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A 2013/2014 northern hemisphere season surface antigen inactivated trivalent influenza vaccine - Assessing the immunogenicity and safety in an open label, uncontrolled study

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The present study evaluated the safety and immunogenicity of the 2013/2014 trivalent surface antigen inactivated subunit seasonal influenza virus vaccine Fluvirin® in healthy adults (18 - ≤ 60 years) and elderly (>60 years). The vaccine contained 15 μg haemagglutinin protein from each of influenza A/California/7/2009 (H1N1)pdm09–like strain, A/Victoria/361/2011 (H3N2)–like strain and B/Massachusetts/2/2012-like strain (B/Yamagata) as recommended by the WHO in the 2013/2014 Northern Hemisphere season. Antibody response to each influenza antigen after vaccination was measured prior to vaccination and 21 d after by single radial hemolysis (SRH) assay or hemagglutination inhibition (HI) assay in accordance with Guidance CPMP/BWP/214/96. 125 subjects (61 adults and 64 elderly) were enrolled in the study. Pre-vaccination protective antibody levels (SRH area ≥ 25 mm^2) against A(H1N1), A(H3N2) and the B strain were detected in 17%, 20% and 57% of adults and in 36%, 20% and 55% of elderly, respectively, Post-vaccination, SRH area ≥ 25 mm^2 was detectable in 95%, 82% and 92% in adult and in 80%, 84% and 92% of the elderly subjects for A(H1N1), A (H3N2) and the B strain, respectively. Geometric mean ratio (GMR) was higher in adult subjects (2.62–7.62) than in elderly subjects (2.33–3.42). All three CHMP licensure criteria were met for all strains contained in the vaccine for both age groups. The most frequently reported solicited local and systemic reactions were pain at the injection side, headache and fatigue. In conclusion, the vaccine demonstrated a good immunogenicity and an acceptable safety profile in both adults and elderly.

Introduction

Vaccines against influenza are considered the most important intervention to reduce the substantial health burden caused by seasonal influenza infection worldwide.[1-3] Due to antigen drift virus strains vary from one year to another.[4] This necessitates extensive surveillance of the circulating strains to match the most prevalent strains with the subsequent seasonal vaccine composition. In Europe, the 2012/2013 influenza season was of longer duration and mortality rates were overall higher than in the 2011/2012 season.[5] In the 2012/2013 season influenza A predominated with 63% of the isolated viruses, subdivided into 2 thirds A(H1N1)pdm09 and one third A(H3N2). Remaining 37% of isolated viruses were influenza B viruses.

For the northern hemisphere's 2013/14 seasonal trivalent influenza vaccine the World Health Organization (WHO) recommended a switch compared to the previous season from a B-Yamagata clade 3-lineage virus (B/Wisconsin/1/2010-like virus) to a B/Yamagata clade 2-lineage virus (B/Massachussets/2/2012-like virus). The switch was necessary as clade 2 viruses of the Yamagata lineage became dominant in many areas and were antigenically different from viruses in the clade 3.[6,7] The recommended A(H3N2) (A/Victoria/361/2011 (H3N2)-like virus) strain was already part of the 2012/2013 composition and the A (H1N1) strain (A/California/7/2009 (H1N1) pdm09-like virus) has been kept unchanged since the pandemic in 2009.[6,7] However, as the duration of vaccine induced protection may wane over time, reassessment of seasonal influenza vaccines has still been considered necessary to ensure vaccine effectiveness and safety/tolerability.[8,9]

This study aimed to evaluate the safety and immunogenicity of the trivalent seasonal 2013/2014 influenza virus vaccine

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Fluvirin® in healthy adult and elderly subjects in the northern hemisphere in compliance with current EU guidelines.

Results

A total of 125 subjects were enrolled in the study in July 2013, 61 in the adult group and 64 in the elderly group. Baseline and demographic data are shown in **Table 1**. All 125 subjects received the study vaccination, provided appropriate serum samples before and after vaccination and were available for final safety assessment on day 22. One subject received additional vaccinations from her primary care physician during the study period and was therefore excluded from immunogenicity analyses.

Immunogenicity

SRH areas at baseline (day 1 prior to vaccination) and day 22 after vaccination are summarized in **Table 2**. Pre-vaccination geometric mean areas (GMAs) for A(H1N1), A(H3N2) and the B strain were measured with 9.2, 12 and 22 in adults and 14, 13, and 22 in elderly subjects, respectively. SRH area $\geq$ 25 mm^2 against A(H1N1), A(H3N2) and the B strain were detected in 17%, 20% and 57% in adults and in 36%, 20% and 55% in elderly, respectively.

Post-vaccination GMAs for A(H1N1), A(H3N2) and the B strain were measured with 70, 46 and 59 in adults and 47, 41, and 52 in elderly subjects, respectively. Seroprotection (SRH area $\geq$ 25 mm^2) after vaccination was detectable for 95%, 82% and 92% in adult and for 80%, 84% and 92% of the elderly subjects for A(H1N1), A(H3N2) and the B strain, respectively. For subjects who had antibody titers of $\leq$ 4 mm^2 prior vaccination seroconversion was observed in 78%–100% in the adult subjects and in 58%–100% in the elderly subjects. In subjects with pre-existing SRH areas > 4 mm^2 65%–79% of the adult subjects and 58%–74% of the elderly subjects had at least a 50% increase in the SRH areas. The geometric mean ratio (GMR) was overall higher in adult subjects (2.62–7.62) than in elderly subjects (2.33–3.42).

In the SHR assay, all 3 CHMP licensure criteria were met for all strains contained in the vaccine in both age groups.

In both age groups, highest GMR on day 22 was measured against the A(H1N1) strain with 7.62 in adults and 3.42 in elderly, while the lowest GMR was observed against the B strain with 2.62 in the adult subjects and 2.33 in elderly subjects.

More than half (58%) of the subjects participating in this trial, had previously been vaccinated against seasonal influenza, 38% of the adult and 77% of the elderly subjects. This included 46 subjects (37%) - 13 adult (21%) and 33 (52%) elderly subjects - who had been vaccinated with the 2012/2013 seasonal influenza vaccine about 1 y before.

Safety and tolerability

In total, 64 subjects (51%) reported any solicited AEs from 6 hours through day 4 after vaccination (40 (60%) were adult and 24 (38%) were elderly subjects). Solicited local AEs were reported by 42% (52 subjects) and solicited systemic AEs were reported by 25% (31 subjects). Across the age groups, local AEs were reported more often than systemic AEs. The most frequent solicited local AE was injection site pain reported by 49 subjects (39%). None of the local injection site AEs were severe. Among systemic AEs, fatigue (16%) and headache (15%) were most common. Fatigue was the only systemic AE that was reported as severe (2%). For a detailed overview of local and systemic AEs see **Table 3**. Most solicited local and systemic AEs resolved within day 4 and all resolved before the subject's study termination. Local and systemic AEs that persisted beyond day 4 were treated as unsolicited adverse events. **Table 4** provides an overview of all AEs between day 1 and 22.

Unsolicited AEs were reported by 6 (5%) of the subjects (3 (5%) of the adult and 3 (5%) of the elderly subjects) of which 4 (3%) were at least possibly related to the study vaccine (decrease of appetite and local injection site reactions). Most unsolicited AEs were mild in intensity and only one was reported as severe and did not resolve until study termination (nasopharyngitis).

Table 1. Demographic and baseline characteristics

	Adults (18–60 years) n = 61	Elderly ≥61 years n = 64	Total n = 125
Age (years, ± SD)	39.2 (± 11.2)	68.2 (± 4.7)	54.1 (± 16.9)
Sex			
Male n (%)	32 (52%)	30 (47%)	62 (50%)
Female n (%)	29 (48%)	34 (53%)	63 (50%)
Weight (kg)	78.69 (± 16.31)	74.65 (± 13.57)	76.62 (± 15.04)
Height (cm)	175.5 (± 9.7)	171.3 (±7.3)	173.4 (± 8.8)
BMI (kg/m^2)	25.4 (± 4.2)	25.3 (± 3.8)	25.4 (± 4.0)
Previous seasonal influenza vaccination			
No n (%)	38 (62%)	15 (23%)	53 (42%)
Yes n (%)	23 (38%)	49 (77%)	72 (58%)
Ethnicity			
White n	60	63	123
Asian n	0	1	1
Other n	1	0	1

Values are means (± SD) if not indicated otherwise.

Table 2. Vaccine Immunogenicity assessment by SHR assay

		18 to ≤60 Years				≥61 Years		
Strains		**A(H1N1)**	**A(H3N2)**	**B**		**A(H1N1)**	**A(H3N2)**	**B**
Pre-vaccination (day 1)								
GMA		9.2	12	22		14	13	22
(95% CI)		(7.11–12)	(10–15)	(17–29)		(10–18)	(11–16)	(17–29)
SRH area ≥ 25 mm^2 n/N, %		10/60 (17%)	12/60 (20%)	34/60 (57%)		23/64 (36%)	13/64 (20%)	35/64 (55%)
(95% CI)		(8%–29%)	(11%–32%)	(43%–69%)		(24%–49%)	(11%–32%)	(42%–67%)
Post-vaccination (day 22)								
	CHMP				CHMP			
Seroconversion n/N, %		24/26 (92%)	7/9 (78%)	5/5 (100%)		14/24 (58%)	5/7 (71%)	5/5 (100%)
Significant increase in antibody titers n/N, %		27/34 (79%)	46/51 (90%)	36/55 (65%)		23/40 (58%)	42/57 (74%)	38/59 (64%)
Seroconversion or significant increase n/N, %	>40%	51/60 (85%)	53/60 (88%)	41/60 (68%)	>30%	37/64 (58%)	47/64 (73%)	43/64 (67%)
(95% CI)		(73%–93%)	(77%–95%)	(55%–80%)		(45%–70%)	(61%–84%)	(54%–78%)
GMA		70	46	59		47	41	52
(95% CI)		(62–80)	(39-54)	(51–68)		(39–58)	(34–48)	(46-59)
GMR	>2.5	7.62	3.66	2.62	>2.0	3.42	3.05	2.33
(95% CI)		(5.83–9.95)	(2.98–4.5)	(2.09–3.27)		(2.65–4.41)	(2.49–3.74)	(1.92–2.83)
SRH area≥ 25 mm^2 n/N, %	>70%	57/6095%	49/60(82%)	55/60(92%)	>60%	51/64(80%)	54/64(84%)	59/64(92%)
(95% CI)		(86%–99%)	(70%–90%)	(82%–97%)		(68%–89%)	(73%–92%)	(83%–97%)

Values are frequencies (%).

Overall, the majority of the possible or probable study vaccine related AEs were solicited local or systemic reactions continuing beyond day 4. There was no study withdrawal due to AEs. None of the subjects suffered from flu like symptoms and no serious adverse events (SAEs) were reported in this study.

Discussion

This study was conducted to evaluate the safety, tolerability and immunogenicity of the egg-derived trivalent inactivated surface antigen influenza vaccine (Fluvirin®) for the 2013/2014 northern hemisphere influenza season. Compared to the previous influenza vaccine, the WHO recommendation for the vaccine composition included an alternative B strain (B/Massachusetts/2/2012-like virus (B/Yamagata lineage)).[7] Until the end of 2013, approximately 75% of the globally circulating strains were A strains (about 52% A(H1N1)pdm09 and about 48% A(H3N2)) and 25% B strains. Even if the 2 Yamagata and Victoria B lineages were co-circulating, the vast majority (93%) of infections were attributed to the B/ Yamagata lineage.[10] This data demonstrates a good match between the 2013/14 trivalent vaccine composition and circulating strains.

Immunogenicity assessment for the vaccine demonstrated that all 3 CHMP licensure criteria were met for all strains in both adult and elderly subjects. For all strains, post-vaccination GMAs were higher in adults than in elderly, which is in line with results of earlier trials.[11] However, for the A(H3N2) and the B strain the 95% - confidence intervals of post-vaccination GMAs from adults and elderly considerably overlap. It is therefore not possible to exclude equal post-vaccination titers in adults and elderly for these 2 strains in the population.

The A(H1N1) strain showed the highest GMR of all strains contained in the vaccine after 21 d These findings are in line with immunogenicity studies for previous influenza vaccines.[12,13]

A high percentage of the subjects (58%) had previously been vaccinated against seasonal influenza. Especially in the elderly group more than half of the pre-vaccinated subjects had even been vaccinated with the 2012/2013 seasonal influenza vaccine about 1 y before, in line with vaccination guidelines in Germany.[11] This may be biased by subject selection as participants may favor the idea of preventive vaccinations. However, the proportion of individuals with pre-vaccination seroprotection (SRH area ≥ 25 mm^2) in this study was only 17–57% depending on strain and age group. Especially for both A strains these pre-vaccination seroprotection rates were considerably lower compared to previously published data.[12-14]

Interestingly, proportions of individuals with seroprotection for A(H3N2) and the B strain were comparable between the age groups despite the fact that more elderly subjects had been vaccinated previously. As data for the 2012/2013 seasonal influenza vaccine demonstrated good post-vaccination antibody levels for elderly, data from the current study could indicate a shorter antibody persistence in elderly compared to adult subjects.[12] Only for the A(H1N1) strain, twice as many elderly subjects compared to adult subjects reached pre-vaccination SRHs areas ≥ 25 $mm.^2$ Furthermore, it is noteworthy that highest pre-vaccination protection was present for the B strain in both age groups. Although the current strain (B/Massachusetts/2/2012) had not been part of the seasonal influenza vaccine the year before, the 2011/12 vaccine already included a strain from the Yamagata lineage (B/Wisconsin/1/2010) as strains from the Victoria lineage stopped being most prominent in 2011 after several years.

Overall, the rather low percentages of pre-vaccination seroprotection support the recommendation of annual influenza vaccination even if the antigen composition is left unchanged in comparison to the previous season, as was the case for both A viruses in the 2013/14 season.

The vaccine demonstrated a robust safety profile. Most AEs possibly or probably related to the vaccine were solicited local or

Table 3. Local injection site and systemic reaction (solicited AEs) between day 1 and day 4

		Adults (18–60 years) n = 125	Elderly ≥61 years n = 64	Elderly ≥61 years n = 64
Any		40 (66%)	24 (38%)	64 (51%)
Local injection site reaction (Day 1 to 4 post vaccination)				
Any	Any Severe	33 (54%) 0	19 (30%) 0	52 (42%) 0
Pain	Any Severe	31(51%) 0	18 (28%) 0	49 (39%) 0
Induration	Any > 50 mm	3 (5%) 0	1 (2%) 0	4 (3%) 0
Ecchymosis	Any > 50 mm	2 (3%) 0	1 (2%) 0	3 (2%) 0
Erythema	Any > 50 mm	0 0	1 (2%) 0	1 (1%) 0
Systemic reaction (Day 1 to 4 post vaccination)				
Any	Any Severe	21 (34%)	10 (16%)	31 (25%)
Fatigue	Any Severe	14 (23%) 1 (2%)	6 (9%) 1 (2%)	20 (16%) 2 (2%)
Headache	Any Severe	14 (23%) 0	5 (8%) 0	19 (15%) 0
Malaise	Any Severe	8 (13%) 0	1 (2%) 0	9 (7%) 0
Arthalgia	Any Severe	5 (8%) 0	1 (2%) 0	6 (5%) 0
Myalgia	Any Severe	2 (3%) 0	2 (3%) 0	4 (3%) 0
Chills/shivering	Any Severe	3 (5%) 0	0 0	3 (2%) 0
Fever (> 38°C)		0	0	0
Other				
Therapeutic use of analgesics/antipyretics		3 (5%)	0	3 (2%)

Values are number (%) of subjects

systemic reactions lasting longer than 3 d after vaccination. Most of the AEs have been mild in severity and all but one resolved until study termination. Adverse events were more often described by adult subjects than by elderly subjects. Local reactions were mostly pain at the injection site, while systemic reactions mainly consisted of fatigue and headache. The overall incidence and

Table 4. Overview of subjects with AEs from day 1 to day 22

	Adults (18–60 years) n = 61	Elderly ≥61 years n = 64	Total n = 125
Any AE	42 (69%)	26 (41%)	68 (54%)
Unsolicited AEs	3 (5%)	3 (5%)	6 (5%)
At least possible related AEs	1 (2%)	3 (5%)	4 (3%)
Serious AEs	0	0	0
AEs leading to withdrawal	0	0	0

characteristics of local and systemic AEs was comparable to the incidences in previous studies with other influenza vaccines.[12-14]

In conclusion, the egg-derived season 2013/2014 trivalent inactivated surface antigen influenza vaccine demonstrated a good immunogenicity and an acceptable safety profile in both adults and elderly. Subjects with increased risks should be vaccinated annually against seasonal influenza even if the vaccine composition is unchanged.

Patients and Methods

All subjects were enrolled at the Bernhard Nocht Center for Clinical Trials (www.bncct.de), University Medical Center Hamburg-Eppendorf, Germany, in July 2013. The study was designed as a single arm, open-label study to evaluate the immunogenicity and safety for the 2013/2014 northern hemisphere's seasonal influenza vaccine. The primary immunogenicity objective was to evaluate the antibody response to each influenza vaccine strain 3 weeks after a single intramuscular injection of the seasonal trivalent influenza vaccine Fluvirin® in adult and elderly subjects. The primary safety objective was to evaluate the safety of the vaccine in compliance with the requirements of the current EU recommendations for clinical trials related to yearly licensing of influenza vaccines (CPMP/BWP/214/96). The trial is registered at EUDRA-CT (2013-000601-23).

It was intended to enrol 126 subjects to obtain a minimum of 100 complete data sets as approximately 10% of subjects were anticipated to be non-valuable for immunogenicity. Subjects were recruited into 2 age groups: 18 and ≤60 years of age (adults, 50%), >60 years of age (elderly, 50%). Main exclusion criteria were allergy against chicken eggs, serious acute or chronic illness, significant immunodeficiency, laboratory confirmed seasonal or pandemic influenza disease or seasonal or pandemic influenza vaccination within 6 month prior to study enrolment, receipt of any other vaccine within 4 weeks prior to study enrolment, cognitive impairment or psychiatric disease that could have interfered with the subject's ability to participate in the study, and women, who were pregnant, breastfeeding or refusing to use an acceptable method of birth control for the whole duration of the study. For women of childbearing potential urine pregnancy test was performed prior to vaccine administration.

After verifying inclusion and exclusion criteria, subjects were vaccinated intramuscularly with a single dose of Fluvirin® (0.5 ml), a trivalent subunit influenza vaccine produced in embryonated chicken eggs. Fluvirin® includes 15 μg hemagglutinin (HA) protein from each of influenza A/California/7/2009 (H1N1) pdm09–like strain used (NIB-74) derived from A/Christchurch/16/2010, A/Victoria/361/2011 (H3N2)–like strain used (NYMC X-223) derived from A/Texas/50/2012 and B/Massachusetts/2/2012-like strain used B/Massachusetts/2/2012 wild type strains.

Blood samples (15 to 20 ml) were obtained on day 1 before vaccination and on day 22 (−1/+3). Serum was obtained by centrifugation of the blood samples at 2000g for 10 minutes and then stored below −20°C until shipment to Novartis Vaccines Clinical Serology Laboratory in Marburg, Germany. Serum antibody titers against the 3 antigens were measured by single radial hemolysis (SRH) assay as described elsewhere[12] as well as by hemagglutination inhibition (HI) assays using WHO antigens[15] for confirmatory purposes.

For the SRH assay, seroprotection was defined as SRH area ≥25 mm^2, seroconversion was defined as (i) day 1 SRH area with a negative result and day 22 SRH area ≥25 mm^2 or day 1 SRH area >4 mm^2 and an increase of at least 50% of the SRH area at day 22. For the HI assay, seroprotection was defined as titer ≥1:40, seroconversion or significant increase was defined as (i) day 1 HI titer <1:10 and day 22 HI titer ≥1:40 or (ii) day 1 titer ≥1: 10 and a ≥4-fold increase in HI titer on day 22.

Separated by age cohort, influenza-antibody HI antibody geometric mean titers (GMTs) or SRH geometric mean areas (GMAs), pre-vaccination (day 1), and post-vaccination (day 22 [−1/+3]), associated 2-sided 95% CIs and median, minimal, and maximal area/titer values were determined using descriptive analyses and presented together with N (number of subjects). Accordingly, the geometric mean of the day 1 to day 22 (−1/+3) individual geometric mean ratios (GMRs) with 2-sided 95% CIs, median, minimum, maximum, and N (total number of subjects) were calculated.

Overall vaccine immunogenicity was evaluated according to CHMP criteria specified in Guidance on Harmonization of Requirements for Influenza vaccines (CPMP/BWP/214/96). Briefly, for adults 18 to ≤60 years: (i) >70% of subjects achieving an SRH area ≥25 mm^2 or HI titer ≥1:40 or (ii) >40% of subjects achieving seroconversion or significant increase in SRH area or HI titer or (iii) GMR day 22 / day 1 >2.5; for elderly ≥61 years: (iv) >60% of subjects achieving an SRH area ≥25 mm^2 or HI titer ≥1:40, (v) >30% of subjects achieving seroconversion or significant increase in SRH area or HI titer or (vi) GMR day 21 / day 0 >2.0.

To assess the safety of the investigational product, all subjects were observed for at least 30 minutes after the vaccination by a physician in order to detect acute systemic or local reactions. Furthermore, diary cards were handed out in order to record all solicited and unsolicited local and systemic reactions. Solicited local and systemic reactions were recorded from day 1 to day 4 and included: ecchymosis (local haematoma), erythema, injection-site induration and pain at injection site as well as fever (oral temperature ≥38°C), chills/shivering, malaise, headache, myalgia, arthralgia, and fatigue. The administration of analgesics/antipyretics as response to pain or headache between day 1 and day 4 was also recorded and considered as solicited event. Furthermore, all subjects were asked to record any reaction persisting after day 4 and all unsolicited events, as well as any events emerging between day 5 and day 22. Adverse events (AEs) were defined as local or systemic reactions persisting after day 4 and all unsolicited events/reactions that (i) were medically attended, (ii) lead to study withdrawal or (iii) meet the criteria of a serious AE (SAE). All AEs, SAEs and concomitant medication were recorded for the whole study period including day 22. The relationship between AE and investigational product was determined by the investigator.

All reactions were classified as mild (transient with no limitation in the normal daily activity), moderate (some limitation in the normal daily activity) or severe (unable to perform normal daily activity). For local injection site reactions a diameter of 10 to ≤25 mm was defined as mild, a diameter of 26 to ≤50 mm as moderate and a diameter >50 mm as severe reaction.

In order to ensure correct recording of all solicited and unsolicited events, on day 1 all subjects were trained on filling out the diary cards and were called on day 3 (±1) and on day 20 (±2) to remind them of the correct use of the diary cards. Thus, safety was assessed from day 1 to day 22 (−1/+3).

The study protocol was approved by the ethics committee of the Medical Council in Hamburg, Germany.

Disclosure of Potential Conflicts of Interest

This vaccine trial has been conducted at the Bernhard Nocht Center for Clinical Trials with financial compensation by Novartis Vaccines and Diagnostics. The authors Seetha Meyer and Kai Witte are employees of the sponsor.

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