Safety and immunogenicity of a quadrivalent, inactivated, split-virion influenza vaccine (IIV4-W) in healthy people aged 3–60 years: a phase III randomized clinical noninferiority trial

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ABSTRACT

**Background:** A quadrivalent split influenza vaccine IIV4-W against both influenza A and B viruses is urgently needed.

**Methods:** To evaluate the safety and immunogenicity of IIV4-W in people aged 3–60 years, 2400 participants recruited in a double-blind phase III trial and were randomly assigned to the IIV4-W, TIV1 and TIV2 groups. The immunogenicity indicators were measured at 28 days postvaccination and for 180 days for safety follow-up.

**Results:** Adverse events (AEs) occurred in 162 (20.28%), 116 (14.55%) and 123 (15.41%) participants in the IIV4-W, TIV1 and TIV2 groups, respectively. All these AEs were mild and self-limiting, and no serious AEs related to the vaccines were observed. IIV4-W elicited a non-inferior immune response for matched strains (the lower limit of 95% CI for GMT ratio >0.67, for SCR and SPR difference >-10%) and superior immune response for the additional B strains (the lower limit of 95% CI for GMT ratio >1.5, for SCR difference >10%) versus TIVs. The lower limit of the 95% confidence interval of the GMT increase fold, the seroconversion rate and the seroprotection rate exceeded 2.5, 40% and 70% for the four strains in IIV4-W respectively.

**Conclusions:** IIV4-W was noninferior to the TIV-matched strains and was superior to the additional B strain. IIV4-W was safe in the participants and elicited high antibody titers.

Introduction

Circulation of various B strains increases human influenza infection in different regions and seasons.1 As trivalent influenza vaccines (TIVs) contain two strains of influenza A lineage (A/H1N1 and A/H3N2) and one strain of influenza B (BV, B/Victoria or BY, B/Yamagata),2 the limited cross-lineage protection of B strains of TIVs poses a long-term threat to human health. There is an urgent need for seasonal quadrivalent influenza vaccines that contain both A (H1N1, H3N2) and B (BV, BY) antigens to provide coverage against influenza.3 Based on the influenza disease surveillance data from the previous year, the World Health Organization (WHO) recommends the dominant influenza stains used in vaccines for the next influenza season.4 The reformulated seasonal quadrivalent influenza vaccine strains will replace the current existing influenza vaccines and may decrease the incidence of influenza-related consultations and hospitalizations in the upcoming influenza season.3

Seasonal quadrivalent influenza vaccines were found to have noninferior immunogenicity and acceptable safety in several phase III trials in infants, children and adults compared to TIV.5-7 IIV4-HL, which is produced by Hualan Biological Engineering, was the first available seasonal quadrivalent split influenza vaccine in China since the 2018/2019 influenza epidemic season for populations aged 3 years old or above (China Drug Approval No.: S20083016). However, there is still an urgent need for IIV4 in China. The phase III trial of IIV4-W by the Wuhan Institute of Biological Products Co. Ltd. was completed to evaluate the tolerability and immunogenicity in elderly individuals aged 60 years above in 2019.8 To investigate the safety and immunogenicity of IIV4-W in children and older adults as a candidate influenza vaccine, we conducted a phase III noninferiority trial that compared IIV4-W with two controls (TIV1 and TIV2 produced by Changchun Institute of Biological Products Co. Ltd. and approved by the National Institutes for Food and Drug Control that included two influenza A strains, TIV1
containing influenza B/Yamagata and TIV2 containing B/Victoria) as the chosen influenza vaccine in China in participants aged 3–60 years.

Materials and methods

Study design

This randomized, double-blind, active-controlled, three-center trial was designed by the Wuhan Institute of Biological Products Co Ltd (WIBP). To evaluate the immunogenicity and safety of IIV4-W to two National Institutes for Food and Drug Control-licensed TIV in adults 3–60 years of age (Clinical approval number 2015L00649 and China clinical trial identifier: CTR20160206), the study was performed in three clinical centers during March 2016 and ended in July 2017: Chaoyang District, Beijing, Chingyuan and Quwo County of Shanxi Province in China. The safety set was evaluated for 180 days after vaccination, and the serological index of the participants was detected before immunization and 28 days postimmunization. The vaccine injection, safety and immunogenicity data collection was performed by the investigators of the Beijing City Centers for Disease Control and Prevention (CDC) and the Shanxi Province CDC. The Department of Health Statistics of the Fourth Military Medical University, as the independent data and safety monitoring board, was responsible for the safety and immunogenicity data monitoring and statistical analysis.

Ethics

The protocols of this trial were approved by the National Medical Products Administration (NMPA). The study was conducted following the principles of the Declaration of Helsinki and was consistent with the Good Clinical Practice (GCP) of China, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). All the participants and the guardians of young children (3–17 years old) signed written informed consent before enrollment.

Participants

Healthy participants aged 3–60 years old without a history of influenza vaccine injection with the last three years or influenza virus infection with the last three months according to inquiry were eligible for enrollment. Participants with an axillary temperature ≤37.0°C who abided by the clinical trial protocols were needed. For randomization, the recruited 2400 participants were sequentially assigned a number by SAS software to stratify them via block randomization, and the participants were randomly assigned to receive intramuscular injections of a single dose of 15 μg IIV4-W, TIV1 or TIV2 (1:1:1). The participants and investigators were both masked to the vaccine that was administered.

Vaccination

IIV4-W contained 60 μg (15 μg for each strain) HA antigen. The production process of IIV4-W includes the influenza vaccine strains grown in embryonated eggs, the harvest, concentration, inactivation, cleavage and purification of the viral liquid, then combination, dilution, and equal division of 0.5 mL inactivated viral liquid into a vial with standard techniques. The four influenza vaccine strains used for the 2015/2016 season (Northern Hemisphere) were recommended by the WHO, were approved by the State Food and Drug Administration (FDA) and were purchased from The National Institute for Biological Standards and Control (NIBSC). IIV4-W (batch number: 20151101) contains four vaccine strains (H1N1 lineage: NYMC X 179A reassortant derived from A/California/7/2009; the H3N2 lineage: NIB 88 reassortant derived from A/Switzerland/9715293/2013; the B/Victoria lineage: NYMC BX 51B reassortant derived from B/Massachusetts/2/2012; and the B/Yamagata lineage: NYMC BX 35 reassortant derived from B/Brisbane/60/2008). Licensed TIV1 (influenza vaccine, split virus, inactivated, 15 μg of each strain, batch number: 20150632) contains the same influenza A strains and one B strain (B/Yamagata lineage: NYMC BX 35 reassortant derived from B/Brisbane/60/2008), and TIV2 (influenza vaccine, split virus, inactivated, 15 μg of each strain, batch number: S20150801) contains the other influenza B strain (B/Victoria lineage: NYMC BX 51B reassortant derived from B/Massachusetts/2/2012) in addition to the same two influenza A strains.

Safety endpoints

Safety was assessed by the incidence, severity and duration of solicited systemic and injection-site adverse events (AEs), unsolicited AEs, and serious AEs (SAEs). All the participants were observed for 30 minutes after vaccination in case of acute AEs. Local AEs, including pain, redness, swelling, local inflammation, itching and systemic reactogenicity, including fever, fatigue, headache, gastrointestinal symptoms, etc., were solicited using memory aids (e.g., diary cards) during the week after vaccination. The adult participants or the children guardians reported any AEs by a contact card within 8–28 days. The unsolicited AEs included any other medical event in addition to the solicited AEs, such as oral herpes, upper respiratory tract infection, ear trauma, poisoning, and surgical complications. SAEs were recorded via a telephone interview 29 days to 6 months after vaccination.

The AEs were graded on a severity scale that ranged from grades 1 to 4. Grade 1 and 2 symptoms were mild and moderate and did not or only partially interfered with normal activity, while grade 3 AEs prevented the participant’s normal daily activity. Grade 4 AEs are life-threatening and require hospitalization.
**General immunogenicity**

For the immunogenicity subset, peripheral blood samples (5 mL/each) were collected and centrifuged for serum before the vaccination and 28 days after the vaccination. Hemagglutination inhibition (HI) assays were performed for the serological antibody assessments using the same procedure as previously reported.² Twenty-five microliters of serum was used for HI antibody detection of one subtype strain, and the HI titer was defined as the dilution factor of serum completely inhibiting hemagglutination.

Seroconversion rate (SCR) was defined as the proportion of the participants with either a prevaccination HI titer of <1:10 with a postvaccination titer ≥1:40 or the proportion of the participants with a prevaccination titer ≥1:10 with a ≥4 -fold increase in the antibody titers after vaccination, and seroprotection rate (SPR) was defined as the proportion of the participants with HI titers ≥1:40. According to the Committee for Human Medicinal Products (CBER) criteria, the immunogenicity indicators included that the lower limit of the two-sided 95% confidence interval (CI) of SCRs and SPRs should exceed 40% and 70% in the IIV4-W group, respectively. In addition, the lower limit of the 95% CI of geometric mean fold increase (GMFI) in the participants who received the vaccine exceeded 2.5-fold from baseline at 28 days postvaccination.

**Noninferiority immunogenicity for the matched strains**

The primary noninferior immunogenicity indicators for the H1N1, H3N2 and matched B strains included the lower limit of the two-sided 95% CI of the geometric mean HI antibody titer (GMT) ratio difference exceeding 0.67 for the new vaccine/registered vaccine and exceeding −10% of the SCR and the SPRs difference for the new vaccine-registered vaccine.

**Inferiority immunogenicity for the additional B strains**

The superior immunogenicity indicators of the additional B strains in IIV4-W were determined as the lower limit of the two-sided 95% CI of the GMT ratio >1.5, and the SCR difference was >10%.

**Statistical analysis**

Considering the participants drop-out rate and the number of invalid blood samples, the sample size was estimated to achieve at least 90% power to demonstrate noninferiority over six statistical tests (GMT ratios and SPRs for the matched strains compared with two TIV controls) using a one-sided alpha of 0.03 for each comparison with PASS15 software.

The safety analyses were conducted with Fisher’s exact probability tests. The HI titer was fitted and log10 transformed, and the significant differences in GMT and the adjusted GMT ratio between the IIV4-W and control groups were analyzed with analysis of covariance (ANOVA) and t-tests, respectively. The 95% CIs of the SPRs and SCRs were calculated by the Clopper-Pearson test, and the statistical analysis was conducted using the chi-square and Fisher’s exact probability tests with SAS software.

**Results**

**Participants**

A total of 2400 participants were enrolled, of which 800 were assigned to the IIV4-W, TIV1, or TIV2 groups. Due to the withdrawal of several participants, 799, 797, and 798 participants in the IIV4-W, TIV1, and TIV2 groups completed the immunizations and scheduled visits, respectively. The safety results of the overall subjects (safety set, SS) were analyzed, and the safety data of the 3- to 8-year-old children were highlighted. Because of incomplete immunogenicity samples (pre- and post-immunization), only 792,
Table 1. Baseline characteristics of the participants.

| Characteristic       | 3-8 years |            |            | 3-17 years |            |            | 18-60 years |            |
|----------------------|-----------|------------|------------|------------|------------|------------|-------------|------------|
|                      | IV4       | IV1        | TIV2       | IV4        | IV1        | TIV2       | IV4         | IV1        | TIV2       |
|                      | n=114     | n=126      | n=107      | n=259      | n=270      | n=257      | n=533       | n=519      | n=532      |
| Age, X(SD)           | 6.55(1.60)| 6.65(1.66)| 6.71(1.58)| .7712      | 9.70(3.48)| 9.46(3.29)| 9.75(3.22)| .5510      | 41.43(9.98)| 40.51(9.93)| 41.13(9.93)| .3120      |
| Age, M(min,max)      | 6.69(3.06,8.99)| 7.02(3.06,8.99)| 6.79(3.03,8.98)| 9.47(3.06,17.97)| 9.34(3.06,17.97)| 9.70(3.06,17.97)| 41.68(18.09,60.80)| 39.83(18.09,59.99)| 41.53(18.60,60.15)| 261(48.97)| 249(47.98)| 269(50.56)| .6984      |
| Male, (n, %)         | 55(48.25)| 69(54.76)| 60(56.07)| .4499      | 130(50.19)| 142(52.59)| 127(49.42)| .7476      | 261(48.97)| 249(47.98)| 269(50.56)| .6984      |
| Female, (n, %)       | 59(51.75)| 57(45.24)| 47(43.93)| 129(49.81)| 128(47.41)| 130(50.58)| 272(51.03)| 270(52.02)| 263(49.44)|           |           |           |            |

IV4-W, inactivated quadrivalent influenza vaccine induced by WIBP; TIV, inactivated trivalent influenza vaccine; SD, standard deviation; M, mean; min, minimum; Max, maximum. ANOVA and chi-square tests were used for age and sex, respectively. P > .05 indicates that no difference was found between any two groups.
Grading scale for the injection site AEs, such as redness and swelling: grade 1, <15 mm; grade 2, 15–30 mm; grade 3, >30 mm in diameter; grade 4, gangrene or exfoliative dermatitis. The systemic AEs included fever: grade 1: 37.1–37.5°C grade 2: 37.6–39.0°C and grade 3 >39°C. The table represents numbers (percentage) of subjects (n) with AEs. The P value was calculated from Fisher exact probability tests for the comparison. P > .05 indicates that no difference was found in any two groups, and P < .05 indicates that there was a difference between participants vaccinated with IVIV-W to TIV1 or TIV2.

Table 3. The frequency and severity of solicited AEs in 3–8 years old cohorts.

| AEs            | IVIV-W(n=114) | TIV1(n=126) | TIV2(n=110) | Total n | p   |
|----------------|---------------|-------------|-------------|---------|-----|
|                | Grade1 n(%)   | Grade2 n(%) | Grade3 n(%) | Total n|     |
| Solicited systemic AEs |               |             |             |         |     |
| Any            | 15(13.16)     | 12(10.53)   | 0(0.00)     | 27(23.68)|     |
| Fever          | 11(9.65)      | 11(9.65)    | 0(0.00)     | 22(19.30)|     |
| Headache       | 1(0.88)       | 0(0.00)     | 0(0.00)     | 1(0.88) |     |
| Fatigue        | 0(0.00)       | 0(0.00)     | 0(0.00)     | 0(0.00) |     |
| Vomiting       | 10(8.88)      | 0(0.00)     | 0(0.00)     | 10(8.88)|     |
| Diarrhea       | 1(0.88)       | 0(0.00)     | 0(0.00)     | 1(0.88) |     |
| Myalgia        | 0(0.00)       | 0(0.00)     | 0(0.00)     | 0(0.00) |     |
| Coughing       | 10(8.88)      | 1(0.88)     | 0(0.00)     | 2(1.75) | 43(1.76) | .0522 |
| Hypersensitivity | 0(0.00)     | 0(0.00)     | 0(0.00)     | 0(0.00) | 1(0.91) |      |
| Solicited local AEs |           |             |             |         |     |
| Any            | 5(4.40)       | 1(0.88)     | 0(0.00)     | 6(5.45) |     |
| Pain           | 3(2.63)       | 0(0.00)     | 0(0.00)     | 3(2.63) |     |
| Redness        | 10(8.88)      | 0(0.00)     | 0(0.00)     | 10(8.88)|     |
| Swelling       | 0(0.00)       | 0(0.00)     | 0(0.00)     | 0(0.00) |     |
| Itching        | 10(8.88)      | 1(0.88)     | 0(0.00)     | 11(9.98)|     |

The table represents the numbers (percentage) of subjects (n) with adverse events.

789, 789 were available for immunogenicity analysis in groups IVIV-W, TIV1 and TIV2, respectively (Figure 1). There were no significant differences in age or sex among the three groups, and the baseline demographic characteristics of the participants are listed in Table 1.

Safety

All AEs (combined solicited and unsolicited AEs) occurred in 162 (20.28%), 116 (14.55%), and 123 (15.41%) participants in the IVIV1, TIV1, and TIV2 cohorts during the follow-up, respectively. The most solicited local and systemic reactions were resolved within 7 days after immunization and were reported to be mild (grade 1) or moderate (grade 2) in severity after vaccination in the three groups; fewer than 0.50% or 0.88% of the patients reported grade 3 systemic or local AEs in the IVIV-W group (Table 2). Four, two, and two vaccine-unrelated SAEs were observed up to six months after the first vaccination in the IVIV-W, TIV1 and TIV2 groups, respectively, and no AEs led to withdrawal from the study.

Although there were no significant differences in the prevalence of total solicited (systemic or local) and unsolicited AEs among the IVIV-W, TIV1 and TIV2 vaccination groups (P > .05), fever as the most common solicited systemic AE was reported by 88 (10.89%), 43 (5.27%), 61 (7.64%) participants in the IVIV-W, TIV1 and TIV2 groups with a significant difference (p = .0002). Headache, as the second most common systematic AE, was reported by 16 (2.00%), 5 (0.63%), and 5 (0.63%) participants in the three vaccination groups, respectively (p = .0175) (Table 3). There were no differences in the incidence of fatigue, vomiting, diarrhea, myalgia, coughing, or hypersensitivity that were observed among the IVIV-W and the two control
Table 4. Immunogenicity measures in the participants.

| Subtype | 3-8y | 3-17y | 18-60y |
|---------|------|-------|-------|
|         | IV-W (n=114) | TV1 (n=126) | TV2 (n=107) | P |
| IV-W (n=259) | TV1 (n=270) | TV2 (n=257) | P |
| IV-W (n=533) | TV1 (n=519) | TV2 (n=532) | P |

**Prevaccination GMT (95% CI)**

- **H1N1**: 32.73 (25.95, 41.27)  
  - 3.8y: 27.22 (22.28, 33.24)  
  - 17.2y: 32.72 (25.90, 41.34)  
  - GMT: 29.88 (26.25, 34.01)  
  - P: .888
- **H3N2**: 105.18  
  - 3.8y: 115.02  
  - 17.2y: 114.24  
  - GMT: 82.39 (73.16, 92.79)  
  - P: .769
- **BY**: 30.24 (24.28, 37.67)  
  - 3.8y: 25.90 (21.18, 31.68)  
  - 17.2y: 28.56 (21.96, 37.14)  
  - GMT: 39.36 (33.99, 45.58)  
  - P: .604
- **BV**: 15.31 (12.87, 18.20)  
  - 3.8y: 17.24 (14.53, 20.48)  
  - 17.2y: 14.75 (12.30, 17.69)  
  - GMT: 15.10 (13.30, 16.89)  
  - P: .419

**Postvaccination GMT (95% CI)**

- **H1N1**: 517.32  
  - 3.8y: 396.58  
  - 17.2y: 493.91  
  - GMT: 702.85  
  - P: .353
- **H3N2**: 1092.83  
  - 3.8y: 885.40  
  - 17.2y: 1109.98  
  - GMT: 803.48  
  - P: .218
- **BY**: 209.08  
  - 3.8y: 190.80  
  - 17.2y: 58.24 (46.53, 72.90)  
  - GMT: 268.91  
  - P: .001
- **BV**: 73.47 (59.58, 90.61)  
  - 3.8y: 70.62 (33.38, 119.32)  
  - 17.2y: 63.77 (50.82, 80.02)  
  - GMT: 85.76 (76.38, 96.30)  
  - P: .001

**GMFI (95% CI)**

- **H1N1**: 15.81 (12.31, 20.29)  
  - 3.8y: 14.57 (11.63, 18.26)  
  - 17.2y: 15.09 (11.71, 19.46)  
  - GMT: 23.52 (19.93, 27.16)  
  - P: .818
- **H3N2**: 10.38 (2.12, 16.80)  
  - 3.8y: 7.97 (4.34, 11.27)  
  - 17.2y: 21.32  
  - GMT: 7.52 (4.68, 10.34)  
  - P: .001
- **BY**: 6.91 (5.55, 8.62)  
  - 3.8y: 7.37 (5.82, 9.32)  
  - 17.2y: 2.04 (1.78, 2.33)  
  - GMT: 6.83 (5.79, 8.07)  
  - P: .001
- **BV**: 4.80 (4.89, 5.92)  
  - 3.8y: 1.61 (1.41, 1.83)  
  - 17.2y: 4.32 (3.47, 5.39)  
  - GMT: 5.68 (4.97, 6.49)  
  - P: .001

**SCR (95% CI)**

- **H1N1**: 84.21 (76.20, 90.37)  
  - 3.8y: 85.71 (78.37, 91.31)  
  - 17.2y: 85.05 (76.86, 91.20)  
  - GMT: 90.35 (86.93, 93.66)  
  - P: .982
- **H3N2**: 81.58 (73.23, 89.82)  
  - 3.8y: 75.40 (66.93, 82.63)  
  - 17.2y: 80.37 (71.58, 88.42)  
  - GMT: 81.85 (76.61, 86.35)  
  - P: .478
- **BY**: 71.93 (67.29, 76.79)  
  - 3.8y: 74.60 (66.08, 81.93)  
  - 17.2y: 18.69 (11.81, 27.38)  
  - GMT: 68.34 (62.30, 73.96)  
  - P: .001
- **BV**: 59.65 (50.05, 68.73)  
  - 3.8y: 10.32 (5.61, 17.07)  
  - 17.2y: 52.34 (42.46, 62.08)  
  - GMT: 69.11 (63.10, 74.68)  
  - P: .001

**SPR (95% CI)**

- **H1N1**: 97.37 (95.20, 99.49)  
  - 3.8y: 90.48 (83.95, 94.98)  
  - 17.2y: 90.65 (83.48, 95.43)  
  - GMT: 98.46 (96.09, 99.55)  
  - P: .072
- **H3N2**: 100.0  
  - 3.8y: 99.07 (97.11, 100.0)  
  - 17.2y: 99.07 (97.11, 100.0)  
  - GMT: 99.07 (97.11, 100.0)  
  - P: .000

The population used for the immunogenicity analysis was those participants who received vaccination with nonmissing pre- or postvaccination antibody data. The GMFI was the ratio of the antibody titers compared to the baseline GMT, and the SPR was the ratio of the antibody titers compared to the baseline GMT, with any difference in any two groups, with P < .05 indicating a difference between IV-W and TV1 or TV2.
groups \((P > .05)\). Pain was the most common local AE and was observed in 37 (4.63%), 18 (2.26%), and 28 (3.51%) participants in the IIV4-W, TIV1 and TIV2 groups, respectively \((P < .05)\). Other injection-site AEs, such as redness, swelling, induration and itching, had similar incidences among the three groups \((P > .05)\).

In 3- to 8-year-old children, solicited systemic AEs were reported by 23.68%, 19.84% and 20.00% of participants in the IIV4-W, TIV1 and TIV2 groups, respectively, which was higher than in the overall cohort (IIV4-W, 16.52%; TIV1, 9.28%; TIV2, 10.78%). The proportion of participants with local AEs in 3- to 8-year-old children was similar to that in the overall cohort (Table 3). Although the solicited systemic AEs in the IIV4-W group seemed to be higher than those in the comparator TIV groups, the results of statistical analysis showed that there was no significant difference in the incidence of solicited AEs among the three groups \((P > .05)\). Most of the solicited AEs were mild and moderate in intensity. Systemic fever and injection-site pain were the most common solicited AEs in 3- to 8-year-old children, the same as in the whole age cohort.

**General immunogenicity**

A total of 2341 serological results were obtained. All the participants had similar prevaccination HI titers \((P > .1)\) among the IIV4-W, TIV1 and TIV2 groups, and over 70% of the participants had detectable HI antibodies \((HI > 1:10)\) at baseline (Table 4). The lower limit of the 2-sided 95% CI of the GMFI in the IIV4-W group and in the comparator TIV group exceeded 2.5-fold among the 3–17 and 18–60 years age cohorts for H1N1, H3N2 or the matched B lineage strains (Table 4). In the 3–17 year cohorts, the HI titer GMTs but not the GMFI was higher than that in the 18–60 year cohort for the four strains among the IIV4-W, comparator TIV1 and TIV2 groups (Table 4). The HI titer and GMFI induced by the IIV4-W additional B lineage strain were significantly higher than those elicited by the comparators TIV1 and TIV2 without the matched B strain \((P = .0001)\) (Table 4).

Higher SCR and SPRs of IIV4-W against H1N1, H3N2, BY and BV were observed in the cohorts aged 3–17 and 18–60 years. More than 60% of the participants had HI titers that seroconverted for the H1N1 and H3N2 strains, and more than 40% of the participants had HI titers that seroconverted for matched BY and BV strains in the IIV4-W, TIV1 and TIV2 strains, which exceeded the CBER criteria and required a lower limit of the two-sided 95% CI of SCR (Table 4). The SCR of IIV4-W, TIV1 and TIV2 for H1N1 and H3N2 in the 3–17 cohort were lower than those of the participants who were over 18 years old. The SCR for the BY strain in the 18–60-year-old cohort were slightly higher than those in the 3–17-year-old cohort. The SCR for the BV strain were similar in the two age cohorts.

The SPRs for the H1N1, H3N2 and BY strains were not significantly different among the 3–17- and 18–60-year age cohorts (Table 4). Higher SPRs for the BV strain were observed in the 3–17-year age cohort than in the 18–60-year age cohort. In the two age cohorts, the SPRs against the H1N1 and H3N2 subtypes exceeded 70% in the trial and in the control vaccine groups, which is the same as the SPRs against the BY strain in the IIV4-W and TIV1 groups and against the BV strain in the IIV1 and TIV2 groups. The SCR and SPRs against the BY strain in the TIV1 and TIV2 group and against the BV strain in the TIV1 group were at low levels because of the lack of BV strain antigen in the TIV1 and BV strain antigen in the TIV2 vaccine.

Among the trial and control vaccine groups, there were no differences in the HI titer GMTs and GMFI for H1N1 and H3N2 in the 3–8-year cohort \((P > .2)\). The GMFI exceeded 15.81 (12.31, 20.29), 10.39 (8.02, 13.46), 6.91 (5.55, 8.62) and 4.80 (3.89, 5.92) for the H1N1, H3N2, BY and BV strains of IIV4-W, respectively (Table 4). The SCR for the BV strain in the 3–8-year-old cohort were statistically equivalent to those in the 18-60-year-old cohort and were slightly higher than those in the 3–17-year-old cohort.

**Noninferiority immunogenicity for the matched strains**

The lower limit of the two-sided 95% CI of the GMT ratio of IIV4-W/TIV1 and IIV4-W/TIV2 exceeded 0.67 for the matched strains in the overall participants. The lower limit of the two-sided 95% CI of the SCR and SPR differences of IIV4-W/TIV1 and IIV4-W/TIV2 for H1N1, H3N2 and matched B strains exceeded –10% in all of the participants, which met the CBER criteria for noninferiority (Table 5).

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**Table 5.** The non-inferiority and superiority comparisons between IIV4-W and TIVs.

| Subtype | IIV4-W vs TIV1 | IIV4-W vs TIV2 |
|---------|---------------|---------------|
| H1N1    |               |               |
| GMT ratio (95%)* | 1.15(1.03,1.29) | 1.01(0.89,1.14) |
| SCR difference (95%)* | -0.23%(-2.76%,2.30%) | 0.53%(-2.06%,3.13%) |
| SPR difference (95%)* | 2.41%(1.00%,3.73%) | 3.30%(1.83%,4.76%) |
| Noninferiority* | Yes | Yes |
| H3N2    |               |               |
| GMT ratio(95%) | 0.91(0.81,1.02) | 0.78(0.69,0.87) |
| SCR difference (95%) | 1.32%(−2.23%,4.87%) | -2.48%(−5.84%,0.88%) |
| SPR difference (95%) | -0.50%(−1.20%,0.19%) | -0.25%(−1.03%,0.53%) |
| Noninferiority | Yes | Yes |
| BY      |               |               |
| GMT ratio(95%) | 0.97(0.87,1.07) | 2.34(2.10,2.61) |
| SCR difference (95%) | 0.48%(−3.88%,4.85%) | 37.11%(32.56%,41.66%) |
| SPR difference (95%) | 0.13%(−1.10%,1.36%) | 10.40%(7.98%,12.81%) |
| Noninferiority* | - | Yes |
| Noninferiority* | - | - |

*GMT ratio: the ratio of GMTs of IIV4-W/GMTs of TIV1, or GMTs of IIV4-W/GMTs of TIV2.
1Scr difference was the difference of sera neutralization rates (IIV4-W minus TIV1 or IIV4-W minus TIV2).
2SPR difference was the difference of sera conversion rates (IIV4-W minus TIV1 or IIV4-W minus TIV2).
3Noninferiority, IIV4-W was non-inferior to TIV1 or TIV2 matched strains if the lower limit of the two-sided 95% CI of GMT ratio, SCR difference and SPR difference was >0.67, −10%, and −10%.
4Noninferiority, IIV4-W was non-inferior to TIV1 or TIV2 unmatched strains if the lower limit of the two-sided 95% CI of GMT ratio, SCR difference was >1.5, 10%.
Superiority immunogenicity for the additional B strains

The lower limit of the two-sided 95% CI of the GMT ratio of IIV4-W/TIV1 for the BV strain and IIV4-W/TIV2 for the BY strain exceeded 1.5 in all the participants. The lower limit of the two-sided 95% CI of the SCR and SPR differences of IIV4-W-TIV1 for the BV strain and IIV4-W-TIV2 of the BY strain exceeded 10%, which met the superiority criterion (Table 5).

Discussion

In phase III clinical trials, IIV4-W elicited a robust amount of humoral antibodies for the additional B strains (B/Yamaga or B/Victoria) compared to TIVs, with noninferior immunogenicity (GMT ratios and differences in SCRs and SPRs) for the H1N1, H3N2 and matched B strain lineages and superior immunogenicity for the additional B strain lineages in the 3-60-year-old population. Most injection-site and systemic AEs were mild to moderate and were self-limiting. Overall, IIV4-W had satisfactory immunogenicity and an acceptable safety profile.

The subjects received IIV4-W provided a strong serological response with serum HI titer GMFI, SCR, and SPR over 2.5, 40%, 70% for young children and adults, respectively. IIV4-W met the noninferiority criteria for the H1N1, H3N2, BY and BV strains for the GMT ratio, SCR and SPR difference in the participants, and these noninferior immunogenicity results are consistent with other clinical trials of IIV4s.10,11 All the above results indicated that IIV4-W could be used as a candidate seasonal tetravalent influenza vaccine.

Although the antibody levels of the four influenza strains were generally higher in 3-17-year-old participants than in the populations aged 18-60 years old, the GMFI was higher in the 18-60-year-old cohorts than in the younger cohorts, which is likely due to the low prevaccination HI titer in the elder adults.12,13 For children, influenza vaccines may be moderately immunogenic because of the limited previous exposure to vaccines and viruses, so two doses of influenza vaccine are recommended for 6-month- to 8-year-old children to achieve protective antibody titers.14 The SCR of one does IIV4-W was closer to that of two doses quadrivalent influenza vaccine in children 3-8 years of age, indicating that IIV4-W could be used not only in adults but also in children.6

Increased HI titers for the BV strain in TIV1 and the BY strain in TIV2 suggest that cross-reactivity of the B strains in TIV may elicit antibodies to heterologous B strains, similar to the previous clinical trial of split-virion trivalent influenza vaccine.15 Regardless, compared to the matched B strains, the extent of cross-reactivity elicited by the heterologous B strains was expected to be low, and there was uncertainty regarding its ability to produce sufficient immune protection; IIV4 could reduce annual cases, hospitalizations and deaths.16

In this clinical trial, the safety profile of IIV4-W was characterized for up to 6 months after vaccination. Although the incidences of local pain, systemic fever and headache were higher in the participants who received IIV4-W than in the participants who received TIV1 and TIV2, the incidences of other AEs, such as local redness, swelling and systemic fatigue, were not significantly higher than those in the control group. The reason for these results may be related to the fact that IIV4-W has one more B lineage strain antigen than TIV, and the total protein content of IIV4-W was 25% higher than that in TIV. The incidence of grade 3 and above AEs in the IIV4-W group was not significantly higher than that in the comparator group, and IIV4-W was not considered to increase the incidence of AEs of a higher severity relative to the comparator vaccine, indicating that IIV4-W was safe and well tolerated.

The frequencies of fever induced by IIV4-W observed here were higher than those in other studies of IIV4, possibly due to the definition of fever grade (grade 1, 37.1–37.5°C grade 2, 37.6–39°C grade 3, ≥39.1°C) based on the guiding principles for grading standards of adverse reactions in clinical trials of preventive vaccines issued by the State Food and Drug Administration being different from other studies (grade 1, 38.0–38.4°C grade 2, 38.5–38.9°C grade 3: ≥39.0°C or fever was defined as above 37.5°C). The other safety results were consistent with the clinical study results of the other quadrivalent influenza vaccine, such as local pain;5,17-19 however, the incidence of redness and swelling was higher than that in other studies, just because of the different definitions of redness and swelling grades.18,19 The frequencies of systemic AEs decreased with increasing age, which was also observed previously.17,20

We took full account of the complexity of the participants in this study and excluded the factors affecting vaccination. In addition to dividing the participants into two age cohorts to assess the safety and immunogenicity of IIV4-W and comparator TIV so that the results were more detailed and reliable, we also analyzed the immunogenicity and safety of IIV4-W in people aged 3–8 years to determine whether IIV4-W is available for children. However, we did not evaluate the effectiveness of IIV4-W, which is not equivalent to its immunogenicity. The immunogenicity evaluation of a single epidemic season makes it challenging to assess the efficacy of IIV4-W against various influenza strains.

Conclusions

We found that the split influenza vaccine IIV4-W has a comparable safety profile with noninferior immunogenicity in individuals aged 3–60 years. IIV4-W containing both of the B lineage influenza strain antigens could decrease influenza hospitalization and could be considered a candidate influenza vaccine against seasonal influenza in children, adults and elderly individuals.6

Abbreviations

| AE          | Adverse Event                        |
|-------------|--------------------------------------|
| ANOVA       | Analysis of Variance                 |
| CBER        | The Center for Biologics Evaluation and Research |
| CDC         | Centers for Disease Control and Prevention |
| CI          | Confidence Interval                  |
| FAS         | Full Analysis Set                    |
| FDA         | Food and Drug Administration         |
| GCP         | Good Clinical Practice               |
| GMFI        | Geometric Mean Fold Increase         |
| GMT         | Geometric Mean Titer                 |
| HI          | Hemagglutination Inhibition          |

References:
10. The authors cite several studies and clinical trials that support their findings.
11. Additional studies and data are included to strengthen the conclusions.
12. The complexities of vaccine development and the clinical impact of IIV4-W are discussed.
13. The implications of the findings for public health and vaccine policy are highlighted.
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Authors’ contributions

Xiaoming Yang, Xiaoyuan Huang, Li Li, Ting Fan and Guohua Li were responsible for the study concept, design and statistical analysis. Xuanxuan Nian and Jiayou Zhang were responsible for the manuscript drafting and revision. Xuefen Gao, Wei Zhao, Wei Chen, Zhaoqing Zhang, Zhihao Yao, and Xixin Han were responsible for the clinical trial follow-up, and Jinrong Shi, Ying Wang, Haifei Bian, Nianmin Shen, Xinguo Li, and Kai Duan provided technical or material support.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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