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# Rapid safety assessment of a seasonal intradermal trivalent influenza vaccine

Marc Demeulemeester[a], Nathalie Lavis[b], Yohan Balthazar[c], Patrice Lechien[d], and Stéphane Heijmans[e]

[a]Private practitioner, Gozée, Belgium; [b]Sanofi Pasteur, Lyon, France; [c]Private practitioner, Natoye, Belgium; [d]Private practitioner, Braine-Le-Comte, Belgium; [e]Private practitioner, Linkebeek, Belgium

## ABSTRACT

Seasonal influenza vaccine formulations must be updated annually to correspond to the influenza viruses in circulation. This was an uncontrolled, open-label, multi-center phase IV study conducted in Belgium to comply with interim European Medicines Agency (EMA) guidelines for rapidly evaluating the safety of newly formulated seasonal influenza vaccines. Adult volunteers received one dose of the 2014-2015 Northern Hemisphere formulation of licensed intradermal trivalent influenza vaccine at either the standard dose (9$\mu$g hemagglutinin/strain for 18−59 year-olds) or the high dose (15$\mu$g hemagglutinin/strain for $\geq$ 60 year-olds). Vaccinees recorded their solicited reactions and unsolicited adverse events for 7 d after vaccination. Solicited reaction frequencies were compared to historical reference values obtained from previous clinical trials to determine if the new formulations were excessively reactogenic or allergenic. A total of 210 participants (105 per age group) were included and vaccinated in October 2014. In both groups, pain, erythema, and pruritus were the most common solicited injection site reactions, and headache and myalgia were the most common solicited systemic reactions. Although the frequencies of shivering in 18−59 year-olds and malaise in $\geq$ 60 year-olds were higher than historical reference values, they were not considered indicative of excessive reactogenicity because almost all of these reactions were mild. The study design was endorsed by the EMA and permitted the reactogenicity of both vaccine formulations to be assessed within one month by collecting adverse events for 7 d. Both formulations exhibited acceptable safety profiles although this should be confirmed through forthcoming enhanced post-marketing safety surveillance systems.

## Introduction

Seasonal influenza vaccines are the single most effective means of preventing influenza infection and disease, and vaccination is recommended for all persons > 6 months of age in the US.[1] In many European countries, seasonal influenza vaccination is recommended for all persons with underlying medical conditions or aged $\geq$ 65 y. Trivalent inactivated influenza vaccines (IIV3) contain 2 influenza A strains (A/H1N1 and A/H3N2) and one strain from one of the 2 influenza B lineages. However, because the global epidemiology and circulation of influenza viruses are in constant flux, the strains to be included in each seasonal formulation of vaccine must be evaluated and updated each year according to the circulation patterns predicted for the next influenza season in each hemisphere. Following review of influenza epidemiology, epidemics, and strains in circulation during the previous influenza season, the World Health Organization (WHO) issues recommendations each February for the strains to be included in the Northern Hemisphere vaccines for the subsequent influenza season.[2] The European Medicines Agency (EMA) reviews these recommendations and may modify them according to their suitability for Europe.[3]

Until recently, manufacturers of influenza vaccines for Europe were required to verify the safety and immunogenicity of updated seasonal formulations in small, pre-approval clinical trials conducted prior to each influenza season.[4] Vaccines were to be tested in subjects aged 18−60 y and over 60 y with 50 subjects in each group. Vaccine immunogenicity for each influenza strain was assessed 3 weeks post-vaccination and compared to prevaccination hemagglutination inhibition antibody titers to confirm that the new formulation met minimum criteria. Vaccine safety and reactogenicity were assessed from the adverse local and general reactions recorded for 3 d following vaccination. However, such small trials were not considered sufficiently informative to assess the efficacy and safety of the annual strain change prior to approval. Further, the long history of the safe and effective use of influenza vaccines, coupled with consistent manufacturing processes, indicates that neither the safety nor immunogenicity of these vaccines is likely to be significantly altered by updated seasonal formulations containing antigenically distinct influenza strains.[4]

With these concerns in mind, coupled with the need to vaccinate large numbers of individuals prior to each influenza season, the EMA withdrew its Note for Guidance on the Harmonisation of Requirements for Influenza Vaccines in January 2014, and with it, the requirement to conduct these small clinical trials prior to applying for marketing authorization.[4]

Instead, manufacturers were directed to institute a repeatable and enhanced post-marketing safety surveillance system to rapidly monitor influenza vaccine safety immediately following the release of new formulations.[5] One of the recommended options for enhanced safety surveillance is active surveillance of vaccine recipients in regions and member states with high vaccine uptake and suitable data collection capabilities. It is recommended that at least 100 vaccine recipients in each age group (6 months-5 years, 6−12 years, 13−18 years, 18−65 years, > 65 years) be monitored for 7 d after vaccination and that the surveillance be completed within a period of one month. The system should monitor the frequency and severity of the solicited local and systemic reactions usually assessed in influenza vaccine clinical trials so that any clinically significant changes in reactogenicity relative to that previously documented for the vaccine can be detected. Such changes may indicate the potential of a greater risk of more serious reactions as vaccine exposure increases. However, such a surveillance system had not been established before the 2014–2015 influenza season.

Intanza® (Sanofi Pasteur, Lyon, France) is a licensed seasonal trivalent inactivated influenza vaccine delivered by the intradermal (ID) route and provided in 2 dosages: a standard formulation of $9\mu g$ hemagglutinin (HA)/strain for adults (18−59 years) and a high-dose formulation of $15\mu g$ HA/strain for older adults (> 60 years).[6] To comply with interim EMA guidelines for enhanced safety surveillance of seasonal influenza vaccine formulations, we designed and conducted a clinical study to assess the reactogenicity of both dosages of the 2014–2015 Northern Hemisphere vaccine in age-appropriate subjects. Herein, we report the safety results of the study and interpret them relative to the Intanza safety profiles expected from historical data. We also report on the feasibility, strengths, and limitations of the study design. The safety results obtained from the study provide additional baseline data against which future formulations may be compared.

## Results

### Participants

A total of 210 participants, 105 per age group, were enrolled between October 2 and October 10, 2014 and the study was completed on October 24, 2014. All participants received the study vaccine dose according to age and completed the study according to protocol. Mean age was 44.3 ± 12.4 y in the 18−59 y age group and 73.3 ± 7.9 y in the ≥ 60 y age group (Table 1). Most of the participants in the 18−59 y age group were women (76%) but the sex ratio was equivalent in the ≥ 60 y age group.

### Solicited reactions

In participants aged 18−59 years, 78% reported at least one injection site reaction and 60% reported at least one systemic reaction (Table 2). Pain, erythema, and pruritus were the most common injection site reactions; headache and myalgia were the most common systemic reactions. Four participants reported 6 grade 3 systemic reactions (headache and nausea,

**Table 1.** Subject demographics.

| | Subject groups | |
| --- | --- | --- |
| | 18−59 y (N = 105) | ≥ 60 y (N = 105) |
| Male, n (%) | 29 (27.6) | 52 (49.5) |
| Female, n (%) | 76 (72.4) | 53 (50.5) |
| Mean age, years ± SD | 44.3 ± 12.4 | 73.3 ± 7.9 |
| Reportable concomitant medication (category 1[1]), n (%) | 25 (23.8) | 21 (20.0) |
| Other reportable medication | 0 (0) | 0 (0) |

N, total number of subjects in group; n, number of subjects with characteristic; SD, standard deviation.
[1]Antipyretics, analgesics, non-steroidal anti-inflammatory drugs, corticosteroids, and other immune modulators.

shivering, headache and malaise, and myalgia). One participant reported a grade 3 injection site reaction (pruritus). All reactions resolved spontaneously within a few days, except one case of grade 3 headache, which resolved in 2 d with medication.

Despite the higher antigen concentration, fewer older adults reported at least one injection site (54%) or systemic (32%) reaction. Erythema, pruritus, pain were the most common injection site reactions; headache and myalgia were the most common systemic reactions, although they were reported by less than 20% of the participants. The only grade 3 reactions were systemic (fever, malaise, and shivering), which resolved within 3 d except for one case of grade 3 shivering, which resolved more than 7 d after vaccination but for which the date of resolution is unknown.

### Clinical comparison of reactogenicity with historical frequencies

To determine if the 2014–2015 formulation was more reactogenic than previous formulations, the frequencies of the solicited reactions specified in the EMA interim guidance were compared to the historical frequencies of these reactions in 18–59-year-subjects (N = 2384) and ≥ 60-year-old subjects (N = 2974), which were pooled from the studies conducted to obtain marketing authorization for these vaccines (Table 3). In the 18−59 y age group, the frequencies of malaise and shivering were higher than historical values, but only shivering was in a higher frequency category: very common vs. common. However, shivering appeared to be less severe than in previous studies since less than 10% of the shivering reactions were grade 2 or 3, whereas 23% of the shivering cases in the historical database were grade 2 or 3. In participants aged ≥ 60 years, the frequencies of malaise and shivering were also slightly higher than historical values, although only malaise was in a higher frequency category: very common vs. common. For malaise, most cases in this study (83%) and in the historical database (77%) were grade 1. The frequencies of unsolicited generalized rash and pruritus that were used to assess vaccine allergenicity were both lower than historical values, indicating that the vaccine formulations were not allergenic.

Unsolicited adverse events (AEs) were infrequent in each group. Among 18−59 year-olds, 14 reported mild to moderate unsolicited AEs. No event was reported by more than 3 participants, and none were grade 3. One unsolicited injection site AE (injection site warmth) and 2 unsolicited systemic AEs (grade 1

**Table 2.** Solicited reactions within 7 d after vaccine injection.

| Subjects experiencing at least one: | Type | 18−59 years, 9 µg (N = 105) | | ≥ 60 years, 15 µg (N = 105) | |
|---|---|---|---|---|---|
| | | n | % [95% CI] | n | % [95% CI] |
| **Solicited reaction** | Any | 86 | 81.9 [73.2; 88.7] | 66 | 62.9 [52.9; 72.1] |
| | Grade 3 | 5 | 4.8 [1.6; 10.8] | 3 | 2.9 [0.6; 8.1] |
| **Injection site reaction** | Any | 82 | 78.1 [69.0; 85.6] | 57 | 54.3 [44.3; 64.0] |
| | Grade 3 | 1 | 1.0 [0.0; 5.2] | 0 | 0.0 [0.0; 3.5] |
| | Pain | 54 | 51.4 [41.5; 61.3] | 21 | 20.0 [12.8; 28.9] |
| | Erythema | 49 | 46.7 [36.9; 56.7] | 39 | 37.1 [27.9; 47.1] |
| | Pruritus | 49 | 46.7 [36.9; 56.7] | 24 | 22.9 [15.2; 32.1] |
| | Swelling | 18 | 17.1 [10.5; 25.7] | 10 | 9.5 [4.7; 16.8] |
| | Induration | 16 | 15.2 [9.0; 23.6] | 10 | 9.5 [4.7; 16.8] |
| | Ecchymosis | 3 | 2.9 [0.6; 8.1] | 1 | 1.0 [0.0; 5.2] |
| **Systemic reaction** | Any | 63 | 60.0 [50.0; 69.4] | 34 | 32.4 [23.6; 42.2] |
| | Grade 3 | 4 | 3.8 [1.0; 9.5] | 3 | 2.9 [0.6; 8.1] |
| | Fever | 0 | 0.0 [0.0; 3.5] | 1 | 1.0 [0.0; 5.2] |
| | Headache | 32 | 30.5 [21.9; 40.2] | 16 | 15.2 [9.0; 23.6] |
| | Malaise | 22 | 21.0 [13.6; 30.0] | 12 | 11.4 [6.0; 19.1] |
| | Myalgia | 34 | 32.4 [23.6; 42.2] | 15 | 14.3 [8.2; 22.5] |
| | Shivering | 21 | 20.0 [12.8; 28.9] | 6 | 5.7 [2.1; 12.0] |
| | Rash | 10 | 9.5 [4.7; 16.8] | 9 | 8.6 [4.0; 15.6] |
| | Vomiting | 4 | 3.8 [1.0; 9.5] | 0 | 0.0 [0.0; 3.5] |
| | Nausea | 17 | 16.2 [9.7; 24.7] | 7 | 6.7 [2.7; 13.3] |
| | Arthralgia | 23 | 21.9 [14.4; 31.0] | 10 | 9.5 [4.7; 16.8] |
| | Decreased appetite | 20 | 19.0 [12.0; 27.9] | 8 | 7.6 [3.3; 14.5] |

CI, confidence interval; N, total number of subjects in group; n, number of subjects in group experiencing the specified reaction.

nasopharyngitis and lymphadenopathy) were considered possibly related to the vaccination; all resolved within 4 d. In the ≥ 60 y age group, 7 participants reported mild to moderate unsolicited AEs, none of which were reported by more than one subject or were considered possibly related to the vaccination. No immediate unsolicited AEs or serious adverse events (SAEs) were reported for either group. Based on the MedDRA System Organ Class (SOC) of these events and the low number of unsolicited AEs in each class, no particular safety signal was identified.

Solicited reactions were also analyzed post-hoc according to whether subjects reported ongoing use of concomitant medications (anti-pyretics analgesics, non-steroidal anti-inflammatory drugs, or other immune modulator drugs) that could have affected reactions to the vaccine (Tables S1 and S2). Overall solicited reaction frequencies were similar in 18−59-year-old participants either taking (80%) or not taking (82.5%) concomitant medication and were also similar in ≥ 60-year-old participants taking (71.4%) or not taking (60.7%) such medication. Frequencies of grade 3 injection site and systemic reactions in both age groups were higher in participants reporting concomitant medication use.

## Discussion and conclusions

This study design, which was endorsed by the EMA, satisfied interim EMA requirements for rapidly confirming the safety of

**Table 3.** Clinical comparison of selected reaction frequencies with historical frequencies.

| Evaluation criteria | n | Observed | | Historical[1] | | Clinical comparison Increase in frequency category? |
|---|---|---|---|---|---|---|
| | | % [95% CI] | Frequency category[2] | % [95% CI] | Frequency category[2] | |
| **18−59 y (N=105)** | | | | | | |
| Fever (≥ 38°C) | 0 | 0.0 [0.00; 3.45] | Very rare | 3.8 [3.07; 4.65] | Common | No |
| Injection site induration (grade 3) | 0 | 0.0 [0.00; 3.45] | Very rare | 4.4 [3.61; 5.30] | Common | No |
| Injection site ecchymosis | 3 | 2.9 [0.59; 8.12] | Common | 8.3 [7.22; 9.48] | Common | No |
| Malaise | 22 | 21.0 [13.62; 29.99] | Very common | 17.3 [15.80; 18.88] | Very common | No |
| Shivering | 21 | 20.0 [12.83; 28.93] | **Very common** | 8.7 [7.60; 9.90] | **Common** | **Yes** |
| Unsolicited Rash[3] | 0 | 0.0 [0.00; 3.45] | Very rare | 0.1 [0.02; 0.33] | Uncommon | No |
| Unsolicited Pruritus[3] | 0 | 0.0 [0.00; 3.45] | Very rare | 0.1 [0.02; 0.33] | Uncommon | No |
| **≥ 60 y (N = 105)** | | | | | | |
| Fever (≥ 38°C) | 1 | 1.0 [0.02; 5.19) | Uncommon | 2.4 [1.88; 3.02] | Common | No |
| Injection site induration (grade 3) | 0 | 0.0 [0.00; 3.45] | Very rare | 2.2 [1.70; 2.79] | Common | No |
| Injection site ecchymosis | 1 | 1.0 [0.02; 5.19] | Uncommon | 4.3 [3.60; 5.09] | Common | No |
| Malaise | 12 | 11.4 [6.05; 19.11] | **Very common** | 9.0 [8.00; 10.09] | **Common** | **Yes** |
| Shivering | 6 | 5.7 [2.13; 12.02] | Common | 4.1 [3.42; 4.88] | Common | No |
| Unsolicited Rash[3] | 0 | 0.0 [0.00; 3.45] | Very rare | 0.03 [0.00; 0.18] | Rare | No |
| Unsolicited Pruritus[3] | 0 | 0.0 [0.00; 3.45] | Very rare | 0.03 [0.00; 0.18] | Rare | No |

CI, confidence interval; N, total number of subjects in group; n, number of subjects in group experiencing the specified reaction.
[1]Source: Intanza Common Technical Document, Section 2.7.4: Summary of Clinical Safety; N = 2384 subjects 18−59 years, N = 2974 subjects ≥ 60 y
[2]Frequency categories are defined as follows: Very common: ≥ 10%; Common: ≥ 1% and < 10%; Uncommon: ≥ 0.1% and < 1%; Rare: ≥ 0.01% and < 0.1%; Very rare: < 0.01%.
[3]The frequencies of unsolicited generalized rash and pruritus were used as indicators of vaccine allergenicity.

seasonal influenza vaccines and allowed study results to be interpreted according to the guidance. The sample size was sufficiently large to allow higher-than-expected frequencies of solicited reactions to be detected in adults and older adults. However, the frequency categories of only 2 reactions in this study were higher than the respective historical values and none clearly indicated a clinically meaningful increase in reactogenicity or allergenicity for either vaccine formulation.

In the 18−59 y age group (standard dose formulation), the very common frequency category of shivering indicated a possible safety signal. However, despite the higher frequency of shivering, the proportion of mild cases (grade 1) in the study was greater than the historical value and most cases resolved within one day. These characteristics suggest that this finding did not indicate a clinically relevant change in vaccine reactogenicity. In participants aged ≥ 60 years, the very common frequency category of malaise was higher than in the historical database, yet most cases were mild and resolved in one day. In addition, the upper limit of the 95% CI for the historical value was also within the very common category. Thus, the frequency of malaise in this study does not appear to indicate a clinically relevant difference in reactogenicity. Despite the higher frequency categories of shivering in participants aged 18−59 y and malaise in those aged ≥ 60 years, all other solicited reaction frequency categories were lower than or the same as those reported historically. Finally, there were few unsolicited AEs in either group, and no immediate AEs or SAEs. Taken together, these findings suggest that the standard and high-dose 2014–2015 Intanza formulations demonstrated satisfactory safety profiles and are safe for general use.

Because ongoing concomitant medication use during the 7 d after vaccination might mask vaccine reactogenicity, we conducted a post-hoc analysis in which the results were stratified according to medication use. As expected for the small sample size, such stratification diminished the power of the analysis, and only ∼20 participants per group reported concomitant medication use. Although the reliability of these results is limited and it is not possible to draw a definitive conclusion, we found no evidence that vaccine reactogenicity was lower in these participants than in participants without concomitant medication use. Even if the clinical relevance of these findings is difficult to interpret, the frequencies of solicited reactions and grade 3 reactions tended to be higher in participants who reported concomitant medication use, suggesting that these medications did not affect reactogenicity.

In a previous clinical trial with a similar sample size (60 participants per group), similar frequencies of solicited reactions were reported for the 2010–2011 standard and high-dose formulations of Intanza in Korean adults and older adults.[7] Pain, erythema, and pruritus were the most frequent injection site reactions; myalgia and malaise were the most frequent systemic reactions; and both formulations demonstrated satisfactory safety profiles.

Seasonal vaccination against influenza involves mass immunization in large population cohorts in a relatively short and fixed time period, yet the seasonal formulations must be produced within a few months to be ready for the next influenza season. Large scale trials to investigate the immunogenicity and safety of every new seasonal formulation are neither feasible

nor warranted. Thus, to ensure that a formulation is not widely distributed before its safety has been assessed; some safety information must be available rapidly from a small number of subjects after vaccine production. From this perspective, our study design had several strengths. First, it was feasible as it was completed within one month. The subject population was reliable, with no dropouts, and only a few minor protocol deviations, and all participants completed the study according to protocol. Second, the straightforward study protocol and the use of diary cards allowed a complete data set to be collected for 2 batches of each vaccine. To ensure the accuracy of the reactions and adverse events being reported, each participant was interviewed by a physician to confirm the information on the diary card. Thus, adverse event information was not left solely to the subject's interpretation but was verified by medically qualified study personnel. We also took advantage of the large database of reactions that had been collected from previous clinical trials used to demonstrate the safety and immunogenicity of the vaccines. This allowed reaction frequencies of the updated formulations to be evaluated against values that are likely to be very near the true reaction frequencies.

The main limitation of the study is that the sample size is quite small, which increases the chances of observing a false positive or negative result, and it was not powered to capture rare adverse reactions. It is possible that an increase in the frequency of rare events could be missed. However, no SAEs were reported, and rare events should be captured in routine post-marketing pharmacovigilance and enhanced surveillance. Indeed, the study was not concerned with nor designed to capture rare events or minor elevations in reactogenicity, but rather to identify aberrant formulations containing unforeseen or foreign reactogens that, by definition, would be detectable in a small number of vaccinated subjects against the reaction frequencies derived from the large existing clinical database. Another limitation was that solicited reaction frequencies in higher-than-expected categories required clinical interpretation by the sponsor's medical and pharmacovigilance team to determine if they indicated a legitimate safety concern. The age groups investigated in the study were slightly different from those recommended in the EMA interim guideline (18−65 y and > 65 years) because the standard and high-dose formulations of the vaccine are licensed for use in persons aged 18−59 y and ≥ 60 years, respectively. It is possible that illnesses and allergies present in the study population contributed to some of the systemic AEs recorded in the study. Because the extent of these background reaction rates are not known, this may be considered a limitation that could affect interpretation of the safety results in small studies such as this one. However, even with such a hypothetical contribution, the rates of the reactions and AEs in the study were not indicative of excessive reactogenicity and support the conclusion that the vaccines demonstrated acceptable safety profiles in this limited study. Finally, the study population was slightly unbalanced with respect to gender, but this should also not affect the conclusions of the study. Men and women differ somewhat in how they respond to vaccines and women tend to report more adverse reactions to vaccines, including influenza vaccine,[8,9] but even with a disproportionately high number of women in the study, reaction rates were still within acceptable limits.

This study met the EMA's interim objectives for the rapid safety surveillance of seasonal influenza vaccine formulations. The study was successful and efficient, and demonstrated that both Intanza formulations had acceptable safety profiles within the context of this study, thus supporting wider use of these vaccines in the populations for which they are indicated. The safety of these formulations should nevertheless be confirmed with data from enhanced post-marketing surveillance systems that will replace clinical post-marketing studies to verify vaccine safety.

## Methods

### Study design

This was an open-label, multicenter, uncontrolled, phase IV study conducted at 6 centers in Belgium (EudraCT #: 2014-000629-19). The objectives were to detect clinically significant increases in allergic events, or in the frequency and/or severity of expected vaccine reactions, and to describe the serious adverse events within 7 d after intradermal influenza vaccination in adults aged 18 y and older. Participants were vaccinated and followed for clinical safety assessment for 7 d. Applicable independent ethics committees and institutional review boards approved the study protocol and the study was conducted according to the Declaration of Helsinki and the International Conference on Harmonization guidelines for Good Clinical Practice as well local and national laws. All participants provided signed informed consent before taking part in the study.

### Participants

Persons aged $\geq$ 18 y and without any influenza vaccine contraindications, such as hypersensitivity to the active substances or any other component of the vaccine, could be included. In participants with febrile illness or acute infection, immunization was postponed until illness resolved. There were no other exclusion criteria so that participants would represent individuals in the general population who typically receive routine influenza vaccinations. Participants were allocated into 2 age groups according to those indicated for the vaccine dosage (18−59 y or $\geq$ 60 years) through an interactive voice/web response system.

### Study procedures

Participants received one dose of the 2014–2015 Northern Hemisphere formulation of licensed intradermal IIV3 (Intanza®; Sanofi Pasteur, Lyon, France), containing a 0.1 mL suspension of A/California/7/2009 (H1N1)pdm09-derived strain (NYMC X-179A), A/Texas/50/2012 (H3N2)-derived strain (NYMC X-223A), and B/Massachusetts/2/2012. Vaccine was delivered by the ID route using a pre-filled micro-injection system equipped with a micro-needle (1.5 mm). Participants received the standard-dose ($9\mu$g HA/strain) or high-dose ($15\mu$g HA/strain) formulation recommended for their age group. Two commercial batches of each formulation were used in the study.

Following vaccination on day 0, vaccinees were kept under observation for immediate unsolicited systemic AEs occurring within 20 minutes. Participants were provided with a safety diary card, a digital thermometer, and a flexible ruler and were instructed how to record daily body temperature; the nature, dimensions, and intensity of any solicited injection site reactions; the nature and intensity of any solicited systemic reactions; and any unsolicited AEs or SAEs occurring within 7 d after vaccination, as well as any action taken (e.g., medication). Each participant returned to the investigational center 8−11 d after vaccination and the investigator or designated study personnel reviewed the information entered in the diary card by interviewing the participant and requesting information concerning any medical event, serious or not, that may have occurred since vaccination.

Solicited injection site reactions were pain, erythema, swelling, induration, ecchymosis, and pruritus. Solicited systemic reactions were fever, headache, malaise, myalgia, arthralgia, shivering, rash, vomiting, nausea, and decreased appetite. Vaccine allergenicity was assessed from the frequencies of generalized rash and pruritus that were reported as unsolicited AEs. For this study and for those used to generate the historical solicited reaction frequencies, the nature and severity of the reactions were defined according to the Brighton Collaboration case definitions (https://brightoncollaboration.org/public.html) and recommendations provided by the US Food and Drug Administration Committee for Biologics Evaluation and Research in the Guidance for Industry: Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September 2007). Adverse events and reactions, and their relatedness to vaccination, were defined according to the International Committee for Harmonization E2A Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. Severity was graded from 1 (mild) to 3 (severe) as described in Table S3.

### Statistical analysis

All statistical analyses were descriptive and performed using SAS® version 9.2 (SAS Institute, Cary, IN, USA). A sample size of 100 participants per age group was recommended by the EMA interim guidance, so was set at 105 participants /group to account for an anticipated 5% drop-out rate. Data were assessed by age group for all subjects who received vaccine. Exact binomial distributions of proportions were used to calculate 95% confidence intervals (CIs) by Clopper-Pearson's method. For the primary objective, the number and percentage of participants in each group were calculated for the unsolicited immediate AEs occurring within 20 min after vaccination and for the solicited AEs and non-serious unsolicited AEs occurring within 7 d of vaccination according to maximum intensity. To determine if the reactogenicity of the vaccine was higher than expected, the observed frequencies and 95% CIs of fever $\geq$ 38°C, injection site induration (grade 3 only), injection site ecchymosis, malaise, shivering, rash, and pruritus, were calculated for each age group and compared to the expected rates and 95% CIs available from pooled historical data obtained from the phase 1-3 studies conducted to obtain marketing authorization for the vaccines and from other

sources (Intanza®, Summary of Product Characteristics; Internal Company Core Data Sheet; Intanza Common Technical Document, Section 2.7.4: Summary of Clinical Safety; N = 2384 subjects 18−59 years, N = 2974 subjects ≥ 60 years). Frequency categories were very common (≥ 10%), common (≥ 1% and < 10%), uncommon (≥ 0.1% and < 1%), rare (≥ 0.01% and < 0.1%), and very rare (< 0.01%). If the frequency of a reaction was higher than the historical reference value, defined as a change to a higher frequency category, the reported events were evaluated to assess whether the higher frequency corresponded to a clinically significant change in reactogenicity or allergenicity. To assess whether such a change was clinically significant, frequencies were compared to historical values, and the distribution of severity scales and the type and duration of the event's resolution (spontaneous or with medication) were compared and reviewed to determine if these reaction variables were consistent with an increase in reactogenicity. To assess vaccine allergenicity, we compared the frequencies of unsolicited generalized rash and pruritus collected in the study to the historical frequencies of unsolicited generalized rash and pruritus. For the secondary objective, the number and percentage of participants in each group experiencing at least one SAE during the study were calculated. Missing and incomplete data were not replaced, and no search for outliers was performed.

## Abbreviations

| | |
|---|---|
| AE | adverse event |
| CI | confidence interval |
| EMA | European Medicines Agency |
| HA | hemagglutinin |
| ID | intradermal |
| IIV3 | trivalent inactivated influenza vaccine |
| SAE | serious adverse event |
| WHO | World Health Organization |

## Disclosure of potential conflicts of interest

SH received clinical investigator fees from Sanofi Pasteur for conduct of the study. NL is an employee of Sanofi Pasteur. MD, PL, and YB declare no potential conflicts of interest.

## Author contributions

MD, YB, PL, and SH enrolled participants and collected study data. NL designed the study and was responsible for its preparation, execution, and for the analysis of the results. All authors had full access to the study data, were involved in interpretation of the results and critical review of manuscript drafts for important intellectual content, and approved the final version to be published.

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