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Licensing the first reduced, 6 μg dose whole virion, aluminum adjuvanted seasonal influenza vaccine – A randomized-controlled multicenter trial ☆

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ABSTRACT

Introduction: Shortages of vaccine supplies repeatedly occur, limiting our abilities to prevent influenza. Therefore, increasing production volume remains a priority. The presently licensed seasonal influenza vaccines contain 15 μg of viral hemagglutinin per strain in adult, and up to 60 μg in elderly patients. Decreasing the amount of viral parts while maintaining efficacy is one way of increasing production capacity.

Methods: This was multicenter, stratified (18–60 years and >60 years of age), prospective, randomized, double-blind, active-controlled, parallel-arm, non-inferiority clinical trial, conducted in the European Union, involving 1206 patients. We used hemagglutination inhibition assay to assess the immunogenicity of a newly developed, whole virion, seasonal trivalent influenza vaccine, containing 6 μg hemagglutinin per strain (FluArt, Hungary) and to assess whether it is non-inferior to the presently licensed vaccine containing 15 μg hemagglutinin per strain. Safety and tolerability of both vaccines were assessed based on EMEA guidelines.

Results: The reduced dose vaccine containing 6 μg of hemagglutinin per strain was safe and non-inferior to the currently licensed 15 μg vaccine, not only in adult, but also in elderly patients, according to the immunogenicity criteria by the FDA and EMEA (seroconversion, seroprotection and post/pre vaccination GMT ratios), and it fulfilled all applicable licensing requirements for both age groups.

Conclusions: Based on the results, the reduced dose vaccine was licensed in the EU member state Hungary and safely administered in over 1.5 million cases so far. The amount of viral hemagglutinin needed can be reduced by using a whole virion vaccine with aluminum phosphate adjuvants.

Registration: This study was registered by the European Clinical Trials Database, EudraCT, number: 2011-003314-16.

1. Introduction

Influenza remains a substantial public health concern, resulting in tens of thousands of deaths, hundreds of thousands of hospitalizations and millions of work days lost during each season in the US alone [1,2]. Immunization by vaccination remains one of the most effective ways to mitigate the enormous burden caused by influenza. However, shortages of vaccine supply continue to occur, limiting our abilities to prevent influenza and its complications [3]. Therefore, finding ways to increase production volume remains a priority.

The presently licensed trivalent or quadrivalent inactivated influenza vaccines contain 15 μg of viral hemagglutinin (HA) per strain in adult, and up to 60 μg in elderly patients [2,4]. Decreasing the amount of viral parts, while maintaining efficacy is one way of increasing production capacity.

We recently demonstrated that when using a whole virion vaccine with aluminum phosphate adjuvant, no additional benefit is seen with doses in excess of 6 μg HA per virus strain and that vaccines with only 6 μg HA per virus strain can fulfill all applicable licensing criteria [5,6]. Thus, an increase of production volume is possible this way. In the present trial, we aimed to show

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non-inferiority of a reduced dose seasonal inactivated vaccine using whole virion and aluminium phosphate adjuvant, containing 6 μg HA per strain compared to a well established and licensed seasonal influenza vaccine with 15 μg of HA per virus strains (FluvalAB, Omninvest, Hungary) [7].

2. Methods

2.1. Trial design and study settings

This was a multicenter, stratified (18–60 years and 60+ years of age), prospective, randomized, double-blind, active-controlled, parallel-arm, non-inferiority study conducted in the European Union (Budapest, Hungary) at 7 primary care clinics, between October 24, 2011 (enrollment of the first participant) and March 21, 2012 (last visit of the last subject participating). The study was registered with the European Union Drug Regulatory Authorities, European Medicines Agency (http://eudract.emea.europa.eu/) under clinical trial registration number: 2011-003314-16. No changes were made to methods after trial commencement.

2.2. Participants, eligibility criteria

Participants were recruited by their primary care physicians. Subjects eligible for enrolment into this study were: male and female adult volunteers aged 18 years or older, mentally competent, able to understand and comply with all study requirements, able to give written informed consent prior to initiation of study procedures, in good health (as determined by the investigator on the basis of medical history and examination) and stable medical condition. Female subjects of childbearing potential were required to have a negative urine pregnancy test prior to vaccination and to use contraception throughout the trial.

Exclusion criteria included: pregnancy, breast feeding, female subjects who are able to bear children but not willing to use contraception for the duration of the study, hypersensivity to eggs, chicken protein, thiomersal, formaldehyde, gentamycin, ciprofloxacin, neomycin or any other components of the vaccine, history of anaphylactic shock or neurological symptoms following administration of any vaccine; history of Guillain-Barre syndrome; malignant tumor, autoimmune disease, advanced arteriosclerotic disease, complicated diabetes mellitus, acute or progressive hepatic disease, acute or progressive renal disease, congestive heart failure; immunosuppressive therapy within 36 months prior to vaccination; concomitant corticosteroid therapy (including high-dose inhaled corticosteroids); receiving immunostimulants; receipt of immunoglobulin, blood products and/or plasma derivates within 3 months prior to vaccination; HIV, HBV or HCV infection; fever of >37C within 3 days prior to vaccination; vaccine therapy within 4 weeks; Influenza vaccination within 6 months prior to vaccination; experimental drug therapy within 4 weeks prior to vaccination; concomitant participation in another clinical study; psychiatric disease that may affect the decision-making ability; alcohol or drug abuse.

The study protocol, the patient information sheet, the informed consent form and other appropriate study-related documents were reviewed by the Ethics Committee for Clinical Pharmacology of the Medical Research Council (ETT KFEB), and approved by the National Institute of Pharmacy of Hungary (OGYI) in accordance with domestic legal requirements. ETT KFEB approval number: 18677-0/2011-EKL, OGYI permission number: OGYI/29399-8/2011. The study was conducted in accordance with the rules of Good Clinical Practice. The conditions were in compliance with the Declaration of Helsinki.

2.3. Vaccines

The investigational vaccine was a whole virus, aluminum phosphate adjuvanted, trivalent vaccine against seasonal influenza containing 6 μg of HA per virus strain. The influenza viruses included in the investigational vaccine were grown in embryonated hen eggs, inactivated by formaldehyde, purified and concentrated, and absorbed on aluminum phosphate gel, as described in detail elsewhere [6]. The virus strains were chosen according to the EU Recommendations for the Seasonal Influenza Vaccine Composition for the Season of 2011/12 as follows: A/California/7/2009(H1N1) derived NYMC X-179A reassortant strain, A/Perth/16/2009(H3N2)-like A/Victoria/210/2009(H3N2) derived NYMC X-187 reassortant strain, and B/Brisbane/60/2008 derived NYMC BX-35 reassortant strain [8]. The reference vaccine was the approved, licensed influenza vaccine for the season of 2011/12, containing 15 μg of HA per virus strain, also a whole virion vaccine and including aluminum phosphate as an adjuvant, as described in detail elsewhere [7]. The HA content of both vaccines was determined before the addition of the aluminum phosphate adjuvant by a single radial immunodiffusion test, using reagents supplied by NIBSC (United Kingdom), as reported previously [9] Purity for both vaccines was evaluated by endotoxin content, which was <0.05 IU/dose, and the amount of ovalbumin, which was <5 ng/dose. Both values are much lower than the concentrations considered acceptable by the European Pharmacopoeia, which are 100 IU and 1000 ng/human dose, respectively [10]. Aluminum phosphate was added as adjuvant, in the amount of 0.31 mg Al/ampoule, meeting the requirements of the European Pharmacopoeia [10]. Both vaccines were manufactured according to GMP requirements by Omninvest LTD (Budapest, Hungary), LOT numbers FL-K-02/11 (for the investigational) and FL-K-04/11 for the reference vaccine.

2.4. Interventions

Subjects were randomly assigned in a 1:1 ratio to one of the following vaccine groups: Group 1 received one 0.5 mL intramuscular injection of the investigational vaccine, while Group 2 was given one 0.5 mL injection of the licensed vaccine. The subjects were divided into two subgroups according to age (18–60 years and over 60 years). All subjects were observed for 30 min after vaccination for any immediate reactions and were instructed to complete a diary card to record local (ecchymosis, erythema, induration, swelling and pain at injection site) and systemic reactions (chills, malaise, myalgia, arthralgia, headache, sweating, fatigue and potential indicators of oculo-respiratory syndrome), and axillary temperature starting on the day of vaccination and for each of the 7 days following the immunization. All adverse events were collected from the day of vaccination until day 120. All adverse events necessitating a physician's visit or consultation and/or leading to premature study discontinuation and all serious adverse events were collected throughout the entire trial and data were reconciled at study termination visit.

Baseline evaluations on day 0 included obtaining demographic data, medical history, and performing a complete physical examination. Blood samples were drawn for baseline hemagglutination inhibition test (HI) for all three vaccine virus strains. Serum antibody titers against the vaccine virus strain were measured by HI, using chicken red blood cells and following standard procedures, as recommended by the CDC and WHO, described in detail elsewhere [11,12]. On day 21, a medical history and the list of medications used during the days since the last visit were obtained, physical examination was performed, and blood samples were drawn for HI.

2.5. Objectives

The primary immunogenicity objective was to assess the immunogenicity of the reduced dose trivalent influenza vaccine containing 6 μg of hemagglutinin per strain as measured by HI test 21 days after vaccination in compliance with the requirements of the European Union recommendations as determined by the The European Agency for the Evaluation of Medicinal Products, as well as the applicable US FDA guidelines [13,14].

The secondary immunogenicity objective was to assess non-inferiority of the investigational trivalent influenza vaccine containing 6 μg of hemagglutinin per strain against the licensed seasonal vaccine (FluvalAB, Omninvest, Hungary) containing 15 μg of hemagglutinin per strain in terms of post-immunization geometric mean titers (GMTs) as measured by HI test 21 days after vaccination.

The safety objective was to evaluate the tolerability and safety of the administration of the two vaccines containing either 6 or 15 μg of HA of seasonal A/H1N1, A/H3N2 and B influenza antigens as determined by the licensing requirements of the European Union [13]. No changes in the outcomes were made after the trial commenced. No interim analyses were performed. The study was funded by Omninvest LTD, Budapest, Hungary. The funding source had no role in the conduct of the study or the preparation of the manuscript.

2.6. Sample size

The sample-size calculation was based on the goal to accomplish the secondary immunogenicity objective. We used a two-sided confidence interval of 95%, an overall statistical power of 80% and non-inferiority margin 1/1.5 [15]. For the age group of 18–60 years, we estimated a maximum GMT ratio of 1.041 and a geometric standard deviation 3.06 and we concluded that 225 evaluable subjects per Vaccine Group were necessary [5]. Considering an approximately 10% drop-out, 252 subjects were needed per Vaccine Group in the age group of 18–60 years. For the age group of 60+ years, we estimated a maximum GMT ratio of 1.087 and a geometric standard deviation 3.29 and we concluded that 328 evaluable subjects per Vaccine Group were necessary [5]. Considering an approximately 10% drop-out, 364 subjects were needed per Vaccine Group in the age group of 60+ years. Altogether, $252 \times 2 + 364 \times 2 = 1232$ subjects were planned to be enrolled in the study. The sample-size calculation was performed using the sample-size formula by Chow and Wang [15].

2.7. Randomization and blinding

Assignment of subjects to vaccination groups was performed by means of stratified blocked randomization using http://randomization.com/. Two randomization lists, one for each age group, was prepared by the Sponsor. On the basis of the randomization lists the ampoules were marked with individual serial numbers during manufacturing, and the investigators were provided with such ampoules and lists of sequence how the ampoules should be used. The ampoules had otherwise identical appearance. Adherence to randomization lists was verified by the Sponsor's Study Monitor by checking the enrolment records against the randomization lists. The only copy of the randomization list was stored in a sealed envelope in a locked filing cabinet which was accessible only for the Study Director. All study participants, including the study subjects, the investigators, the study monitor and the test laboratory were kept blinded during the entire duration of the study. The blinding was revealed only after the test laboratory performing the immunogenicity examinations had completed the HI tests and had issued the final certificate of analysis. Premature unblinding was not necessary during the study.

2.8. Statistical analysis

All data analyses were carried out according to a pre-established analysis plan. Descriptive statistics (mean, standard deviation, median, minimum and maximum) for age at enrolment were calculated overall and by vaccine group and age group. Distributions of subjects by sex and previous influenza vaccination were summarized overall and by vaccine group and age group.

Distributions of the logarithms of 0, and 21 day titers in each subgroup were visualized with normal quantile-quantile plot to ensure normality. Distributions of the logarithms of pre-vaccination titers were summarized and visualized in each subgroup to ensure that they don't vary between Vaccine Groups in the same Age Group.

The Primary immunogenicity objective was to evaluate the immunogenicity of the reduced dose influenza vaccine containing 6 μg of HA according to the applicable EU (CHMP) and US (FDA) requirements [13,14]. During the assessment, the following serological indices were considered: (i) seroconversion or ≥4-fold increase in HI antibody titer in subjects; (ii) post/pre vaccination Geometric Mean Titers (GMT) ratios; and (iii) proportion of subjects achieving an HI titer of >40 after immunization [13,14].

The measures of immunogenicity were calculated as GMTs (with 95% confidence intervals), which were determined for study day 0, study day 21–28. Post/pre vaccination Geometric Mean Titer Ratio: GMTRs (with 95% confidence interval) of the study day 21–28 titers to the study day 0 titers were calculated. Percentages of Subjects with Seroconversion or Significant Increase in HI Titer: The number and proportion of subjects achieving seroconversion or at least a four-fold increase in HI titers from pre-immunization to 21–28 days after the immunization were tabulated.

The secondary immunogenicity objective was to determine non-inferiority of the reduced dose, 6 μg vaccine, compared to the licensed standard dose 15 μg vaccine. For that, we followed the Guidance for Industry of U.S. Department of Health and Human Services, Food and Drug Administration on Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines [14], which states that non-inferiority of a new seasonal influenza vaccine as compared to a licensed seasonal inactivated influenza vaccine can be concluded, if the upper bound of the two-sided 95% CI on the ratio of the GMTs ($GMT_{licensed\ vaccine}/GMT_{new\ vaccine}$) does not exceed 1.5, which is equivalent to the requirement that lower bound of the two-sided 95% CI on the ratio of the GMTs ($GMT_{new\ vaccine}/GMT_{licensed\ vaccine}$) does not exceed 1/1.5, i.e. 0.67. Thus, the null hypothesis for the secondary immunogenicity objective stated that the lower limit of the two-sided 95% confidence interval for the ratio of geometric mean titers measured 21 days after vaccination in Group 1 and Group 2 was below 1/1.5 [14]. Instead of using the raw titer values, the log-transformed titers were calculated and the GMT ratio was tested as the difference between the means of the log-transformed titers. The corresponding hypotheses was that $\log(GMT_{new\ vaccine}) - \log(GMT_{licensed\ vaccine}) > -0.405$.

The 95 percent two-sided confidence interval for $\log(GMT_{new\ vaccine}) - \log(GMT_{licensed\ vaccine})$ was calculated using the built-in R method which implements Welch-confidence intervals. If the lower limit of the interval is greater than −0.405, non-inferiority is concluded. This calculation was performed for the data from the three different strains and for the two age-groups.

In terms of serocinversion rates, non-inferiority immunogenicity of HI antibody responses to the new vaccine as compared to a licensed seasonal inactivated influenza vaccine was concluded, if

the upper bound of the two-sided 95% CI on the difference between the seroconversion rates ($Seroconversion_{licensed\ vaccine} - Seroconversion_{new\ vaccine}$) does not exceed 10 percentage points, based on FDA guidelines [14].

For the safety objective, the chi square test was used to determine if there was a significant difference between the two vaccine groups regarding the occurrence rates of vaccine related adverse events.

3. Results

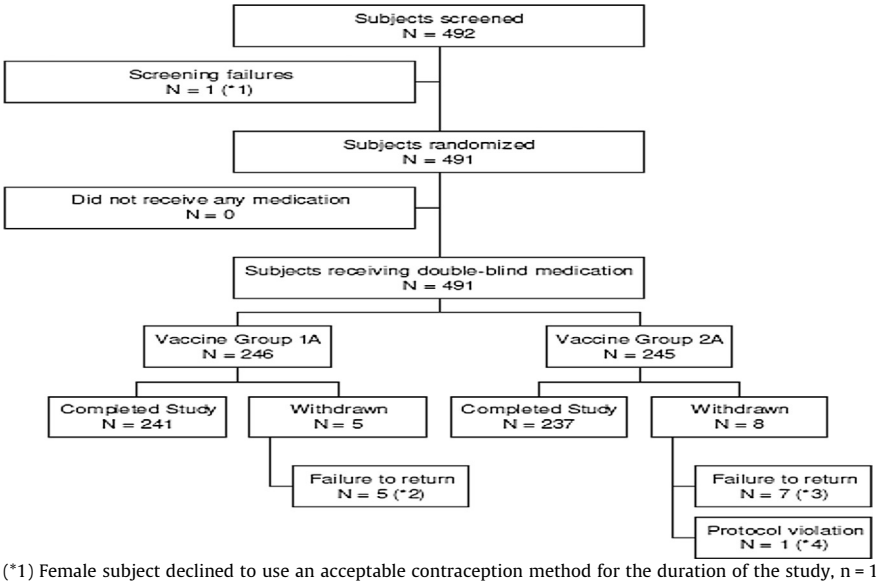
The study initiation date (enrolment of first participant) was Oct. 24, 2011, and the study completion date (last follow-up visit) was March 21, 2012.

1206 subjects were actually enrolled in the study, randomly assigned to two vaccine groups, and vaccinated by double-blind medication (ITT population). The data of these 1206 subjects were included in the safety evaluation. Out of the 1206 subjects vaccinated 1179 subjects attended both Visit 2 at Day 21 and Visit 3 at Day 120 (PP population). The data of these 1179 subjects were included in the immunogenicity evaluation (Table 1). The baseline characteristics, including age, gender distribution and pre-vaccination HA titers were not significantly different between the two vaccination groups (Table 2). Individual HA titers are available upon request.

Both vaccines fulfilled all applicable immunogenicity criteria by EMA and FDA guidelines (Table 3).

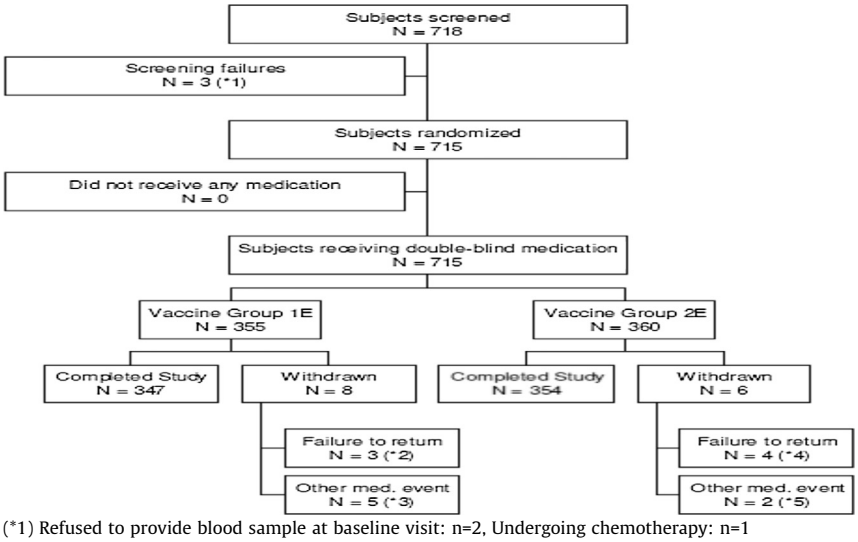
Table 1
Study flow chart.

Adult subjects, age 18–60 years



(*1) Female subject declined to use an acceptable contraception method for the duration of the study, n = 1.
(*2) Declined to attend follow up visit: n = 3; unable to attend follow up visit due to travel: n = 2
(*3) Declined to attend follow up visit: 5, unable to attend follow up visit due to having moved: n = 2
(*4) attended Visit, but the blood sample was forwarded to the test laboratory beyond time frame, therefore it was not tested for HI, n = 1.

Elderly subjects, age >60 years



(*1) Refused to provide blood sample at baseline visit: n=2, Undergoing chemotherapy: n=1
(*2) Did not attend follow up visit: n=3
(*3) Deceased due to unrelated reason before attending Visit: n=2; did not attend Visit, n=3
(*4) Declined to attend Visit: n=3; did not attend Visit due to travel: n=1.
(*5) Deceased due to unrelated reason before attending Visit n=1; Hospitalized due to unrelated event: n=1

Table 2
Demographic data of the subjects.

Population	N =	Mean age ± SD (years)	Median (years)	Min age in years	Max age in years	Received seasonal influenza vaccine during the previous season: n (%)
Vaccine group 6 μg HA	601	59.8 ± 16.3	62	18	96	32 (5.32%)
Vaccine group 15 μg HA	605	60.0 ± 16.4	63	18	92	31 (5.12%)

Table 3
Licensing criteria and immunogenicity results for both age groups and vaccine doses. Both vaccines fulfilled all three CPMP criteria for licensing (seroconversion, post/pre vaccination GMT Ratio and seroprotection) for both age groups.

Age group	Strain	Critetion for licensing (lower bound of 95% CI)	Results for the 6 μg vaccine (95% CI)	Results for the 15 μg vaccine (95% CI)
18–60 years	H1N1	Seroconversion ≥ 40%	66.0% (59.6, 71.9)	69.2% (62.9, 75.0)
		GMT ratio ≥ 2.5	5.39 (4.69, 6.18)	6.12 (5.26, 7.12)
		Seroprotection ≥ 70%	92.9 (88.9, 95.8)	94.5 (90.8, 97.0)
	H3N2	Seroconversion ≥ 40%	65.1% (58.8, 71.1)	68.8% (62.5, 74.6)
		GMT ratio ≥ 2.5	5.39 (4.70, 6.19)	5.53 (4.81, 6.37)
		Seroprotection ≥ 70%	98.8 (96.4, 99.7)	98.7 (96.3, 99.7)
	B	Seroconversion ≥ 40%	67.2% (60.9, 73.1)	74.7% (68.6, 80.1)
		GMT ratio ≥ 2.5	5.23 (4.57, 6.00)	5.45 (4.80, 6.20)
		Seroprotection ≥ 70%	90.0 (85.5, 93.5)	89.5 (84.8, 93.1)
60+ years	H1N1	Seroconversion ≥ 30%	68.3% (63.1, 73.2)	67.8% (62.7, 72.6)
		GMT ratio ≥ 2.0	5.52 (4.95, 6.15)	5.24 (4.68, 5.86)
		Seroprotection ≥ 60%	82.7 (78.3, 86.5)	83.3 (79.0, 87.1)
	H3N2	Seroconversion ≥ 30%	60.2% (54.9, 65.4)	58.2% (52.9, 63.4)
		GMT ratio ≥ 2.0	4.54 (4.08, 5.05)	4.36 (3.91, 4.85)
		Seroprotection ≥ 60%	95.4 (92.6, 97.3)	96.9 (94.5, 98.4)
	B	Seroconversion ≥ 30%	56.2% (50.8, 61.5)	64.4% (59.2, 69.4)
		GMT ratio ≥ 2.0	4.00 (3.61, 4.43)	4.24 (3.84, 4.68)
		Seroprotection ≥ 60%	75.5 (70.6, 79.9)	82.2 (77.8, 86.0)

The non-inferiority analysis showed that on the basis of the Guidance for Industry of U.S. Department of Health and Human Services, Food and Drug Administration on Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines [14], the investigational influenza vaccine containing 6 μg of HA/strain was non-inferior to the licensed 15 μg vaccine in terms of HI titers and seroconversion rates 21 days after vaccination, meaning that the upper bound of the two-sided 95% CI on the difference between the seroconversion rates ($Seroconversion_{licensed\ vaccine} - Seroconversion_{new\ vaccine}$) did not exceed 10 percentage points (Table 4) [14]. Also, the $\log(GMT_{new\ vaccine}) - \log(GMT_{licensed\ vaccine})$ values and their 95% confidence intervals were calculated in each age group for each outcome. Since the lower limits of the confidence intervals were greater than −0.405 in every case, it can be concluded that the new vaccine is non-inferior to the licensed vaccine based on this criterion also [14]. This was met for both age groups in terms of all virus strains.

Administration of both the investigational and the reference vaccines was well tolerated by the study subjects. No serious and no severe possibly or probably related adverse event was observed. Both the investigational and the reference vaccines proved to be safe, no vaccine-related clinically significant changes in the physical condition or vital signs of the volunteers were observed. There was no significant difference between the safety profiles of the investigational and the reference vaccines (Table 5).

4. Discussion

4.1. Interpretation

The currently available seasonal intramuscular inactivated influenza vaccines contain 15–60 μg of viral hemagglutinin per virus strain [2,16]. Using a microinjection system for intradermal delivery, it has been shown that the amount of the active ingredient can be reduced to 9 μg HA per strain, however, only in adult patients, aged 18–64 years [17]. Obtaining optimal acceptability of intradermal vaccines may represent an additional asset to help increase the coverage of influenza vaccination in young adults. In the present trial, we evaluated a whole virion, aluminum phos-

Table 4
Non-inferiority evaluation: The differences between the upper bound (UB) of the two-sided 95% CI of the seroconversion (SC) rates of the licensed trivalent influenza vaccine with 15 mcg of HA per strain, versus the investigational trivalent influenza vaccine with 6 mcg HA per strain in both age groups (A.G.). All values were<10% and thus, non-inferiority is concluded [14].

Age group	Virus strain	Day 21 after vaccination			Day 120 after vaccination		
		UB of 95% CI of SC (15 mcg vaccine)	UB of 95% CI of SC (6 mcg vaccine)	Difference of UBs	UB of 95% CI of SC (15 mcg vaccine)	UB of 95% CI of SC (6 mcg vaccine)	Difference of UBs
18–60 years	H1N1	75.0	71.9	3.1	57.6	53.8	3.8
	H3N2	74.6	71.1	3.5	60.5	57.5	3.0
	B	80.1	73.1	7.0	62.1	56.3	5.8
>60 years	H1N1	72.6	73.2	−0.6	55.9	56.4	−0.5
	H3N2	63.4	65.4	−2.0	45.4	43.7	1.7
	B	69.4	61.5	7.9	55.3	46.9	8.4

Age group	Virus strain	Day 21 after vaccination	Day 120 after vaccination
18–60 years	H1N1	−0.05083 (−0.2393; 0.1376)	−0.03279 (−0.2349; 0.1693)
	H3N2	−0.04055 (−0.229; 0.148)	−0.02137 (−0.2279; 0.1851)
	B	−0.02912 (−0.2153; 0.1571)	0.0002184 (−0.1883; 0.1887)
>60 years	H1N1	−0.02131 (−0.1916; 0.149)	−0.09745 (−0.2692; 0.07429)
	H3N2	−0.09805 (−0.2611; 0.06502)	−0.1082 (−0.278; 0.06168)
	B	−0.1395 (−0.3018; 0.02279)	−0.1714 (−0.3275; −0.01532)

$\log(GMT_{\text{new vaccine}}) - \log(GMT_{\text{licensed vaccine}})$ values and their 95% confidence intervals in each age group for each outcome. Since the lower limits of the confidence intervals were greater than −0.405 in every case, it can be concluded that the new vaccine is non-inferior to the licensed vaccine based on this criterion also [14].

Table 5
Possibly or probably related Adverse Events by Vaccine Group.

System	Reaction	Mild	Moderate	Severe	Total
Vaccine group 6 mcg					
Gastrointestinal	Nausea	1	–	–	1
General disorders and administration site conditions	Chills	5	3	–	8
	Malaise	18	2	–	20
	Pyrexia	10	–	–	10
	Vaccination site erythema	58	2	–	60
	Vaccination site hematoma	3	–	–	3
	Vaccination site induration	49	5	–	54
	Vaccination site pain	132	3	–	135
	Vaccination site swelling	41	6	–	47
Musculoskeletal	Myalgia	17	2	–	19
Nervous system	Dizziness	1	–	–	1
	Headache	14	1	–	15
	Hypoesthesia	1	–	–	1
Skin	Urticaria	–	–	–	–
Vaccine group 15 mcg					
Gastrointestinal	Nausea	–	–	–	–
General disorders and administration site conditions	Chills	9	–	–	9
	Malaise	18	2	–	20
	Pyrexia	11	–	–	11
	Vaccination site erythema	54	7	–	61
	Vaccination site hematoma	3	–	–	3
	Vaccination site induration	51	7	–	58
	Vaccination site pain	121	4	–	125
	Vaccination site swelling	36	10	–	46
Musculoskeletal	Myalgia	16	4	–	20
Nervous system	Dizziness	–	–	–	–
	Headache	14	4	–	18
	Hypoesthesia	–	–	–	–
Skin	Urticaria	–	1	–	1

phate adjuvanted inactivated influenza vaccine with only 6 μg HA per strain in both adult and elderly patients.

Both the investigational vaccine and the reference vaccine were well tolerated by the participants. No statistically significant difference was found in frequency of adverse reactions between the two vaccine groups. This finding is as expected, since the only difference between the two vaccines was the amount of viral hemagglutinin, which was lower in the investigational vaccine.

Both vaccines fulfilled all immunogenicity criteria for the evaluation of seasonal influenza vaccines as determined European Union (EMA) and US (FDA) guidelines in terms of all three virus strains and both age groups 21 days after vaccination [13,14]. This is consistent with our previous clinical studies where we found that whole virion, aluminum phosphate adjuvanted trivalent seasonal, and monovalent pandemic influenza vaccines containing 6 μg hemagglutinin per virus strain fulfill all applicable EMA and FDA criteria for licensure [5,6,18].

Furthermore, the non-inferiority analysis demonstrated that the trivalent seasonal influenza vaccine containing 6 μg hemagglutinin was not inferior to the approved and licensed 15 μg vaccine. Based on the above results, the 6 μg vaccine was approved and licensed by the National Institute of Pharmacy in the EU member state Hun-

gary under the name of 3Fluart (OGYI-T-8998/03). To our knowledge, this is the lowest dose influenza vaccine currently licensed [2,16]. This can have significant economic and public health implications. Post-licensure safety data are available, and have been partly published [19]. They agree with the safety and tolerability results seen in this study. Furthermore, the frequency and severity of adverse events were not different from what had been seen with the 15 μg vaccine reference vaccine, which has been licensed since 1997 and used in more than 20 million cases without known safety issues [7,18]. This is not unexpected, since the only difference between the reference and the novel vaccine was the amount of the antigen. Decreasing the antigen amount needed for adequate immunogenicity by using a whole virion vaccine with aluminum phosphate as an adjuvant has been proven during the 2009 pandemic [18]. Aluminum adjuvant containing vaccines have a demonstrated safety profile of over six decades of use and have only uncommonly been associated with severe local reactions. Scientific research has shown the amount of aluminum exposure in people from vaccination is low and is not readily absorbed by the body [20]. Whole virion vaccines were the first to be used in widespread seasonal influenza vaccination campaigns. However, these early whole virion formulations caused local and systemic adverse effects upon administration. This was likely due to the presence of impurities, such as egg proteins, in the early whole virion vaccines. Therefore, whole virion vaccines were mostly abandoned when split and subunit vaccines were developed, which were thought to be less reactogenic. However, the use of modern vaccine production technologies results in better defined and pure whole virion vaccines than previously, which give rise to very low levels of side effects [21]. Both vaccines studied in the present trial contained much lower amounts of ovalbumin and endotoxin than currently required by the European Pharmacopea [10].

4.2. Limitations and generalizability

Our study involved only white Caucasian subjects. Although there is at least some published evidence that there might be ethnic differences in the immune response to vaccination [22], there are currently no licensing requirements to indicate such [13,14]. Also, we only studied adult and elderly patients, and thus, we do not know if our results are generalizable to pediatric populations. Some data suggest that pediatric patients might react differently to influenza vaccination [23,24]. Therefore, studies investigating the immunogenicity and safety of the reduced dose vaccine in pediatric and adolescent patients are underway. Furthermore, while routine annual influenza vaccination is recommended for all persons aged ≥6 months who do not have contraindications, we only studied a relatively healthy population [25]. Further studies that include pregnant women, persons with impaired immune systems, and other chronic conditions are also needed.

Conflict of interest and author contributions

The authors declare that they have no conflict of interest. ZV prepared the manuscript, reviewed the literature and created the tables, LK provided supervision of the study process, analysis, reviewed the literature and was involved in writing the paper, PV provided statistical analysis, GB, NR and PT recruited and followed patients, and edited the manuscript.

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