

A phase II, randomised clinical trial to demonstrate the non-inferiority of low-dose MF59®-adjuvanted pre-pandemic A/H5N1 influenza vaccine in adult and elderly subjects

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Key words

H5N1 • Influenza • Pandemic • MF59 • Adjuvant

Summary

Background. *Effective planning and preparedness against a possible future A/H5N1 influenza pandemic is a major global challenge. Because dose sparing strategies are required to meet the global demand for vaccine, efforts have focused on the development of adjuvanted vaccine formulations of relatively lower antigen content.*

Aim. *This study aimed to demonstrate the non-inferiority of a low-antigen-dose (3.75 mg) A/H5N1 pre-pandemic vaccine compared with a licensed, higher-dose (7.5 mg) formulation in adult and elderly subjects. Immunogenicity was assessed according to European and U.S. licensure criteria.*

Methods. *A total of 722 subjects were randomized in equal numbers to receive either the licensed or low-dose formulation. All subjects received two vaccine doses administered three weeks apart. Immunogenicity was assessed three weeks after the administration of each vaccine dose by hemagglutination inhibition*

(HI), single radial haemolysis (SRH) and microneutralization assays (MN). Local and systemic reactions were assessed over a seven day period post-vaccination. Adverse events were recorded throughout.

Results. *The low-dose vaccine was demonstrated to be non-inferior to the licensed formulation in terms of antibody titres against the vaccine strain. All three European licensure criteria were met by adult subjects in response to the low-dose vaccine; two criteria were met by the elderly age group. Cross-reactive antibodies were detected against the heterologous A/H5N1 antigen strains A/Indonesia/05/05 and A/turkeyTurkey/01/05. Both vaccines were generally well tolerated by both age groups.*

Conclusion. *These data demonstrate that a low antigen dose in combination with MF59® adjuvant is adequate for the routine pre-pandemic immunization of adult and elderly subjects.*

Introduction

The A/H5N1 influenza virus is highly pathogenic and remains a significant pandemic threat to the human population. Effective vaccination programmes against A/H5N1 influenza are essential [1-3]. Mortality rates as high as 60% have been observed in humans infected with the virus [4]. In the event of a pandemic, most of the world's population would have no existing immunity to the virus, and would therefore, urgently require a pandemic vaccine. Current estimates suggest that it would take four to six months to produce enough pandemic-strain-specific vaccine to meet global demand [2].

Adjuvants are substances with the ability to enhance innate immune responses and antigen presentation [5]. Adjuvants serve to heighten the immunogenicity of vaccines while decreasing the required antigen dose,

thereby helping to increase manufacturing capacity and ensure the widest possible population coverage from a limited vaccine supply. MF59® (Novartis Vaccines, Marburg, Germany) was the first oil-in-water emulsion licensed as an adjuvant for human use [6]. As well as heightening antigen-specific antibody production in response to vaccination, MF59 has been shown to promote cross-reactive antibody production [6-9], essential qualities for an effective vaccine against pandemic influenza [1-3, 10, 11]. Clinical trials and post-marketing safety surveillance have established a good safety profile for MF59 across age groups [6, 8, 12, 13]. MF59-adjuvanted influenza vaccines have proven to be effective and well tolerated in the elderly and other high-risk populations [14-16].

We report the results of a Phase II, randomised, double-blind, controlled clinical trial, conducted in adult and

elderly subjects to assess the immunogenicity and safety of two MF59-adjuvanted A/H5N1 pre-pandemic vaccine formulations, containing either 3.75 mg or 7.5 mg antigen. This study aimed to demonstrate the low-dose vaccine to be non-inferior to the licensed formulation in both age groups.

Materials and methods

STUDY DESIGN AND OBJECTIVES

This Phase II, randomized, controlled, double-blind clinical trial, was conducted between September and December 2009 across twelve study sites – six in Poland and six in Turkey. The protocol was approved by the Ethics Committee of each participating centre, and the study performed according to the principles of the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all participants before enrolment. The primary objective of the study was to demonstrate the non-inferiority of antibody responses resulting from two vaccine doses containing 3.75 mg antigen per dose, when compared with two 7.5 mg vaccine doses in a pooled study population of adult and elderly subjects. The secondary study objective was to evaluate the immunogenicity and reactogenicity profiles of low- and high-dose vaccine formulations for the adult and elderly study populations separately. Participants were randomly assigned in equal numbers to receive two doses of vaccine containing either 3.75 mg or 7.5 mg antigen. First and second vaccine doses were administered three weeks apart (Day 1 and Day 22). Blood samples (~10 mL per sample) were collected for immunogenicity analysis at baseline (Day 1), and three weeks after administration of first (Day 22) and second (Day 43) vaccine doses.

SUBJECTS

A total of 385 healthy adult (18-60 years) and 337 healthy elderly (≥ 61 years) participants were enrolled. The main exclusion criteria were: receipt of another investigational agent < 4 weeks prior to study enrolment; confirmed influenza disease < 6 months prior to study enrolment; fever (≥ 38°C) within 3 days prior to each study vaccination, or infection requiring systemic antibiotic or antiviral therapy < 6 days prior to study enrolment; female subjects either pregnant, breastfeeding, or refusing to use an acceptable method of birth control for the duration of the study; any serious disease; hypersensitivity to eggs, chicken protein, influenza viral protein, neomycin, polymyxin or any other vaccine component; a history of anaphylactic shock; an impaired or altered immune system; receipt of a non-study vaccine < 4 weeks before receiving the first dose of study vaccine; and use of antipyretic/analgesic medication within 24 hours of each vaccination.

VACCINES

The investigational, egg-derived, monovalent, MF59-adjuvanted, pre-pandemic vaccine (Aflunov®, Novartis Vaccines) contained haemagglutinin and neuraminidase surface antigens derived from the influenza strain

A/H5N1 Vietnam/1194/04. Each 0.5 mL vaccine dose contained either 3.75 mg or 7.5 mg antigen. Both low-dose (3.75-MF59) and high-dose (7.5-MF59) vaccine formulations were identical except for the difference in quantity of antigen per dose. One dose of MF59 adjuvant contained 9.75 mg squalene, 1.175 mg polysorbate 80, 1.175 mg sorbitan trioleate, 0.66 mg sodium citrate dehydrate, and 0.04 mg citric acid monohydrate. All vaccines were administered in the deltoid muscle of the non-dominant arm.

IMMUNOGENICITY ASSESSMENT

Antibody responses against the A/H5N1 vaccine antigen strain (Vietnam/1194/04) were measured by haemagglutination inhibition (HI), microneutralization (MN), and single radial haemolysis (SRH) assays according to standard protocol [17-19]. Cross-reactive antibody responses were measured against the heterologous A/H5N1 influenza strains Indonesia/05/05 and turkey/Turkey/01/05 by MN assay alone. HI and MN assays were performed at the Clinical Serology Laboratory of Novartis Vaccines in Marburg, Germany. SRH assays were performed at the University of Siena, Department of Pathophysiology, Experimental Medicine and Public Health. Seroconversion, as assessed by HI assay, was defined as a negative pre-vaccination antibody titre of < 10 to a positive post-vaccination titre of ≥ 40; as measured by MN assay, titre < 20 to ≥ 40; as measured by SRH assay, area ≤ 4 mm² to ≥ 25 mm². A significant increase in antibody titre, as assessed by HI and MN assays, was defined as ≥ 4-fold increase; by SRH assay, ≥ 50% increase in area. HI and MN titres below the detection limits of 1:10 and 1:20, respectively, were arbitrarily assigned to half that limit for the purpose of analysis. All SRH areas below the lower limit of detection (4 mm²) were set to 4 for analysis.

SAFETY ASSESSMENT

Subjects were monitored for 30 minutes after each vaccination for possible immediate adverse reactions. Solicited reports of local and systemic adverse reactions were collected for a seven-day period after the administration of each vaccine dose using diary cards. Solicited local reactions were ecchymosis, erythema, induration, swelling, and pain at the site of injection. Solicited systemic reactions were headache, arthralgia, chills, fatigue, malaise, myalgia, nausea, sweating, and fever (axillary temperature ≥ 38°C). All adverse events (AE) and serious adverse events (SAE) were recorded throughout the entire study period (Day 1 to Day 43). The investigator used a standard scale to grade AE, in which symptoms were defined as mild, moderate or severe if they resulted in no limitation of, some limitation of, or inability to perform normal daily activities, respectively.

STATISTICAL ANALYSES

A sample size of 326 subjects per group was considered sufficient to test the null hypothesis with 80% power, taking into account a 15% dropout rate. Statistical analyses of HI, SRH and MN data were performed on

logarithmically (base 10) transformed values. The non-inferiority of the low-dose vaccine was demonstrated by the ratio of low- to high-dose Day 43 geometric mean areas (GMAs) from either SRH data alone, or SRH and HI data together. The antibody response to the low-dose vaccine was considered to be non-inferior to that of the high-dose formulation if the lower limit of the two-sided 95% confidence interval (CI) was above 0.667. Immunogenicity was assessed according to criteria established by the European Committee for Medicinal Products for Human Use (CHMP). The following CHMP criteria applied: the number of subjects achieving seroconversion or significantly increased antibody titres should be > 40% and > 30% for adult and elderly subjects, respectively; geometric mean ratio (GMR) should be > 2.5 for adults and > 2.0 for the elderly; and for seroprotection, the proportion of subjects achieving an HI titre $\geq 1:40$ or SRH titre > 25mm² should be > 70% and > 60% for

adults and the elderly, respectively. Immunogenicity was also assessed according to criteria established by the US Centre for Biologics Evaluation and Research (CBER) criteria. The following CBER criteria applied: the lower limit of the two-sided 95% CI for the percentage of subjects achieving seroconversion for HI antibody should meet or exceed 40% in adult and 30% in elderly subjects; the lower limit of the two-sided 95% CI for the percentage of subjects achieving an HI antibody titre ≥ 40 should meet or exceed 70% in adult and 60% in elderly subjects (seroprotection). Statistical analyses were performed using SAS 9.1[®] software.

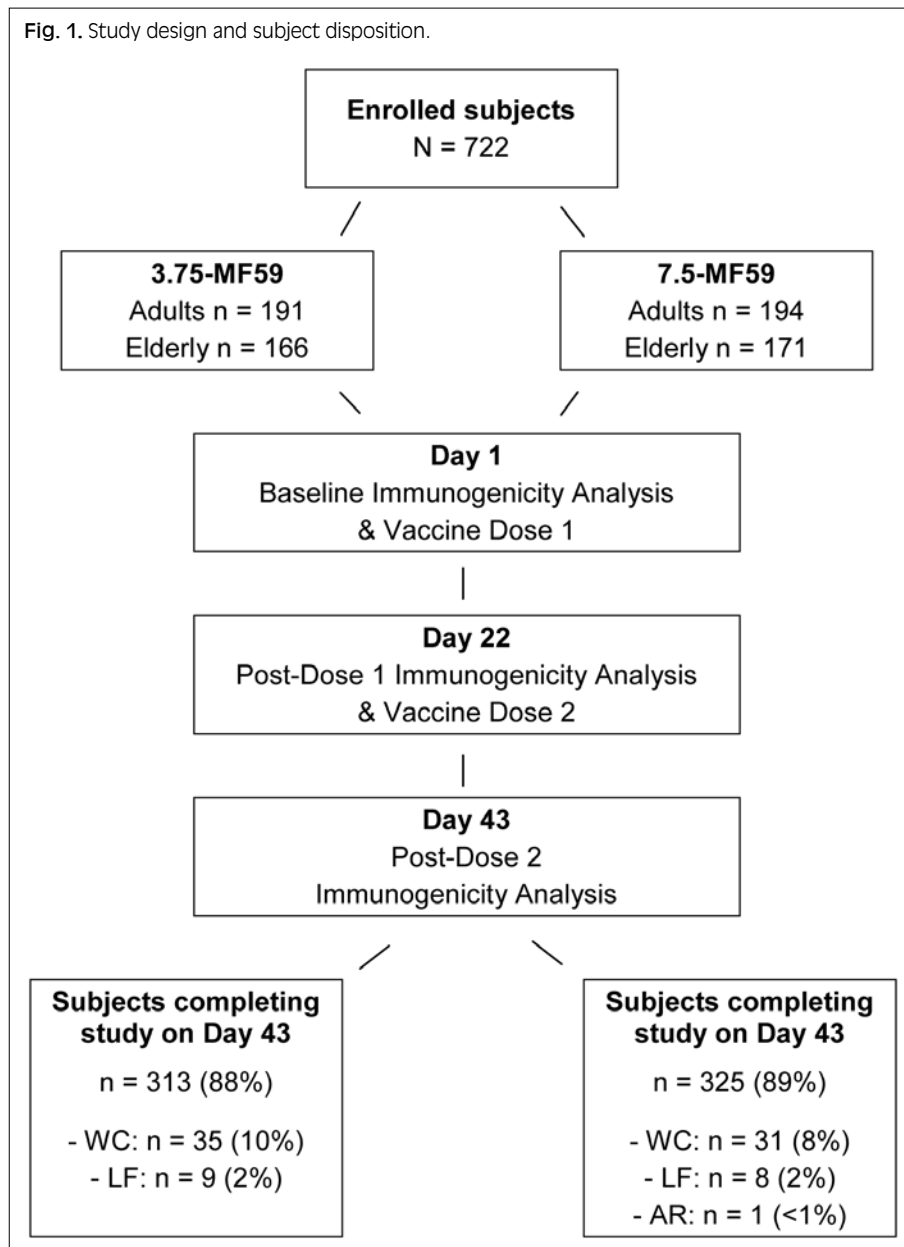
Results

Of the 357 subjects assigned to the 3.75-MF59 study group, and the 365 subjects assigned to 7.5-MF59 study group, 88% and 89% completed the study on Day 43, respectively. Subject disposition and study design are illustrated in Figure 1. The mean ages of subjects within the adult and elderly study groups were 36 and 68 years, respectively. The ratio of male to female subjects was approximately equal in both age cohorts. On average, 20% of adult and 43% of elderly subjects had previously been immunised against influenza. Study population demographics are presented in Tab. I. Per Protocol Set (PPS) analyses are reported throughout, but as there were no major protocol deviations, the Full Analysis Set (FAS) showed similar results.

IMMUNOGENICITY ANALYSIS

Combined antibody responses for adults and elderly subjects against the 3.75 µg and 7.5 µg MF59-H5N1 vaccines are shown in Figure 2. The ratio of GMAs for the low and high antigen dose groups (3.75µg: 7.5 µg) was 0.94 (CI 0.82-1.07), thereby establishing the non-inferiority of the 3.75 µg formulation (lower limit of 95% CI > 0.67). When assessed by HI assay, the ratio of geometric mean titres (GMT) (3.75µg: 7.5 µg) was 0.9 (95% CI 0.66-1.22), narrowly missing the non-inferiority criterion. Analysis of antibody responses against the vaccine strain, A/

Fig. 1. Study design and subject disposition.



Tab. I. Study population demographics.

	Adults (18-60 yrs)		Elderly (≥ 61 yrs)	
	3.75 µg (N = 191)	7.5 µg (N = 194)	3.75 µg (N = 166)	7.5 µg (N = 171)
Mean age (years, SD)	35.1 (11.1)	36.9 (11.3)	68.2 (5.6)	68.7 (6.1)
Female (%)	48	43	53	50
Mean weight (kg, SD)	72.1 (14.3)	72.9 (13.0)	73.4 (12.2)	73.5 (11.7)
Mean height (cm, SD)	169.6 (9.5)	170.4 (9.5)	163.7 (8.7)	164.6 (8.9)
Mean BMI (kg/m ² , SD)	25.03 (4.1)	25.06 (3.5)	27.34 (3.4)	27.11 (3.5)
Prior influenza vaccination (%)	17	23	45	41

BMI: body mass index

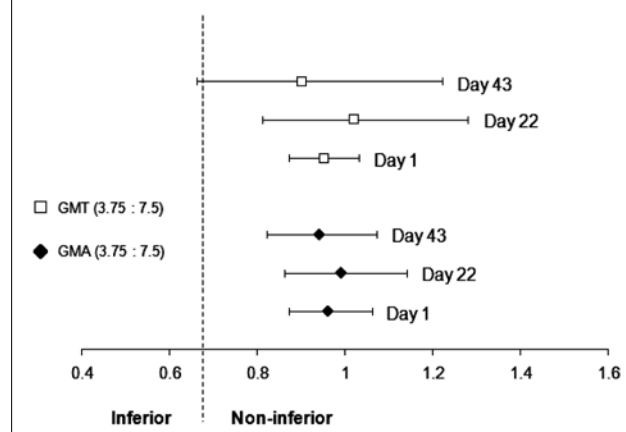
H5N1/Vietnam/1194/04, by SRH, HI and MN assays are shown in Figure 3 and Table II. Adult SRH antibody responses to both the 3.75 µg and 7.5 µg formulations on Day 43 met all three CHMP licensure criteria. In the elderly subjects, GMR and seroconversion criteria were met in the 3.75 µg vaccine group, while all three criteria were met in the 7.5 µg group (Fig. 2). At baseline, HI titres against the vaccine strain were low in both study groups. The mean HI titres at Day 43 were higher in adult than elderly subjects in both vaccine groups (Tab. II). On Day 43, two of three CHMP criteria (GMR and seroconversion) were met by adult subjects in both vaccine groups. In the elderly subjects, two CHMP criteria were met in the 3.75 µg vaccine group (GMR and seroconversion), while all three criteria were met in the 7.5 µg group (Tab. II). A four-fold increase in antibody titre was observed in 62% and 55% of adult and elderly subjects in the 3.75-MF59 group, respectively. In the 7.5-MF59 group, 63% and 61% of adult and elderly subjects achieved a four-fold increase in antibody titres. The CBER criteria for HI seroconversion was met by adult and elderly subjects after two doses (Day 43) of either 3.75 mg or 7.5 mg vaccine. The CBER criteria for seroprotection was not met by any vaccination group on Day 43.

Microneutralization assays were used to assess cross-reactive antibody responses against the heterologous A/H5N1/Indonesia/05/05 and A/H5N1/turkey/Turkey/01/05 antigen strains three weeks (Day 43) after a second vaccine dose. Four-fold increases in MN titres against A/ turkey/Turkey/01/05 antigen were observed in 27% of adult and 15% of elderly 3.75-MF59 group subjects, and 28% and 18% of adult and elderly 7.5-MF59 group subjects, respectively. In the 3.75-MF59 group, 34% of adult and 28% of elderly subjects seroconverted against Indonesia/05/05 antigen, compared with 19% of adult and 24% of elderly subjects in the 7.5-MF59 group. Seroprotective cross-reactive antibody titres against both heterologous strains were detected in 10–15% of adults in both vaccine groups.

SAFETY ANALYSIS

All subjects who received at least one vaccine dose were included in the safety analysis. Both A/H5N1 vaccine formulations were well-tolerated. In both the 3.75-MF59 and 7.5-MF59 groups, similar percentages of adult and elderly subjects reported at least one lo-

Fig. 2. Non-inferiority of 3.75-MF59 vaccine compared with the 7.5-MF59 formulation. Combined adult and elderly subject SRH and HI data three weeks after a second vaccine dose (Day 43).

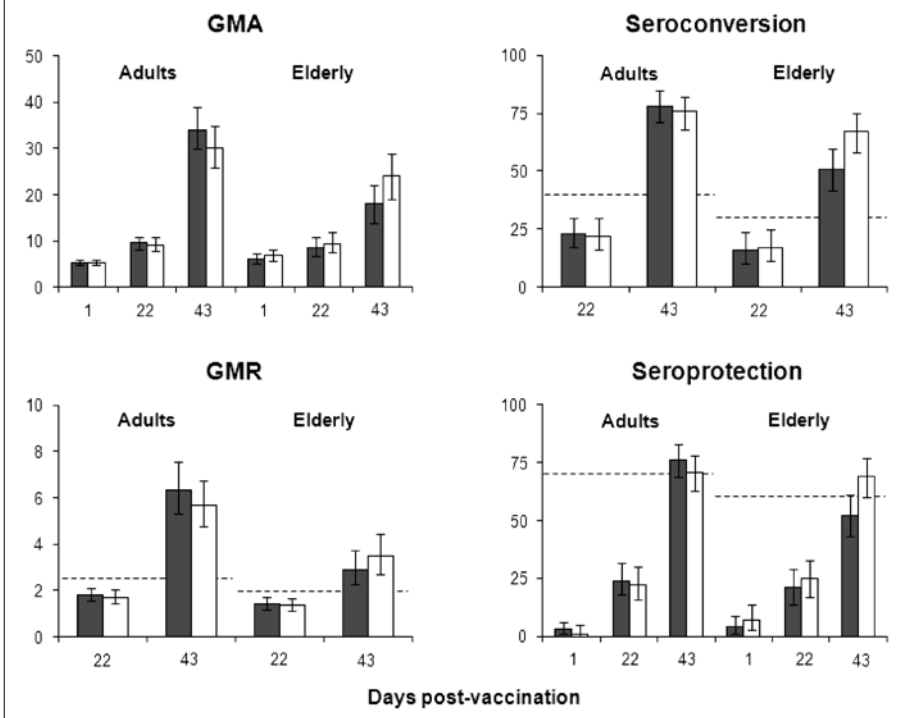


cal or systemic reaction. Overall, reactions were less common in elderly than adult subjects. Pain at the site of injection was the most commonly reported reaction, followed by induration and erythema in all groups (Tab. III). Fatigue was the most common systemic reaction, followed by myalgia, malaise and headache in all groups (Tab. III). Fever was reported in ≤ 3% of adult and elderly subjects in both groups. The percentages of subjects with AEs were similar across groups after both first and second doses. Overall, 9-15% of subjects had at least one AE. The most commonly reported AEs were nasopharyngitis, malaise, headache, myalgia and erythema. Three subjects experienced SAEs, none of which were related to vaccination.

Discussion

Pre-pandemic influenza vaccines should be highly immunogenic, induce cross-reactive antibody responses, and be well tolerated [1-3]. A pre-pandemic vaccine achieving even moderate rates of seroprotection can significantly reduce infection rates during a pandemic [20, 21]. The present study demonstrated that SRH antibody responses to MF59-adjuvanted A/H5N1 vaccine containing 3.75 mg antigen were non-inferior to those of a 7.5 mg formulation (Aflunov®, Novartis Vac-

Fig. 3. SRH antibody responses against the vaccine antigen strain, A/Vietnam/1194/04, at baseline (Day 1), and three weeks after first (Day 22) and second (Day 43) doses of 3.75-MF59 (grey bars) and 7.5-MF59 (white bars) vaccines. CHMP licensure criteria are represented by broken lines.



cines). Microneutralization titres are increasingly being considered important in the assessment of antibody responses against H5 viruses [10, 22, 23], and were therefore, evaluated in this study. The development of effective A/H5N1 vaccines requiring a minimal amount of antigen per dose is a global priority [1-3, 11, 24]. Our study found that two 3.75 µg doses of adjuvanted vaccine were needed to meet at least two CHMP licensure criteria. The administration of two doses of pandemic influenza vaccine is recommended and common practice [25]. Minimizing the amount of antigen required per dose is essential to ensure the widest possible population coverage from a limited vaccine supply. Our data demonstrating the adequacy of a 3.75 mg dose vaccine is supported by studies such as that of Langley et al., who also found two doses of an

Tab. II. Haemagglutination inhibition (HI) and microneutralization (MN), antibody responses against A/Vietnam/1194/04 virus strain at baseline (Day 1), and after one (Day 22) and two (Day 43) doses of MF59-H5N1 vaccine.

	HI ASSAY				MN ASSAY			
	Adults		Elderly		Adults		Elderly	
	3.75 µg (N = 155)	7.5 µg (N = 151)	3.75 µg (N = 124)	7.5 µg (N = 121)	3.75 µg (N = 157)	7.5 µg (N = 153)	3.75 µg (N = 124)	7.5 µg (N = 121)
	GMT (95% CI)				GMT (95% CI)			
Day 1	5.19 (4.85-5.56)	5.32 (4.96-5.7)	5.6 (4.77-6.58)	6.15 (5.24-7.22)	5 (4.84-5.17)	5.12 (4.95-5.29)	5.09 (4.74-5.46)	5.34 (4.98-5.73)
Day 22	10 (7.82-13)	8.69 (6.73-11)	9.13 (6.39-13)	10 (7.22-15)	8.87 (7.6-10)	6.82 (5.84-7.95)	7.41 (5.94-9.25)	7 (5.62-8.73)
Day 43	64 (44-93)	55 (38-80)	33 (21-51)	47 (30-74)	45 (36-56)	32 (25-39)	19 (15-26)	24 (18-31)
	GMR (95% CI)				GMR (95% CI)			
Day 22	1.94 (1.53-2.48)	1.63 (1.28-2.08)	1.63 (1.19-2.23)	1.67 (1.22-2.29)	1.77 (1.53-2.06)	1.33 (1.15-1.55)	1.46 (1.19-1.78)	1.31 (1.07-1.6)
Day 43	12 (8.46-18)	10 (7.15-15)	5.83 (3.78-9)	7.69 (4.99-12)	9.04 (7.24-11)	6.17 (4.94-7.7)	3.8 (2.9-4.98)	4.42 (3.38-5.79)
	(% titre ≥ 40 (95% CI))				(% titre ≥ 40 (95% CI))			
Day 1	1 (0-5)	2 (0-6)	3 (1-8)	6 (2-12)	0 (0-2)	1 (0.02-4)	0 (0-3)	2 (0-6)
Day 22	23 (17-31)	19 (13-26)	20 (13-28)	25 (17-33)	7 (4-12)	3 (1-7)	8 (4-14)	8 (4-15)
Day 43	65 (56-72)	64 (55-71)	56 (46-65)	64 (55-73)	54 (45-61)	43 (35-51)	32 (24-41)	38 (29-47)
	(% displaying 4-fold increase)				(% displaying 4-fold increase)			
Day 22	22 (16-29)	18 (12-25)	17 (11-25)	19 (12-27)	20 (14-27)	7 (4-12)	15 (9-22)	11 (6-18)
Day 43	65 (56-72)	63 (55-71)	53 (44-62)	61 (52-70)	78 (71-85)	65 (57-73)	52 (43-61)	58 (49-67)

GMT, geometric mean titre; GMR, geometric mean ratio

Tab. III. Percentage of subjects experiencing any and severe (brackets) solicited local* and systemic adverse reactions occurring within one week of any vaccination.

	Adults		Elderly	
	3.75 µg (N = 180)	7.5 µg (N = 184)	3.75 µg (N = 151)	7.5 µg (N = 155)
Ecchymosis*	1 (0)	1 (0)	1 (0)	1 (0)
Erythema*	5 (0)	5 (0)	3 (0)	3 (0)
Induration*	6 (0)	6 (0)	5 (0)	3 (0)
Swelling*	3 (0)	3 (0)	5 (0)	1 (0)
Pain*	59 (3)	62 (3)	33 (2)	37 (1)
Headache	24 (5)	22 (4)	18 (3)	17 (1)
Arthralgia	13 (2)	10 (1)	9 (4)	8 (0)
Chills	13 (1)	7 (2)	10 (1)	5 (1)
Fatigue	35 (3)	25 (2)	20 (3)	21 (1)
Malaise	24 (4)	22 (3)	20 (3)	16 (1)
Myalgia	26 (4)	23 (1)	15 (5)	17 (0)
sweating	15 (2)	13 (1)	9 (2)	5 (1)
Nausea	7 (1)	9 (1)	5 (0)	3 (0)
Fever	2 (0)	2 (0)	3 (0)	1 (0)

AS03®-adjuvanted (GlaxoSmithKline, Wavre, Belgium) A/H5N1 vaccine containing 3.75 mg antigen sufficient to meet the European licensure criteria in adult and elderly subjects [26].

Antibody responses to the 3.75 mg vaccine were higher in adults than the elderly. The lower antibody responses observed in elderly subjects may be due to a general decline in immune function with age [27, 28]. In adults, both 3.75 µg and 7.5 µg vaccines induced similar antibody titres. Both vaccines were well tolerated and demonstrated a favourable safety profile. The majority of adverse reactions were mild to moderate in severity. The most frequently reported reaction was pain at the site of injection, this finding is consistent with previous trials of MF59-adjuvanted vaccines [6-9, 14-16, 29].

Both 3.75 µg and 7.5 µg vaccine formulations induced cross-reactive antibodies against the heterologous strains A/Indonesia/05/05 and A/turkey/Turkey/01/05. The broad serological response induced by MF59 has been demonstrated by several studies [6-9, 28-30]. In primed subjects, seroprotective antibody titres against

heterologous H5 strains can be achieved with a single booster dose of MF59-adjuvanted A/H5N1 vaccine [30, 31], even in subjects vaccinated and primed six years prior to booster administration³². Although this study has shown a 3.75 mg formulation to be highly immunogenic, Aflunov, as licensed, contains a 7.5 mg antigen dose in order to provide optimal levels of homologous and heterologous protection. The levels of cross-reactive antibody production observed in the present study were atypically low. Several trials have demonstrated MF59 to promote seroprotective cross-reactive antibody levels in adults and the elderly. A study by Fragapane et al. found MF59-adjuvanted A/H5N1 vaccine to induce cross-reactive antibody at titres sufficient to meet CHMP licensure criteria in adult and elderly subjects [30, 33].

These and other data demonstrate MF59-adjuvanted A/H5N1 vaccine to be highly immunogenic, well tolerated, and to provide heterologous immunity, as well as an immune platform from which booster vaccination rapidly results in optimal levels of seroprotection [29, 34].

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■ Received on December 13, 2011. Accepted on April 23, 2012.

■ Abbreviations used in this paper: HI, haemagglutination inhibition; MN, microneutralization; SRH, single radial haemolysis; GMT, geometric mean titre; GMA, geometric mean area; GMR, geometric mean ratio; CI, confidence interval; AE, adverse event; SAE, serious adverse event; CHMP, European Committee for Medicinal Products for Human Use.

■ Author contributions: All authors participated in the conception, design and implementation of the trials. All authors were involved in the interpretation of analyzed data and the decision to submit for publication.

■ Conflict of Interests: RB, IA, JB, and EF are permanent employees of Novartis Vaccines and Diagnostics. All other authors declare no conflict of interests.

■ Funding statement: This clinical trial was supported by Novartis Vaccines and Diagnostics.

■ Trial registration: (www.clinicaltrials.gov) NCT00914771

■ Acknowledgements. The authors wish to acknowledge and thank the following co-investigators for their significant contributions to this study: Dr Ewa Smukalska, Bydgoszcz, Poland; Dr Ireneusz Szymczyk, Katowice, Poland; Dr Basak Dokuzoguz, Sample Training and Research Hospital, Ankara, Turkey HM, Infectious Diseases and Clinical Microbiology Clinic, Ankara, Turkey; and Dr Dilek Arman, Gazi University Faculty of Medicine, Department of Clinical Microbiology and Infectious Diseases, Ankara, Turkey. The authors are grateful to study coordinators, Jozsef Kovacs and Csaba Kovacs (both Novartis Vaccines and Diagnostics); and Dr Jamie Stirling, Dr Shivani Vadapalli (both Novartis Vaccines and Diagnostics) and Bilal Bham (Scripsi Scriptum Ltd) for editorial assistance in the preparation of this manuscript.

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