

Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomised controlled phase 2 trials



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Summary

Background Data are needed from large clinical trials of paediatric, adult, and elderly people to find the appropriate antigen dose and vaccination schedule for the 2009 pandemic influenza A H1N1. We therefore report preliminary safety and immunogenicity results after one injection of a licensed monovalent pandemic H1N1 vaccine in the USA.

Methods We randomly assigned healthy children (aged 6–35 months and 3–9 years) and adults (18–64 years and ≥65 years) to vaccine containing per dose 7·5 µg (children and adults), 15 µg (children and adults), or 30 µg (adults only) haemagglutinin in two placebo-controlled, observer-masked, multicentre phase 2 studies done in the USA. Participants were allocated with an interactive voice-response system or computer-generated randomisation lists with opaque scratchable patches. Primary outcome was haemagglutination inhibition antibody response 21 days after the first of two planned vaccinations (interim analysis of studies in progress). Analyses were by full-analysis set. The trials are registered with ClinicalTrials.gov as NCT00953524 and NCT00952419.

Findings 410 of 423 children and 724 of 750 adults given an active vaccine, and 50 of 51 children and 95 of 99 adults given placebo were assessed for immunogenicity on day 21. After active vaccination, 45 of 101 (45%; 95% CI 35–55) to 47 of 94 (50%; 40–61) infants aged 6–35 months, 75 of 109 (69%; 59–77) to 80 of 106 (75%; 66–83) 3–9-year-old children, 134 of 141 (95%; 90–98) to 144 of 144 (100%; 98–100) of 18–64-year-old adults, and 93 of 100 (93%; 86–96) to 93 of 98 (95%; 89–98) elderly adults were seroprotected (proportion with titres ≥1:40). No vaccine-related serious adverse events occurred. Injection-site and systemic reactions were reported by up to about 50% of every age and vaccine group, with no noticeable differences between vaccine and placebo groups.

Interpretation One dose of vaccine was highly immunogenic in adults, suggesting that it afforded sufficient protection against this pandemic influenza A H1N1 virus. Two doses of vaccine will probably be needed in children younger than 9 years. Safety and reactogenicity of the vaccine were acceptable and similar to those of seasonal vaccine.

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Introduction

The emergence and rapid spread of a novel influenza A H1N1 virus with potential to cause death in healthy adults and children, and substantial socioeconomic disruption, prompted health authorities to call for the rapid development of vaccines to mitigate the effect of the first influenza pandemic of this century.^{1,2}

Between first identification in the USA in April and end of August, the 2009 pandemic influenza A H1N1 virus was estimated to have caused at least 1 million infections, 9000 hospital admissions, and almost 600 deaths nationwide.³ Human infections have mostly been reported as uncomplicated influenza-like illnesses from which patients have recovered.^{3,4} Most people with H1N1 infections were children and young adults. The median age of patients was 20–25 years, or even younger, depending on the case series.^{4,5} On the basis of surveillance data from Australia, Kelly and colleagues⁵ argue that the young age of patients is not specific to this pandemic H1N1 strain, but is characteristic of seasonal and pandemic influenza A H1N1 infections, and that

influenza in elderly adults is caused predominantly by influenza A H3N2.⁵

Severe cases of pandemic H1N1 infection and deaths as a result have been reported in individuals in high-risk groups, such as those with underlying diseases, pregnant women, and young children, and also in children of any age and young adults with no known health risk.^{1,6,7} Severe cases were initially reported mainly in children,⁴ but in an analysis of 343 fatal cases up to July 16, the median age was 37 years and 51% of cases were aged 20–49 years.⁷ The pandemic H1N1 strain was more pathogenic and replicated more efficiently than did the seasonal H1N1 strains in ferrets and mice.^{8–10}

Simulation models have been used to predict the health and cost benefits of vaccination against pandemic H1N1 infection.^{11,12} Estimates based on US data suggest that a severe epidemic could be mitigated if vaccine was available early in autumn, and if children and adults were vaccinated with an overall coverage of 70%.¹² The US Food and Drug Administration (FDA) has approved four H1N1 vaccines, produced by four different manufacturers using the same

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See [Comment](#) pages 6 and 9

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	Influenza A H1N1 vaccine haemagglutinin			Placebo	All
	7.5 µg	15 µg	30 µg		
18–64 years					
Number	150	151	148	48	497
Mean age (years; SD)	40.0 (13.3)	40.8 (13.9)	42.3 (13.3)	41.0 (13.7)	41.0 (13.5)
Men	64 (43%)	62 (41%)	68 (46%)	24 (50%)	218 (44%)
≥65 years					
Number	99	103	99	51	352
Mean age (years; SD)	72.2 (5.3)	71.9 (5.0)	72.5 (5.9)	71.0 (4.4)	72.0 (5.3)
Men	45 (46%)	49 (48%)	34 (34%)	18 (35%)	146 (41%)
6–35 months					
Number	105	98	..	26	229
Mean age (months; SD)	21.2 (8.1)	22.0 (8.9)	..	18.2 (9.2)	21.2 (8.7)
Boys	52 (50%)	53 (54%)	..	16 (62%)	121 (53%)
3–9 years					
Number	110	110	..	25	245
Mean age (years; SD)	6.4 (2.1)	6.5 (2.1)	..	6.0 (1.8)	6.4 (2.0)
Boys	52 (47%)	56 (51%)	..	11 (44%)	119 (49%)

Data are number or number (%), unless otherwise indicated.

Table 1: Baseline demographic characteristics by age group

licensed processes as those used for the production of seasonal influenza vaccines. However, we need clinical data from large trials of paediatric, non-elderly, and elderly populations to establish the appropriate antigen dose and vaccination schedule.¹³

We present the interim immunogenicity and safety results from two clinical trials of different doses of the 2009 pandemic influenza A H1N1 monovalent vaccine in different age groups of children and adults 21 days after the first vaccination.

Methods

Study design

We administered the first of two planned vaccine injections in two randomised, observer-masked, placebo-controlled, multicentre phase 2 trials. In the first study, we enrolled 497 adults aged 18–64 years and 352 older than 65 years on Aug 6 and 7, 2009, and administered three antigen doses (7.5 µg, 15 µg, and 30 µg haemagglutinin). In the second study, we enrolled 229 children aged 6–35 months and 245 aged 3–9 years between August 6–13, 2009, and administered two antigen doses (7.5 µg and 15 µg haemagglutinin).

Each study was reviewed by the US Center for Biologics Evaluation and Research, Rockville, MD, and was approved by applicable US institutional review boards (Western Institutional Review Board [Olympia, WA], Eastern Virginia Medical School Institutional Review Board [Norfolk, VA], and Chesapeake Research Review, [Columbia, MD]) before enrolment. Both studies were done in accordance with the Declaration of Helsinki, Good Clinical Practice (as defined by the International Conference on Harmonisation), and federal regulations.

All participants or parents provided written informed consent, and children older than 7 years of age provided their assent before enrolment.

Participants

Participants were enrolled from 35 clinical sites within 18 US states, including three in which H1N1 was particularly prevalent (CA, TX, NJ). The participating clinical research investigators screened their clinical research volunteer databases for potentially eligible participants, who were then contacted and invited to participate. Healthy individuals were eligible for participation in the study. The main exclusion criteria were known or suspected influenza infection since March, 2009; any vaccination in the past 4 weeks or planned in the following 6 weeks; known or suspected immunodeficiency; recent history (<6 months) of immunosuppressive treatment or long-term use of systemic corticosteroids; self-reported HIV/AIDS, or hepatitis B or C infection; receipt of blood or blood-derived products in the past 3 months; and febrile or acute illness on the day of enrolment. Women who were pregnant or breastfeeding were also excluded. Contact with an individual infected with pandemic influenza A H1N1 and vaccination with the influenza A/New Jersey/76 vaccine were recorded at enrolment, but were not exclusion criteria.

Randomisation and masking

We used an interactive voice-response system to assign participants after enrolment to one of the study groups (table 1) according to a centralised, non-stratified randomisation list (adults), or opaque scratchable randomisation lists, stratified by age group and trial centre (children). The sponsor's biostatistics department generated the randomisation lists using block permutation. Participants were then vaccinated in an observer-masked manner—ie, study staff who were not involved in any other aspect of the study prepared and injected vaccine or placebo without informing the participant, parents, and investigator which product had been administered. The investigators were not present during the vaccine preparation and injection steps. Investigators, and participants or their parents were unaware of which product participants were assigned to and will remain so until the end of the study.

Vaccines

Consistent with WHO's recommendation, the vaccine was an inactivated split-virion preparation of the New York Medical College X-179A reassortant of the A/California/07/2009 H1N1 and PR8/8/34 strains, distributed by the Centers for Disease Control and Prevention, Atlanta, GA, USA.² Seed virus was propagated in embryonated chicken eggs, inactivated, and split in accordance with the process used to produce a seasonal influenza vaccine (Fluzone, Sanofi-Pasteur, Swiftwater, PA, USA) licensed for individuals older than

6 months. Vaccine was supplied by the manufacturer as vials containing one dose without preservative for children aged 6–35 months or multidose vials containing 0·01% thiomersal preservative for all other ages. Batches of vaccines were prepared specifically for each group such that when a 0·25 mL (age group 6–35 months) or 0·5 mL (all other age groups) dose was withdrawn for injection, it contained the assigned dose of haemagglutinin (ie, 7·5 µg, 15 µg, or 30 µg). Without calibration reagents that were needed for the single-radial immunodiffusion (SRID) assay—the standard method for assessment of the potency of inactivated influenza vaccine—when the clinical lots were manufactured, potency was assessed with high-performance liquid chromatography. Haemagglutinin content was quantified again with the SRID assay when reagents became available; according to this method, the assigned vaccine doses of 7·5 µg, 15 µg, and 30 µg contained, 11 µg, 24 µg, and 50 µg haemagglutinin, respectively. We injected vaccines or saline placebo intramuscularly in the thigh (infants <1 year) or deltoid (all other age groups).

Immunological response

We took serum samples before and 21 days after vaccination for antibody titration against the vaccine strain using a standard haemagglutination inhibition assay with turkey erythrocytes.¹⁴ Each sample was tested in duplicate, with the geometric mean of the two results recorded as the final titre. Assays were done at the sponsor's central clinical immunology laboratory (Swiftwater, PA, USA) with masking. Haemagglutination inhibition antibody titres were summarised with the criteria conventionally used to assess the immunogenicity of influenza vaccines—ie, geometric mean titre (GMT), geometric mean titre ratio (GMTR), seroprotection rate (proportion with titres $\geq 1:40$), seroconversion rate (proportion with prevaccination titre <1:10 and a postvaccination titre $\geq 1:40$, or a prevaccination titre $\geq 1:10$, and ≥ 4 -fold increase after vaccination).^{13,15}

Safety and reactogenicity

Participants or their parents recorded body temperature and the appearance of solicited injection-site and systemic reactions every day, for 7 days after injection, in their safety diaries. Solicited injection-site reactions were pain (adults and children ≥ 2 years) or tenderness (children <2 years), erythema, swelling, induration, or ecchymosis. Solicited systemic reactions were fever, headache, malaise, myalgia, and shivering (participants ≥ 2 years) or fever, vomiting, abnormal crying, drowsiness, loss of appetite, and irritability (participants <2 years). Unsolicited adverse events occurring up to day 21 and serious adverse events were recorded. Unsolicited adverse events that were judged to be related to the vaccine by the investigator were adverse reactions. To maintain the masking, safety data were presented to ensure that the group allocation of participants could not be identified,

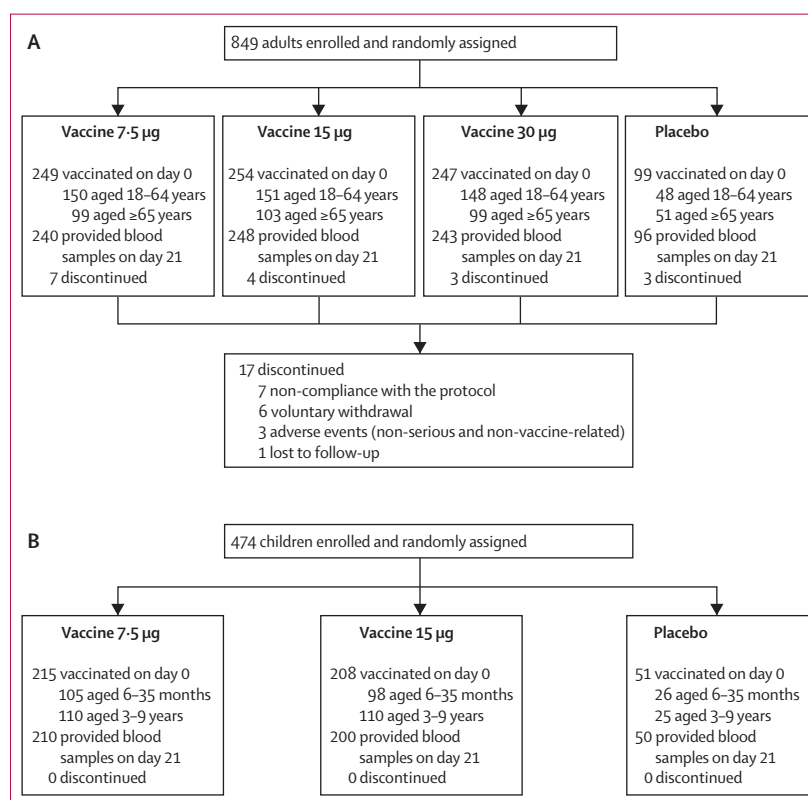


Figure 1: Trial profiles
(A) Study 1: adults. (B) Study 2: children.

and the incidence of solicited reactions was the only information provided by vaccine group.

Statistical analysis

We did not do a formal calculation of sample size. The sample size was chosen pragmatically rather than statistically because of the need to obtain robust estimates of vaccine immunogenicity and safety, and to complete the study as quickly as possible because the pandemic H1N1 was a public health priority.

The sample size was defined in collaboration with the Center for Biologics Evaluation and Research. The chosen sample size for each vaccine group and age group provided a roughly 95% probability of any adverse event arising with a true incidence of 2% in every 150 adults, and 3% in every 100 children and elderly adults. With an assumption of a true seroprotection rate of 70% in elderly individuals, and 80% in children and adults, and a dropout rate of 10%, the half-width of the 95% CIs was estimated to be 8·3% in every age and vaccine group of children, 6·7% in every vaccine group of non-elderly adults, and 9·5% in elderly individuals. On the basis of these assumptions, the study was sufficiently powered to meet FDA's immunogenicity requirement that the lower limit of the CI for the seroprotection rate should be 60% or more in elderly people and 70% or more in children and non-elderly adults.¹³

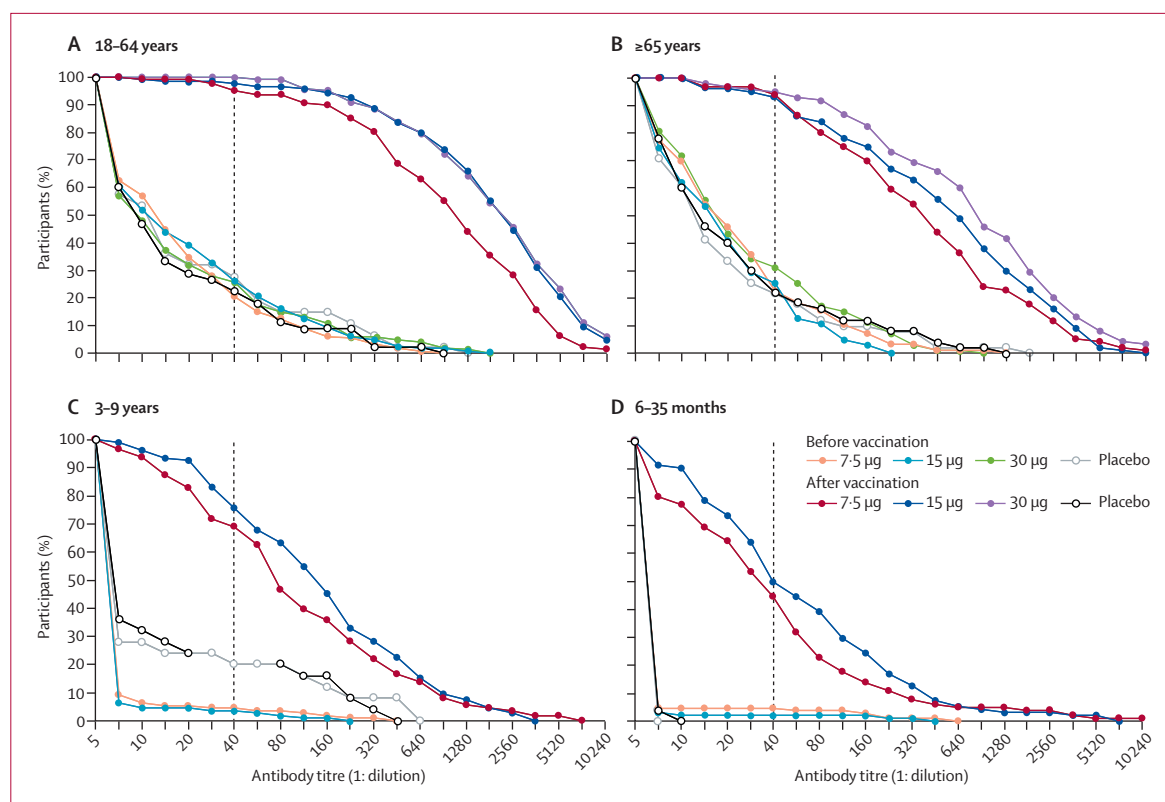


Figure 2: Reverse cumulative distribution of haemagglutination inhibition antibody titre against pandemic 2009 influenza A H1N1 before and 21 days after one injection of vaccine or placebo in different age groups

Vertical dashed lines indicate the 1:40 titre associated with seroprotection.

For each endpoint, the 95% CI was calculated with the exact binomial distribution (Clopper-Pearson method) for proportions. GMTs and 95% CIs were calculated by use of the mean, and lower and upper limits of the 95% CIs of log-transformed titres. These statistical analyses were done by a statistician aware of treatment assignment, not involved in any other aspect of the study, and was the only person to have unmasked access to the data.

Post-hoc analyses were *t* tests for the effect of seasonal influenza vaccination history and date of birth before 1957 on log GMTs before and after vaccination; χ^2 tests for the effect of these two parameters on seroprotection rates; regression for the effect of age on log GMTs before and after vaccination; and logistic regression for the effect of age on seroprotection rates before and after vaccination. All reported *p* values were two-sided, without adjustment for multiple testing. Analyses were done for the full-analysis set, with SAS software (version 9.1).

The trials are registered with ClinicalTrials.gov as NCT00953524 and NCT00952419.

Role of the funding source

The US Department of Health and Human Services had no role in the study or this report. The study sponsor Sanofi-Pasteur was involved in the design and

monitoring of the study, serology, and statistical analyses, interpretation of data, and writing this report.

Results

Figure 1 shows the trial profiles. Table 1 shows the age and sex in each age and vaccine group. Seasonal influenza vaccine for 2008–09 was given to 179 (36%) of 497 individuals aged 18–64 years and 217 (62%) of 352 aged 65 years or older. 13 (3%) and 15 (4%) participants, respectively, recalled being vaccinated during the 1976 mass vaccination campaign against swine influenza A/New Jersey/1976 H1N1, and 6 (1%) and none of 352 said they had been in contact with someone with a confirmed or probable pandemic H1N1 infection in the previous 8 months. 135 (55%) of 245 children aged 3–9 years, and 104 (45%) of 229 aged 6–35 months had been given the 2008–09 seasonal influenza vaccine. None were known to have been in contact with an individual with pandemic H1N1 infection.

At day 0 (baseline), cross-reactive antibodies against the vaccine strain were detected (titre $\geq 1:10$) in 71 (48%) of 147 to 71 (72%) of 99 participants of every age and vaccine group of adults, and 26 (5%) of 474 children. Titres were 1:40 or more in 20–31% of adults (figure 2; table 2).

In the exploratory, post-hoc analysis, administration of seasonal influenza vaccine to adults in the past five

	H1N1 vaccine haemagglutinin			Placebo
	7.5 µg	15 µg	30 µg	
18–64 years				
Participants assessed (n)				
Day 0	147	150	147	47
Day 21	141	145	144	45
Seroprotection rate				
Day 0	30 (20%; 14–28)	39 (26%; 19–34)	38 (26%; 19–34)	13 (28%; 16–43)
Day 21	134 (95%; 90–98)	142 (98%; 94–100)	144 (100%; 98–100)	10 (22%; 11–37)
Seroconversion rate	129* (92%; 86–96)	139 (96%; 91–99)	140 (97%; 93–99)	0 (0; 0–8)
GMT on day 21 (95% CI)	747 (589–947)	1405 (1120–1763)	1493 (1210–1843)	13.2 (8.97–19.4)
GMTR (95% CI)	38.9 (30.4–49.7)	64.3 (50.9–81.2)	73.2 (57.0–94.1)	0.912 (0.745–1.12)
≥65 years				
Participants assessed (n)				
Day 0	98	103	99	51
Day 21	96	100	98	50
Seroprotection rate				
Day 0	22 (22%; 15–32)	26 (25%; 17–35)	31 (31%; 22–41)	11 (22%; 11–35)
Day 21	90 (94%; 87–98)	93 (93%; 86–97)	93 (95%; 89–98)	11 (22%; 12–36)
Seroconversion rate	79† (83%; 74–90)	89 (89%; 81–94)	90 (92%; 85–96)	1 (2%; 0–11)
GMT on day 21 (95% CI)	297 (217–405)	390 (283–537)	588 (429–806)	17.3 (11.8–25.3)
GMTR (95% CI)	13.6 (10.1–18.3)	21.3 (15.7–29.0)	25.5 (19.0–34.3)	1.04 (0.867–1.24)
3–9 years				
Participants assessed (n)				
Day 0	110	110	0	25
Day 21	109	106	0	25
Seroprotection rate				
Day 0	5 (5%; 2–10)	4 (4%; 1–9)	..	5 (20%; 7–41)
Day 21	75 (69%; 59–77)	80 (75%; 66–83)	..	5 (20%; 7–41)
Seroconversion rate	73 (67%; 57–76)	80 (75%; 66–83)	..	0 (0; 0–14)
GMT on day 21 (95% CI)	77.5 (57.2–105)	111 (83.6–147)	..	11.6 (6.45–21.0)
GMTR (95% CI)	7.13 (5.46–9.33)	10.4 (8.02–13.5)	..	0.973 (0.802–1.18)
6–35 months				
Participants assessed (n)				
Day 0	105	98	0	26
Day 21	101	94	0	25
Seroprotection rate				
Day 0	5 (5%; 2–11)	2 (2%; 0–7)	..	0 (0; 0–13)
Day 21	45 (45%; 35–55)	47 (50%; 40–61)	..	0 (0; 0–14)
Seroconversion rate	44 (44%; 34–54)	47 (50%; 40–61)	..	0 (0; 0–14)
GMT on day 21 (95% CI)	31.1 (22.8–42.6)	47.0 (34.4–64.4)	..	5.07 (4.93–5.22)
GMTR (95% CI)	3.17 (2.47–4.07)	4.69 (3.59–6.12)	..	1.00 (1.00–1.00)

Data are number (%; 95% CI), unless otherwise indicated. GMT=geometric mean titre. GMTR=geometric mean of postvaccination to prevaccination titre ratios. *One participant had missing data for this criterion (n=140). †One participant had missing data for this criterion (n=95).

Table 2: Haemagglutination inhibition antibody response against 2009 influenza A H1N1 before and 21 days after one injection of vaccine or placebo

seasons was associated with a significantly higher GMT and seroprotection rate at baseline (table 3). In children, this pattern was not seen, and the GMT and seroprotection rate seemed lower in those vaccinated in previous years than in those not vaccinated. However differences were very small and were only significant when the effect of vaccination in 2007–08 was assessed. Age, date of birth before 1957 (GMT was 15.9 in

participants born before 1957 and 15.6 in those born in 1957 or after; *t* test *p*=0.8), or receipt of influenza A/New Jersey/76 vaccine had no significant effect on baseline titre (regression analysis) or seroprotection (logistic-regression analysis) in adults or children. Numbers were too small for meaningful analysis of the other factors that might have affected the baseline immune response (influenza infection in 2009

	Adults (≥18 years)				Children (6 months to 9 years)			
	GMT	t test p value	Seroprotection rate	χ ² p value	GMT	t test p value	Seroprotection rate	χ ² p value
2008–09		<0.0001		0.0003		0.1400		0.2598
Yes	20.5		120/394 (30.5%)		5.61		8/239 (3.3%)	
No	12.1		83/423 (19.6%)		6.21		11/198 (5.6%)	
2007–08		<0.0001		0.0138		0.0274		0.0373
Yes	18.5		124/438 (28.3%)		5.43		3/154 (1.9%)	
No	13.0		79/379 (20.8%)		6.36		13/196 (6.6%)	
2006–07		0.0002		0.0170		0.3591		ND
Yes	18.9		119/411 (29.0%)		5.78		3/98 (3.1%)	
No	13.5		85/393 (21.6%)		6.29		10/157 (6.4%)	
2005–06		0.0012		0.0457		0.9038		ND
Yes	18.8		112/387 (28.9%)		6.16		3/70 (4.3%)	
No	14.0		94/413 (22.8%)		6.24		8/125 (6.4%)	
2004–05		0.0011		0.0760		0.5989		ND
Yes	18.8		105/371 (28.3%)		5.88		2/49 (4.1%)	
No	14.0		96/421 (22.8%)		6.28		7/117 (6.0%)	

Data are n/N (%), unless otherwise indicated. ND=not determined because sample size too small.

Table 3: Effect of seasonal influenza vaccination history on baseline immune responses to pandemic influenza A H1N1 strain

or contact with an individual with pandemic H1N1 infection).

Strong immune responses to all vaccine formulations were noted in both age groups of adults on day 21. Titres were higher in individuals aged 18–64 years than in those older than 64 years (table 2). A dose response was apparent in both age groups of adults, and also in children (figure 2). In adults, seroprotection rates after one vaccination were 93% or higher, and seroconversion was noted in at least 83% of each vaccine group (table 2). After placebo, seroconversion was recorded in one elderly adult only. In both age groups of adults and in each vaccine group, seroprotection and seroconversion rates, including the lower limit of the two-sided 95% CIs, were greater than the criteria set by the FDA for adults younger than 65 years, and 65 years or older—ie, 70% and 60% for seroprotection, respectively, and 40% and 30% for seroconversion, respectively.¹³

In exploratory, post-hoc analyses, immune responses after vaccination decreased with increasing age (regression analysis for the full-analysis set, $p < 0.0001$ for all groups combined) and the GMT after vaccination was lower in individuals born before 1957 than those born in 1957 or after (292 vs 929; t test $p < 0.0001$). Influenza vaccination in the five previous seasons did not affect the seroprotection rate on day 21 (data not shown), but seemed to be associated with a lower GMT than in individuals not vaccinated: the difference in GMT was significant for seasonal influenza vaccine given every year between 2004–05 (GMT 368 vs 671; $p < 0.0001$) and 2007–08 (356 vs 697; $p < 0.0001$), but not for 2008–09 (425 vs 537; $p = 0.0963$).

At day 21, immune responses to vaccination in children were lower than those in adults (table 2; figure 2). Immune responses elicited with the 15 µg vaccine met the FDA's requirement for children—ie, the lower limit of the 95% CI seroconversion should meet or exceed 40%.¹³ This lower limit was exactly 40% in children aged 6–35 months and greater in those aged 3–9 years. In the age group 3–9 years, the point estimate of the seroprotection rate, but not the lower limit of the CI, was higher than 70%.

No immune response to placebo was noted, and all five recipients (aged 3–9 years) with seroprotective titres on day 21 were already seroprotected before injection. In an exploratory analysis, influenza vaccination in previous seasons had no significant effect on the seroprotection rate or the GMT on day 21 (data not shown).

No deaths, vaccine-related serious adverse events, immediate unsolicited adverse reactions, or new onset of chronic illness occurred during the studies. In adults, the rate of solicited injection-site reactions in each vaccine group was higher than that in the placebo group, whereas in children the rates were similar in the vaccine and placebo groups (table 4). Injection-site pain or tenderness was the most frequent reaction in all groups—ie, 51 (38%) of 134 children aged 6–23 months, 33 (36%) of 92 aged 24–35 months, and 118 (49%) of 242 aged 3–9 years; and 150 (31%) of 482 adults aged 18–64 years, and 39 (11%) of 346 older than 64 years. Other solicited injection-site reactions (erythema, swelling, induration, or ecchymosis) were reported by no more than 1% of each group of adults, and by no more than 16% of each group of children. Most injection-site reactions were of grade 1 (tenderness: infant with a minor reaction to the injection site being touched; pain: easily tolerated or no interference with daily activity; redness and other measurable reactions: 0–2.5 cm for children or 2.5–5 cm for adults), and grade-3 reactions (tenderness: infant cried when injected limb was moved, or reduced movement of the injected limb; pain: prevention of usual daily activity; redness and other measurable reactions: ≥5 cm for children or >10 cm adults) were reported by fewer than 1% of adults and no more than 2% of children.

The rates of solicited systemic reactions in the vaccine and placebo groups were in the same range (table 3). The most commonly reported systemic reactions were headache, myalgia, and malaise in adults, and children older than 24 months (data not shown). Irritability (52 [39%] of 134), abnormal crying (34 [25%]), loss of appetite (23 [17%]), and drowsiness (22 [16%]) were the most commonly reported systemic reactions for children younger than 24 months.

Four (<1%) of 486 adults, two (<1%) of 346 elderly adults, four (2%) of 242 children aged 3–9 years, three (3%) of 92 aged 24–35 months, and eight (6%) of 134 aged 6–23 months had fever. Most solicited systemic reactions were grade 1. 18 (2%) of 833 adults and 17 (4%) of 469 children had grade-3 reactions.

Within 21 days after injection of vaccine or placebo, 20 (4%) of 497 adults aged 18–64 years, six (2%) of 352 adults 65 years or older, six (2%) of 245 children aged 3–9 years, and 16 (7%) of 229 infants aged 6–35 months had unsolicited adverse reactions. Most common reactions were respiratory disorders (21 [2%] of 849 adults and five [1%] of 474 children), such as cough, rhinorrhoea, or nasal congestion.

Discussion

One vaccination with one of three antigen doses was highly immunogenic and well tolerated in US adults, with seroprotection rates of 93–100%. Even the immune responses elicited with the lowest dose exceeded FDA's requirements for non-elderly and elderly adults, supporting the recommendation of just one vaccination in these age groups.¹³ One injection was also immunogenic in children (particularly in age group 3–9 years), but insufficiently immunogenic to meet FDA's criteria, confirming the need for a second vaccination to provide sufficient protection. Noteworthy is that although a titre of 1:40 for haemagglutination inhibition is generally used as a surrogate marker to assess the effectiveness of the influenza vaccines, and the proportion of individuals with titres at least equal to 1:40 is generally referred to as the seroprotection rate, it is not an absolute correlate of protection against laboratory confirmed disease.

To inform public health decision makers as quickly as possible, and in agreement with FDA's recommendation, vaccination was not specifically assessed in the age group 10–17 years.¹³ Immunogenicity and safety in this age group can be inferred from data gathered for younger and older age groups. Since the immunogenicity of the influenza A/California/07/2009 vaccine strain was not known, our trials included a two-dose vaccination schedule and an antigen dose-ranging design (standard dose 15 µg used in seasonal vaccines, and two-fold higher and lower dilutions).

Because of the urgent need for pandemic vaccine and the absence of reagents needed for the SRID assay, we, like other manufacturers, used high-performance liquid chromatography to prepare the clinical batches.¹⁶ SRID testing after receipt of the calibration reagents showed that potency was slightly higher than the target value. On the basis of the high immune responses elicited in adults by the lowest tested dose in our studies (11 µg as assessed with SRID assay), we can assume that the licensed vaccine containing 15 µg of haemagglutinin (as assessed with the SRID assay) will also be highly immunogenic.

Baseline seroprotection rates were similar to those reported in a study of Australian adults aged 18–64 years (30–33%), but rates of baseline haemagglutination inhibition seroprotection were higher than those reported in a UK study (4–12%).^{16,17} The pandemic H1N1 strain is antigenically distinct from the circulating 2009 seasonal influenza A H1N1 viruses, and seasonal influenza vaccination elicits little or no cross-reactive responses

	Influenza A H1N1 vaccine haemagglutinin			Placebo
	7.5 µg	15 µg	30 µg	
Solicited injection-site reactions				
18–64 years	53/144 (37%; 29–45)	41/146 (28%; 21–36)	48/145 (33%; 26–41)	8/47 (17%; 8–31)
≥65 years	12/97 (12%; 7–21)	13/101 (13%; 7–21)	18/98 (18%; 11–28)	0/50 (0; 0–7)
6–23 months	28/61 (46%; 33–59)	16/55 (29%; 18–43)	..	7/18 (39%; 17–64)
24–35 months	14/42 (33%; 20–50)	14/42 (33%; 20–50)	..	5/8 (63%; 25–92)
3–9 years	54/109 (50%; 40–59)	50/108 (46%; 37–56)	..	14/25 (56%; 35–76)
Solicited systemic reactions				
18–64 years	71/146 (49%; 40–57)	65/148 (44%; 36–52)	61/145 (42%; 34–51)	19/47 (40%; 26–56)
≥65 years	16/97 (16%; 10–25)	17/101 (17%; 10–26)	26/98 (27%; 18–36)	7/51 (14%; 6–26)
6–23 months	26/61 (43%; 30–56)	27/55 (49%; 35–63)	..	8/18 (44%; 22–69)
24–35 months	10/42 (24%; 12–40)	9/42 (21%; 10–37)	..	3/8 (38%; 9–76)
3–9 years	32/110 (29%; 21–39)	25/108 (23%; 16–32)	..	8/25 (32%; 15–54)
Data are n/N (%; 95% CI). n=number of participants reporting events. N=number of participants with available data.				
Table 4: Participants with at least one solicited injection-site or systemic reaction within 7 days after vaccination with 2009 influenza A H1N1 vaccine or placebo by age group				

with the pandemic strain.^{2,18} Our finding that the baseline cross-reactive immune response was significantly higher in adults vaccinated with seasonal vaccine in any of the five preceding influenza seasons would seem to contradict this result, although our studies were not designed to investigate this association and analyses were exploratory and done post-hoc. We recorded the history of influenza vaccination for the past five seasons, but the source of the cross-reactive immune response might have been an earlier seasonal vaccination. Indeed, individuals vaccinated in the past 5 years were probably also vaccinated before that time. In children, a history of seasonal vaccination was not associated with a higher cross-reactive immune response and the proportion with detectable baseline titres was much lower than that in adults.

Seasonal influenza vaccination might therefore have played a part in the baseline response, but might also have been a confounder of some other, unknown factor. Exposure to one or more influenza strains through infection or vaccination, or both, possibly occurring before the 2004–05 influenza season, with antigenic epitopes similar to the 2009 H1N1 strain contributed to the baseline response in adults.

H1N1 subtype viruses circulated among people between the 1918 pandemic and 1957, when they were replaced by the H2N2 subtype, and then re-emerged in the 1970s. Exposure to virus in circulation before 1957 could therefore potentially provide an immunological advantage against the 2009 strain. However, we detected no difference in the baseline response to the 2009 H1N1 strain between individuals born before or after 1957. Another potential factor contributing to the rates of seroprotection noted at baseline was asymptomatic infection with pandemic H1N1 strain that was circulating in the USA at the time of this study. However, fewer than 1% of adults reported having been in contact with

someone with confirmed or suspected infection, and volunteers with known or suspected influenza infection since March, 2009, were excluded from these studies. Importantly, the presence of pandemic H1N1-specific antibodies before vaccination did not adversely affect the response to vaccination, as indicated by the rates of seroconversion and mean titre increases.

At the time of analysis, these trials were in progress. Consequently, few safety data were available by vaccine group. Nevertheless, the available results show that the safety and reactogenicity profile of the H1N1 vaccine is consistent with the known safety profile of licensed trivalent inactivated seasonal vaccine.^{19–21}

On the basis of our findings, one vaccination should be sufficient to protect all adults against the 2009 pandemic influenza A H1N1 virus. These preliminary results also show that a substantial proportion of children are already seroprotected after their first vaccination. We will report the immunogenicity and safety of a two-dose vaccination schedule in children as soon as all the study results are available.

Contributors

MD, MKRH, and EP designed the trials. All authors reviewed and approved the trials. EP did the two clinical trials. ES and MB were each coordinating investigators for one of these trials and participated in the recruitment of participants. MD, MKRH, and EP interpreted the results. All authors contributed to the critical review and revision of the report, and have seen and approved the final version.

Conflicts of interest

MD, MKRH, and EP are employed by Sanofi-Pasteur. ES has been an investigator of clinical trials sponsored by Sanofi-Pasteur, Novartis, GlaxoSmithKline, MedImmune, and Wyeth. MB has been an investigator of clinical trials sponsored by Sanofi-Pasteur, Novartis, Merck Sharp and Dohme, Commonwealth Serum Laboratories, MedImmune, and Wyeth, and has served on speaker's bureaus for Sanofi-Pasteur and GlaxoSmithKline.

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References

- 1 National Center for Immunization and Respiratory Diseases, CDC; Centers for Disease Control and Prevention (CDC). Use of influenza A (H1N1) 2009 monovalent vaccine: recommendations of the (ACIP), 2009. *MMWR Recomm Rep* 2009; **58**: 1–8.

- 2 WHO. Characteristics of the emergent influenza A (H1N1) viruses and recommendations for vaccine development. May 26, 2009. <http://www.who.int/csr/resources/publications/swineflu/H1N1Vaccinevirusrecommendation26May2009.pdf> (accessed Sept 23, 2009).
- 3 Centers for Disease Control and Prevention. Update: influenza activity—United States, April–August 2009. *MMWR Morb Mortal Wkly Rep* 2009; **58**: 1009–12.
- 4 World Health Organization. New influenza A (H1N1) virus: global epidemiological situation, June 2009. *Wkly Epidemiol Rec* 2009; **84**: 249–60.
- 5 Kelly HA, Grant KA, Williams S, Fielding J, Smith D. Epidemiological characteristics of pandemic influenza H1N1 2009 and seasonal influenza infection. *Med J Aust* 2009; **191**: 146–49.
- 6 Centers for Disease Control and Prevention (CDC). Surveillance for pediatric deaths associated with 2009 pandemic influenza A (H1N1) virus infection—United States, April–August 2009. *MMWR Morb Mortal Wkly Rep* 2009; **58**: 941–47.
- 7 Vaillant L, La Ruche G, Tarantola A, Barboza P; epidemic intelligence team at InVS. Epidemiology of fatal cases associated with pandemic H1N1 influenza 2009. *Euro Surveill* 2009; **14**: 19309.
- 8 Itoh Y, Shinya K, Kiso M, et al. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. *Nature* 2009; **460**: 1021–25.
- 9 Munster VJ, de Wit E, van den Brand JM, et al. Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. *Science* 2009; **325**: 481–83.
- 10 Maines TR, Jayaraman A, Belser JA, et al. Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. *Science* 2009; **325**: 484–87.
- 11 Brouwers L, Cakici B, Camitz M, Tegnell A, Boman M. Economic consequences to society of pandemic H1N1 influenza 2009—preliminary results for Sweden. *Euro Surveill* 2009; **14**: 19333.
- 12 Yang Y, Sugimoto JD, Halloran ME, et al. The transmissibility and control of pandemic influenza A (H1N1) virus. *Science* 2009; **326**: 729–33.
- 13 Food and Drug Administration Center for Biologics Evaluation and Research Office of Vaccines Research and Review. Regulatory considerations regarding the use of novel influenza A (H1N1) virus vaccines. July 23, 2009 <http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/VaccinesandRelatedBiologicalProductsAdvisoryCommittee/UCM172424.pdf> (accessed Sept 30, 2009).
- 14 WHO. WHO manual on animal influenza diagnosis and surveillance. 2002. http://www.who.int/vaccine_research/diseases/influenza/WHO_manual_on_animal-diagnosis_and_surveillance_2002_5.pdf (accessed Oct 21, 2009).
- 15 Committee for Proprietary Medicinal Products (CPMP). Note for guidance on harmonisation of requirements for influenza vaccines. CPMP/BWP/214/96. The European Agency for the Evaluation of Medicinal Products (EMA), March 1997. <http://www.emea.europa.eu/pdfs/human/bwp/021496en.pdf> (accessed Sept 25, 2009).
- 16 Clark TW, Pareek M, Hoschler K, et al. Trial of influenza A (H1N1) 2009 monovalent MF59-adjuvanted vaccine—preliminary report. *N Engl J Med* 2009; published online Sept 10. DOI:10.1056/NEJMoa0907650.
- 17 Greenberg ME, Lai MH, Hartel GF, et al. response after one dose of a monovalent influenza A (H1N1) 2009 vaccine—preliminary report. *N Engl J Med* 2009; published online Sept 10. DOI:10.1056/NEJMoa0907413.
- 18 Hancock K, Veguilla V, Lu X, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. *N Engl J Med* 2009; **361**: 1945–52.
- 19 Falsey AR, Treanor JJ, Tornieporth N, Capellan J, Gorse GJ. Randomized, double-blind controlled phase 3 trial comparing the immunogenicity of high-dose and standard-dose influenza vaccine in adults 65 years of age and older. *J Infect Dis* 2009; **200**: 172–80.
- 20 Ruben FL. Inactivated influenza virus vaccines in children. *Clin Infect Dis* 2004; **38**: 678–88.
- 21 Frey S, Poland G, Percell S, Podda A. Comparison of the safety, tolerability, and immunogenicity of a MF59-adjuvanted influenza vaccine and a non-adjuvanted influenza vaccine in non-elderly adults. *Vaccine* 2003; **21**: 4234–37.