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Safety and immunogenicity of high-dose quadrivalent influenza vaccine in adults ≥65 years of age: A phase 3 randomized clinical trial

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ABSTRACT

Background: A high-dose, split-virion inactivated trivalent influenza vaccine (IIV3-HD; Fluzone® High-Dose, Sanofi Pasteur) is available for adults ≥65 years of age. This study examined the safety and immunogenicity of a quadrivalent high-dose split-virion inactivated influenza vaccine (IIV4-HD).

Methods: This was a randomized, modified double-blind, active-controlled, multi-center trial in healthy adults ≥65 years of age. Subjects were randomized in a 4:1:1 ratio to receive a single intramuscular injection of IIV4-HD, the licensed IIV3-HD, or an IIV3-HD containing the alternate B-lineage strain. Hemagglutination inhibition (HAI), seroneutralisation, and anti-neuraminidase antibody titers were measured at baseline and day 28. Solicited reactions were collected for up to 7 days, unsolicited adverse events up to 28 days, and serious adverse events up to 180 days. The primary immunogenicity objective was to demonstrate that IIV4-HD induces HAI geometric mean titers (GMTs) and seroconversion rates that are non-inferior to those induced by IIV3-HD. Secondary objectives were to describe the safety of IIV4-HD and IIV3-HD and to demonstrate that IIV4-HD induces HAI GMTs and seroconversion rates that are superior to those induced by IIV3-HD not containing the same B-lineage strain.

Results: The study included 2670 adults ≥65 years of age. For all four strains, HAI GMTs and seroconversion rates induced by IIV4-HD were non-inferior to those induced by IIV3-HDs containing the same strains. For both B strains, HAI GMTs and seroconversion rates induced by IIV4-HD were superior to those induced by IIV3-HD not containing the same B–lineage strain. Seroneutralisation and anti-neuraminidase antibody responses, measured in a subset of subjects, were similar. No new safety concerns were identified, and the safety profiles of IIV4-HD and IIV3-HD were similar.

Conclusions: Adding a second B strain in IIV4-HD resulted in improved immunogenicity against the added strain without compromising the immunogenicity of the other strains or the vaccine's tolerability.

Clinical trial registration: NCT03282240.

1. Introduction

Adults ≥65 years of age are particularly vulnerable to complications associated with influenza and are at the highest risk for seasonal influenza-related hospitalizations and deaths [1]. This appears to be due to decreasing immune responses and increased comorbidities [2,3]. Approaches to improve immune responses have included increasing the antigen dose, including an adjuvant, and exploring alternative routes of administration [4–6].

A high dose, split-virion inactivated trivalent influenza vaccine (IIV3-HD[1]; Fluzone® High–Dose, Sanofi Pasteur) [6] has been licensed in adults ≥65 years of age in the United States since 2009, Canada since 2015, Australia since 2017, Brazil since 2018, and the United Kingdom since 2019. IIV3-HD contains 60 μg hemagglutinin

[1] Abbreviations: AE, adverse event; CI, confidence interval; FDA, Food and Drug Administration; GMT, geometric mean titre; GMTR, geometric means of individual post-/pre-vaccination HAI titre ratio; HAI, hemagglutination inhibition; IIV3-HD, high-dose trivalent inactivated influenza vaccine; IIV3-HD1, licensed Northern Hemisphere 2017–2018 formulation of IIV3-HD containing the A/H1N1, A/H3N2, and B Victoria-lineage strains; IIV3-HD2, alternate formulation of IIV3-HD containing the A/H1N1, A/H3N2, and B Yamagata-lineage strains; IIV3-SD, standard-dose trivalent inactivated influenza vaccine; IIV4-HD, high-dose quadrivalent inactivated influenza vaccine; IIV4-SD, standard-dose quadrivalent inactivated influenza vaccine; SAE, serious adverse event; WHO, World Health Organisation.

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per influenza strain, which is four times the antigen content of standard-dose inactivated influenza vaccines. In a phase III multicenter efficacy study including 31,989 adults ≥65 years of age in the US and Canada, IIV3-HD was 24.2% more effective in preventing laboratory-confirmed influenza caused by any strain relative to a standard-dose trivalent split-virion inactivated influenza vaccine (IIV3-SD) [7]. Based on results from this trial, IIV3-HD was predicted to be cost-saving compared to IIV3-SD, mostly due to reduced hospitalizations [8]. A phase IV cluster-randomized trial in long-stay nursing home residents ≥65 years of age further showed that the risk of respiratory-related hospital admissions, pneumonia-related hospital admissions, and all-cause hospitalizations were lower in facilities where residents received IIV3-HD than in those where residents received IIV3-SD [9]. Other post-licensure studies have demonstrated a benefit of IIV3-HD over IIV3-SD for the prevention of pneumonia and other cardio-respiratory events, post-influenza death, and healthcare utilization (e.g., hospitalization) in this age group [10–15]. Increased hemagglutination inhibition, seroneutralisation, and anti-neuraminidase antibody titers further support the improved efficacy of IIV3-HD [16,17].

For the last two decades, two distinct B influenza lineages (Victoria and Yamagata) have co-circulated, making it difficult to predict which of the two B lineages will predominate during the next season. As a result, the B-lineage strain in trivalent vaccines and the dominant circulating B-lineage strain have differed in about 25% of influenza seasons [18]. To improve protection against influenza infection, quadrivalent influenza vaccines incorporating a strain from each B lineage have therefore been developed [19]. The current study assessed the safety and immunogenicity of a quadrivalent formulation of the high-dose split-virion inactivated influenza vaccine (IIV4-HD) in adults ≥65 years of age.

2. Methods

2.1. Overall study design

This was a randomized, modified double-blind, active-controlled, multicenter trial in adults ≥65 years of age conducted between September 2017 and April 2018 at 35 sites in the US (NCT03282240)[2]. The primary objectives were to assess the safety of IIV4-HD as measured by solicited reactions and unsolicited adverse events (AEs) and to demonstrate that IIV4-HD induces hemagglutination inhibition (HAI) geometric mean titers (GMTs) and seroconversion rates that are non-inferior to responses induced by the licensed IIV3-HD (IIV3-HD1) and IIV3-HD containing the alternate B-lineage strain (IIV3-HD2).

2.2. Ethics

The study was approved by the independent ethics committee or institutional review board for each institution and was conducted in compliance with the standards established by the Declaration of Helsinki, International Conference for Harmonisation guidelines for Good Clinical Practice, and all local and national regulations and directives. All subjects provided signed, informed consent before taking part in the study.

2.3. Subjects

The study enrolled adults ≥65 years of age who were not vaccinated against influenza in the previous 6 months. Individuals were excluded if they had any condition or were receiving any treatment that, in the opinion of the investigator, could interfere with the trial conduct, completion, or assessments. Other exclusions are listed in Supplementary Table 1.

2.4. Vaccines

IIV4-HD contained the World Health Organization (WHO)/US Food and Drug Administration (FDA)-recommended strains for the 2017–2018 Northern Hemisphere influenza season (batch UD19520). IIV3-HD1 (Fluzone High-Dose, Sanofi Pasteur) was the licensed 2017–2018 Northern Hemisphere formulation and contained the WHO/FDA-recommended A/H1N1, A/H3N2, and B Victoria-lineage strains (batch UD19521). IIV3-HD2 was an alternate formulation of IIV3-HD and contained the WHO/FDA-recommended A/H1N1, A/H3N2, and B Yamagata-lineage strains for the 2017–2018 Northern Hemisphere influenza season (batch UD19522). All vaccines were administered by intramuscular injection as sterile suspensions. The vaccines, their antigen content, and the volumes injected are summarized in Table 1. The volume administered was 0.7 mL for IIV4-HD and 0.5 mL for IIV3-HD1 and IIV3-HD2.

2.5. Randomization

Subjects were randomized by interactive response technology in a 4:1:1 ratio to receive a single intramuscular injection in the upper arm of IIV4-HD, IIV3-HD1, or IIV3-HD2. Permuted block randomization with block sizes 6 and 12 was applied for all subjects within strata by sites. In addition, approximately 100 subjects per treatment group were randomly selected by interactive response technology for measurement of neuraminidase and seroneutralisation antibody titers.

2.6. Blinding

The study was modified double-blind, meaning that an unblinded administrator at each site administered the vaccine but that the investigators (or delegates) in charge of safety assessment, the trial staff who collected the safety data, the laboratory personnel who analyzed the blood samples, and the subjects did not know which product was administered. The vaccine administrator was not involved in any of the blinded study assessments (e.g., safety).

2.7. Measurement of HAI antibody titers

All subjects were to provide a pre-vaccination (baseline) blood sample at day 0 and a post-vaccination blood sample at the end of the active phase of the trial (day 28–35) for HAI testing. HAI titers were detected as described previously [20]. Briefly, test and control sera were incubated with Type III neuraminidase to eliminate non-specific inhibitors and then with a suspension of Turkey red blood cells to adsorb spontaneous anti-species agglutinins. Ten two-fold dilutions of each sera were mixed and incubated with 4 hemagglutination units/25 μL of influenza virus. A red blood cell suspension was added to the mixture and, following incubation, titers were recorded as the highest serum dilution in which complete inhibition of hemagglutination occurred. If the lowest/first serum dilution used in the assay (1:10) did not result in complete inhibition of hemagglutination, the serum HAI titer was reported as <10. If the highest/last serum dilution used in the assay (1:10,240) exhibited complete inhibition of hemagglutination, the serum HAI titer was reported as ≥10,240. The primary endpoints for the evaluation of immunogenicity were HAI antibody titers obtained on day 28 and seroconversion, defined as either (i) a HAI titer <10 (1/dilution) at day 0 and a post-vaccination

[2] A summary of the study protocol is available at https://clinicaltrials.gov/ct2/show/NCT03282240.

Table 1
Influenza vaccines, antigen content, and injection volumes.

Content	IIV4-HD	IIV3-HD1	IIV3-HD2
A/H1N1 strain	A/Michigan/45/2015 X-275	A/Michigan/45/2015 X-275	A/Michigan/45/2015 X-275
A/H3N2 strain	A/Hong Kong/4801/2014 [NYMC X-263B]	A/Hong Kong/4801/2014 [NYMC X-263B]	A/Hong Kong/4801/2014 [NYMC X-263B]
B Victoria-lineage strain	B/Brisbane/60/2008	B/Brisbane/60/2008	–
B Yamagata-lineage strain	B/Phuket/3073/2013	–	B/Phuket/3073/2013
Total hemagglutinin	240 μg	180 μg	180 μg
Injection volume	0.7 mL	0.5 mL	0.5 mL

(day 28–35) HAI titer ≥40 or (ii) a HAI titer ≥10 at day 0 and a ≥4-fold increase in HAI titer between day 0 and post-vaccination.

2.8. Measurement of neuraminidase antibody titers

Neuraminidase antibody titers were measured as an exploratory endpoint. H6Nx virus reassortants were used in the assay as a source of neuraminidase from A/California/7/2009 (H1N1) and A/Victoria/361/2011 (H3N2) [21,22]. Serum samples, quality control sera, and previously titred influenza virus were added into duplicate wells of a fetuin-coated 96-well plate and incubated overnight. The plate was washed and incubated with peroxidase-conjugated peanut agglutinin. After washing, o-phenylenediamine dihydrochloride was added and color detected. The titer of each determination was the reciprocal of the last dilution with an optical density less than or equal to the midpoint between the mean optical density of the virus-only control wells and the mean optical density of the background wells on each plate. The lower limit of quantitation was the reciprocal of the lowest dilution used in the assay (1:10). Titers below this level were reported as <10.

2.9. Measurement of seroneutralisation antibody titers

Seroneutralisation antibody titers were measured as an exploratory endpoint. Serially diluted, heat-inactivated human serum samples were pre-incubated with a fixed amount of challenge virus and then incubated overnight with Madin-Darby canine kidney cells. Viral nucleoprotein production was measured by enzyme-linked immunosorbent assay using a monoclonal antibody specific to either influenza A or influenza B nucleoprotein. The neutralization titer (1/dilution) was calculated from the intersection of the optical density curve of the test sample and the 50% neutralization point of the control optical density curve. The lower limit of quantitation was the reciprocal of the lowest dilution used in the assay (1:10). Titers below this level were reported as <10.

2.10. Solicited reactions

For 7 days after the vaccination, using a diary card, subjects recorded daily temperature, with the route by which it was taken; daily measurement or intensity grade of all other solicited injection site and systemic reactions; and action taken. Injection-site reactions recorded included pain, erythema, swelling, induration, and bruising. Systemic reactions recorded included fever, headache, malaise, myalgia, and shivering. Injection-site erythema, swelling, induration and bruising were scored as grade 1 if 25–50 mm in diameter, grade 2 if 51–100 mm in diameter, and grade 3 if >100 mm in diameter. Fever was scored as grade 1 if 100.4–101.1°F (38.0–38.4 °C), grade 2 if 101.2–102.0°F (38.5–38.9 °C), and grade 3 if ≥102.1°F (≥39.0 °C). All other solicited reactions were scored as grade 1 if transient, requiring minimal therapeutic intervention, or did not interfere with daily activities; grade 2, if they required additional therapeutic intervention or interfered with daily activities but posed no significant permanent risk; and grade 3 if they interrupted usual daily activities, significantly affected clinical status, or required intensive therapeutic intervention.

2.11. Unsolicited AEs

Unsolicited AEs and serious adverse events (SAEs) were defined as described in the International Conference for Harmonisation E2A Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting [23] and encoded using the Medical Dictionary for Regulatory Activities [24]. Unsolicited AEs were collected up to the end of the active phase of the study (day 28–35). Any unsolicited systemic AEs occurring during the first 30 min after vaccination were recorded as immediate unsolicited systemic AEs. SAEs were collected up to end of the study (day 180–194). SAEs included any medical occurrence resulting in death, was life-threatening, required inpatient hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability/incapacity, was a congenital anomaly/birth defect, or was an AE of special interest. AEs of special interest included new onset of Guillain-Barré syndrome, encephalitis/myelitis (including transverse myelitis), Bell's palsy, optic neuritis, and brachial neuritis. For all AEs and SAEs, investigators recorded duration and the potential relationship to vaccination.

2.12. Statistical analysis

The primary endpoints, HAI antibody titer and seroconversion rate, were analyzed in both the per-protocol analysis set and the full analysis set. The full analysis set was defined as all subjects who received the trial vaccine and had a post-vaccination blood sample HAI result for at least one strain. The per-protocol analysis set was defined as all subjects in the full analysis set who did not have a major protocol deviation. Seroneutralisation and anti-neuraminidase antibody responses were measured in the expanded immunogenicity subset, which was defined as all subjects in the full analysis set who were randomized to the subset and who had a post-vaccination seroneutralisation assay result for at least one strain. The 95% confidence intervals (CIs) were calculated using normal approximation of log-transformed titers for ratio of GMTs and using the Wilson score method [25] without continuity correction for differences in seroconversion rates.

The primary objective of non-inferiority used margins of 1.5 for GMTs and 10% for seroconversion rates, which were agreed on with the US FDA. Non-inferiority of IIV4-HD to each IIV3-HD group in terms of HAI GMTs was demonstrated if, for each of the three common strains, the lower limit of the 2-sided 95% CI for the difference of the $\log_{10}$ (GMT) for IIV4-HD vs. IIV3-HD was $>\log_{10}$ (1/1.5). The non-inferiority of IIV4-HD to each IIV3-HD group in terms of seroconversion rates was demonstrated, if for each of the three common strains, the lower limit of the 2-sided 95% CI for the difference of seroconversion rates was >−10%. For non-inferiority comparisons, the IIV3 comparator was the pooled IIV3-HD1 and IIV3-HD2 groups for the A/H1N1 and A/H3N2 strains; IIV3-HD1 for the B Victoria-lineage strain; and IIV-HD2 for the B Yamagata-lineage strain. The primary immunogenicity

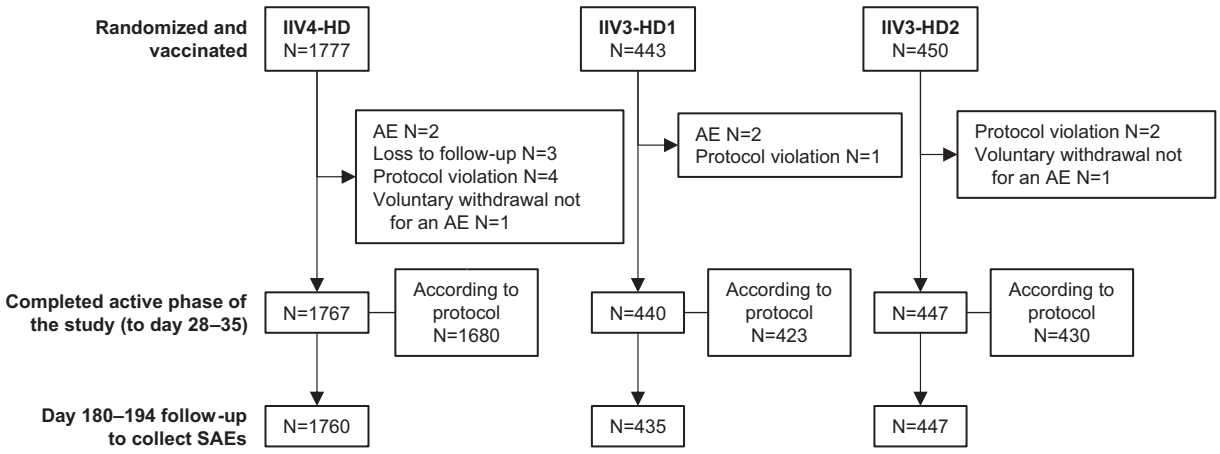


Fig. 1. Study design and subject disposition. Subjects were randomized in a 4:1:1 ratio to receive a single intramuscular injection of IIV4-HD, IIV3-HD1, or IIV3-HD2. All subjects were to provide a pre-vaccination (baseline) blood sample at day 0 and a post-vaccination blood sample at the end of the active phase of the trial (day 28–35) for HAI testing. Serious adverse events were collected up to the end of the study (day 180–194). Immunogenicity was analyzed in the subjects completing the active phase of the trial according to protocol. Solicited reactions and unsolicited AEs were analyzed in the full analysis set. Abbreviations: AE, adverse event; HAI, hemagglutination inhibition; IIV3-HD1, high-dose trivalent inactivated influenza vaccine containing the Victoria-lineage B strain; IIV3-HD2, high-dose trivalent inactivated influenza vaccine containing the Yamagata-lineage B strain; IIV4-HD, high-dose quadrivalent inactivated influenza vaccine.

objective was achieved if non-inferiority was demonstrated for all four strains and for both GMTs and seroconversion rates in the per-protocol analysis set. Sensitivity of non-inferiority of HAI GMTs was assessed by analysis of covariance of post-vaccination HAI titers adjusted for baseline HAI titers.

Superiority was assessed as a secondary objective and was demonstrated if the 2-sided 95% CI for the difference of the $\log_{10}$ (GMT) for IIV4-HD vs. IIV3-HD was $>\log_{10}$ (1.5) and for the difference of seroconversion rates was >10%. For superiority comparisons, the IIV3 comparator was IIV3-HD1 for the B Yamagata-lineage strain and IIV-HD2 for the B Victoria-lineage strain. The secondary objective of superiority was achieved if superiority was demonstrated for both B strains and for both GMTs and seroconversion rates in the full analysis set.

Solicited reactions and AEs were analyzed in the safety analysis set, defined as all subjects who received a study vaccine; analyzed according to the vaccine received. Only descriptive analyses were performed for safety endpoints.

Statistical analyses were performed using SAS version 9.4 or later (SAS Institute, Cary, NC, USA). Missing data were not imputed, and no search for outliers was performed.

2.13. Sample size estimation

A sample size of 2616 subjects was determined based on an overall power of 90% for demonstrating non-inferiority for both the HAI GMTs and seroconversion rates comparing IIV4-HD vs. IIV3-HD1 and/or IIV3-HD2 for all four virus strains. The non-inferiority margins were defined as 1.5 for GMTs and 10% for seroconversion rates. This assumed (i) an 8% attrition rate in the per-protocol analysis set, (ii) seroconversion rates of 45% for the A/H1N1 strain, 70% for the A/H3N2 strain, and 40% for both B (B1 and B2) strains, and (iii) standard deviations for HAI GMTs of 0.63 for both A strains and 0.55 for both B strains.[3]

Based on the planned sample size of 2616, there was 55.4% power to conclude the superiority of each B strain comparing IIV4-HD groups versus either the IIV3-HD1 or IIV3-HD2 group assuming (i) a 5% attrition rate in the full analysis set, (ii) a GMT ratio of 1.8 with a standard deviation of 0.55, (iii) a seroconversion rate of 8% increase in the group without the B strain and a 22% increase in the group with the B strain.[4]

3. Results

3.1. Subjects

The study included 2670 adults ≥65 years of age enrolled between September 8, 2017 and September 15, 2017. The study was completed on April 19, 2018. All subjects were vaccinated as randomized (1777 to IIV4-HD, 443 to IIV3-HD1, and 450 to IIV3-H2) (Fig. 1). Of the 2670 enrolled subjects, 2654 completed the active study period (day 28–35). The most common reason for early discontinuation was a protocol deviation (did not complete the day 28–35 visit [n = 6] and participating in another clinical trial [n = 1]). The primary analysis of non-inferiority was conducted in the 2533 subjects who completed the active study period according to protocol. Safety follow-up to month 6 was completed by 2642 subjects.

Baseline characteristics were similar in the three vaccine groups (Table 2). In all three groups, just over half of the subjects were female, most were White and not Hispanic or Latino. Mean ages and age distributions were similar, with approximately two-thirds of subjects <75 years of age. In all three groups, over 70% were vaccinated the previous year for influenza.

3.2. Immunogenicity

3.2.1. Non-inferiority and superiority of HAI GMTs and seroconversion rates

For all four strains, HAI GMTs and seroconversion rates induced by IIV4-HD were non-inferior to those induced by IIV3-HDs containing the same strains (Fig. 2). For both B strains, HAI GMTs and seroconversion rates induced by IIV4-HD were superior to those induced by IIV3-HD not containing the same B-strain lineage (Table 3). These results were confirmed by repeating the analysis in the alternative analysis set (full analysis set for non-inferiority, per-protocol analysis set for superiority) and by analysis of covariance using post-vaccination HAI titers adjusted for baseline HAI

[3] Based on previous clinical trials using high-dose formulations of trivalent split-virion inactivated influenza vaccines.

[4] Ibid.

Table 2
Subject baseline characteristics.

Characteristic	IIV4-HD N = 1680	IIV3-HD1 N = 423	IIV3-HD2 N = 430
Sex, n (%)			
Male	703 (41.8)	172 (40.7)	191 (44.4)
Female	977 (58.2)	251 (59.3)	239 (55.6)
Age (years)			
Mean (SD)	72.9 (5.66)	72.8 (5.82)	73.2 (5.50)
≥75 years, n (%)	594 (35.4)	141 (33.3)	164 (38.1)
Racial origin, n (%)			
American Indian or Alaska Native	9 (0.5)	2 (0.5)	3 (0.7)
Asian	12 (0.7)	2 (0.5)	3 (0.7)
Black or African American	114 (6.8)	36 (8.5)	32 (7.4)
White	1532 (91.2)	380 (89.8)	385 (89.5)
Multiple	6 (0.4)	1 (0.2)	2 (0.5)
Not reported	4 (0.2)	1 (0.2)	4 (0.9)
Ethnicity, n (%)			
Hispanic or Latino	47 (2.8)	9 (2.1)	13 (3.0)
Not Hispanic or Latino	1630 (97.0)	413 (97.6)	415 (96.5)
Unknown	0 (0.0)	0 (0.0)	1 (0.2)
Body mass index categories, n (%)			
Underweight (<18.5 kg/m^2)	17 (1.0)	0 (0.0)	1 (0.2)
Normal weight (18.5–24.9 kg/m^2)	355 (21.1)	91 (21.5)	82 (19.1)
Overweight (25–29.9 kg/m^2)	565 (33.6)	156 (36.9)	151 (35.1)
Obese (≥30)	691 (41.1)	164 (38.8)	183 (42.6)
Missing	52 (3.1)	12 (2.8)	13 (3.0)
Smoking habits, n (%)			
Never	1033 (58.1)	251 (56.7)	284 (63.1)
Current	110 (6.2)	22 (5.0)	23 (5.1)
Former	634 (35.7)	170 (38.4)	143 (31.8)
History of chronic medical conditions, n (%)	992 (55.8)	256 (57.8)	222 (49.3)
Ongoing chronic medical conditions at inclusion, n (%)	835 (47.0)	225 (50.8)	185 (41.1)
Concomitant medications, n (%)			
Any	1345 (75.7)	332 (74.9)	333 (74.0)
Category 1[a]	1011 (56.9)	254 (57.3)	264 (58.7)
Category 2[b]	24 (1.4)	3 (0.7)	3 (0.7)
Category 3[c]	44 (2.5)	6 (1.4)	12 (2.7)
Category 4[d]	876 (49.3)	216 (48.8)	217 (48.2)
Influenza vaccination the previous year, n (%)	1339 (75.4)	308 (69.5)	333 (74.0)

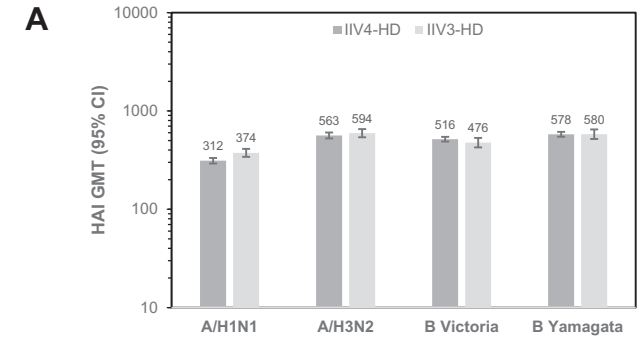
Values are for the full analysis set. Abbreviations: IIV3-HD1, high-dose trivalent inactivated influenza vaccine containing the Victoria-lineage B strain; IIV3-HD2, high-dose trivalent inactivated influenza vaccine containing the Yamagata-lineage B strain; IIV4-HD, high-dose quadrivalent inactivated influenza vaccine; SD, standard deviation.

[a] Medications affecting or that may affect safety evaluation (e.g. antipyretics, analgesics, non-steroidal anti-inflammatory drugs).

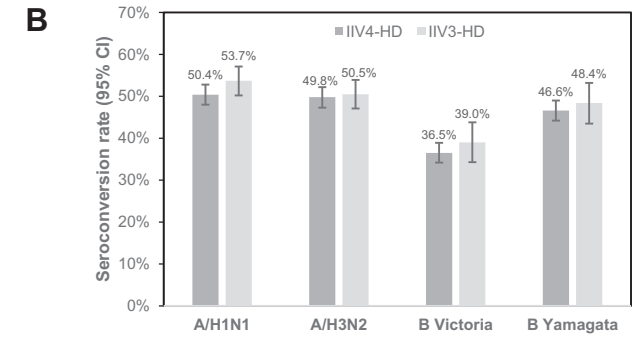
[b] Medications affecting or that may affect immune response (e.g. other vaccines, blood products, antibiotic classes that may interfere with bioassays, immune suppressors, immune modulators with immunosuppressive properties, and anti-proliferative drugs such as DNA synthesis inhibitors).

[c] Medications affecting or that may affect both safety and immune response (e.g., steroids/corticosteroids).

[d] Statins.



Ratio of day 28 HAI GMT (IIV4-HD/IIV3-HD)	0.83	0.95	1.08	1.00
95% CI	(0.744, 0.932)	(0.842, 1.066)	(0.958, 1.224)	(0.881, 1.129)
Non-inferior	Yes	Yes	Yes	Yes



Difference in seroconversion rate (IIV4-HD-IIV3-HD)	-3.27	-0.71	-2.41	-1.75
95% CI	(−7.37, 0.86)	(−4.83, 3.42)	(−7.66, 2.70)	(−7.04, 3.53)
Non-inferior	Yes	Yes	Yes	Yes

Fig. 2. Non-inferiority of HAI GMTs (A) and seroconversion rates (B). Values are for the per-protocol analysis set. (A) The non-inferiority of IIV4-HD to each IIV3-HD group in terms of post-vaccination (day 28–35) HAI GMTs was demonstrated, if for each of the three common strains, the lower limit of the 2-sided 95% CI for the difference of $\log_{10}$ (GMTs) > $\log_{10}$ (1/1.5). For the A/H1N1 and A/H3N2 strains, the comparator was the pooled IIV3-HDs, and for the B strains, the comparator was the IIV3-HD containing the same B-lineage strain. (B) Seroconversion was defined as (i) HAI titer <10 (1/dilution) at day 0 and a post-vaccination HAI titer ≥40 or (ii) a HAI titer ≥10 at day 0 and a ≥4-fold increase in titer at day 28. The non-inferiority of IIV4-HD to each IIV3-HD group in terms of seroconversion rates was demonstrated, if for each of the three common strains, the lower limit of the 2-sided 95% CI for the difference of seroconversion rates was >−10%. For the A/H1N1 and A/H3N2 strains, the comparator was the pooled IIV3-HDs, and for the B strains, the comparator was the IIV3-HD containing the same B-lineage strain. Abbreviations: CI, confidence interval; GMT, geometric mean titer; HAI, hemagglutinin inhibition titer; IIV3-HD1, high-dose trivalent inactivated influenza vaccine containing the Victoria-lineage B strain; IIV3-HD2, high-dose trivalent inactivated influenza vaccine containing the Yamagata-lineage B strain; IIV4-HD, high-dose quadrivalent inactivated influenza vaccine.

titers (data not shown). Thus, the primary immunogenicity objective of non-inferiority for all four strains for both GMTs and seroconversion rates was confirmed.

3.2.2. HAI antibody response to influenza A strains

Post-vaccination HAI GMTs were similar between IIV4-HD and the pooled IIV3-HDs for strains A/H1N1 (312 [95%CI: 292–332] for IIV4-HD vs. 374 [95%CI: 341–411] for IIV3-HD) and A/H3N2 (563 [95%CI: 525–603] for IIV4-HD vs. 594 [95%CI: 540–653] for IIV3-HD) (Table 4). Seroconversion rates for the A/H1N1 strain were 50.4% (95%CI: 48.0%–52.8%) for IIV4-HD and 53.7% (95%CI: 50.2%–57.1%) for the pooled IIV3-HDs. For the A/H3N2 strain, seroconversion rates were 49.8% (95%CI: 47.3%–52.2%) for IIV4-HD and 50.5% (95%CI: 47.1%–53.9%) for the pooled IIV3-HDs.

3.2.3. HAI antibody response to the B Victoria-lineage strain

For the B Victoria-lineage strain, the post-vaccination HAI GMT was similar for IIV4-HD (516 [95%CI: 488–545]) and IIV3-HD containing the B Victoria-lineage strain (IIV3-HD1) (476 [95%CI: 426–532]) but lower for IIV3-HD containing the B Yamagata-lineage strain (IIV3-HD2) (253 [95%CI: 226–283]) (Table 4). Seroconversion rates were 36.5% (95%CI: 34.2%–38.9%) for IIV4-HD, 39.0% (95%CI: 34.3%–43.8%) for IIV3-HD1, and 15.2% (95%CI: 11.9%–18.9%) for IIV3-HD2.

Table 3
Superiority of HAI GMTs and seroconversion rates.

Measure	B-strain lineage	Vaccine	N	Value (95% CI)	Comparison	Superiority
					Ratio of day 28 GMTs (IIV4–HD/IIV3–HD)	
HAI GMT	Victoria	IIV4-HD	1763	515 (488, 543)	2.03 (1.802, 2.288)	Yes
		IIV3-HD2	446	253 (227, 283)		
	Yamagata	IIV4-HD	1763	573 (542, 605)	2.04 (1.804, 2.315)	Yes
		IIV3-HD1	439	280 (249, 316)		
					Difference of seroconversion rates (IIV4–HD–IIV3–HD)	
Seroconversion rate, %	Victoria	IIV4-HD	1751	36.3 (34.1, 38.6)	20.78 (16.5, 24.61)	Yes
		IIV3-HD2	444	15.5 (12.3, 19.3)		
	Yamagata	IIV4-HD	1751	46.7 (44.3, 49.0)	29.27 (24.78, 33.29)	Yes
		IIV3-HD1	437	17.4 (13.0, 21.3)		

Values are for the full analysis set. Seroconversion was defined as (i) HAI titer <10 (1/dilution) at day 0 and a post-vaccination (day 28–35) HAI titer ≥40 or (ii) a HAI titer ≥10 at day 0 and a ≥4-fold increase HAI titer between day 0 and post-vaccination. Abbreviations: CI, confidence interval; IIV3-HD1, high-dose trivalent inactivated influenza vaccine containing the Victoria-lineage B strain; IIV3-HD2, high-dose trivalent inactivated influenza vaccine containing the Yamagata-lineage B strain; IIV4-HD, high-dose quadrivalent inactivated influenza vaccine.

Table 4
HAI GMTs, GMTRs, and seroconversion rates.

Strain	Day	Measure	IIV4-HD	Pooled IIV3-HDs	IIV3-HD1	IIV3-HD2
A/H1N1	0	N	1669	848		
		HAI GMT (95% CI)	70.9 (66.1, 75.9)	72.8 (66.0, 80.3)		
		HAI titer <10, n (%)	140 (8.4)	56 (6.6)		
	28	N	1680	853		
		HAI GMT (95% CI)	312 (292, 332)	374 (341, 411)		
	28/0	N	1669	848		
		GMTR (95% CI)	4.38 (4.11, 4.66)	5.14 (4.67, 5.66)		
		Seroconversion rate, % (95% CI)[a]	50.4 (48.0, 52.8)	53.7 (50.2, 57.1)		
A/H3N2	0	N	1668	848		
		HAI GMT (95% CI)	121 (111, 130)	122 (109, 135)		
		HAI titer <10, n (%)	99 (5.9)	52 (6.1)		
	28	N	1679	853		
		HAI GMT (95% CI)	563 (525, 603)	594 (540, 653)		
	28/0	N	1668	848		
		GMTR (95% CI)	4.65 (4.35, 4.98)	4.88 (4.45, 5.35)		
		Seroconversion rate, % (95% CI)[a]	49.8 (47.3, 52.2)	50.5 (47.1, 53.9)		
B Victoria	0	N	1669		421	428
		HAI GMT (95% CI)	162 (152, 172)		142 (125, 160)	153 (136, 172)
		HAI titer <10, n (%)	26 (1.6)		8 (1.9)	6 (1.4)
	28	N	1680		423	430
		HAI GMT (95% CI)	516 (488, 545)		476 (426, 532)	253 (226, 283)
	28/0	N	1669		421	428
		GMTR (95% CI)	3.17 (2.99, 3.35)		3.35 (2.99, 3.76)	1.65 (1.52, 1.78)
		Seroconversion rate, % (95% CI)[a]	36.5 (34.2, 38.9)		39 (34.3, 43.8)	15.2 (11.9, 18.9)
B Yamagata	0	N	1669		421	428
		HAI GMT (95% CI)	150 (142, 160)		150 (132, 170)	152 (135, 171)
		HAI titer <10, n (%)	33 (2.0)		12 (2.9)	2 (0.5)
	28	N	1680		423	430
		HAI GMT (95% CI)	578 (547, 612)		282 (250, 318)	580 (519, 649)
	28/0	N	1669		421	428
		GMTR (95% CI)	3.82 (3.62, 4.03)		1.86 (1.73, 2.02)	3.82 (3.43, 4.24)
		Seroconversion rate, % (95% CI)[a]	46.6 (44.2, 49.0)		17.6 (14.1, 21.6)	48.4 (43.5, 53.2)

Values are for the per-protocol analysis set. Abbreviations: CI, confidence interval; GMT, geometric mean titer; HAI, hemagglutination inhibition; IIV3-HD1, high-dose trivalent inactivated influenza vaccine containing the Victoria-lineage B strain; IIV3-HD2, high-dose trivalent inactivated influenza vaccine containing the Yamagata-lineage B strain; IIV4-HD, high-dose quadrivalent inactivated influenza vaccine.

[a] HAI titer <10 (1/dilution) at day 0 and a post-injection HAI titer ≥40 at day 28; or a HAI titer ≥10 at day 0 and a ≥4-fold increase in HAI titer at day 28.

3.2.4. HAI antibody response to the B Yamagata-lineage strain

For the B Yamagata-lineage strain, the post-vaccination HAI GMT was similar for IIV4-HD (578 [95%CI: 547–612]) and IIV3-HD containing the B Yamagata-lineage strain (IIV3-HD2) (580 [95%CI: 519–649]) but lower for IIV3-HD containing the B Victoria-lineage strain (IIV3-HD1) (282 [95%CI: 250–318]) (Table 4). Seroconversion rates were 46.6% (95%CI: 44.2%–49.0%) for IIV4-HD, 17.6% (95%CI: 14.1%–21.6%) for IIV3-HD1, and 48.4% (95%CI: 43.5%–53.2%) for IIV3-HD2.

3.2.5. Influence of baseline antibodies and previous vaccination on post-vaccination HAI antibody responses

In all vaccine groups and for all strains, at least 91.6% of subjects were seropositive before vaccination as defined by a HAI titer ≥10 (Table 4). For all strains in all vaccine groups except B Victoria lineage for IIV3-HD1, post-vaccination HAI GMTs were higher and seroconversion rates lower for subjects who were seropositive at baseline (Supplementary Tables 2 and 3). Post-vaccination HAI GMTs and seroconversion rates were generally lower in subjects

who were vaccinated for influenza the previous year than those who were not (Supplementary Tables 4 and 5).

3.2.6. Seroneutralisation and anti-neuraminidase antibody responses

In a random subset of approximately 100 subjects from each group, seroneutralisation was measured for all four vaccine strains (Fig. 3 and Supplementary Table 6) and anti-neuraminidase antibody titers for the two A strains (Fig. 4 and Supplementary Table 7). For the A/H1N1 and A/H3N2 strains, post-vaccination seroneutralisation and anti-neuraminidase GMTs and GMTRs were similar for IIV4-HD and the pooled IIV3-HDs. For the two B-lineage strains, post-vaccination seroneutralisation GMTs and geometric mean post-/pre-vaccination titer ratios (GMTRs) were similar for IIV4-HD and IIV3-HD when it contained the same B-lineage strain but higher for IIV4-HD when it contained the B-lineage strain not included in the IIV3-HD. Seroneutralisation post-/pre-vaccination GMTRs for IIV4-HD were 5.40 (95%CI: 3.90–7.48) for A/H1N1, 2.83 (95%CI: 2.31–3.46) for A/H3N2, 2.81 (95%CI: 2.21–3.58) for B Victoria, and 3.51 (95%CI: 2.80–4.39) for B Yamagata. Anti-neuraminidase post-/pre-vaccination GMTRs were 1.61 (95%CI: 1.44–1.81) for A/H1N1 and 2.12 (95%CI: 1.84–2.45) for A/H3N2.

3.3. Solicited reactions

The most common solicited reactions for both IIV3-HD and IIV4-HD were injection-site pain, myalgia, malaise, and headache (Fig. 5). Proportions reporting all injection-site reactions, as well as myalgia, shivering, and headache were higher for IIV4-HD than for IIV3-HD. For each solicited reaction, fewer than 1% of subjects reported it as grade 3. Most reactions began within 3 days of vaccination (Supplementary Table 8) and resolved within 3 days of onset (Supplementary Table 9).

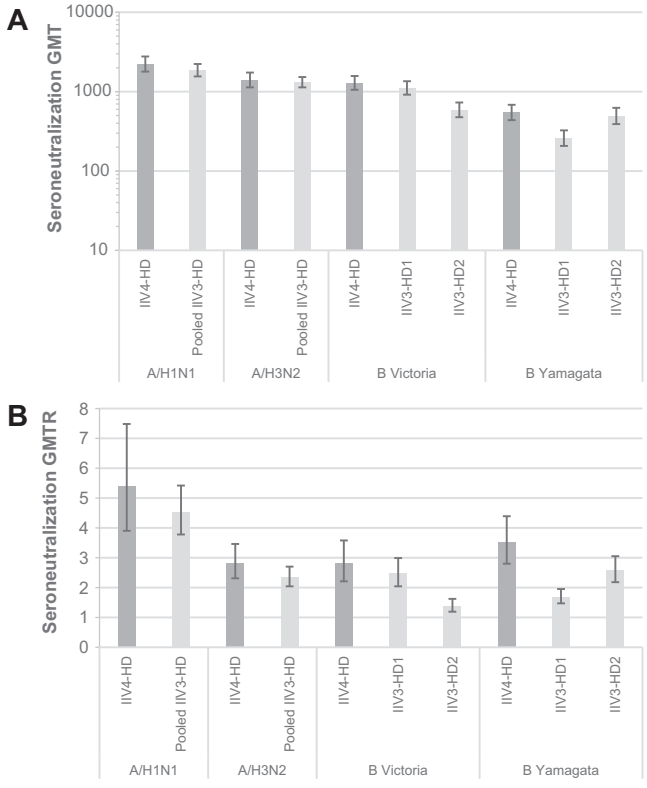


Fig. 3. Seroneutralisation antibody GMTs (A) and GMTRs (B). Values are for the expanded immunogenicity subset. Abbreviations: CI, confidence interval; GMT, geometric mean titer; GMTR, geometric mean post-/pre-vaccination titer ratio; IIV3-HD1, high-dose trivalent inactivated influenza vaccine containing the Victoria-lineage B strain; IIV3-HD2, high-dose trivalent inactivated influenza vaccine containing the Yamagata-lineage B strain; IIV4-HD, high-dose quadrivalent inactivated influenza vaccine.

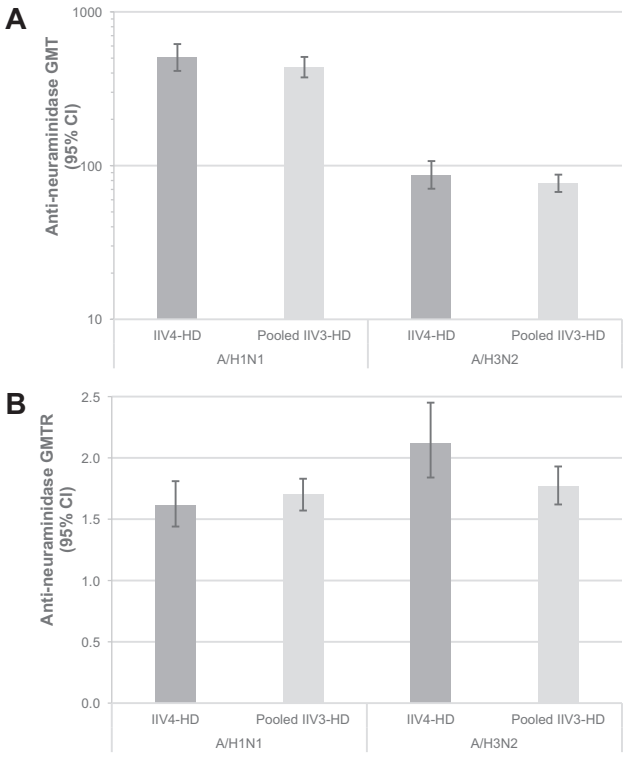


Fig. 4. Anti-neuraminidase antibody GMTs (A) and GMTRs (B). Values are for the expanded immunogenicity subset. Abbreviations: CI, confidence interval; GMT, geometric mean titer; GMTR, geometric mean post-/pre-vaccination titer ratio; IIV3-HD1, high-dose trivalent inactivated influenza vaccine containing the Victoria-lineage B strain; IIV3-HD2, high-dose trivalent inactivated influenza vaccine containing the Yamagata-lineage B strain; IIV4-HD, high-dose quadrivalent inactivated influenza vaccine.

3.4. Unsolicited AEs

For unsolicited AEs, AEs leading to study discontinuation, SAEs, deaths, and AEs of special interest, proportions were similar between IIV4-HD and the IIV3-HDs (Table 5). An AE of special interest, considered by the investigator as possibly vaccine related, was reported for one subject. The event was a grade 2 (moderate severity) small-fiber neuropathy involving both sensory and autonomic fibers diagnosed 42 days after vaccination with IIV4-HD. The subject had a recent viral infection and was deficient in vitamin B12.

4. Discussion

The high-dose trivalent inactivated influenza vaccine, IIV3-HD, was licensed in adults ≥65 years of age based on its ability to provide a clinically relevant improvement in protection against laboratory-confirmed influenza and influenza-related medical events over the standard-dose trivalent vaccine, IIV3-SD [7]. Post-licensure studies have further demonstrated a benefit of IIV3-HD over IIV3-SD for serious influenza-related events and death [9–15,26].

The current study, performed in 2670 adults ≥65 years of age, showed that a quadrivalent formulation of the high-dose vaccine, IIV4-HD, induced HAI antibody responses that were non-inferior

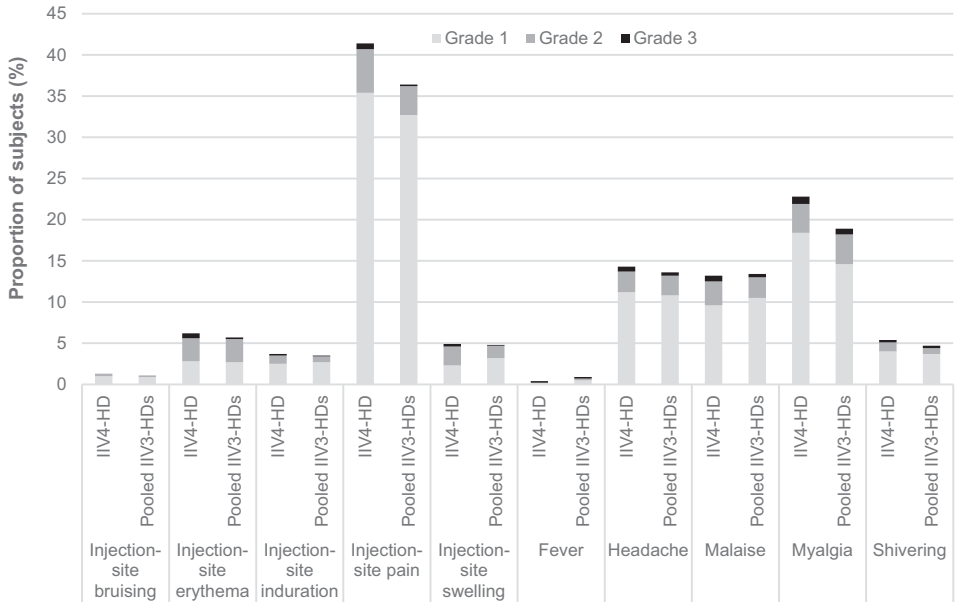


Fig. 5. Solicited reactions. Values are for the full analysis set. Solicited reactions were recorded by subjects for 7 days after vaccination. Injection-site erythema, swelling, induration and bruising were scored as grade 1 if 25–50 mm in diameter, grade 2 if 51–100 mm in diameter, and grade 3 if >100 mm in diameter. Fever was scored as grade 1 if 100.4–101.1°F (38.0–38.4 °C), grade 2 if 101.2–102.0°F (38.5–38.9 °C), and grade 3 if ≥102.1°F (≥39.0 °C). All other solicited reactions were scored as grade 1 if transient, requiring minimal therapeutic intervention, does not interfere with daily activities; grade 2, if they required additional therapeutic intervention, interfered with daily activities but posed no significant permanent risk; and grade 3 if they interrupted usual daily activities, significantly affected clinical status, or required intensive therapeutic intervention. Abbreviations: IIV3-HD, high-dose trivalent inactivated influenza vaccine containing; IIV4-HD, high-dose quadrivalent inactivated influenza vaccine.

Table 5
Unsolicited AEs and SAEs.

	n (%)	
Event	IIV4-HD N = 1777	Pooled IIV3-HDs N = 893
Any unsolicited AE	292 (16.4)	147 (16.5)
Immediate unsolicited AE (<30 min)	5 (0.3)	2 (0.2)
AE leading to study discontinuation	1 (<0.1)	2 (0.2)
SAE within 28 days	19 (1.1)	12 (1.3)
SAE during the entire study (up to 6 months)	80 (4.5)	48 (5.4)
SAE considered related to the study vaccine	1 (<0.1)[a]	0 (0.0)
Fatal SAEs	3 (0.2)	2 (0.2)
AEs of special interest [b]	1 (<0.1)	2 (0.2)

Values are for the full analysis set. Abbreviations: AE, adverse event; IIV3-HD, high-dose trivalent inactivated influenza IIV4-HD, high-dose quadrivalent inactivated influenza vaccine; SAE, serious adverse event.

[a] Grade-2 small-fiber neuropathy 42 days after vaccination with IIV4-HD, an AE of special interest.

[b] Included new onset of Guillain-Barré syndrome, encephalitis/myelitis (including transverse myelitis), Bell's palsy, optic neuritis, and brachial neuritis.

to responses induced by IIV3-HD for the three shared strains and superior responses for the additional B-lineage strain. IIV4-HD also induced seroneutralisation and anti-neuraminidase antibody responses that were similar to those induced by IIV3-HD for the three shared strains and higher for the additional B-lineage strain. Thus, despite the higher dose of antigen, adding a second B strain to the high-dose split-virion influenza vaccine did not compromise the immunogenicity induced by the other three strains or limit the immune response to the added B-lineage strain.

Similar findings were obtained in a study comparing the immunogenicity of standard-dose quadrivalent split-virion influenza vaccine (IIV4-SD) and IIV3-SDs in adults ≥65 years of age [27]. In that study, the current one, and some others examining standard-dose trivalent influenza vaccines [20,28], significant low-level B-strain cross-reactivity has been detected. This has also been observed in children vaccinated with trivalent influenza vaccines [29,30]. However, antibody titers induced by B-strain cross-reactivity have been suboptimal and may require priming by natural infection or previous vaccination [29,30]. Adding a second B-lineage strain in IIV4-HD should therefore broaden protection against influenza B infection in adults ≥65 years of age.

In this study, the HAI GMT for strain A/H1N1 was slightly lower for IIV4-HD than for the pooled IIV3-HDs. This has been observed previously with the standard-dose quadrivalent influenza vaccine [31] and quadrivalent intradermal influenza vaccine [32] but is not considered to be clinically relevant because the HAI GMTs and seroconversion rates for the quadrivalent vaccines were statistically non-inferior to those induced by the trivalent vaccines containing the same strains.

Despite the higher dose of antigen in IIV4-HD, previous exposure to influenza vaccine reduced antibody titers and seroconversion rates. This phenomenon is well known for influenza vaccines [33] and may be due, in part, to absorption of antigen by circulating pre-existing antibodies [34].This study demonstrated that, despite the increase in total antigen dose, IIV4-HD was well tolerated and had a similar safety profile as IIV3-HD. As expected with the higher dose of antigen in IIV4-HD, proportions of subjects reporting solicited injection-site reactions, and some solicited systemic reactions (myalgia, shivering, and headache) were slightly higher than with IIV3-HD, although the severity of solicited events and the severity and occurrence of unsolicited AEs did not appear to increase. However, firm conclusions about rates of individual safety events cannot be drawn because the study was not designed or powered to make comparisons. Post-licensure monitoring will be conducted to confirm the safety of IIV4-HD.

A single AE of special interest, small-fiber neuropathy in a subject 42 days after vaccination with IIV4-HD, was recorded by the investigator as possibly related to the study vaccine. Small-fiber neuropathy has been reported following vaccination for other diseases [35]. The subject also had a recent viral infection and was diagnosed with vitamin B12 deficiency, either of which might have caused the event [35,36].

The study should be interpreted in the context of certain limitations of the study design. Importantly, the population was limited to healthy, community-dwelling older adults. Further investigation is warranted to determine whether these results will be generalizable to frail or institutionalized older adults. Another limitation is that this study did not directly measure protection against influenza infection, although findings from the HAI, seroneutralisation, and anti-neuraminidase antibody measurements consistently indicated that IIV4-HD should provide improved protection over IIIV3-HD against influenza infection. Finally, based on safety data collected from more than 25,000 individuals during clinical development of IIV3-HD [6,16] and pharmacovigilance data collected from more than 115 million doses of IIV3-HD distributed since 2009 [37], no safety concerns with IIV4-HD are anticipated.

In conclusion, the current study demonstrated that, as with IIV4-SD, adding a second B strain in IIV4-HD resulted in improved immunogenicity against the added strain without compromising the immunogenicity of the other strains or the vaccine's tolerability. Thus, transitioning from the trivalent formulation, IIV3-HD, to the quadrivalent formulation, IIV4-HD, is expected to further extend the benefits of the high-dose vaccine, which include improved protection against influenza infection and influenza-related cardio-respiratory events, death, and health care utilization [9–15].

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All authors acquired, analyzed, or interpreted data; drafted or critically revised the manuscript; approved the submitted version; and agreed to be accountable for its accuracy and integrity. In addition, LC, YM, HJ, and VL conceived and designed the study.

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Declaration of Competing Interest

LC, YM, HJ, and VL are employees of Sanofi Pasteur, the study sponsor. HKT received research funding from Sanofi Pasteur to conduct work related to this study and is on the Safety Board of Seqirus.

Author contributions and compliance with ICMJE criteria for authorship

All authors helped conceive or design the study; acquired, analyzed, and interpreted data; participated in editing the manuscript; approved the final version; and agreed to be accountable for its contents. Thus, all authors attest they meet the ICMJE criteria for authorship.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.08.016.

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