.5–5.1)	4.7*	(3.9–5.7)	4.7*	(3.9–5.7)
	% with ≥4-fold rise from D0 to D30[a]	41%	(34–48%)	48%	(40–55%)	54%*	(46–61%)	56%*	(48–63%)
MN	A/Hong Kong/4801/2014 (H3N2) cell-like								
	Day 0 GMT	38	(31–46)	43	(35–53)	34	(28–41)	48	(39–59)
	Day 30 GMT	87	(72–106)	125*	(102–152)	116*	(95–141)	223*	(189–263)
	Mean fold rise from D0 to D30	2.3	(2.0–2.6)	2.9*	(2.5–3.4)	3.4*	(2.9–4.0)	4.7*	(3.9–5.6)
	% with ≥4-fold rise from D0 to D30[a]	28%	(22–35%)	39%*	(32–46%)	47%*	(40–54%)	57%*	(50–64%)
HAI	B/Brisbane/60/2008 (Victoria lineage)								
	Day 0 GMT	24	(20–28)	31*	(26–38)	31*	(26–37)	29*	(25–35)
	Day 30 GMT	89	(75–105)	95	(81–112)	132*	(112–157)	90	(76–107)
	Mean fold rise from D0 to D30	3.7	(3.2–4.4)	3.0	(2.6–3.5)	4.2	(3.5–5.0)	3.1	(2.7–3.5)
	% with ≥4-fold rise from D0 to D30[a]	48%	(41–56%)	44%	(37–51%)	52%	(45–60%)	44%	(37–51%)
HAI	B/Phuket/3073/2013[b] (Yamagata lineage)								
	Day 0 GMT	37	(31–44)	41	(35–49)	41	(34–50)	45	(38–54)
	Day 30 GMT	121	(104–141)	63*	(54–74)	68*	(57–81)	131	(111–155)

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		Vaccination Group							
		Standard-dose QIV, n = 200		MF59-adjuvanted TIV, n = 200		High-dose TIV, n = 200		Recombinant-HA QIV, n = 200	
Assay	**Strain**	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**	**Estimate**	**(95% CI)**
	Mean fold rise from D0 to D30	3.3	(2.8–3.8)	1.5*	(1.4–1.7)	1.6*	(1.5–1.8)	2.9	(2.5–3.3)
	% with ≥4-fold rise from D0 to D30[a]	42%	(36–50%)	12%*	(8–18%)	15%*	(10–21%)	42%	(36–50%)

*Values that are significantly different from the corresponding value in the standard-dose group. Statistical significance was designated at *P* value <.05.

Abbreviations: CI, confidence interval; D, day; GMT, geometric mean titer; HA, hemagglutinin; HAI, hemagglutination inhibition assay; MN, microneutralization assay; QIV, quadrivalent influenza vaccine; TIV, trivalent influenza vaccine.

[a]At least a 4-fold rise from D0 to D30 with D30 titer ≥40.

[b]Note that a B/Yamagata lineage virus was not included in the MF59-adjuvanted TIV and the high-dose TIV.

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Table 4.

Proportions of Participants With Postvaccination Antibody Titers ≥40, ≥80, and ≥160 in Each Group

		Vaccination Group							
		Standard-dose QIV, n = 200		MF59-adjuvantedTIV, n = 200		High-doseTIV, n = 200		Recombinant-HA QIV, n = 200	
Assay	**Strain**	**Prop.**	**(95% CI)**	**Prop.**	**(95% CI)**	**Prop.**	**(95% CI)**	**Prop.**	**(95% CI)**
Postvaccination (D30) titer ≥40									
HAI	A/Michigan/45/2015 (H1N1)	73%	(66–79%)	82%*	(76–87%)	83%*	(77–88%)	76%	(69–81%)
HAI	A/Hong Kong/4801/2014 (H3N2)	94%	(90–97%)	96%	(92–98%)	96%	(92–98%)	96%	(92–98%)
MN	A/Hong Kong/4801/2014 (H3N2)	78%	(72–84%)	85%	(79–90%)	82%	(77–87%)	94%*	(90–97%)
HAI	B/Brisbane/60/2008	84%	(78–88%)	88%	(83–93%)	90%	(85–94%)	88%	(82–92%)
HAI	B/Phuket/3073/2013[a]	94%	(90–97%)	77%*	(71–83%)	78%*	(72–84%)	90%	(86–94%)
Postvaccination (D30) titer ≥80									
HAI	A/Michigan/45/2015 (H1N1)	54%	(47–61%)	64%*	(57–71%)	69%*	(62–75%)	62%	(54–68%)
HAI	A/Hong Kong/4801/2014 (H3N2)	81%	(75–86%)	88%*	(83–93%)	88%	(82–92%)	92%*	(8%–96%)
MN	A/Hong Kong/4801/2014 (H3N2)	63%	(56–70%)	72%	(65–78%)	74%*	(67–79%)	88%*	(83–92%)
HAI	B/Brisbane/60/2008	69%	(62–75%)	70%	(63–76%)	78%	(71–83%)	64%	(57–71%)
HAI	B/Phuket/3073/2013[a]	76%	(69–81%)	58%*	(51–65%)	58%*	(51–65%)	77%	(71–83%)
Postvaccination (D30) titer ≥160									
HAI	A/Michigan/45/2015 (H1N1)	35%	(28–42%)	45%*	(38–52%)	55%*	(48–62%)	46%*	(39–53%)
HAI	A/Hong Kong/4801/2014 (H3N2)	64%	(56–70%)	72%	(66–79%)	75%*	(68–81%)	81%*	(75–86%)
MN	A/Hong Kong/4801/2014 (H3N2)	48%	(41–56%)	54%	(47–61%)	58%	(51–65%)	74%*	(68–80%)
HAI	B/Brisbane/60/2008	40%	(34–48%)	44%	(37–52%)	54%*	(46–61%)	39%	(32–46%)
HAI	B/Phuket/3073/2013[a]	52%	(45–59%)	32%*	(26–39%)	33%*	(27–40%)	56%	(49–63%)

*Significant differences in comparison to standard-dose QIV.

Abbreviagtions: D, day; HA, hemagglutinin; HAI, hemagglutination inhibition assay; MN, microneutralization assay; Prop., proportion; QIV, quadrivalent influenza vaccine; TIV, trivalent influenza vaccine.

[a]Note that a B/Yamagata lineage virus was not included in the MF59-adjuvanted TIV and the high-dose TIV.