Immunogenicity and safety of cell-derived MF59®-adjuvanted A/H1N1 influenza vaccine for children

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Mass immunization of children has the potential to decrease infection rates and prevent the transmission of influenza. We evaluated the immunogenicity, safety, and tolerability of different formulations of cell-derived MF59-adjuvanted and nonadjuvanted A/H1N1 influenza vaccine in children and adolescents. This was a randomized, single-blind, multicenter study with a total of 666 healthy subjects aged 6 months–17 y in one of 3 vaccination groups, each receiving formulations containing different amounts of influenza A/H1N1 antigen with or without MF59. A booster trivalent seasonal MF59 vaccine was administered one year after primary vaccinations. Antibody titers were assessed by hemagglutination inhibition (HI) and microneutralization assays obtained on days 1, 22, 43, 366, and 387 (3 weeks post booster). Safety was monitored throughout the study. One vaccination with 3.75 μg of A/H1N1 antigen formulated with 50% MF59 (3.75_halfMF59) or 7.5 μg of A/H1N1 antigen formulated with 100% MF59 (7.5_fullMF59) induced an HI titer $\geq$1:40 in >70% of children in the 1–<3, 3–8, and 9–17 y cohorts; however, 2 vaccinations with nonadjuvanted 15 μg A/H1N1 antigen were needed to achieve this response in the 1–<3 and 3–8 y cohorts. Among children aged 6–11 months, 1 dose of 7.5_fullMF59 resulted in an HI titer $\geq$1:40 in >70% while 2 doses of 3.75_halfMF59 were required to achieve this result. All vaccines were well tolerated. Our findings support the immunogenicity and safety of the 3.75_halfMF59 (2 doses for children <12 months) and 7.5_fullMF59 vaccine formulations for use in children and adolescents aged 6 months to 17 y The use of the 3.75_halfMF59 could have the benefit of antigen and adjuvant sparing, increasing the available vaccine doses allowing vaccination of more people.

Introduction

Young people from birth to 18 y of age were at significantly higher risk from the 2009 A/H1N1 influenza pandemic than older adults,[1-3] with the most severe infections initially reported in children.[4] Estimates suggest that the cumulative rate of developing clinical illness was increased by 30- to 80-fold among people aged $\leq$24 years compared with individuals aged $\geq$65 years.[1] In addition to their heightened susceptibility to seasonal and pandemic influenza viruses, children are also important contributors to virus transmission.[5,6]

Mass immunization of children is considered essential to achieve disease control that will decrease infection rates and the risk of viral transmission within families and communities.[6-9]

Health authorities and vaccine manufacturers have focused on the development of vaccines based on the A/California/7/2009 viral strain, with various adjuvanted and nonadjuvanted formulations produced using both traditional and novel methods.[10] One important advance in the manufacture of influenza vaccines is the use of cell-culture to replace traditional egg-based production. This technology is not dependent on the availability of eggs, and facilitates enhanced manufacturing control and potentially reduction in production lead times, all of which could be pivotal in the event of another influenza pandemic. A number of controlled clinical trials confirm that oil-in-water adjuvants, such as MF59, meet the qualities required of a safe vaccine for diverse populations.[11-19] In addition, MF59 has been shown to heighten antibody response to the A/H1N1 pandemic virus[18,20–24] and

has been endorsed by the World Health Organization (WHO) for this indication.[25] This study evaluated the immunogenicity, safety, and tolerability of different formulations of cell-derived MF59-adjuvanted and nonadjuvanted A/H1N1 vaccine in healthy children and adolescents.

Results

A total of 666 subjects were enrolled (cohort 1; n = 159; cohort 2; n = 184; cohort 3; n = 172; cohort 4; n = 151). Data from one site (n = 86) were excluded from the analysis as a result of noncompliance with protocol requirements for a different clinical study. Subject disposition and demographics are shown in **Figure 1** and **Table 1**, respectively.

Immunogenicity
Figure 2 shows the cumulative percentage of subjects by hemagglutination inhibition (HI) titer following the initial injections (up to 43 days) in the 4 cohorts. In cohort 1 (9–17 years), there were 13- and 24-fold increases in geometric mean titer (GMT) in the 3.75_halfMF59 and 7.5_fullMF59 vaccine groups, respectively, 3 weeks following the first vaccination (**Table 2**). By day 43 (3 weeks following the second vaccination), GMT had increased 47-fold in the 3.75_halfMF59 group and 80-fold in the 7.5_fullMF59 group (**Table 2**). There were 6- to 14-fold increases in GMT across the 3 vaccine groups at day 22 for cohort 2 (3–8 years), with 28- to 115-fold increases across the 3 vaccine groups by day 43 (**Table 2**). Higher GMTs were evident for both adjuvanted groups compared with the nonadjuvanted group (**Table 2**). Three weeks following the first vaccination for cohort 3 (1–<3 years), there was a 6- to 7-fold increase in GMT from baseline in the adjuvanted vaccine groups compared with a 2-fold increase in the nonadjuvanted group (**Table 2**). By day 43, there was an 89- to 108-fold increase in GMT in the adjuvanted groups and an 11-fold increase in the nonadjuvanted group (**Table 2**). At day 22, there was an approximate 5- to 10-fold increase in GMT in the 3.75_halfMF59 and 7.5_fullMF59 groups, respectively, in cohort 4 (6–11 months), with a 92- to 129-fold increase by day 43 (**Table 2**).

All three European Committee for Medicinal Products for Human Use (CHMP) criteria were met for both the nonadjuvanted and adjuvanted groups following the second vaccination (day 43) in all cohorts (**Tables 3 and 4**). Following the first vaccination (day 22) for the adjuvanted formulations, the 3 CHMP criteria were met in cohorts 1, 2, and 3 and in the 7.5_fullMF59 group in cohort 4. The 3.75_halfMF59 group in cohort 4 did not meet the criterion of HI ≥1:40 at day 22 but met the other 2
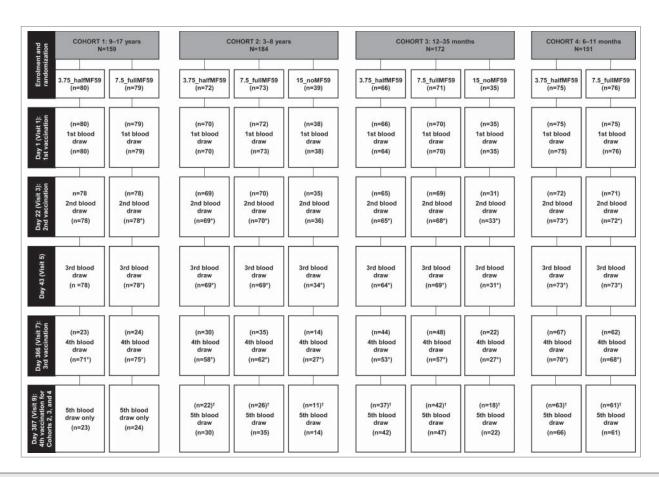

Figure 1. Subject disposition in each age cohort and vaccine group. *N values include blood draws outside the specified time window; †Fourth vaccination to be done only on the subset of naïve subjects <9 years of age. HI – hemagglutination inhibition.

Table 1. Demographics and other baseline characteristics of subjects in cohorts 1, 2, 3 and 4 (enrolled population, excluding non-compliant site)

	Cohort 1 (9–17 years)			Cohort 2 (3–8 years)			
	3.75 μg +halfMF59 (n = 80)	7.5 μg +fullMF59 (n = 79)	Total (n = 159)	3.75 μg +halfMF59 (n = 72)	7.5 μg +fullMF59 (n = 73)	15 μg +noMF59 (n = 39)	Total (n = 184)
Age (years), mean ± SD	13.2 ±2.7	13.2 ±2.7	13.2 ±2.7	5.5 ±1.9	5.3 ±1.7	5.2 ±1.5	5.3 ±1.7
Age (months), mean ± SD	166.9 ±32.7	167.5 ±31.4	167.2 ±32.0	71.6 ±24.7	69.6 ±21.6	68.4 ±20.1	70.1 ±22.5
Sex, n (%)							
Male	39 (49)	42 (53)	81 (51)	27 (38)	42 (58)	14 (36)	83 (45)
Female	41 (51)	37 (47)	78 (49)	45 (63)	31 (42)	25 (64)	101 (55)
Ethnic origin, n (%)							
Asian	2 (3)	0	2 (1)	0	1 (1)	1 (3)	2 (1)
Black	0	0	0	3 (4)	2 (3)	0	5 (3)
Caucasian	77 (96)	79 (100)	156 (98)	64 (89)	70 (96)	34 (87)	168 (91)
Hispanic	0	0	0	2 (3)	0	0	2 (1)
Other	1 (1)	0	1 (<1)	3 (4)	0	4 (10)	7 (4)
Height (cm), mean ± SD	158.8 ±16.0	159.8 ±15.6	159.3 ±15.7	116.8 ±13.5	114.8 ±11.5	113.0 ±11.4	115.2 ±12.3
Weight (kg), mean ± SD	51.4 ±15.7	52.5 ±16.2	51.9 ±15.9	22.1 ±6.5	21.6 ±5.3	21.1 ±5.9	21.7 ±5.9
Body mass index (kg/m²), mean ± SD	19.9 ±3.3	20.2 ±4.6	20.0 ±4.0	16.0 ±3.0	16.1 ±1.7	16.2 ±2.1	16.1 ±2.4
Previous influenza vaccination, n (%)	14 (18)	22 (28)	36 (23)	18 (25)	16 (22)	8 (21)	42 (23)

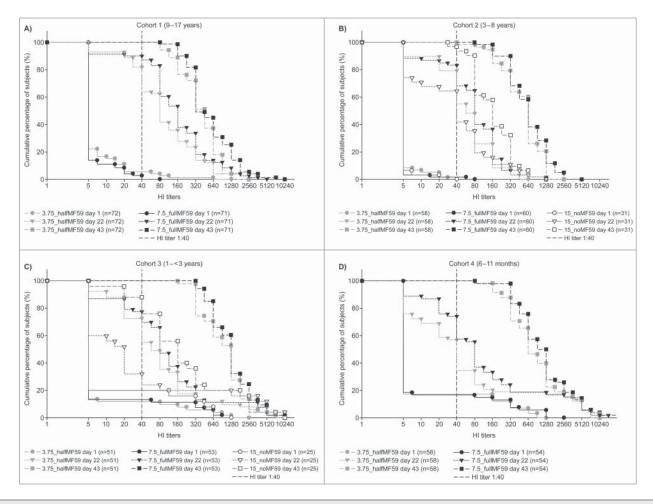|	Cohort 3 (1–<3 years)				Cohort 4 (6–11 months)		
	3.75 μg +halfMF59 (n = 66)	7.5 μg +fullMF59 (n = 71)	15 μg +noMF59 (n = 35)	Total (n = 172)	3.75 μg +halfMF59 (n = 75)	7.5 μg +fullMF59 (n = 76)	Total (n = 151)
Age (months), mean ± SD	21.8 ±7.3	23.1 ±7.1	23.1 ±7.8	22.6 ±7.3	8.9 ±1.5	8.8 ±1.7	8.9 ±1.6
Sex, n (%)							
Male	32 (48)	46 (65)	20 (57)	98 (57)	35 (47)	34 (45)	69 (46)
Female	34 (52)	25 (35)	15 (43)	74 (43)	40 (53)	42 (55)	82 (54)
Ethnic origin, n (%)							
Asian	2 (3)	2 (3)	2 (6)	6 (3)	0	0	0
Black	2 (3)	1 (1)	1 (3)	4 (2)	0	3 (4)	3 (2)
Caucasian	37 (56)	42 (59)	19 (54)	98 (57)	12 (16)	10 (13)	22 (15)
Hispanic	24 (36)	25 (35)	13 (37)	62 (36)	63 (84)	62 (82)	125 (83)
Other	1 (2)	1 (1)	0	2 (1)	0	1 (1)	1 (<1)
Height (cm), mean ± SD	83.9 ±7.0	85.7 ±7.8	85.0 ±7.2	84.9 ±7.4	71.4 ±4.8	71.4 ±4.0	71.4 ±4.4
Weight (kg), mean ± SD	12.5 ±2.4	13.0 ±2.6	12.0 ±2.1	12.6 ±2.4	9.1 ±1.3	9.1 ±1.4	9.1 ±1.4
Body mass index (kg/m²), mean ± SD	17.7 ±2.6	17.8 ±3.1	16.7 ±2.2	17.5 ±2.7	18.0 ±2.4	17.9 ±2.5	17.9 ±2.4
Previous influenza vaccination, n (%)	5 (8)	5 (7)	3 (9)	13 (8)	0	0	0

SD, standard deviation

Figure 2. Reverse cumulative distribution of hemagglutination inhibition (HI) titers in cohort 1 (**A**), cohort 2 (**B**), cohort 3 (**C**) and cohort 4 (**D**) on days 1, 22 and 43.

CHMP criteria. At day 22, the 15 μg nonadjuvanted group met the CHMP geometric mean ratio (GMR) and seroconversion criteria but not the HI ≥1:40 criterion in cohort 2, and did not meet any CHMP criteria in cohort 3.

One year after the primary vaccinations, hemagglutinating antibodies were still present in all vaccine groups in subjects of all 4 cohorts, with a trend to higher HI titers in subjects primed with 7.5_fullMF59 (**Table 3**). In addition, greater persistence was achieved in cohorts 2 and 3 in response to both doses of the adjuvanted formulation vs. the nonadjuvanted vaccine (**Table 3**). Following the booster vaccination, all vaccine groups and cohorts achieved an HI titer ≥1:40.

For the post-hoc analysis using an HI titer cutoff of ≥1:330, at day 43, 49–94% of subjects receiving the adjuvanted vaccines achieved this titer (**Table 5**), while only 10% and 24% of subjects achieved this titer following vaccination with the nonadjuvanted vaccines in cohorts 2 and 3, respectively. There was no apparent difference between the 2 adjuvanted formulations in the percentages of subjects achieving HI titers ≥1:330. Following the booster vaccination, 90–100% of subjects achieved an HI titer

≥1:330 with no difference between subjects primed with adjuvanted or non-adjuvanted formulations.

Analysis of results from the microneutralization (MN) titer assay revealed findings consistent with those for the HI assay, with the percentage of subjects with MN titer ≥1:40 substantially increased after the first and second vaccinations. The MN results for cohorts 2 and 3 were consistently lower for the nonadjuvanted groups. However, almost all subjects in the 3 vaccine groups in cohorts 2 and 3 demonstrated a 4-fold increase in MN titers following the second vaccination. A 4-fold increase in MN titers was evident 3 weeks after the first vaccination for approximately 50% of subjects in both vaccine groups in cohort 4, increasing to 100% at 3 weeks following the second vaccination (**Table 6**).

Safety

For all formulations of study vaccine, the majority of solicited local and systemic reactions were considered to be mild or moderate and most subjects recovered within 7 d of onset without sequelae (**Tables 7 and 8**). The rate of local and systemic reactions was lower after the second vaccination for all

Table 2. Geometric mean titers (GMT) and geometric mean ratios (GMR) – hemagglutination inhibition assay, per-protocol set*

| | Cohort 1 (9–17 years) | | Cohort 2 (3–8 years) | | | Cohort 3 (1–<3 years) | | | Cohort 4 (6–11 months) | |
	3.75 µg +halfM F59 (n = 72)	7.5 µg +fullM F59 (n = 71)	3.75 µg +halfM F59 (n = 58)	7.5 µg +fullM F59 (n = 60)	15 µg +noM F59 (n = 31)	3.75 µg +halfM F59 (n = 51)	7.5 µg +fullM F59 (n = 53)	15 µg +noM F59 (n = 25)	3.75 µg +halfM F59 (n = 58)	7.5 µg +fullM F59 (n = 54)
GMT day 1	6.72 (5.58–8.1)	5.86 (4.89–7.01)	5.65 (5.1–6.26)	5.39 (4.9–5.94)	5.38 (4.75–6.08)	7.33 (4.08–13)	8.52 (5.01–14)	12 (5.79–23)	8.35 (3.37–21)	8.33 (3.35–21)
GMT day 22	90 (61–131)	140 (97–203)	61 (41–90)	73 (50–107)	35 (21–56)	43 (21–91)	63 (32–123)	22 (9.26–54)	43 (13–148)	82 (24–282)
GMR day 22/day 1	13 (9.09–20)	24 (17–35)	11 (7.29–16)	14 (9.46–20)	7 (4.07–10)	6 (3.53–9.97)	7 (4.6–12)	2 (1.04–3.56)	5 (2.63–10)	10 (5.01–20)
GMT day 43	314 (254–387)	466 (380–572)	487 (379–626)	622 (492–788)	151 (112–204)	650 (419–1009)	917 (616–1366)	131 (77–221)	770 (431–1376)	1074 (600–1921)
GMR day 43/day 1	47 (36–61)	80 (61–103)	86 (67–111)	115 (91–147)	28 (21–38)	89 (54–145)	108 (69–169)	11 (6.23–20)	92 (51–166)	129 (72–232)
Pre-booster	**n = 21**	**n = 22**	**n = 24**	**n = 31**	**n = 11**	**n = 41**	**n = 41**	**n = 19**	**n = 52**	**n = 46**
GMT day 366	99 (49–198)	150 (78–286)	162 (106–248)	171 (118–248)	89 (52–151)	154 (81–295)	235 (130–425)	56 (26–119)	142 (76–267)	254 (129–501)
GMR day 366/day 1	17 (8.33–33)	25 (13–47)	28 (18–44)	29 (20–43)	17 (9.58–29)	23 (12–46)	32 (17–60)	5 (2.22–11)	18 (8.78–37)	33 (15–72)
Post-booster	**n = 19**	**n = 20**	**n = 20**	**n = 29**	**n = 10**	**n = 38**	**n = 38**	**n = 17**	**n = 47**	**n = 40**
GMT day 387	1701 (1114–2599)	1339 (934–1918)	4577 (2815–7443)	4496 (2982–6776)	2618 (1439–4764)	3389 (2021–5684)	3783 (2371–6034)	1852 (1007–3407)	5076 (3436–7499)	5994 (3920–9165)
GMR day 387/day 1	319 (198–515)	226 (151–340)	777 (448–1349)	760 (477–1211)	496 (251–977)	478 (200–1144)	548 (249–1205)	151 (54–424)	621 (215–1790)	712 (225–2253)
GMR day 387/366	15 (6.89–33)	8.47 (4.34–17)	28 (15–51)	26 (16–43)	30 (14–62)	19 (9.73–39)	17 (8.88–31)	36 (16–81)	33 (17–63)	23 (11–46)

*Data presented for days 1–366 are derived from a re-analysis of the original dataset following protocol violation regarding the administration of the booster. This violation required the retrospective removal of the non-compliant site data from the original data set. Data presented for the booster correspond to the original dataset as the non-compliant site did not contribute post-booster data.

Table 3. Number (percentage and 95% confidence interval) of subjects with hemagglutination inhibition titer ≥1:40 (per-protocol set)*

	Cohort 1 (9–17 years)		Cohort 2 (3–8 years)			Cohort 3 (1–<3 years)			Cohort 4 (6–11 months)	
	3.75 μg +halfMF59 (n = 72)	7.5 μg +fullMF59 (n = 71)	3.75 μg +halfMF59 (n = 58)	7.5 μg +fullMF59 (n = 60)	15 μg +noMF59 (n = 31)	3.75 μg +halfMF59 (n = 51)	7.5 μg +fullMF59 (n = 53)	15 μg +noMF59 (n = 25)	3.75 μg +halfMF59 (n = 58)	7.5 μg +fullMF59 (n = 54)
Day 1	4 (6)	2 (3)	0 (0)	1 (2)	0 (0)	6 (12)	7 (13)	5 (20)	10 (17)	9 (17)
	(2–14)	(0–10)	(0–6)	(0.042–9)	(0–11)	(4–24)	(5–25)	(7–41)	(9–29)	(8–29)
Day 22	59 (82)†	64 (90)†	46 (79)†	50 (83)†	20 (65)	37 (73)†	41 (77)†	8 (32)	33 (57)	40 (74)†
	(71–90)	(81–96)	(67–89)	(71–92)	(45–81)	(58–84)	(64–88)	(15–54)	(43–70)	(60–85)
Day 43	72 (100)†	71 (100)†	58 (100)†	60 (100)†	30 (97)†	51 (100)†	53 (100)†	22 (88)†	58 (100)†	54 (100)†
	(95–100)	(95–100)	(94–100)	(94–100)	(83–100)	(93–100)	(93–100)	(69–97)	(94–100)	(93–100)
Pre-booster	n = 21	n = 22	n = 24	n = 31	n = 11	n = 36	n = 41	n = 19	n=52	n = 46
Day 366	18 (86)†	21 (95)†	24 (100)†	31 (100)†	10 (91)†	36 (100)†	41 (100)†	14 (74)†	49 (94)†	46 (100)†
	(64–97)	(77–100)	(86–100)	(89–100)	(59–100)	(90–100)	(91–100)	(49–91)	(84–99)	(92–100)
Post-booster	n = 19	n = 20	n = 20	n = 29	n = 10	n = 32	n = 38	n = 17	n = 47	n = 40
Day 387	19 (100)†	20 (100)†	20 (100)†	29 (100)†	10 (100)†	32 (100)†	38 (100)†	17 (100)†	47 (100)†	40 (100)†
	(82–100)	(83–100)	(83–100)	(88–100)	(69–100)	(89–100)	(91–100)	(80–100)	(92–100)	(91–100)

*Data presented for days 1–366 are derived from a re-analysis of the original data set following protocol violation regarding the administration of the booster. This violation required the retrospective removal of the non-compliant site data from the original dataset. Data presented for the booster correspond to the original data set as the non-compliant site did not contribute post-booster data.
†Percentage of subjects achieving hemagglutination inhibition titer ≥1:40 is >70%.

Table 4. Number (percentage and 95% confidence interval) of subjects who achieved seroconversion or significant increase in hemagglutination inhibition (per-protocol set)*

	Cohort 1 (9–17 years)		Cohort 2 (3–8 years)			Cohort 3 (1–<3 years)			Cohort 4 (6–11 months)	
	3.75 µg +halfM F59 (n = 72)	7.5 µg +fullM F59 (n = 71)	3.75 µg +halfM F59 (n = 58)	7.5 µg +fullM F59 (n = 60)	15 µg +noM F59 (n = 31)	3.75 µg +halfM F59 (n = 51)	7.5 µg +fullM F59 (n = 53)	15 µg +noM F59 (n = 25)	3.75 µg +halfM F59 (n = 58)	7.5 µg +fullM F59 (n = 54)
Day 22	56 (78)† (66–87)	63 (89)† (79–95)	46 (79)† (67–89)	50 (83)† (71–92)	20 (65)† (45–81)	37 (73)† (58–84)	41 (77)† (64–88)	8 (32) (15–54)	33 (57)† (43–70)	40 (74)† (60–85)
Day 43	71 (99)† (93–100)	71 (100)† (95–100)	58 (100)† (94–100)	60 (100)† (94–100)	30 (97)† (83–100)	50 (98)† (90–100)	53 (100)† (93–100)	21 (84)† (64–95)	57 (98)† (91–100)	54 (100)† (93–100)
Pre-booster	n = 21	n = 22	n = 24	n = 31	n = 11	n = 36	n = 41	n = 19	n = 52	n = 46
Day 366 (from day 1)	18 (86) (64–97)	21 (95) (77–100)	24 (100) (86–100)	31 (100) (89–100)	10 (91) (59–100)	33 (92) (78–98)	37 (90) (77–97)	9 (47) (24–71)	43 (83) (70–92)	42 (91) (79–98)
Post-booster	n = 19	n = 20	n = 20	n = 29	n = 10	n = 32	n = 38	n = 17	n = 47	n = 40
Day 387 (from day 1)	19 (100) (82–100)	20 (100) (83–100)	20 (100) (83–100)	29 (100) (88–100)	10 (100) (69–100)	32 (100) (89–100)	38 (100) (91–100)	17 (100) (80–100)	46 (98) (89–100)	40 (100) (91–100)
Day 387 (from day 366)	17 (89)† (67–99)	16 (80)† (56–94)	19 (95)† (75–100)	29 (100)† (88–100)	10 (100)† (69–100)	30 (94)† (79–99)	36 (95)† (82–99)	17 (100)† (80–100)	40 (85)† (72–94)	36 (90)† (76–97)

*Data presented for days 1–366 are derived from a re-analysis of the original dataset following protocol violation regarding the administration of the booster. This violation required the retrospective removal of the non-compliant site data from the original data set. Data presented for the booster correspond to the original dataset as the non-compliant site did not contribute post-booster data.
†Percentage of subjects achieving seroconversion or significant increase in hemagglutination inhibition is ≥40 %.

Table 5. Number (percentage and 95% confidence interval) of subjects with hemagglutination inhibition ≥1:330 (per-protocol set)

	Cohort 1 (9–17 years)		Cohort 2 (3–8 years)			Cohort 3 (1–<3 years)			Cohort 4 (6–11 months)	
	3.75 µg +halfM F59 (n = 72)	7.5 µg +fullM F59 (n = 71)	3.75 µg +halfM F59 (n = 58)	7.5 µg +fullM F59 (n = 60)	15 µg +noM F59 (n = 31)	3.75 µg +halfM F59 (n = 51)	7.5 µg +fullM F59 (n = 53)	15 µg +noM F59 (n = 25)	3.75 µg +halfM F59 (n = 58)	7.5 µg +fullM F59 (n = 54)
Day 1	1 (1) (0.035–7)	0 (0) (0–5)	0 (0) (0–6)	0 (0) (0–6)	0 (0) (0–11)	3 (6) (1–16)	4 (8) (2–18)	3 (12) (3–31)	4 (7) (2–17)	4 (7) (2–18)
Day 22	10 (14) (7–24)	13 (18) (10–29)	2 (3) (0–12)	2 (3) (0–12)	2 (6) (1–21)	6 (12) (4–24)	7 (13) (5–25)	5 (20) (7–41)	11 (19) (10–31)	10 (19) (9–31)
Day 43	37 (51) (39–63)	35 (49) (37–61)	37 (64) (50–76)	41 (68) (55–80)	3 (10) (2–26)	38 (75) (60–86)	50 (94) (84–99)	6 (24) (9–45)	41 (71) (57–82)	45 (83) (71–92)
Pre-booster	n = 21	n = 22	n = 24	n = 31	n = 11	n = 36	n = 41	n = 19	n = 52	n = 46
Day 366 (6–46)	4 (19) (5–42)	5 (23) (8–45)	1 (4) (0–21)	4 (13) (4–30)	0 (0) (0–28)	5 (14) (5–29)	10 (24) (12–40)	4 (21)		
	13 (25) (14–39)	17 (37) (23–52)								
Post-booster	n = 19	n = 20	n = 20	n = 29	n = 10	n = 32	n = 38	n = 17	n = 47	n = 40
Day 387	18 (95) (74–100)	19 (95) (75–100)	20 (100) (83–100)	29 (100) (88–100)	9 (90) (55–100)	30 (94) (79–99)	38 (100) (91–100)	16 (94) (71–100)	47 (100) (92–100)	40 (100) (91–100)

Table 6. Summary of immunogenicity data based on the microneutralization (MN) assay (per-protocol set)[*]

Cohort 1 (9–17 years)		3.75 µg +halfMF59 (n = 72)	7.5 µg +fullMF59 (n = 71)
GMTs and GMRs (95% CI)	Day 1	5.84 (5.06–6.74)	5.21 (4.53–5.99)
	Day 22	84 (61–116)	150 (109–205)
	Day 22 over day 1	14 (11–20)	29 (21–39)
	Day 43	323 (260–402)	557 (452–687)
	Day 43 over day 1	55 (44–70)	107 (85–134)
	Pre-booster	**n = 21**	**n = 22**
	Day 366	63 (35–112)	66 (38–112)
	Day 366 over day 1	11 (6.66–20)	12 (7.27–20)
	Post-booster	**n = 19**	**n = 20**
	Day 387	1239 (616–2495)	1351 (746–2446)
	Day 387 over day 1	227 (98–526)	245 (120–499)
	Day 387 over day 366	16 (5.75–43)	20 (8.37–46)
Subjects (n,%) with MN titers ≥1:40 (95% CI)		**n = 72**	**n = 71**
	Day 1	3 (4) (1–12)	0 (0) (0–5)
	Day 22	55 (76) (65–86)	67 (94) (86–98)
	Day 43	72 (100) (95–100)	71 (100) (95–100)
	Pre-booster	**n = 21**	**n = 22**
	Day 366	15 (71) (48–89)	18 (82) (60–95)
	Post-booster	**n = 19**	**n = 20**
	Day 387	(95) (74–100)	20 (100) (83–100)
Subjects (n,%) with an at least 4-fold increase in MN titers (95% CI)		**n = 72**	**n = 71**
	Day 22	62 (86) (76–93)	68 (96) (88–99)
	Day 43	71 (99) (93–100)	71 (100) (95–100)
	Pre-booster	**n = 21**	**n = 22**
	Day 366 to day 1	17 (81) (58–95)	19 (86) (65–97)
	Post-booster	**n = 19**	**n = 20**
	Day 387 to day 1	(95) (74–100)	20 (100) (83–100)
	Day 387 to day 366	(89) (67–99)	(85) (62–97)

Cohort 2 (3–8 years)		3.75 µg +halfMF59 (n = 58)	7.5 µg +fullMF59 (n = 60)	15 µg +noMF59 (n = 31)
GMTs and GMRs (95% CI)	Day 1	5.08 (4.59–5.62)	5.16 (4.69–5.68)	5.37 (4.76–6.07)
	Day 22	50 (31–82)	64 (41–100)	33 (19–60)
	Day 22 over day 1	9.93 (6.16–16)	12 (7.89–19)	6.22 (3.51–11)
	Day 43	536 (400–719)	668 (507–879)	160 (113–228)
	Day 43 over day 1	106 (78–144)	129 (97–173)	30 (21–43)
	Pre-booster	**n = 24**	**n = 31**	**n = 11**
	Day 366	56 (33–93)	100 (64–156)	40 (21–76)
	Day 366 over day 1	10 (6.13–18)	19 (12–29)	7.49 (3.89–14)
	Post-booster	**n = 20**	**n = 29**	**n = 10**
	Day 387	4190 (2442–7188)	4411 (2797–6956)	2232 (1149–4338)
	Day 387 over day 1	775 (416–1444)	817 (483–1381)	416 (193–895)
	Day 387 over day 366	75 (43–132)	45 (28–72)	53 (27–106)
Subjects (n,%) with MN titers ≥1:40 (95% CI)		**n = 58**	**n = 60**	**n = 31**
	Day 1	0 (0) (0–6)	1 (2) (0.042–9)	0 (0) (0–11)
	Day 22	35 (60) (47–73)	44 (73) (60–84)	18 (58) (39–75)
	Day 43	57 (98) (91–100)	60 (100) (94–100)	26 (84) (66–95)
	Pre-booster	**n = 24**	**n = 31**	**n = 11**
	Day 366	17 (71) (49–87)	28 (90) (74–98)	6 (55) (23–83)
	Post-booster	**n = 20**	**n = 29**	**n = 10**
	Day 387	20 (100) (83–100)	29 (100) (88–100)	10 (100) (69–100)
Subjects (n,%) with an at least 4-fold increase in MN titers (95% CI)		**n = 58**	**n = 60**	**n = 31**
	Day 22	46 (79) (67–89)	49 (82) (70–90)	17 (55) (36–73)
	Day 43	58 (100) (94–100)	60 (100) (94–100)	30 (97) (83–100)
	Pre-booster	**n = 24**	**n = 31**	**n = 11**
	Day 366 to day 1	22 (92) (73–99)	30 (97) (83–100)	8 (73) (39–94)
	Post-booster	**n = 20**	**n = 29**	**n = 10**
	Day 387 to day 1	20 (100) (83–100)	29 (100) (88–100)	10 (100) (69–100)
	Day 387 to day 366	20 (100) (83–100)	29 (100) (88–100)	10 (100) (69–100)

(continued on next page)

Table 6. Summary of immunogenicity data based on the microneutralization (MN) assay (per-protocol set)* (*Continued*)

Cohort 3 (12–35 months)		3.75 μg +halfMF59 (n = 51)	7.5 μg + fullMF59 (n = 54)	15 μg +noMF59 (n = 25)
GMTs and GMRs (95% CI)	Day 1	5.05 (2.88–8.88)	6.14 (3.68–10)	8.71 (4.45–17)
	Day 22	21 (8.17–52)	32 (14–73)	14 (4.74–43)
	Day 22 over day 1	4.07 (2.19–7.56)	5.15 (2.94–9.02)	1.63 (0.78–3.42)
	Day 43	580 (352–957)	764 (486–1203)	100 (55–181)
	Day 43 over day 1	115 (76–174)	125 (85–182)	11 (6.96–19)
	Pre-booster	**n = 36**	**n = 41**	**n = 19**
	Day 366	175 (82–370)	292 (147–579)	71 (30–171)
	Day 366 over day 1	26 (13–53)	42 (22–79)	5.98 (2.64–14)
	Post-booster	**n = 31**	**n = 38**	**n = 17**
	Day 387	5645 (3371–9452)	6505 (4091–10343)	2983 (1628–5464)
	Day 387 over day 1	735 (308–1754)	998 (456–2182)	232 (83–643)
	Day 387 over day 366	28 (12–62)	23 (11–48)	46 (18–119)
Subjects (n,%) with MN titers ≥1:40 (95% CI)		**n = 51**	**n = 54**	**n = 25**
	Day 1	4 (8) (2–19)	6 (11) (4–23)	5 (20) (7–41)
	Day 22	24 (47) (33–62)	30 (56) (41–69)	7 (28) (12–49)
	Day 43	51 (100) (93–100)	54 (100) (93–100)	20 (80) (59–93)
	Pre-booster	**n = 36**	**n = 41**	**n = 19**
	Day 366	34 (94) (81–99)	40 (98) (87–100)	13 (68) (43–87)
	Post-booster	**n = 31**	**n = 38**	**n = 17**
	Day 387	31 (100) (89–100)	38 (100) (91–100)	17 (100) (80–100)
Subjects (n,%) with an at least 4-fold increase in MN titers (95% CI)		**n = 51**	**n = 54**	**n = 25**
	Day 22	30 (59) (44–72)	40 (74) (60–85)	9 (36) (18–57)
	Day 43	51 (100) (93–100)	54 (100) (93–100)	22 (88) (69–97)
	Pre-booster	**n = 36**	**n = 41**	**n = 19**
	Day 366 to day 1	34 (94) (81–99)	41 (100) (91–100)	9 (47) (24–71)
	Post-booster	**n = 31**	**n = 38**	**n = 17**
	Day 387 to day 1	31 (100) (89–100)	38 (100) (91–100)	17 (100) (80–100)
	Day 387 to day 366	(90) (74–98)	(87) (72–96)	(94) (71–100)

Cohort 4 (6–11 months)		3.75 μg +halfMF59 (n = 58)	7.5 μg +fullMF59 (n = 54)	
GMTs and GMRs (95% CI)	Day 1	8.78 (3.34–23)	8.59 (3.25–23)	
	Day 22	25 (5.77–105)	46 (11–197)	
	Day 22 over day 1	2.8 (1.32–5.97)	5.36 (2.51–11)	
	Day 43	759 (387–1486)	1039 (529–2038)	
	Day 43 over day 1	86 (49–153)	121 (68–214)	
	Pre-booster	**n = 52**	**n = 46**	
	Day 366	195 (105–364)	388 (198–758)	
	Day 366 over day 1	24 (12–50)	50 (23–110)	
	Post-booster	**n = 47**	**n = 39**	
	Day 387	9302 (6597–13118)	9482 (6492–13849)	
	Day 387 over day 1	1097 (354–3397)	1122 (323–3899)	
	Day 387 over day 366	43 (22–84)	23 (11–48)	
Subjects (n,%) with MN titers ≥1:40 (95% CI)		**n = 58**	**n = 54**	
	Day 1	10 (17) (9–29)	9 (17) (8–29)	
	Day 22	17 (29) (18–43)	25 (46) (33–60)	
	Day 43	58 (100) (94–100)	54 (100) (93–100)	
	Pre-booster	**n = 52**	**n = 46**	
	Day 366	50 (96) (87–100)	46 (100) (92–100)	
	Post-booster	**n = 47**	**n = 39**	
	Day 387	47 (100) (92–100)	39 (100) (91–100)	
Subjects (n,%) with an at least 4-fold increase in MN titers (95% CI)		**n = 58**	**n = 54**	
	Day 22	25 (43) (30–57)	32 (59) (45–72)	
	Day 43	58 (100) (94–100)	54 (100) (93–100)	
	Pre-booster	**n = 52**	**n = 46**	
	Day 366 to day 1	47 (90) (79–97)	43 (93) (82–99)	
	Post-booster	**n = 47**	**n = 39**	
	Day 387 to day 1	(98) (89–100)	(97) (87–100)	
	Day 387 to day 366	(83) (69–92)	(79) (64–91)	

*Data presented for days 1–366 are derived from a re-analysis of the original dataset following protocol violation regarding the administration of the booster. This violation required the retrospective removal of the non-compliant site data from the original dataset. Data presented for the booster correspond to the original dataset as the non-compliant site did not contribute post-booster data. CI, confidence interval; GMR, geometric mean ratio; GMT, geometric mean titer.

Table 7. Numbers (%) of subjects with any (and severe/>100 mm) local reaction within 7 days after each vaccination (safety population)

Cohort 1 (9–17 years)		First vaccination		Second vaccination		Booster vaccination	
		3.75 μg +halfM F59 (n = 79)	7.5 μg +fullM F59 (n = 79)	3.75 μg +halfM F59 (n = 77)	7.5 μg +fullM F59 (n = 78)	3.75 μg +halfM F59 → trivalent seasonal MF59 vaccine (n = 23)	7.5 μg +fullM F59 → trivalent seasonal MF59 vaccine (n = 24)
Ecchymosis	Any	3 (4)	5 (6)	3 (4)	2 (3)	2 (9)	0
	>100 mm	0	0	0	0	0	0
Erythema	Any	6 (8)	7 (9)	4 (5)	10 (13)	2 (9)	3 (13)
	>100 mm	0	0	0	0	0	0
Induration	Any	9 (11)	12 (15)	7 (9)	5 (6)	5 (22)	3 (13)
	>100 mm	0	0	0	0	0	0
Swelling	Any	5 (6)	10 (13)	4 (5)	7 (9)	0	4 (17)
	>100 mm	0	0	0	0	0	0
Pain	Any	54 (68)	65 (82)	42 (55)	43 (55)	17 (74)	19 (79)
	Severe	0	1 (1)	0	1 (1)	3 (13)	2 (8)

Cohort 2 (3–8 years)		First vaccination			Second vaccination			Booster vaccination			Second seasonal vaccination		
		3.75 μg +halfMF59 (n = 70)	7.5 μg +fullMF59 (n = 72)	15 μg +noMF59 (n = 39)	3.75 μg +halfMF59 (n = 70)	7.5 μg +fullMF59 (n = 70)	15 μg +noMF59 (n = 36)	3.75 μg +halfMF59 → trivalent seasonal MF59 vaccine (n = 30)	7.5 μg +fullMF59 → trivalent seasonal MF59 vaccine (n = 35)	15 μg +noMF59 → trivalent seasonal MF59 vaccine (n = 14)	3.75 μg +halfMF59 → 2× trivalent seasonal MF59 vaccine (n = 22)	7.5 μg +fullMF59 → 2× trivalent seasonal MF59 vaccine (n = 26)	15 μg +noMF59 → 2× trivalent seasonal MF59 vaccine (n = 11)
Ecchymosis	Any	4 (6)	6 (8)	7 (18)	6 (9)	5 (7)	4 (11)	1 (3)	1 (3)	0	0	1 (4)	0
	>100 mm	0	0	0	0	0	0	0	0	0	0	0	0
Erythema	Any	11 (16)	14 (19)	7 (18)	7 (10)	14 (20)	3 (8)	8 (27)	5 (14)	0	4 (18)	6 (23)	0
	>100 mm	0	0	0	0	0	0	0	0	0	0	0	0
Induration	Any	6 (9)	11 (15)	3 (8)	4 (6)	10 (14)	3 (8)	4 (13)	5 (14)	0	4 (18)	7 (27)	0
	>100 mm	0	0	0	0	0	0	0	0	0	0	0	0
Swelling	Any	5 (7)	7 (10)	2 (5)	5 (7)	5 (7)	2 (6)	3 (10)	5 (14)	1 (7)	4 (18)	5 (19)	1 (9)
	>100 mm	0	0	0	0	0	0	0	0	0	0	0	0
Pain	Any	30 (43)	33 (46)	14 (36)	26/69 (38)	30 (43)	10 (28)	23 (77)	24 (69)	9 (64)	13 (59)	13 (50)	7 (64)
	Severe	1 (1)	2 (3)	0	1/69 (1)	0	0	1 (3)	1 (3)	0	0	1 (4)	0

Cohort 3 (1–<3 years)

		First vaccination			Second vaccination			First booster vaccination			Second seasonal vaccination		
		3.75 μg + halfMF59 (n = 65)	7.5 μg + fullMF59 (n = 73)	15 μg + noMF59 (n = 33)	3.75 μg + halfMF59 (n = 64)	7.5 μg + fullMF59 (n = 71)	15 μg + noMF59 (n = 29)	3.75 μg + halfMF59 → trivalent seasonal MF59 vaccine (n = 42)	7.5 μg + fullMF59 → trivalent seasonal MF59 vaccine (n = 51)	15 μg + noMF59 → trivalent seasonal MF59 vaccine (n = 21)	3.75 μg + halfMF59 → 2× trivalent seasonal MF59 vaccine (n = 36)	7.5 μg + fullMF59 → 2× trivalent seasonal MF59 vaccine (n = 45)	15 μg + noMF59 → 2× trivalent seasonal MF59 vaccine (n = 17)
Ecchymosis (mm)	Any	3 (5)	8 (11)	5 (15)	1 (2)	5 (7)	2 (7)	2 (5)	5 (10)	0	0	1 (2)	1 (6)
	>100 mm	0	0	0	0	0	0	0	0	0	0	0	0
Erythema (mm)	Any	8 (12)	12 (16)	6 (18)	6 (9)	11 (15)	4 (14)	6 (14)	13 (25)	4 (19)	10 (28)	10 (22)	2 (12)
	>100 mm	0	0	0	0	1 (1)	0	0	0	0	0	0	0
Induration (mm)	Any	6 (9)	10 (14)	3 (9)	2 (3)	4 (6)	2 (7)	4 (10)	7 (14)	3 (14)	8 (22)	8 (18)	3 (18)
	>100 mm	0	0	0	0	0	0	0	0	0	0	0	0
Swelling (mm)	Any	1 (2)	4 (5)	2 (6)	1 (2)	4 (6)	1 (3)	6 (14)	4 (8)	1 (5)	7 (19)	7 (16)	2 (12)
	>100 mm	0	1 (1)	0	0	0	0	0	0	0	0	0	0
Tenderness	Any	7 (11)	30 (41)	10 (30)	5 (8)	21 (30)	9 (31)	8 (19)	18 (35)	7 (33)	8 (22)	11 (24)	4 (24)
	Cried when injected limb was moved	0	3 (4)	1 (3)	0	1 (1)	0	1 (2)	0	0	0	0	1 (6)

Cohort 4 (6–11 months)

		First vaccination		Second vaccination		Booster vaccination		Booster vaccination	
		3.75 μg + halfMF59 (n = 75)	7.5 μg + fullMF59 (n = 74)	3.75 μg + halfMF59 (n = 71)	7.5 μg + fullMF59 (n = 70)	3.75 μg + halfMF59 → trivalent seasonal MF59 vaccine (n = 67)	7.5 μg + fullMF59 → trivalent seasonal MF59 vaccine (n = 61)	3.75 μg + halfMF59 → 2× trivalent seasonal MF59 vaccine (n = 63)	7.5 μg + fullMF59 → 2× trivalent seasonal MF59 vaccine (n = 60)
Ecchymosis (mm)	Any	2 (3)	0	3 (4)	0	1 (1)	1 (2)	1 (2)	1 (2)
	>100 mm	0	0	0	0	0	0	0	0
Erythema (mm)	Any	6 (8)	5 (7)	9 (13)	3 (4)	5 (7)	9 (15)	6 (10)	6 (10)
	>100 mm	0	0	0	0	0	1 (2)	0	0
Induration (mm)	Any	4 (5)	3 (4)	3 (4)	7 (10)	5 (7)	4 (7)	6 (10)	5 (8)
	>100 mm	0	0	0	0	0	0	0	0
Swelling (mm)	Any	0	0	1 (1)	0	1 (1)	5 (8)	3 (5)	3 (5)
	>100 mm	0	0	0	0	0	0	0	0
Tenderness	Any	9 (12)	17 (23)	12 (17)	18 (26)	11 (16)	19 (31)	14 (22)	13 (22)
	Cried when injected limb was moved	0	2 (3)	0	1 (1)	2 (3)	2 (3)	2 (3)	3 (5)

Table 8. Numbers (%) of subjects with any (and severe) systemic reaction within 7 d after each vaccination (safety population)

Cohort 1 (9–17 years)

		First vaccination		Second vaccination		Booster vaccination	
		3.75 μg + halfMF59 (n=79)	7.5 μg + fullMF59 (n=79)	3.75 μg + halfMF59 (n=77)	3.75 μg + halfMF59 (n=78)	3.75 μg + halfMF59 → trivalent seasonal MF59 vaccine (n=23)	7.5 μg + fullMF59 → trivalent seasonal MF59 vaccine (n=24)
Chills	Any	1 (1)	3 (4)	2 (3)	7 (9)	1 (4)	1 (4)
	Severe	0	0	0	0	0	0
Malaise	Any	6 (8)	9 (11)	6 (8)	8 (10)	5 (22)	3 (13)
	Severe	1 (1)	0	0	0	0	0
Myalgia	Any	16 (20)	16 (20)	8 (10)	8 (10)	7 (30)	8 (33)
	Severe	1 (1)	0	0	0	0	0
Arthralgia	Any	6 (8)	8 (10)	6 (8)	4 (5)	1 (4)	2 (8)
	Severe	0	0	0	0	0	0
Headache	Any	18 (23)	23 (29)	11 (14)	10 (13)	5 (22)	5 (21)
	Severe	2 (3)	2 (3)	2 (3)	0	1 (4)	0
Sweating	Any	4 (5)	3 (4)	1 (1)	0	2 (9)	2 (8)
	Severe	0	0	0	0	0	0
Fatigue	Any	16 (20)	21 (27)	12 (16)	11 (14)	4 (17)	2 (8)
	Severe	1 (1)	1 (1)	3 (4)	1 (1)	1 (4)	0
Nausea	Any	5 (6)	6 (8)	4 (5)	3 (4)	4 (17)	2 (8)
	Severe	1 (1)	0	0	0	0	0
Fever (≥38°C)	Yes	1 (1)	0	2 (3)	1 (1)	1 (4)	1 (4)
Other							
Temperature (°C)	<38.0°C	78 (99)	79 (100)	75 (97)	77 (99)	22 (96)	24 (100)
	≥40.0°C	0	0	0	0	0	0
Stayed home	Yes	2 (3)	0	3 (4)	3 (4)	3 (13)	2 (8)
Analgesic/antipyretic medications used	Yes	7 (9)	7 (9)	5 (6)	5 (6)	4 (17)	3 (13)

Cohort 2 (3–8 years)

		First vaccination			Second vaccination			Booster vaccination			Second seasonal vaccination		
		3.75 μg + halfMF59 (n = 70)	7.5 μg + fullMF59 (n = 72)	15 μg + noMF59 (n = 39)	3.75 μg + halfMF59 (n = 69)	7.5 μg + fullMF59 (n = 70)	15 μg + noMF59 (n = 36)	3.75 μg + halfMF59 → trivalent seasonal MF59 vaccine (n = 30)	7.5 μg + fullMF59 → trivalent seasonal MF59 vaccine (n = 35)	15 μg + noMF59 → trivalent seasonal MF59 vaccine (n = 14)	3.75 μg + halfMF59 → 2× trivalent seasonal MF59 vaccine (n = 22)	7.5 μg + fullMF59 → 2× trivalent seasonal MF59 vaccine (n = 26)	15 μg + noMF59 → 2× trivalent seasonal MF59 vaccine (n = 11)
Chills	Any	3 (4)	2 (3)	0	1 (1)	5 (7)	2 (6)	3 (10)	5 (14)	0	0	1 (4)	0
	Severe	0	0	0	0	0	0	0	2 (6)	0	0	0	0
Malaise	Any	9 (13)	3 (4)	6 (15)	4 (6)	4 (6)	4 (11)	8 (27)	11 (31)	0	1 (5)	2 (8)	1 (9)
	Severe	2 (3)	2 (3)	0	0	0	0	0	1 (3)	0	1 (5)	0	0
Myalgia	Any	5 (7)	3 (4)	2 (5)	2 (3)	8 (11)	2 (6)	4 (13)	9 (26)	4 (29)	1 (5)	2 (8)	1 (9)
	Severe	0	0	0	0	0	0	0	1 (3)	0	0	0	0

Arthralgia	Any	2 (3)	3 (4)	0	3 (4)	2 (6)	5 (17)	5 (14)	2 (14)	1 (5)	2 (8)	1 (9)
	Severe	0	0	0	0	0	0	0	0	0	0	0
Headache	Any	7 (10)	5 (7)	1 (3)	1 (1)	4 (6)	6 (17)	10 (33)	11 (31)	1 (7)	3 (14)	3 (12)	1 (9)
	Severe	2 (3)	1 (1)	0	0	0	0	0	1 (3)	0	0	0	0
Sweating	Any	1 (1)	1 (1)	0	0	0	0	2 (7)	3 (9)	0	0	1 (4)	0
	Severe	0	0	0	0	0	0	0	1 (3)	0	0	0	0
Fatigue	Any	17 (24)	15 (21)	7 (18)	8 (12)	10 (14)	5 (14)	7 (23)	10 (29)	1 (7)	3 (14)	6 (23)	2 (18)
	Severe	2 (3)	3 (4)	0	0	0	0	0	1 (3)	0	0	0	0
Nausea	Any	2 (3)	5 (7)	2 (5)	3 (4)	1 (1)	2 (6)	4 (13)	6 (17)	1 (7)	1 (5)	2 (8)	1 (9)
	Severe	1 (1)	1 (1)	0	0	0	0	0	1 (5)	0	1 (5)	0	0
Fever (≥38 °C)	Yes	7 (10)	4 (6)	0	3 (4)	3 (4)	3 (8)	0	8 (23)	0	1 (5)	2 (8)	1 (9)
Other													
Temperature	<38.0 °C	63 (90)	68 (94)	39 (100)	66 (96)	67 (96)	33 (92)	30 (100)	27 (77)	14 (100)	21 (95)	24 (92)	10 (91)
	≥40.0 °C	0	0	0	0	0	0	0	0	0	0	0	0
Stayed home	Yes	5 (7)	10 (14)	0	1 (1)	6 (9)	3 (8)	3 (10)	10 (29)	0	1 (5)	0	0
Analgesic/ antipyretic medications used	Yes	9 (13)	11 (15)	4 (10)	2 (3)	7 (10)	2 (6)	4 (13)	8 (23)	1 (7)	2 (9)	6 (23)	1 (9)

Cohort 3 (1–<3 years)

		First vaccination			Second vaccination			Booster vaccination			Second seasonal vaccination		
		3.75 μg + halfMF59 (n = 65)	7.5 μg + fullMF59 (n = 73)	15 μg + noMF59 (n = 33)	3.75 μg + halfMF59 (n = 64)	7.5 μg + fullMF59 (n = 71)	15 μg + noMF59 (n = 29)	3.75 μg + halfMF59 → trivalent seasonal MF59 vaccine (n = 42)	7.5 μg + fullMF59 → trivalent seasonal MF59 vaccine (n = 51)	15 μg + noMF59 → trivalent seasonal MF59 vaccine (n = 21)	3.75 μg + halfMF59 → 2× trivalent seasonal MF59 vaccine (n = 36)	7.5 μg + fullMF59 → 2× trivalent seasonal MF59 vaccine (n = 45)	15 μg + noMF59 → 2× trivalent seasonal MF59 vaccine (n=17)
Sleepiness	Present	14 (22)	23 (32)	7 (21)	11 (17)	13 (18)	4 (14)	9 (21)	6 (12)	1 (5)	1 (3)	1 (2)	1 (6)
Diarrhea	Present	15 (23)	17 (23)	6 (18)	10 (16)	10 (14)	4 (14)	6 (14)	4 (8)	1 (5)	3 (8)	3 (7)	0
Vomiting	Present	7 (11)	11 (15)	2 (6)	5 (8)	8 (11)	1 (3)	1 (2)	2 (4)	0	1 (3)	1 (2)	0
Irritability	Present	17 (26)	17 (23)	5 (15)	9 (14)	6 (8)	2 (7)	5 (12)	3 (6)	3 (14)	2 (6)	3 (7)	3 (18)
Change in eating habits	Present	14 (22)	18 (25)	4 (12)	12 (19)	11 (15)	4 (14)	5 (12)	4 (8)	1 (5)	3 (8)	2 (4)	1 (6)
Shivering	Present	3 (5)	2 (3)	1 (3)	4 (6)	4 (6)	1 (3)	1 (2)	3 (6)	0	1 (3)	0	0
Unusual crying	Present	15 (23)	22 (30)	7 (21)	11 (17)	11 (15)	3 (10)	3 (7)	4 (8)	1 (5)	1 (3)	3 (7)	1 (6)
Fever (≥38 °C)	Yes	8 (12)	8 (11)	4 (12)	12 (19)	7 (10)	1 (3)	6 (14)	15 (29)	2 (10)	3 (8)	4 (9)	0
Other													
Temperature	<38.0° C	57 (88)	65 (89)	29 (88)	52 (81)	64 (90)	28 (97)	36 (86)	36 (71)	19 (90)	33 (92)	41 (91)	17 (100)
	≥40.0° C	0	0	0	0	1 (1)	0	1 (2)	0	1 (5)	0	0	0
Stayed home	Yes	6 (9)	8 (11)	2/32 (6)	8 (13)	2 (3)	3 (10)	5/41 (12)	1/50 (2)	2 (10)	1 (3)	0	0
Analgesic antipyretic medications used	Yes	15 (23)	21 (29)	8/32 (25)	18 (28)	15 (21)	8 (28)	8 (19)	15 (29)	5 (24)	3 (8)	6 (13)	1 (6)

Table 8. Numbers (%) of subjects with any (and severe) systemic reaction within 7 d after each vaccination (safety population) *(Continued)*

Cohort 4 (6–11 months)		First vaccination		Second vaccination		Booster vaccination		Second seasonal vaccination	
		3.75 µg + halfMF59 (n = 75)	7.5 µg + fullMF59 (n = 74)	3.75 µg + halfMF59 (n = 71)	7.5 µg + fullMF59 (n = 70)	3.75 µg + halfMF59 → trivalent seasonal MF59 vaccine (n = 67)	7.5 µg + fullMF59 → trivalent seasonal MF59 vaccine (n = 61)	3.75 µg + halfMF59 → 2× trivalent seasonal MF59 vaccine (n = 63)	7.5 µg + fullMF59 → 2× trivalent seasonal MF59 vaccine (n = 60)
Sleepiness	Present	12 (16)	15 (20)	10 (14)	13 (19)	5 (7)	10 (16)	3 (5)	5 (8)
Diarrhea	Present	20 (27)	22 (30)	13 (18)	20 (29)	8 (12)	6 (10)	7 (11)	7 (12)
Vomiting	Present	12 (16)	11 (15)	8 (11)	10 (14)	2 (3)	1 (2)	5 (8)	2 (3)
Irritability	Present	9 (12)	11 (15)	9 (13)	6 (9)	3 (4)	6 (10)	4 (6)	2 (3)
Change in eating habits	Present	13 (17)	21 (28)	10 (14)	12 (17)	2 (3)	7 (11)	6 (10)	5 (8)
Shivering	Present	3 (4)	2 (3)	2 (3)	4 (6)	3 (4)	2 (3)	2 (3)	1 (2)
Unusual crying	Present	20 (27)	16 (22)	15 (21)	17 (24)	4 (6)	9 (15)	8 (13)	3 (5)
Fever ($\geq$38 °C)	Yes	7 (9)	7 (9)	7 (10)	12 (17)	9 (13)	13 (21)	7 (11)	10 (17)
Other									
Temperature	<38.0°C	68 (91)	67 (91)	64 (90)	58 (83)	58 (87)	48 (79)	56 (89)	50 (83)
	$\geq$40.0°C	0	1 (1)	1 (1)	0	1 (1)	0	0	0
Stayed home	Yes	8 (11)	5 (7)	7 (10)	7 (10)	5 (7)	5 (8)	2 (3)	3 (5)
Analgesic/antipyretic medications used	Yes	16 (21)	21 (28)	16 (23)	17 (24)	13 (19)	19 (31)	10 (16)	11 (18)

groups and cohorts. Within each cohort, the occurrence of local and systemic reactions was higher in the 7.5_fullMF59 groups than the 3.75_halfMF59 groups. Systemic reactions were reported by more subjects in the adjuvanted groups than in the nonadjuvanted group in cohort 3, but there was no clear difference in the occurrence of systemic reactions between adjuvanted and non-adjuvanted groups in cohort 2. There was lower reactogenicity in all cohorts following the administration of the seasonal MF59 vaccine after one year.

Unsolicited adverse events (AEs) through day 43 were reported by 32–34% of subjects in cohort 1 and 35–36% of cohort 2, with higher rates for cohort 3 (37–61%) and for cohort 4 (63–64%). Nasopharyngitis, diarrhea, vomiting, eating disorder, and pyrexia were the most frequently reported unsolicited AEs for cohorts 3 and 4 that were considered possibly related to vaccine. The rate of unsolicited AEs following booster vaccinations was low, ranging from 5% in cohort 1, 7% cohort 2, 10% cohort 3, and 24% of subjects in cohort 4.

From day 1–546, 48 subjects experienced 65 serious AEs (SAEs), with higher rates evident for cohorts 3 and 4. A SAE considered possibly related to vaccine was reported for 3 subjects: multiple seizure episodes at day 98 affecting one subject in cohort 1 (7.5_fullMF59); vomiting in one subject in cohort 3, 26 d after the third vaccination (7.5_fullMF59); and febrile convulsion at day 10 in one subject in cohort 4 (3.75_halfMF59). Eight subjects, 2 in each of the 4 cohorts (3.75_halfMF59 n = 4; 7.5_fullMF59 n = 2; 15_noMF59 n = 2), discontinued study participation from day 1–546 due to at least one AE, with all withdrawals occurring before administration of the booster. No deaths occurred during the study.

Discussion

Effective, well-tolerated A/H1N1 vaccines are needed for children, particularly those aged 6–12 months, who are at high risk of contracting the virus due to lack of protection and priming of the immune system by exposure to the influenza virus. Children also contribute to virus transmission to family members and the community at large.[5,6] Our results demonstrate that nonadjuvanted A/H1N1 vaccines are less immunogenic than adjuvanted formulations for children. Low immunogenicity of nonadjuvanted vaccines in children has also been observed in other studies; for example, HI titers ≥1:40 were observed in 45% and 69% of subjects aged 6–35 months and 3–9 years, respectively, following a single dose of nonadjuvanted A/California/07/2009 vaccine, compared with rates of 95% and 94% in subjects aged 18–64 y and ≥65 years, respectively.[26]

Our study showed that one vaccination with 3.75_halfMF59 or 7.5_fullMF59 induced an HI titer of ≥1:40 in >70 % of children and adolescents in the 1–<3 year, 3–8 year, and 9–17 y cohorts; however, 2 vaccinations with nonadjuvanted 15 μg A/H1N1 antigen were needed to achieve a similar response in the 1–<3 and 3–8 y cohorts. Among children aged 6–11 months, 1 dose of the fully adjuvanted vaccine was associated with an HI titer of ≥1:40 in

>70% of children while 2 doses of the half-adjuvant formulation were required to achieve the same result. On a practical level, and considering cost effectiveness, administering 1 vaccine dose rather than 2 doses would be preferable. However, this approach would require commercial availability of the 2 presentations (3.75_halfMF59 and 7.5_fullMF59) which, during the very hectic times of a pandemic, may not be easy to achieve. A study conducted in children and adolescents aged 6 months to 18 y found that a single 15 μg dose of A/H1N1 monovalent vaccine resulted in HI titers ≥1:40 in >70% of those aged 9 to <18 years of age; however a single 7.5 or 15 μg dose did not achieve the same result in subjects 6 months to 8 y of age.[27] Together, these results suggest that the number and formulation of adjuvant vaccine doses should be tailored to children's age in order to achieve HI titers ≥1:40.

Our study established that the 3 CHMP criteria for immunogenicity were met 3 weeks following administration of the booster adjuvanted vaccine in the 4 cohorts across all vaccine groups. This was the case regardless of the priming formulation and irrespective of HI titers at day 366. However, one concern with the CHMP criteria is that they are based on studies conducted in adults and the applicability of these criteria for studies in children is not clear. While we were conducting this study, a separate study in 777 vaccine-naïve children aged 6–72 months 50 d following administration of 2 doses of MF59-adjuvanted seasonal vaccine was published.[28] Findings from this study suggested that the conventional adult HI titer of ≥1:40 is only associated with 22% protection in children aged 6–72 months.[28] Notably, titers of 1:110, 1:215, 1:330, and 1:629 were associated with protection rates of 50%, 70%, 80%, and 90%, respectively. The investigators concluded that a titer of 1:330 would have more favorable public health implications. To address this, we conducted a post-hoc analysis to establish the percentages of subjects achieving an HI titer ≥1:330. We observed that at day 43, 49–94% of subjects receiving the adjuvanted vaccines achieved this titer, compared with only 10% and 24% of subjects in cohorts 2 and 3 following vaccination with the nonadjuvanted vaccines. Following the booster, ≥90 % of subjects achieved an HI titer ≥1:330, with no difference between those subjects primed with adjuvanted or non-adjuvanted vaccine.

All study vaccines were safe and well tolerated, with the majority of solicited reactions mild and self-limiting. The safety profile of the adjuvanted vaccine was generally similar to that of the nonadjuvanted vaccine, including in subjects who received repeat doses of the adjuvanted formulation; in line with previous studies, local reactogenicity was higher with the adjuvanted formulation, especially in older age groups.[29] Furthermore, the safety profile of the cell-derived vaccine was similar to the safety profile previously reported for an equivalent egg-derived vaccine.[30] Together, these findings support the acceptable safety profile of the cell-derived halfMF59 and fullMF59 formulations when administered with 3.75 μg or 7.5 μg of A/H1N1 antigen to children aged 6 months to 17 y

Our findings support the immunogenicity and acceptable safety of the cell-derived MF59-adjuvanted A/H1N1 influenza vaccine in children aged 6 months to 17 y.

Methods

Study design and objectives

This randomized, single-blind, dose-ranging study was conducted between 2009 and 2011 at 8 centers in Germany, 2 in Belgium, 2 in the Dominican Republic, and one in the Netherlands. The protocol was approved by the Institutional Review Board or Ethics Committee at each center and the study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from each subject or their legal guardians before enrollment. The study was registered with the National Institutes of Health database, ClinicalTrials.gov (NCT00971100).

The primary study objective was to identify the preferred vaccine formulation (with or without MF59), dosage of antigen and adjuvant, and schedule (1 or 2 administrations) of the cell-derived A/H1N1 monovalent vaccine in healthy children and adolescents. Secondary objectives included evaluation of long-term persistence of antibodies and vaccine safety throughout the study.

Subjects

Eligible subjects were healthy males and females aged 6 months to 17 y on the day of enrollment without documented influenza in the 3 months preceding enrollment. Influenza was considered documented by positive serology test, viral culture, or rapid antigen tests. Exclusion criteria are detailed in the supplementary section.

Four cohorts were enrolled: children aged 9–17 y (cohort 1), 3–8 y (cohort 2), 1–<3 years (cohort 3), and 6–11 months (cohort 4).

Vaccines

Each subject received 2 doses of one of 3 vaccine formulations (3.75_halfMF59, 7.5_fullMF59 and 15_noMF59 containing 3.75, 7.5 and 15 μg A/H1N1 and 0.125, 0.25 and 0 mL MF59 adjuvant, respectively), (**Supplementary Table 1**) administered approximately 3 weeks apart. One year after the first vaccination, subjects received a booster with the trivalent seasonal MF59 vaccine (Fluad®) recommended for the 2010/11 influenza season (15 μg antigen from each influenza strain [including A/H1N1] + 0.35 mL MF59). A second dose of trivalent seasonal vaccine was administered 3 weeks after the booster to naïve subjects <9 years of age, to complete the primary immunization against influenza according to WHO recommendations. Vaccinations were administered by intramuscular injection in the deltoid muscle of the non-dominant arm and the anterolateral thigh for children ≥24 months and <24 months, respectively.

Immunogenicity analysis

Blood samples were taken on day 1 prior to vaccination, on days 22, 43, and 366 following the first vaccination, and on day 387 (3 weeks after the booster). Immunogenicity was evaluated by homologous (A/California/7/2009) H1N1 strain-specific HI and MN assays. Immunogenicity measures were: 1) GMT for HI and MN for the primary vaccine and for the booster; 2) GMR of HI and MN; 3) percentage of subjects achieving seroconversion (HI ≥1:40 for subjects negative at baseline [HI <1:10]), or a significant increase in HI (minimum 4-fold increase in HI titer for subjects positive at baseline); 4) percentage of subjects with a MN titer ≥1:40, 1:80, and 1:160; 5) percentage of subjects with an HI titer ≥1:40; and 6) percentage of subjects achieving a minimum 4-fold increase in MN titer. Following publication of HI antibody titers necessary for protection for inactivated influenza vaccines in children, a further analysis was conducted to evaluate the percentage of subjects with an HI titer ≥1:330.[28]

Safety analysis

Safety reactions were collected on diary cards and reviewed at each follow-up visit. The safety analysis included the number of subjects exposed to study vaccine with solicited local and systemic reactions and unsolicited AEs reported per group. Solicited reactions occurring within 7 d post each vaccination were considered indicators of reactogenicity that might possibly be related to study vaccine. These included local and systemic reactions collected for 7 d following each vaccination.

All unsolicited AEs were collected for 3 weeks following each vaccination. All AEs resulting in study discontinuation, SAEs, onset of chronic health conditions, and associated medications were collected for the whole study (i.e., days 1–546).

Statistical analysis

Approximately 720 subjects (80 per age group receiving adjuvanted vaccines; 40 per age group in cohorts 2 and 3 receiving non-adjuvanted vaccines) were planned for study enrollment to provide adequate power for evaluation of the primary endpoint. Sample size was defined as sufficient to provide adequate estimates for the endpoints specified in the CHMP criteria, and was calculated to demonstrate non-inferiority with a power of 90%, if the real GMT ratio is 0.93. The full analysis set included all subjects who received a study vaccination and provided at least one evaluable serum sample before and after baseline. The per-protocol set (PPS) comprised all subjects who received the correct doses of vaccine, provided evaluable serum samples at the appropriate time points, and had no major protocol violations. The PPS was the primary analysis set for the immunogenicity analyses.

Immunogenicity criteria comprised those of the CHMP for adults,[31] including: 1) the percentage of subjects with seroconversion or significant increase in HI antibody is >40%; 2) the percentage of subjects achieving an HI titer ≥1:40 is >70%; and 3) GMR is ≥2.5. All three criteria assessed at day 43 and at least one of the 3 criteria assessed 3 weeks post-booster dose had to be met by each cohort to fulfill regulatory requirements. Results

from day 22 following the first vaccination were also evaluated against these criteria. Vaccine group differences were assessed using 2-way analysis of variance with factors for vaccine group and center.

The safety population included all subjects who received study vaccination and provided post-baseline safety data. Descriptive statistics were calculated for all safety data and expressed as the proportion and number of subjects with solicited local or systemic reactions, unsolicited AEs, SAEs, and AEs resulting in study discontinuation. All statistical analyses were performed by Novartis Vaccines using SAS, version 9.1 or higher.

Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

Author Contributions

MK, GL-R, HR, LR and DK participated in the conduct of the study, data acquisition and interpretation, and contributed to the development of the initial draft of the manuscript, reviewed and revised the manuscript, and approved the final manuscript as submitted. PP participated in the conception, design, statistical analyses, implementation of the study, interpretation of analyzed data, contributed to the development of the initial draft of the manuscript, reviewed and revised the manuscript, and approved the final manuscript as submitted. AKA, ML and GDC participated in the conception, design and implementation of the study, interpretation of analyzed data, and contributed to the development of the initial draft of the manuscript, reviewed and revised the manuscript, and approved the final manuscript as submitted.

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