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Safety and immunogenicity of an MF59®-adjuvanted A/H1N1 pandemic influenza vaccine in children from three to seventeen years of age

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ABSTRACT

Objectives: This study was designed to identify the optimal dose of an MF59®-adjuvanted, monovalent, A/H1N1 influenza vaccine in healthy paediatric subjects.
Methods: Subjects aged 3–8 years (n = 194) and 9–17 years (n = 160) were randomized to receive two primary doses of A/H1N1 vaccine containing either 3.75 μg antigen with half a standard dose of MF59 adjuvant, 7.5 μg antigen with a full dose of MF59, or (children 3–8 years only), a non-adjuvanted 15 μg formulation. A booster dose of MF59-adjuvanted seasonal influenza vaccine including homologous A/H1N1 strain was given one year after priming. Immunogenicity was assessed by haemagglutination inhibition (HI) and microneutralization assays. Vaccine safety was assessed throughout the study (up to 18 months).
Results: A single priming dose of either MF59-adjuvanted formulation was sufficient to meet the European licensure criteria for pandemic influenza vaccines (HI titres ≥1:40 > 70%; seroconversion > 40%; and GMR > 2.5). Two non-adjuvanted vaccine doses were required to meet the same licensure criteria. After first and second doses, percentage of subjects with HI titres ≥1:40 were between 97% and 100% in the adjuvanted vaccine groups compared with 68% and 91% in the non-adjuvanted group, respectively. Post-vaccination seroconversion rates ranged from 91% to 98% in adjuvanted groups and were 68% (first dose) and 98% (second dose) in the non-adjuvanted group. HI titres ≥1:330 after primary doses were achieved in 69% to 90% in adjuvanted groups compared with 41% in the non-adjuvanted group. Long-term antibody persistence after priming and a robust antibody response to booster immunization were observed in all vaccination groups. All A/H1N1 vaccine formulations were generally well tolerated. No vaccine-related serious adverse events occurred, and no subjects were withdrawn from the study due to an adverse event.
Conclusions: An MF59-adjuvanted influenza vaccine containing 3.75 μg of A/H1N1 antigen was well tolerated and sufficiently immunogenic to meet all the European licensure criteria after a single dose in healthy children 3–17 years old.

1. Introduction

Pandemic outbreaks of influenza can occur when an influenza virus emerges containing haemagglutinin of a novel subtype to which the human population has little or no existing immunity. In June 2009, the World Health Organization (WHO) declared an influenza pandemic following the rapid spread of a novel, reassortant, A/H1N1 influenza virus of swine origin [1].

According to the last update released by the WHO on August 6, 2010 (when the 2009 pandemic was officially declared as terminated), laboratory-confirmed cases of A/H1N1 influenza disease in humans had been reported in more than 214 countries

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worldwide, resulting in over 18,400 fatalities [2]. More recent data has estimated the number of deaths from the 2009 A/H1N1 pandemic at more than 284,000, approximately 15 times the number of laboratory-confirmed cases [3]. Children were particularly susceptible to A/H1N1 disease, due to relatively higher levels of exposure within communities [4–7]. In the United States, more than 45% of hospitalizations due to A/H1N1 occurred in children under eighteen years of age [6].

Safe and effective A/H1N1 pandemic vaccines providing long-term immunity were urgently needed in large quantities to protect the particularly susceptible paediatric population, and to reduce levels of transmission within communities [8]. Ideally, pandemic influenza vaccines should require a minimal quantity of antigen per dose in order to ensure the widest possible population coverage given a limited global capacity for vaccine production. Previous experience with candidate avian (A/H5N1) influenza prepandemic vaccines has shown that the addition of oil-in-water adjuvants, such as MF59® (Novartis Vaccines), allows for a significant reduction in antigen content per dose and increased levels of immunogenicity [9–11].

A good safety profile for MF59-adjuvanted seasonal and pandemic influenza vaccines has been established [12–15]. The egg-derived monovalent, MF59-adjuvanted, pandemic vaccine Focetria® (Novartis Vaccines) was approved for use by the European Medicines Agency with an antigen content of 7.5 μg per dose [16]. In a previous study it was demonstrated that a single dose of this vaccine was sufficient to meet the European Committee for Medicinal Products for Human Use (CHMP) licensure criteria for pandemic influenza vaccines in adults and elderly [17].

In this study we aimed to identify priming antigen and adjuvant doses resulting in optimal antibody levels shortly after primary immunization in pediatric subjects 3–17 years of age. In addition, long-term antibody persistence and responses to a one-year booster dose were evaluated. Vaccine safety was assessed up to 18 months after vaccination.

2. Materials and methods

This multinational, single-blind, randomized, dose-range study was conducted between September 2009 and July 2011 across 11 study sites in Germany, 2 sites in Belgium, 1 site in The Netherlands, 2 sites in The Dominican Republic, and 2 sites in Chile. One German site was excluded from analyses due to noncompliance with the protocol requirements for safety reporting for a different study. The trial was performed in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The protocol was reviewed and approved by the Ethics Committee of each participating institution. Written informed consent was obtained from the parent or legal guardian of each subject before enrolment in the study, and, where applicable according to local requirement, assent was obtained from the subject.

2.1. Subjects

A total of 684 healthy children 6 months to 17 years of age were included in this clinical study, of which 354 children in one of the age cohorts 3–8 years and 9–17 years are presented here. The results of the younger age cohorts (6–11 months and 12–35 months) will be reported separately.

Main exclusion criteria included any serious illness; hypersensitivity or previous adverse reaction to vaccination; influenza disease, or previous receipt of any adjuvanted influenza vaccine, investigational agent or blood/plasma derivate within three months prior to enrolment, and an impaired immune function.

2.2. Study procedures

Subjects were divided into 3–8 and 9–17 year-old cohorts. Subjects in the 3–8 year-old age group were randomized in a 2:2:1 ratio to receive vaccine containing either 3.75 μg antigen with half (4.875 mg squalene) the standard dose of MF59 adjuvant (3.75-Half MF59), 7.5 μg antigen with a standard/full dose (9.75 mg squalene) of MF59 (7.5-Full MF59), or a non-adjuvanted 15 μg formulation (15-No MF59), respectively. Subjects in the 9–17 year-old age group were randomized in equal numbers to receive either 3.75-Half MF59 or 7.5-Full MF59 vaccine. Subjects were randomly assigned to a vaccination group using 'Hidden Entry Envelopes', in which the vaccine information was contained in a sealed envelope, preventing tampering and reading of the assigned group before the subject number was provided. The identity of the assigned vaccine formulation was not revealed to the subject or their parent/legal guardian. All subjects received two primary vaccine doses with monovalent A/H1N1 vaccine given three weeks apart in the deltoid muscle of the non-dominant arm. One year after primary immunization a booster dose of MF59-adjuvanted trivalent seasonal influenza vaccine was administered to all subjects. Children who had not previously received seasonal influenza vaccine and were <9 years-old at the time of booster administration were given a second dose of the adjuvanted seasonal vaccine three weeks later, according to national recommendations.

Blood samples (~10 mL per sample) were collected for immunogenicity analyses on Day 1 (baseline), Day 22 (three weeks after first primary dose, window period 18–28 days), Day 43 (three weeks after second primary dose, window period 18–28 days), Day 366 (one year after primary immunization, window period 350–380 days), and Day 387 (three weeks after first booster dose, window period 18–28 days). Immunogenicity following the second seasonal vaccine dose was not assessed. The safety follow up was up to 6 months after the booster, for an overall duration of 18 months.

2.3. Vaccines

The investigational MF59-adjuvanted, pandemic subunit vaccine (Focetria) contained haemagglutinin and neuraminidase surface antigens derived from the A/H1N1/California/7/2009 influenza strain [18]. Each dose of the seasonal, MF59-adjuvanted, trivalent influenza subunit vaccine (MF59-TIV), Fluad® (Novartis Vaccines), contained a standard dose of MF59, and 15 μg antigen from each of the WHO reference strains recommended for the 2010–2011 influenza season: A/California/7/2009 (H1N1, homologous strain); A/Perth/16/2009 (H3N2); and B/Brisbane/60/2008. Vaccines were supplied in pre-filled syringes. Single doses of 7.5-Full MF59, 15-No MF59 and of the seasonal vaccines were administered in a volume of 0.5 mL. A single dose of the 3.75-Half MF59 formulation was administered in a volume of 0.25 mL.

2.4. Immunogenicity assessments

Blood samples were obtained by venipuncture and analysed using validated methods at the Novartis Serology Laboratory (Marburg, Germany). Antibody responses against the homologous A/California/7/2009 (H1N1) strain were assessed by haemagglutination (HI) and MN (microneutralization) assays, as described previously [19,20]. Antibody responses were expressed as geometric mean titre (GMTs) at Days 1, 22, 43, 366 and 387, geometric mean ratio (GMRs) of the postvaccination to prevaccination titres (Day 22/Day 1; Day 43/Day 1 and Day 387/Day 366), percentage of subjects with HI titres ≥1:40, HI titres ≥1:330, and seroconversion rates. Seroconversion was defined as the percentage of subjects per group achieving at least a 4-fold increase in HI titre from a seropositive prevaccination titre (≥1:10) or a rise from <1:10 to ≥1:40 in

those who were originally seronegative. Percentages of subjects with HI titres ≥1:330 were also calculated, as this cut-off titre has been shown to predict an 80% clinical protective level in young children, as compared with only 22% protection for the conventional HI titre of ≥1:40 [21].

2.5. Safety assessment

Subjects were monitored for 30 minutes after each vaccination for possible immediate adverse reactions. Parents/legal guardians were instructed to complete diary cards to record specified local and systemic reactions for seven days, starting on the day of each vaccination [22]. Solicited local reactions were ecchymosis, erythema, induration, swelling, and pain at injection site. Solicited systemic reactions included headache, arthralgia, chills, fatigue, malaise, myalgia, nausea, sweating, and fever (≥38 °C, severe fever ≥40°). Reports of any unsolicited adverse events (AE) were recorded for three weeks after each vaccination. The onset of new chronic diseases, serious adverse events (SAEs), and AEs leading to withdrawal were recorded up to 18 months. The investigator rated AEs as mild, moderate or severe if resulting in no limitation of, some limitation of, or inability to perform normal daily activities, respectively.

2.6. Statistical analyses

Sample sizes were chosen to meet or exceed the minimum requirements of the European guidelines for influenza vaccine clinical trials. No formal statistical hypothesis was tested, immunogenicity endpoints being based on CHMP licensure criteria [23]. There are currently no CHMP criteria for vaccinees under 18 years of age, therefore, the following adult licensure criteria applied: the percentage of subjects achieving seroconversion for HI antibody should be >40%; the percentage of subjects achieving HI titre ≥1:40 should be >70% and the GMR should be >2.5. Percentages of subjects with HI titres ≥1:330 were also calculated.

Immunogenicity data reflecting the above endpoints, GMTs, their ratios, and corresponding 2-sided 95% confidence intervals (CI) were calculated for each vaccine group and age cohort. Safety data were evaluated descriptively and expressed as the percentage or number of subjects with AEs in each group.

Immunogenicity analyses were run on the per-protocol (PP) set, which consisted of subjects who received all the relevant doses of vaccine correctly, provided at least one evaluable serum sample at the relevant time points, and had no major protocol violations. Safety was analyzed for all subjects exposed to at least one study vaccination and provided safety data.

3. Results

After exclusion of one study site, 354 subjects were included in the two age cohorts 3–8 years (n = 194) and 9–17 years (n = 160). Across the study groups, 96–100% completed the primary vaccinations and 33–65% of study groups completed the study protocol (Fig. 1). The German Ethical Committee did not allow booster with adjuvanted TIV. Hence, 21–26% and 54–55% of subjects in age cohorts 3–8 years and 9–17 years, respectively, were withdrawn from the study at Day 366. Other reasons for non-study completion were withdrawal of consent (6–15% across groups), loss to

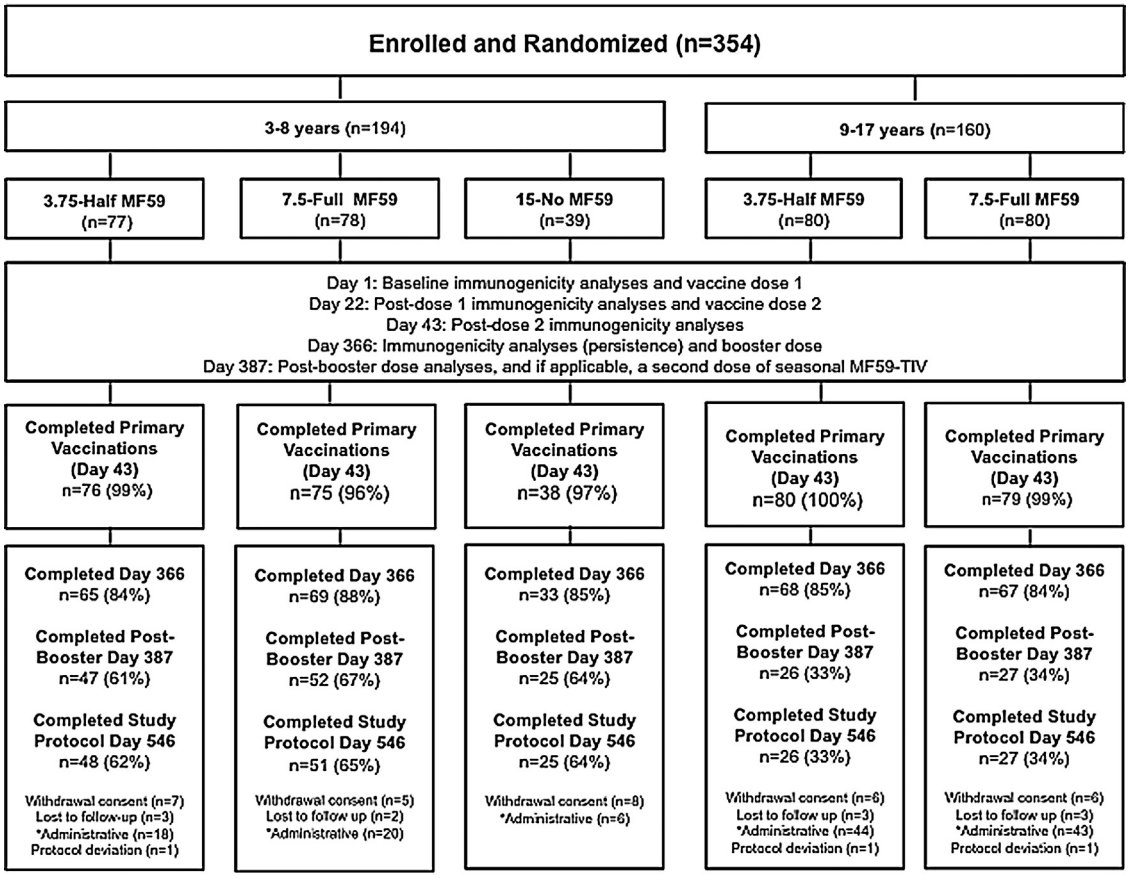

Fig. 1. Study design and subject disposition.

follow-up (up to 4% across groups) and protocol deviations (0–1% across groups). There were no study withdrawals due to AE.

Vaccine groups were comparable with respect to age, body size characteristics, and the majority of study subjects were Caucasian (Table 1). Mean age of the subjects was 5.3 years and 12.4 years in cohorts 3–8 years and 9–17 years, respectively. The majority of subjects (68–85%) had not previously been vaccinated against influenza.

3.1. Immunogenicity

Immunogenicity analyses were performed on the Per Protocol data set, which included 80–89% of subjects across groups after the primary vaccination schedule (Day 43), 27–57% of subjects for persistence analyses (Day 366), and 24–50% of subjects for post-booster analyses (Day 387).

HI antibody responses to vaccination are summarized in Table 2. GMTs were low at baseline (varying between 7.5 and 13), with 12–24% of subjects across groups having antibody titres ≥1:40. In both age cohorts, all subjects in the adjuvanted vaccine groups met all three CHMP criteria after the first priming dose, and continued to meet the criteria after receiving the second priming dose. Subjects aged 3–8 years who received non-adjuvanted vaccine only met all three licensure criteria after the second priming dose.

In subjects aged 3–8 years, geometric mean HI antibody titres (GMTs) increased 34-fold and 37-fold from baseline values after the first priming dose, and increased 88-fold and 81-fold after the second vaccine dose in the 3.75-Half MF59 and 7.5-Full MF59 study groups, respectively (Fig. 2). In this age group, the non-adjuvanted vaccine formulation induced a 12-fold increase in HI GMTs after the first priming dose and a 20-fold increase after the second dose.

In subjects aged 9–17 years, corresponding increases in GMTs were 43-fold and 67-fold after the first priming dose, and 53-fold and 90-fold after the second priming dose. In both age groups, seroconversion rates in the adjuvanted groups were ≥91% after the first priming dose and ≥92% after the second priming dose. Seroconversion rates in subjects aged 3–8 years who received non-adjuvanted vaccine were 68% and 85% after first and second doses, respectively. In both age groups, the proportion of subjects in the adjuvanted groups achieving HI titres ≥1:40 was ≥97% after the first priming dose and 100% after the second priming dose. In subjects aged 3–8 years who received non-adjuvanted vaccine, HI titres ≥1:40 were achieved in 68% after the first dose and 91% after the second dose.

Long-term antibody persistence was demonstrated in subjects receiving MF59-adjuvanted vaccines; 84–100% of subjects across age groups retained HI titres ≥1:40 one year after immunization, as compared with 63% in those receiving the non-adjuvanted formulation (Table 2). HI GMTs remained 4-fold to 25-fold higher than baseline values across groups, with the highest antibody responses seen in subjects who received the MF59-adjuvanted vaccine formulations. All three CHMP licensure criteria were met by all study groups following booster vaccination. HI immunogenicity data were supported by similar MN data (Supplemental Table 1). As the study was conducted during and after the pandemic, the immunogenicity data could be confounded by previous clinical or undiagnosed H1N1 infection. GMTs and percentage of subjects with HI titres ≥1:40 were also assessed by baseline seropositive status. Results suggest that adjuvanted vaccine formulations are able to induce higher immune responses compared to the non-adjuvanted vaccine, also in subjects seronegative at baseline (Supplemental Tables 2–3).

Percentages of subjects with HI titres ≥1:330 are presented in Table 2. In the 3–8 years cohort, more subjects in the adjuvanted groups achieved HI titres ≥1:330 compared with those receiving the nonadjuvanted vaccine. HI titres ≥1:330 after primary vaccinations were achieved in 77–78% of subjects receiving

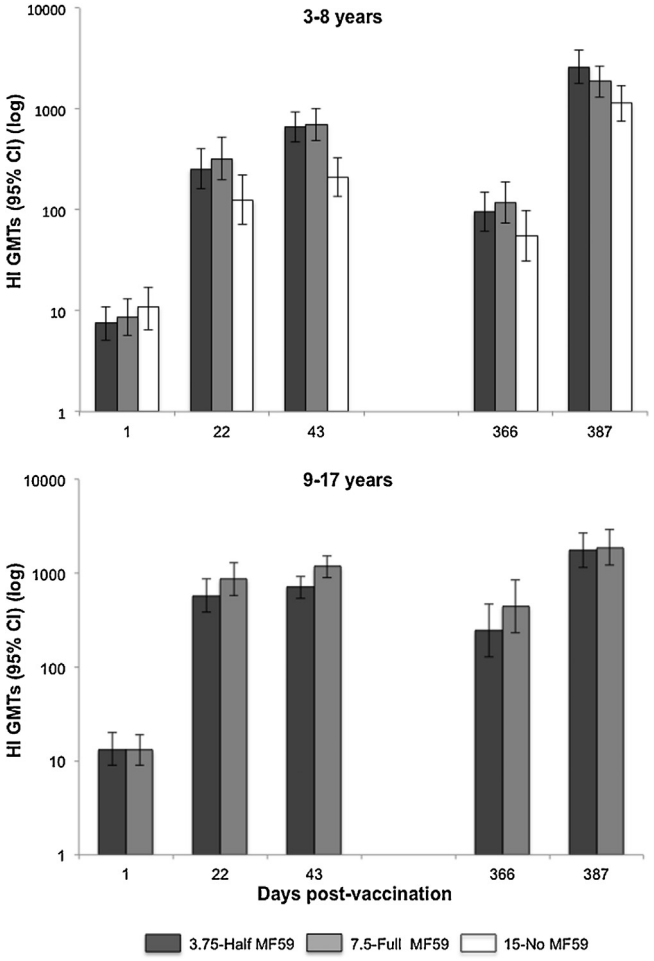

Fig. 2. Geometric mean antibody titres (GMTs) against the vaccine strain, A/H1N1/California/7/2009, measured by HI assay three weeks after first (Day 22) and second (Day 43) vaccine doses, twelve months after primary immunization (Day 366), and three weeks after booster administration (Day 387).

adjuvanted formulation and in 41% of subjects receiving the non-adjuvanted formulation. Following booster, 97–100% (adjuvanted priming formulations) and 86% (nonadjuvanted priming formulation) of subjects exhibited HI titres ≥1:330. In the 9–17 years cohort, HI titres ≥1:330 were observed in 69% (3.75-Half MF59) and 90% (7.5 Full MF59) after primary vaccinations and all subjects (100%) after the booster dose.

3.2. Safety

All subjects were exposed to at least one study vaccination and contributed to the safety analyses. In both age groups after both vaccinations, slightly lower proportions of subjects experienced solicited reactions following the 3.75-Half MF59 vaccine compared with the 7.5-Full MF59 formulation. In subjects aged 3–8 years, rates of solicited reactions were lower with the non-adjuvanted vaccine formulation than with either of the adjuvanted formulations. In both age groups, rates of solicited reactions were higher after the booster dose than after either primary dose. In the 3–8 years age group after the second seasonal vaccination, incidence of solicited reactions decreased. Local and systemic reactions were typically mild to moderate in severity and of short duration. Few subjects experienced severe reactions following vaccinations. The most common local reaction in all study groups was mild to moderate pain at the site of injection. Fatigue and myalgia were the most

Table 1
Study population demographics and baseline characteristics.

	3–8 Years 3.75-Half MF59 (*n* = 77)	3–8 Years 7.5-Full MF59 (*n* = 78)	3–8 Years 15-No MF59 (*n* = 39)	9–17 Years 3.75-Half MF59 (*n* = 80)	9–17 Years 7.5-Full MF59 (*n* = 80)
Mean age (years, SD)	5.2 ± 1.7	5.4 ± 1.7	5.1 ± 1.7	12.3 ± 2.5	12.4 ± 2.4
Male subjects (%)	51	56	36	48	49
Mean weight (kg, SD)	21.8 ± 6.2	21.8 ± 6.3	21.7 ± 6.4	50.9 ± 15.8	51.8 ± 15.9
Mean height (cm, SD)	114 ± 13	116 ± 11	115 ± 12	156 ± 14	158 ± 15
Mean BMI (kg/m^2, SD)	16.3 ± 1.9	16.0 ± 2.6	16.1 ± 2.4	20.4 ± 4.0	20.3 ± 4.2
Caucasian (%)	68	65	64	93	89
Prior influenza vaccination (%)	29	32	15	25	29

SD, standard deviation; BMI, body mass index

Table 2
Immunogenicity analysis (95% CI) by haemagglutination inhibition (HI) assay against A/H1N1/California/7/2009, at baseline (Day 1), three weeks after first (Day 22) and second (Day 43) primary doses, one year after vaccination (Day 366) and three weeks after booster vaccination (Day 387). **Bold:** CHMP criterion met.

	3–8 Years 3.75-Half MF59 (Prime *n* = 66) (Boost *n* = 36)	**3–8 Years 7.5-Full MF59** (Prime *n* = 60) (Boost *n* = 37)	**3–8 Years 15-No MF59** (Prime *n* = 34) (Boost *n* = 22)	**9–17 Years 3.75-Half MF59** (Prime *n* = 65) (Boost *n* = 23)	**9–17 Years 7.5-Full MF59** (Prime *n* = 70) (Boost *n* = 23)
Geometric mean titre					
Day 1	7.53 (5.08–11)	8.65 (5.67–13)	11 (6.4–17)	13 (8.96–20)	13 (8.81–18)
Day 22	254 (162–399)	322 (199–522)	125 (71–221)	576 (382–869)	868 (579–1301)
Day 43	661 (469–934)	703 (486–1017)	210 (136–324)	710 (541–931)	1174 (899–1533)
Day 366	96 (61–151) (*n* = 44)	119 (74–191) (*n* = 43)	55 (31–97) (*n* = 24)	244 (128–468) (*n* = 26)	447 (232–858)(*n* = 27)
Day 387	2616 (1776–3855)	1876 (1311–2685)	1135 (749–1720)	1745 (1142–2666)	1873 (1210–2899)
Geometric mean ratio					
Day 22/1 (1st dose)	**34** (22–51)	**37** (24–58)	**12** (7–20)	**43** (26–71)	**67** (41–108
Day 43/1 (2nd dose)	**88** (56–138)	**81** (50–132)	**20** (11–35)	**53** (34–84)	**90** (58–142)
Day 387/366 (post-booster)	**18** (12–29)	**13** (8–19)	**17** (10–28)	**9.5** (4.6–20)	**5.3** (2.5–11)
Seroconversion (%)					
Day 22	**95** (87–99)	**98** (91–100)	**68** (49–83)	**91**(81–97)	**96** (88–99)
Day 43	**97** (89–100)	**98** (91–100)	**85** (69–95)	**92** (83–97)	**94** (86–98)
Day 387	**92** (78–98)	**95** (82–99)	**95** (77–100)	**83** (61–95)	**61** (39–80)
% subjects HI titre 1:40					
Day 1	12 (5–22)	18 (10–30)	24 (11–41)	14 (7–25)	17 (9–28)
Day 22	**97** (89–100)	**100** (94–100)	68 (49–83)	**97** (89–100)	**99** (92–100)
Day 43	**100** (95–100)	**100** (94–100)	**91** (76–98)	**100** (94–100)	**100** (95–100)
Day 366	84 (70–93) (*n* = 44)	100 (92–100) (*n* = 43)	63 (41–81) (*n* = 24)	96 (80–100) (*n* = 26)	100 (87–100) (*n* = 27)
Day 387	**100** (90–100)	**100** (91–100)	**100** (85–100)	**100** (85–100)	**100** (85–100)
% subjects HI titre 1:330					
Day 1	2 (0.04–8)	0 (0–6)	0 (0–10)	3 (0–11)	3 (0–10)
Day 22	36 (25–49)	33 (22–47)	35 (20–54)	46 (34–59)	66 (53–77)
Day 43	77 (65–87)	78 (66–88)	41 (25–59)	69 (57–180)	90 (80–96)
Day 366	11 (4–25) (*n* = 44)	7 (1–19) (*n* = 43)	4 (0–21) (*n* = 24)	31 (14–52) (*n* = 26)	41 (22–61) (*n* = 27)
Day 387	100 (90–100)	97 (86–100)	86 (65–97)	100 (85–100)	100 (85–100)

Table 3
Percentages of 3–8 year-old children experiencing mild to moderate and (severe) solicited local and systemic reactions within one week of vaccine administration.

	First dose			Second dose			MF59-TIV (booster)			MF59 TIV (2nd seasonal dose)		
	3.75-Half MF59 (*n* = 78)	**7.5-Full MF59** (*n* = 77)	**15-No MF59** (*n* = 39)	**3.75-Half MF59** (*n* = 77)	**7.5-Full MF59** (*n* = 75)	**15-No MF59** (*n* = 38)	**3.75-Half MF59** (*n* = 48)	**7.5-Full MF59** (*n* = 52)	**15-No MF59** (*n* = 25)	**3.75-Half MF59** (*n* = 28)	**7.5-Full MF59** (*n* = 30)	**15-No MF59** (*n* = 14)
Ecchymosis[*]	9 (0)	10 (0)	10 (0)	5 (0)	3 (0)	5 (0)	6 (0)	10 (0)	12 (0)	0 (0)	7 (0)	0 (0)
Erythema[*]	21 (0)	26 (0)	13 (0)	27 (0)	20 (0)	11 (0)	35 (2)	29 (0)	24 (0)	32 (0)	30 (0)	7 (0)
Induration[*]	8 (0)	5 (0)	10 (0)	9 (0)	11 (0)	0 (0)	19 (2)	27 (0)	12 (0)	4 (0)	17 (0)	7 (0)
Swelling[*]	4 (0)	10 (0)	8 (0)	8 (0)	7 (0)	0 (0)	10 (2)	19 (0)	12 (0)	11 (0)	20 (0)	7 (0)
Pain[*]	37 (1)	52 (1)	26 (3)	26 (0)	43 (1)	29 (0)	71 (4)	71 (8)	64 (0)	54 (0)	50 (0)	43 (0)
Chills	4 (0)	4 (0)	10 (0)	1 (0)	7 (0)	3 (0)	6 (0)	15 (0)	4 (0)	11 (0)	3 (0)	0 (0)
Malaise	15 (0)	13 (0)	10 (0)	4 (0)	11 (0)	3 (0)	17 (0)	17 (0)	8 (0)	7 (0)	7 (0)	7 (0)
Myalgia	8 (0)	13 (0)	8 (0)	6 (0)	11 (0)	0 (0)	19 (2)	31 (4)	8 (0)	0 (0)	13 (0)	0 (0)
Arthralgia	4 (0)	8 (0)	8 (0)	3 (0)	7 (0)	0 (0)	8 (0)	15 (2)	8 (0)	4 (0)	17 (0)	7 (0)
Headache	9 (0)	19 (0)	3 (0)	5 (0)	13 (0)	5 (0)	8 (0)	29 (2)	16 (0)	7 (0)	20 (0)	0 (0)
Sweating	1 (1)	3 (0)	8 (0)	0 (0)	5 (1)	3 (0)	6 (0)	6 (0)	0 (0)	4 (0)	3 (0)	0 (0)
Fatigue	27 (0)	17 (0)	21 (0)	8 (0)	19 (0)	11 (3)	21 (0)	19 (0)	12 (0)	11 (0)	23 (0)	7 (0)
Nausea	12 (1)	5 (0)	3 (0)	6 (1)	5 (1)	3 (0)	8 (2)	13 (0)	12 (0)	4 (4)	13 (0)	7 (7)
Fever (≥38 °C)	4 (0)	4 (0)	5 (0)	0 (0)	3 (0)	0 (0)	6 (0)	12 (0)	0 (0)	7 (0)	7 (0)	0 (0)
Use Analgesic/ antipyretic	13	12	13	5	13	5	13	15	16	14	13	7

[*] Solicited local reaction.
[§] Severe fever defined as a body temperature ≥40 °C.

Table 4
Percentages of 9–17 year-old children experiencing mild to moderate and (severe) solicited local and systemic reactions within one week of vaccine administration.

	First dose		Second dose		MF59 TIV (booster)	
	3.75-Half MF59 (*n* = 79)	**7.5-Full MF59** (*n* = 80)	**3.75-Half MF59** (*n* = 79)	**7.5-Full MF59** (*n* = 79)	**3.75-Half MF59** (*n* = 26)	**7.5-Full MF59** (*n* = 27)
Ecchymosis*	9 (0)	8 (0)	4 (0)	4 (0)	4 (0)	4 (0)
Erythema*	14 (0)	11 (0)	14 (0)	18 (0)	19 (4)	19 (0)
Induration*	13 (0)	15 (0)	11 (0)	9 (0)	23 (0)	19 (0)
Swelling*	9 (0)	9 (0)	5 (0)	5 (0)	19 (0)	15 (0)
Pain*	53 (0)	60 (1)	43 (1)	52 (1)	85 (4)	81 (15)
Chills	3 (0)	4 (0)	4 (0)	3 (0)	4 (0)	15 (0)
Malaise	10 (0)	13 (1)	9 (1)	6 (0)	27 (0)	15 (0)
Myalgia	15 (1)	21 (0)	9 (0)	10 (0)	38 (0)	41 (4)
Arthralgia	5 (0)	6 (0)	6 (1)	5 (0)	19 (0)	26 (0)
Headache	20 (0)	18 (0)	16 (1)	14 (0)	15 (0)	48 (0)
Sweating	1 (0)	1 (0)	3 (0)	1 (0)	8 (4)	26 (0)
Fatigue	18 (1)	24 (0)	14 (1)	10 (0)	27 (0)	26 (0)
Nausea	4 (0)	5 (1)	6 (0)	10 (0)	15 (0)	19 (0)
Fever (≥38 °C)	3 (0)	3 (0)	3 (0)	1 (0)	4 (0)	4 (0)
Use Analgesic/antipyretic	5	13	10	9	4	22

* Solicited local reaction.
§ Severe fever defined as a body temperature ≥40 °C.

common reported systemic reactions (Tables 3 and 4). No subjects experienced severe fever (≥40 °C) at any time during the study.

For spontaneously reported AEs, no differences in overall frequency of AEs were observed between the two adjuvanted formulations and between adjuvanted and nonadjuvanted formulations.

Rates of reported unsolicited AEs were generally low, 34–46% and 1–15% of subjects across age and vaccine groups after primary and booster vaccinations, respectively.

Between Days 1–366, there were 14 subjects with SAEs (similarly distributed across groups), none of which related to study vaccination. There were 9 new onsets of chronic diseases, one of which of mild severity was considered at least possibly related to study vaccination (malabsorption in 9–17 years cohort, 7.5-Full MF59 group) (Table 5). No premature study withdrawals were observed. After the booster vaccination (Days 366 to 546), only 2 subjects in age cohort 3–8 years experienced an SAE (one case of asthma in 3.75-Half MF59 group and one case of adenoidal hypertrophy in 7.5-Full MF59 group), none of which was considered related to study vaccination. During this period, there were no onsets of chronic diseases and no subjects were withdrawn due to an AE.

4. Discussion

This study was performed to identify which quantities of priming A/H1N1 antigen and MF59 adjuvant resulted in optimal antibody levels after primary and booster influenza vaccinations in healthy paediatric subjects aged 3–17 years. During any future influenza pandemic, the global demand for vaccine would undoubtedly exceed current manufacturing capacity. Dose-sparing strategies, such as the use of adjuvants in order to reduce antigen content per dose, are critical to ensure that vaccine supply is able to meet public demand. This study found all three doses of A/H1N1 vaccine formulations to be highly immunogenic and well tolerated in healthy children and adolescents. Enhanced antibody titres were observed in response to the MF59-adjuvanted vaccines compared with the non-adjuvanted formulation after primary immunization. The MF59-adjuvanted vaccines also resulted in enhanced long-term antibody persistence and higher antibody titres following booster administration, with no safety concerns.

Previous experience with pre-pandemic A/H5N1 (avian) influenza vaccines suggested that at least two vaccine doses would be required to induce adequate levels of seroprotection against novel influenza strains in naïve populations [20,24–26]. However, the present study demonstrated that a single dose of A/H1N1 vaccine containing 3.75 μg of antigen and half a dose of MF59 was sufficient to meet all the CHMP licensure criteria for pandemic influenza vaccines. Findings of this study are in agreement with previously published results of MF59-adjuvanted A/H1N1 vaccine in adults and children [17,27–30]. Similar results were also reported in paediatric and adult populations using the AS03®-adjuvanted H1N1 pandemic vaccine (GlaxoSmithKline Biologicals, Wavre, Belgium) [31,32], providing further proof of the benefits of adjuvanted A/H1N1 vaccines.

Table 5
Listing of new onset of chronic diseases throughout the study (Day 1 to 366).

	Vaccine formulation	Preferred term	Onset day	Severity	Outcome	Relatedness
3–8 years	3.75-Half MF59	Attention deficit/hyperactivity disorder	201	Moderate	AE persistent	None
		Rhinitis allergic	329	Mild	AE persistent	None
	7.5-Full MF59	Urinary tract infection	45	Moderate	Alive/sequela*	None
		Seasonal allergy	173	Moderate	AE persistent	None
		Attention deficit/hyperactivity disorder	300	Mild	AE persistent	None
	15-No MF59	Attention deficit/hyperactivity disorder	180	Mild	AE persistent	None
9–17 years	3.75-Half MF59	Irritable bowel syndrome	271	Moderate	AE persistent	None
		Polycystic ovaries	306	Mild	AE persistent	None
	7.5-Full MF59	Scoliosis	39	Mild	AE persistent	None
		Malabsorption	65	Mild	AE persistent	Possibly related

Results are presented for individual subjects.
* The event was resolved, but a complication resulted as a consequence of the event.

The HI titre of 1:40, which has been determined as an immunologic correlate corresponding to a 50% reduction in the risk of contracting influenza, is based on studies in adults and may not be generalizable to children. Indeed, a recent study in a large pediatric population indicated that the conventional HI titre of 1:40 was only associated with 22% protection, whereas titres of 1:110 or 1:330 could predict a 50% or an 80% clinical protective level [21]. Our results demonstrate that in children receiving MF59-adjuvanted formulations, a high proportion reached the stringent HI cut-off titre ≥1:330 postvaccination, i.e. 69–90% and 97–100% after primary and booster doses, respectively, as compared with 41% in children receiving the non-adjuvanted vaccine. Following booster vaccination, 86% of subjects primed with the nonadjuvanted formulation reached the HI cut-off titre ≥1:330, demonstrating the ability of seasonal MF59 vaccine to boost the immune responses also in the subjects who previously received the nonadjuvanted vaccine. These findings provide further support for the use of MF59-adjuvanted vaccines in the pediatric population.

No vaccine-related SAEs were reported during the whole study duration of approximately 18 months, and no subjects were withdrawn due to AEs. These findings support the good safety profile of MF59-adjuvanted vaccines observed during other studies [10,11,26,33–36] and after the mass A/H1N1 pandemic vaccinations of 2009 [12].

In conclusion, a monovalent A/H1N1 vaccine containing 3.75 μg antigen and half the standard amount of MF59 per dose was highly immunogenic and well tolerated in healthy paediatric subjects aged 3–17 years, inducing antibody responses sufficient to meet all three CHMP licensure criteria after a single dose.

Author contributions

All authors participated in the conception, design and implementation of this clinical trial. All authors were involved in the interpretation of analysed data and the decision to submit for publication.

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Conflict of interest statement

ML, PP, AA, and GCD are permanent employees of Novartis Vaccines. MK received honoraria for contribution in advisory boards and received travel grants and honoraria for presentations. All other authors declare no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.10.085.

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