

Immunogenicity, large scale safety and lot consistency of an intradermal influenza vaccine in adults aged 18–60 years

Randomized, controlled, phase III trial

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Background: Vaccination is the most effective way of reducing the large health and economic burden of influenza, yet vaccination coverage remains low, particularly among non-elderly adults. Intradermal influenza vaccine produce an effective immune response and represents an alternative to intramuscular influenza vaccination.

Results: The three industrial lots of intradermal vaccine were equivalent in terms of post-vaccination titres elicited by day 21. The intradermal and intramuscular vaccines induced similar post-vaccination titres, and satisfied all three immunogenicity criteria set out in the European regulatory guidelines for influenza vaccines for each of the three influenza strains. The solicited systemic reaction profile and the incidence and type of spontaneously reported adverse events were similar in the two vaccine groups and in line with the known safety profile of inactivated influenza vaccines. Injection site reactions were more frequent with intradermal vaccination.

Methods: A Phase III multicentre, randomised, controlled, double-blind (for the three different lots of intradermal vaccine) study assessed lot-to-lot consistency, immunogenicity and safety of an intradermal inactivated trivalent split-virion influenza vaccine in 2,255 adults aged 18–60 years. Participants received one of three lots of intradermal vaccine containing 9 µg of haemagglutinin per influenza strain, or a licensed intramuscular control vaccine containing 15 µg haemagglutinin/strain.

Conclusions: This intradermal vaccine containing 9 µg per influenza strain, provides an alternative to conventional intramuscular vaccination, has a reliable production method and is equally immunogenic and well tolerated in adults. The study was registered at clinicaltrials.gov (identifier NCT00383539).

Introduction

Influenza continues to be a serious infectious disease and is associated with significant morbidity and mortality.^{1,2} The ability of vaccination to reduce the burden of disease in all adult age groups is well established.^{3,4} Inactivated influenza vaccines, which have been in use for more than 50 years, are safe and effective in preventing laboratory confirmed illness in 70–90% of healthy adults.^{5–7} Traditionally however, healthy adults in the age group 18–60/65 have been excluded from the targeted priority groups for seasonal influenza vaccination, and vaccination coverage in this age-range remains low.^{8–11} A series of population-based studies of influenza vaccination coverage in five European countries has shown that, while there has been a slight increase in coverage rates in recent years, vaccine uptake remains around 10% among adults in their 20s and 30s, 15% among those in their 40s

and 21% among those in their 50s.⁹ Influenza vaccination rates among health-care workers remain similarly low, despite a specific vaccination recommendation for health-care professionals due to their increased risk of infection and the risk of transmission to their susceptible patients.^{9–12} In healthy adult populations, the main impact of influenza, which typically leads to 3 to 4 days of bed rest and restricted activity for several more days, is work absenteeism and productivity losses, which account for 80–90% of the total cost of the disease.^{4,12–16} Vaccination is the most effective way of reducing the large economic burden of influenza.

Increased vaccination uptake in healthy adults would reduce the health and economic burden of disease. Vaccination uptake may be facilitated by the availability of alternative modes of vaccination to complement current methods. Intradermal immunization exploits the skin's unique immune system, delivering antigen directly to dendritic cells in the skin

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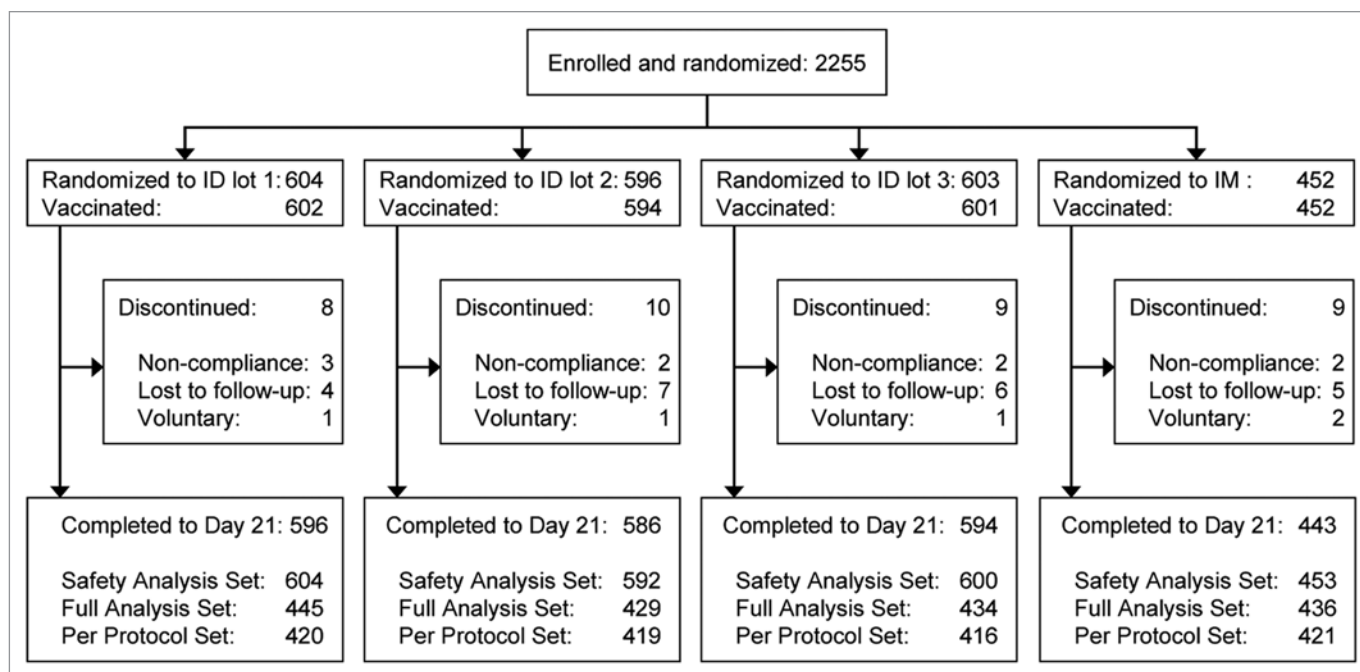


Figure 1. Randomization flow chart.

(antigen presenting cells) and the array of draining lymphatic vessels to initiate an immune response.^{17,18} Historically the use of intradermal immunization has been limited by the lack of an appropriate reliable, easy-to-use, disposable and safe intradermal injection system and technique.¹⁷ A previously described intradermal microinjection system designed to overcome these limitations was used in this Phase III study examining the immunogenicity, safety and lot-to-lot consistency of a trivalent split virion intradermal influenza vaccine intended for non-elderly adults.¹⁹ The primary objective was to test the equivalence of the immune response to intradermal vaccine from three successive industrial lots. This demonstration of lot consistency to confirm the reliability of the production process is a mandatory step in the clinical development of a new vaccine. Secondary objectives were to compare the immunogenicity of the intradermal vaccine with that of an intramuscular control vaccine, to establish whether the intradermal vaccine satisfies the European Medicines Agency (EMA) immunogenicity requirements, and to assess the reactogenicity of the intradermal vaccine and its safety profile compared with that of the intramuscular control.²⁰

Results

Trial participants. A total of 2,255 participants aged 18 to 60 years were included in the study in September and October 2006 and randomized to one of four study groups (Fig. 1). All except 36 participants completed the study to day 21: 5 withdrew voluntarily, 22 were lost to follow-up between day 0 and day 21, and 9 were discontinued for non-compliance with the protocol. The number of participants in each of the analysis subsets is summarized in Figure 1.

Demographic and baseline characteristics of the participants were well-matched across the four groups in the per-protocol population (Table 1) and in the full analysis and safety populations (not shown). There were more women than men in all groups. A similarly high proportion of participants in each group (more than 45%) had been previously vaccinated against influenza, mostly in 2005. The exact date of vaccination was not collected, however it is highly probable that the large majority of these the seasonal influenza vaccinations in 2005 were in the last trimester of the year, approximately one year before this study. Between 40 and 47% of participants per group had a medical condition considered as a risk condition for complications to influenza infection.

Immunogenicity. Before vaccination, antibody responses were comparable across the four groups, with GMTs of between 18.8 and 20.0 for A/H1N1, 22.4 and 24.1 for H3N2 and 10.4 and 10.9 for B.

Lot consistency. The equivalence of the day 21 immune responses to the three industrial lots of intradermal vaccine was demonstrated for each of the three strains, the two-sided 90% CI of the log difference in GMT between pairs of lots were between -0.176 and 0.176. For the A/H1N1, A/H3N2 and B strains, respectively, these 90% CIs were -0.065 to 0.078, -0.113 to 0.025, and 0.102 to 0.008 between lots 1 and 2; -0.051 to 0.096, -0.068 to 0.070, and 0.020 to 0.090 between lots 1 and 3; and -0.057 to 0.090, -0.025 to 0.115, and 0.025 to 0.139 between lots 2 and 3. Equivalence of the immune responses was also demonstrated when considering a more stringent two-sided 95% CI (data not shown) and was confirmed in the full analysis set. Lot-to-lot consistency allowed data from the three intradermal vaccine groups to be pooled for further analysis.

Table 1. Demographic and baseline characteristics of the per-protocol population

	ID 9 µg Lot 1	ID 9 µg Lot 2	ID 9 µg Lot 3	ID 9 µg Pooled lots	IM 15 µg
Per-protocol analysis set for immunogenicity, n	420	419	416	1255	421
Age at inclusion, years					
Mean ± SD	42 ± 12	43 ± 12	42 ± 12	43 ± 12	42 ± 12
Range	(18–60)	(18–60)	(18–60)	(18–60)	(18–60)
Gender, n (%)					
Male	167 (39.8)	164 (39.1)	169 (40.6)	500 (39.8)	171 (40.6)
Female	253 (60.2)	255 (60.9)	247 (59.4)	755 (60.2)	250 (59.4)
Male/female ratio	0.7	0.6	0.7	0.7	0.7
Previous influenza vaccination, n (%)					
Yes	199 (47.4)	199 (47.5)	191 (45.9)	589 (46.9)	192 (45.6)
No	214 (51.0)	211 (50.4)	221 (53.1)	646 (51.5)	220 (52.3)
Unknown	7 (1.7)	9 (2.1)	4 (1.0)	20 (1.6)	9 (2.1)
Year of previous influenza vaccine, n (%)^a					
2006	0 (0)	5 (2.5)	0 (0)	5 (0.8)	1 (0.5)
2005	149 (74.9)	146 (73.4)	145 (75.9)	440 (74.7)	131 (68.2)
2004	17 (8.5)	17 (8.5)	18 (9.4)	52 (8.8)	19 (9.9)
2003	9 (4.5)	8 (4.0)	6 (3.1)	23 (3.9)	7 (3.6)
Earlier than 2003	20 (10.1)	19 (9.5)	20 (10.5)	59 (10.0)	29 (15.1)
Reaction to the previous influenza vaccination, n (%)^a					
Yes	10 (5.0)	16 (8.0)	14 (7.3)	40 (6.8)	16 (8.3)
No	178 (89.4)	175 (87.9)	171 (89.5)	524 (89.0)	167 (87.0)
Unknown	11 (5.5)	8 (4.0)	6 (3.1)	25 (4.2)	9 (4.7)
At-risk participants, n (%)^b					
Yes	176 (41.9)	198 (47.3)	166 (39.9)	540 (43.0)	177 (42.0)
No	244 (58.1)	221 (52.7)	250 (60.1)	715 (57.0)	244 (58.0)

^aFor the percentage calculation, the denominator was the number known to have received a previous influenza vaccination. ^bParticipants were declared at-risk if at least one of the following medical conditions was reported in their medical history: lung disease, heart disease, diabetes, renal disease, neurological disease or any other significant history. ID, intradermal; IM, intramuscular; n/N, number of participants; SD, standard deviation.

Table 2. Haemagglutination inhibition antibody response in serum, 21 days after vaccination with either intradermal or intramuscular vaccine

	EMEA criteria*	ID 9 µg pooled lots			IM 15 µg		
		A/New Caledonia /20/99 (H1N1)	A/Wisconsin /67/2005 (H3N2)	B/Malaysia /2506/2004	A/New Caledonia /20/99 (H1N1)	A/Wisconsin /67/2005 (H3N2)	B/Malaysia /2506/2004
Seroprotection rate	>70%	87.2%	93.5%	72.9%	86.2%	95.4%	74.8%
95% CI		85.2; 89.0	92.0; 94.8	70.4; 75.3	82.6; 89.3	93.0; 97.2	70.4; 78.8
Geometric mean titre ratio post-/pre-vaccination	>2.5	9.17	11.5	6.39	9.71	11.2	6.63
95% CI		8.33; 10.1	10.4; 12.7	5.96; 6.84	8.19; 11.5	9.58; 13.1	5.90; 7.46
Seroconversion or significant increase rate	>40%	57.5%	66.5%	56.7%	56.4%	69.3%	60.8%
95% CI		54.7; 60.2	63.8; 69.0	54.0; 59.4	51.6; 61.1	64.7; 73.6	56.0; 65.4

*European Union Committee for Medicinal Products for Human Use (EMA) immunogenicity criteria of influenza vaccines in adults 18–60 years. ID, intradermal; IM, intramuscular; CI, confidence interval.

Table 3. Reactogenicity of intradermal and intramuscular vaccination: number and proportion of participants experiencing solicited reactions between day 0 and 7 after vaccination

	ID 9 µg pooled lots			IM 15 µg		
	n/N	%	(95% CI)	n/M	%	(95% CI)
Any solicited systemic reaction	788/1774	44.4	(42.1; 46.8)	213/445	47.9	(43.1; 52.6)
Headache	517/1773	29.2	(27.1; 31.3)	133/444	30.0	(25.7; 34.5)
Myalgia	417/1773	23.5	(21.6; 25.6)	131/444	29.5	(25.3; 34.0)
Malaise	323/1773	18.2	(16.4; 20.1)	86/444	19.4	(15.8; 23.4)
Shivering	167/1773	9.4	(8.1; 10.9)	33/444	7.4	(5.2; 10.3)
Fever	69/1774	3.9	(3.0; 4.9)	15/445	3.4	(1.9; 5.5)
Any solicited injection site reaction	1636/1774	92.2	(90.9; 93.4)	295/445	66.3	(61.7; 70.7)
Erythema	1497/1774	84.4	(82.6; 86.0)	113/444	25.5	(21.5; 29.8)
Swelling	1097/1773	61.9	(59.6; 64.1)	92/444	20.7	(17.0; 24.8)
Induration	1078/1773	60.8	(58.5; 63.1)	116/444	26.1	(22.1; 30.5)
Pain	765/1774	43.1	(40.8; 45.5)	215/444	48.4	(43.7; 53.2)
Pruritus	795/1774	44.8	(42.5; 47.2)	58/444	13.1	(10.1; 16.6)
Ecchymosis	177/1773	10.0	(8.6; 11.5)	44/444	9.9	(7.3; 13.1)

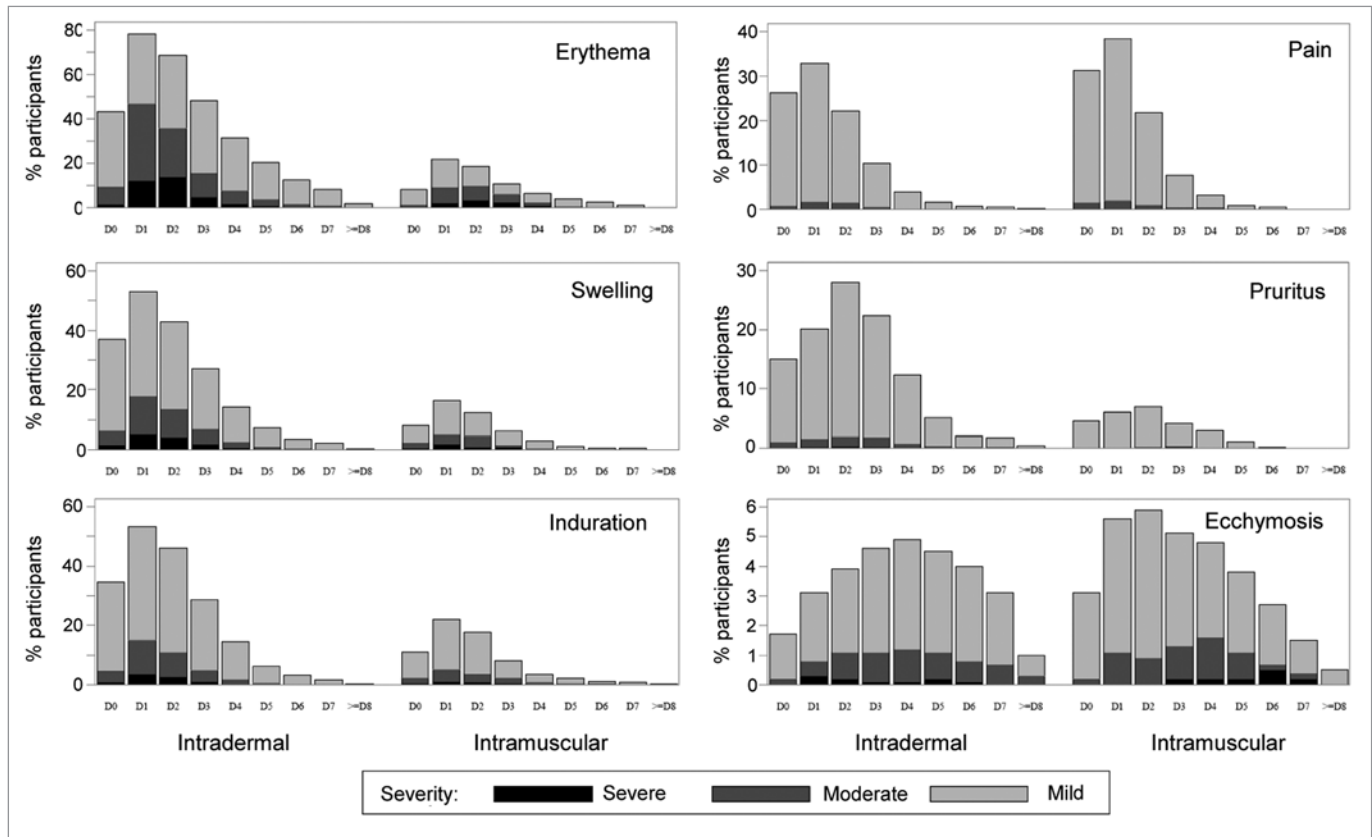


Figure 2. Daily incidence of injection site reactions in individuals with reactions (%).

Non-inferiority versus intramuscular control. Post-vaccination GMTs and their 95% CIs were high in both groups: 182 (168, 197) and 187 (162, 216) for the A/H1N1 strain, 278 (257, 301) and 274 (244, 309) for the A/H3N2 strain, 68.3 (64.1, 72.7) and 69.8 (62.7, 77.8) for the B strain, in the intradermal and intramuscular groups, respectively. The non-inferiority of the day 21 immune

response to intradermal vaccination versus intramuscular vaccination was demonstrated, since the two-sided 95% CIs of the log difference in GMT between intradermal and intramuscular groups was above -0.176 (lower bound of -0.084 for the A/H1N1 strain, -0.059 for the A/H3N2 strain, and -0.064 for the B strain) for each strain. These results were confirmed in the full analysis set.

Table 4. Seroprotection rates before and 21 days after intradermal vaccination in subgroups of participants reporting either no injection site reactions or mild, moderate or severe* solicited injection site reactions in the period 0–7 days after vaccination

	A/New Caledonia/20/99 (H1N1)		A/Wisconsin/67/2005 (H3N2)		B/Malaysia/2506/2004	
	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
No injection site reactions						
Seroprotection %	26.2	79.2	33.0	93.1	7.8	58.4
(95% CI)	(18.0; 35.8)	(70.0; 86.6)	(24.1; 43.0)	(86.2; 97.2)	(3.4; 14.7)	(48.2; 68.1)
N analysed	103	101	103	101	103	101
'Mild' reactions only						
Seroprotection %	34.4	89.3	40.0	92.7	9.9	75.4
(95% CI)	(29.8; 39.1)	(86.0; 92.1)	(35.3; 44.8)	(89.7; 95.0)	(7.3; 13.2)	(71.0; 79.4)
N analysed	425	422	425	422	423	422
'Mild' or 'moderate' reactions only						
Seroprotection %	26.3	85.1	32.3	93.9	9.0	72.9
(95% CI)	(22.5; 30.4)	(86.0; 92.1)	(28.2; 36.6)	(91.5; 95.9)	(6.7; 11.9)	(68.8; 76.8)
N analysed	498	495	498	495	498	495
At least one 'severe' reaction						
Seroprotection %	41.4	91.2	44.8	94.4	13.5	74.4
(95% CI)	(35.7; 47.3)	(87.3; 94.2)	(39.0; 50.8)	(91.0; 96.8)	(9.8; 18.0)	(68.9; 79.4)
N analysed	290	284	290	285	289	285

*Severity of injection site reactions was assessed according to the following scale: Pain or pruritus: mild = easily tolerated, moderate = sufficiently discomforting to interfere with normal activities, severe = unable to perform usual activities or led to medical care or absenteeism; erythema, swelling, induration, ecchymosis: mild <2.5 cm, moderate 2.5–4.9 cm, severe >5 cm.

EMEA criteria. After vaccination, all three EMEA criteria were met in both the intradermal and intramuscular groups for all the three strains and results were very similar in both vaccine groups (Table 2).

Safety and reactogenicity. Serious adverse events. During the six month period after vaccination, 39/1797 intradermal vaccinees (2.2%) and 8/452 intramuscular vaccinees (1.8%) experienced SAEs, none of which were considered to be related to vaccination by either the investigators or the sponsor's pharmacovigilance department. These unrelated SAEs included three deaths occurring between day 21 and month 6 (adenocarcinoma of the colon, acute left ventricular failure and liver adenocarcinoma).

Reactogenicity according to the EMEA note for guidance. A similar proportion of participants in the two groups experienced at least one of the reactions listed in the EMEA Note for Guidance [25.5% (95% CI: 23.5 to 27.6) in the intradermal group, 28.1% (95% CI: 24.0 to 32.5) in the intramuscular group]. The most frequently reported reactions were malaise (experienced by 15.2 and 17.6% of participants in the intradermal and intramuscular groups, respectively), injection site ecchymosis (6.7 and 9.2%, respectively), and shivering (7.8 and 6.8%, respectively). Pyrexia (fever: rectal equivalent temperature >38.0°C for 24 h or more) was reported by 2.7% of participants in the intradermal group and 1.3% of participants in the intramuscular group. Injection site induration of >5 cm was reported by 0.1% of participants in the intradermal group and by no participants in the intramuscular group.

Solicited injection site reactions within 7 days of vaccination. Injection site erythema, swelling, induration and pruritus were more frequent after intradermal vaccination than after

intramuscular vaccination (Table 3 and Fig. 2). In contrast, injection site pain and ecchymosis were reported by a comparable proportion of participants in each group, with a trend for fewer reports of pain after intradermal vaccination. Most injection site reactions appeared on the day of vaccination or the following day and most had disappeared by day 4 (Fig. 2). Ecchymosis after intradermal vaccination tended to appear later than other reactions, with the peak incidence observed on day 4 after vaccination. Injection site pain and pruritus were judged to be 'mild—easily tolerated' in almost all cases in both groups (Fig. 2). Pain and pruritus were judged to be 'severe—unable to perform usual activities or led to medical care or absenteeism' by respectively 0.1 and 0.5% of intradermal vaccinees and 0.2 and 0.2% of intramuscular vaccinees. Erythema, swelling and induration were more frequently classed as 'severe', particularly in the intradermal vaccine group, although the severity of these reactions was not judged by the participants but was assigned at statistical analysis based on the size of the reaction, with reactions of at least 5 cm being classed as severe.

Solicited systemic reactions within 7 days of vaccination. Each of the solicited systemic reactions occurred at very similar rates in the two vaccine groups with the exception of myalgia which appeared to affect a lower proportion of participants in the intradermal vaccine group (Table 3). The most frequently reported systemic reactions were headache, myalgia and malaise. Severe solicited systemic reactions also occurred at similar rates in the two groups: between 0.6 and 1.7% of participants in each group reported severe occurrences of headache, myalgia, malaise and shivering reactions (defined as a reaction that 'prevents daily activities'). Severe fever (body temperature >39.5°C) affected 0.2% of

intradermal vaccinees and 0.4% of intramuscular vaccinees. As with injection site reactions, systemic reactions usually appeared within the 3 days of vaccination and lasted 3 days or fewer and showed no differences between groups (data not shown).

Unsolicited adverse reactions occurring within 21 days of vaccination. The incidence and type of unsolicited adverse reactions occurring within 21 days of vaccination were comparable in the two vaccine groups, and revealed no safety issues. These reactions were reported by 6.0 and 4.9% of participants in the intradermal and intramuscular groups respectively. The reported reactions were most frequently coded to the following system organ classes: general disorders and administration site conditions (ID group: 2.2%, IM group: 1.1%), infections and infestations (ID group: 1.2%, IM group 0.9%) such as rhinitis or other upper respiratory tract infections, and respiratory, thoracic and mediastinal disorders (ID group: 0.7%, IM group: 1.8%) such as cough and nasal congestion.

Relationship between injection site reactogenicity and antibody response after intradermal vaccination. An exploratory post-hoc analysis was performed to determine whether there was a direct relationship between the occurrence of injection site reactions and seroprotection. The seroprotection rate against each of the three strains was determined in four subsets of intradermal vaccine recipients: (1) participants reporting no injection site reactions, (2) participants reporting only mild injection site reactions, (3) participants reporting at least one moderate injection site reaction but none that were severe, and (4) participants reporting at least one severe injection site reaction.

For the H3N2 influenza A strain, the seroprotection rate in the subset with no injection site reactions was similarly high (93.1%) to rates in the other three subsets with mild, moderate or severe reactions (Table 4). For the H1N1 and B strains, seroprotection rates in the subset with no injection site reactions were respectively 79.2 and 58.4%. These values were lower than in the other three subsets by no more than 17 percentage points (Table 4). For each strain, seroprotection rates were comparable between the three groups with mild, moderate or at least one severe injection site reaction. In summary, while lower seroprotection rates were observed for two out of three strains among vaccinees reporting no injection site reactions, there was no direct correlation injection site reactogenicity and seroprotection.

Discussion

This phase III trial in adults aged 18–60 years demonstrated immunogenic consistency of three consecutive production batches of the trivalent inactivated split-virion intradermal influenza vaccine, confirming the reliability and robustness of the manufacturing process. Using a statistical non-inferiority testing approach, the intradermal vaccine containing a reduced dose of antigen was also demonstrated to be as immunogenic as the reference intramuscular vaccine which has proven its safety and effectiveness after many years of use.^{21,22} This finding confirms the conclusions of a previous Phase II trial in 18–60-year-olds reported by Leroux-Roels and colleagues, and confirms that intradermal immunization results in robust immune responses.²³

In our trial both the intradermal and the intramuscular vaccines fulfilled every EMEA immunogenicity criterion for all three influenza strains.²⁰ Leroux-Roels and colleagues also showed that antibody persistence up to one year after vaccination was comparable with the intradermal and intramuscular vaccines, lending further support to the use of this intradermal vaccine as an alternative to intramuscular vaccination.

This study also evaluated the safety and reactogenicity of the intradermal vaccine in a large sample size of more than 1,800 participants. The safety profile of the intradermal vaccine was similar to that of the reference vaccine in terms of both solicited systemic reactions and the number and type of spontaneously reported adverse events. After many years of use and the distribution of billions of doses, the safety profile of inactivated influenza vaccines is well known and is considered to be good. Findings in this study were in line with this known safety profile. Compared with an intramuscular vaccination, intradermal injections are inherently safer as there is no risk of vein or nerve damage.

Intradermal vaccination caused more visible injection site reactions (mainly erythema, swelling and induration and mild pruritus) than the intramuscular vaccine. This finding was expected and consistent with previous reports.^{23,25} Increased local reactogenicity is consistent with the fact that the immune and inflammatory responses activated by the vaccine, as well as by the physical act of injection, occur close to the skin surface after an intradermal vaccination. Importantly, these reactions were transient and were not associated with an increase in injection site pain: pain occurred at comparable rates in the two groups, with a trend for a lower rate of pain in the intradermal group.

As intradermal injections are historically associated with the Mantoux tuberculin test and allergen testing, where a wheal or induration of a certain size is considered as a positive test result, it may be tempting to interpret the occurrence of injection site reactions after intradermal influenza vaccination as a direct indicator of immunization success. Indeed, in our study the overall injection site reactogenicity rate after intradermal vaccination (proportion of participants reporting at least one injection site reaction: 92.2%) was very similar to the observed seroprotection rate (range of strain specific seroprotection rate: 72.9–93.5%). However, analyses performed to explore the relationship between reactogenicity and seroprotection found that there was no direct relationship between the two, i.e., the similarity of the observed seroprotection and reactogenicity rates was coincidental. While seroprotection rates among intradermal vaccinees with injection site reactions were higher for two out of three strains than among those without injection site reaction, they were not 100%. Likewise, most of the participants who reported no injection site reactions after intradermal vaccination, developed seroprotective titres (up to 93.1% against H3N2).

Another concept associated with intradermal vaccination is antigen dose-sparing, which can be particularly relevant in situations where vaccine is in short supply. This association is related to the practice of intradermally injecting a fraction—typically a fifth—of a standard intramuscular vaccine, due to the physiology of the dermis which limits the volume that can be injected. The dose of antigen injected intradermally is reduced in direct

proportion with the reduced volume of vaccine. This practice is most frequently used with rabies vaccine.²⁴ In our study, the intradermal vaccine was not simply a fraction of the intramuscular vaccine but was specially formulated with 3/5th of the antigen content in 1/5th of the volume, and the vaccine was not designed specifically to spare antigen.

Intradermal injections were performed using a new microinjection system, designed specifically to ensure a reliable and accurate injection into the human adult dermis. This main features of this microinjection system and the clinical investigations performed to validate the accuracy of fluid delivery have been described in detail elsewhere.¹⁹ Briefly, the system is ready to use and includes an integrated 1.5 mm-long needle that is inserted perpendicular into the skin, enabling intradermal injections to be performed with no specific training. To help protect the healthcare professional against accidental sharps injury, the microinjection system has an integrated needle shielding system: after the vaccination is done, a protective sheath located within the body of the microinjection system is released by a further push on the plunger to extend forwards to cover the needle.

While the microinjection system is not needle-free and may not convince the truly needle-phobic patient to be vaccinated, patients who are reluctant to receive an intramuscular injection may more readily accept injection into the skin of a smaller volume and lower antigen dose, using a needle that is less than one tenth of the length of a standard intramuscular needle. The complementary offer of both types of vaccine has therefore the potential to contribute to the WHO objective of increasing influenza vaccination in populations such as the healthy adult population studied here. This extent of this contribution can only be evaluated after the vaccine has been in use under normal field conditions.

This Phase III study formed part of the dossier submitted to health authorities to request marketing authorization. Marketing authorization has since been granted by the European Commission for all countries in this region, where the vaccine will be distributed under the trade names Intanza[®] and IDflu[®]. Two formulations of the vaccine were developed: the formulation tested here, containing 9 µg of haemagglutinin per strain per dose, developed for adults under the age of 60 years, and a second formulation, containing 15 µg of haemagglutinin per strain per dose version for adults older than 60.²⁵

In conclusion, this large-scale Phase III trial conducted in 18–60 year olds in four European countries confirmed that a new intradermal, trivalent, inactivated split-virion vaccine against seasonal influenza is consistently immunogenic across successive production batches and is as immunogenic and safe as an intramuscular influenza vaccine control. The easy-to-use intradermal microinjection system with a 1.5 mm long, narrow gauge needle to ensure the accurate delivery of vaccine into the dermis, opens up the possibility of using the intradermal route for the routine prevention of influenza for the first time.

Materials and Methods

Trial design. A Phase III multicentre, randomized, controlled study was designed to assess lot-to-lot consistency, immunogenicity

and safety of an intradermal inactivated trivalent split-virion influenza vaccine in participants aged 18–60 years. The study was double blind for the three different lots of intradermal vaccine and open-label for the administration route.

The study was approved by ethics committees in each country (Comité de Protection des Personnes Ouest II, Angers, France; Comitato Etico Azienda Osp. Univ. San Martino, Genova, Italy; Comitato Etico Azienda USL n.7 di Ragusa, Ragusa, Italy; Universiteit Antwerp Commissie Medische Ethiek, Antwerpen, Belgium; Central Lithuanian Bioethics Committee, Vilnius, Lithuania) and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. All participants gave written informed consent before enrolment. The study was registered at clinicaltrials.gov (identifier NCT00383539).

Trial participants and procedures. Eligible participants were aged 18 to 60 years. Women were eligible with either a confirmed inability to conceive or a negative urine pregnancy test at the first visit. The main exclusion criteria were systemic hypersensitivity to egg proteins, chick proteins, or any of the vaccine components; febrile illness on the day of inclusion; congenital or acquired immunodeficiency, immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding six months or long-term systemic corticosteroids therapy; blood or blood-derived products received in the past three months; any vaccination in the four weeks preceding the trial vaccination or planned vaccination in the four weeks following the trial vaccination; or vaccination against influenza in the previous six months. After verification of eligibility, participants were enrolled and randomized to receive either the intramuscular control vaccine or intradermal vaccine from one of three lots (Fig. 1). Assignment to intramuscular or intradermal vaccination was open, but assignment to the three intradermal lot groups was double blinded. An interactive voice or internet response system (IVRS) was used to randomize participants to one of the intradermal vaccine groups or the intramuscular control group. For each participant number, the IVRS returned one of seven letters corresponding to letters on the labelled syringes (two possible letters per intradermal vaccine lot). The IVRS also assigned participants to an immunogenicity analysis subset or to a safety only subset. Blood samples were obtained from those randomized to the immunogenicity subset only. Randomization lists were generated using a permuted block method with stratification on investigational centre. After blood samples had been obtained if applicable, participants received a single injection with the assigned vaccine in the deltoid region of the non-dominant arm. A second blood sample was obtained at the next study visit, 21 days later. A third study visit occurred at month six for safety follow-up.

Vaccines. All vaccines were inactivated trivalent split-virion influenza vaccines (2006–2007 northern Hemisphere formulation) containing the following strains: A/New Caledonia/20/99 (H1N1)-like virus (H1N1), A/Wisconsin/67/2005 (H3N2)-like virus (H3N2), and B/Malaysia/2506/2004-like virus. The intradermal vaccine contained 9 µg HA/strain in each 0.1 ml dose (Intanza[®] Sanofi Pasteur, Lyon, France) administered via the intradermal route with the ID microinjection system (Soluvia[™], BD, Becton, Dickinson and Company, Franklin Lakes, NJ,

USA), described by Laurent et al.¹⁹ Briefly, the microinjection system includes an integrated 1.5 mm-long narrow gauge needle that is inserted perpendicularly into the skin to deliver 0.1 ml of vaccine directly into the dermis. The control vaccine was a licensed intramuscular (IM) vaccine (Vaxigrip®, Sanofi Pasteur, Lyon, France) containing the standard dosage of 15 µg HA/strain in each 0.5 ml dose. The manufacturing process used to produce the intradermal influenza vaccine was based on that used to produce Vaxigrip, with the addition of a concentration step to produce concentrated monovalent bulks containing an aqueous suspension of inactivated, split virions, propagated in embryonated eggs and purified by zonal centrifugation. This concentration step allows the formulation of vaccine in a lower volume in phosphate buffered saline (PBS), which is necessary to allow its administration via the intradermal route.

Immunogenicity assessment. Immunogenicity was evaluated in serum samples obtained before (on day 0) and 21 days post-vaccination (day 21 ± 3) using the haemagglutination inhibition (HI) technique in a subset of participants for the intradermal investigational vaccine group and in all participants for the intramuscular control vaccine group.²⁷ Antibody titres were determined against each of the three influenza strains in the vaccine. Each serum sample was tested in duplicate and the final titre, expressed in inverse of dilution (1/dil), was the geometric mean of the duplicates.

The primary criteria were the post-vaccine geometric mean titres (GMTs) against each of the three strains in each of the three intradermal vaccine lot groups. Secondary criteria were the pre- and post-vaccination GMTs in the pooled intradermal vaccine group and the intramuscular control group, as well as the European Medicines Agency (EMA) immunogenicity criteria in both these groups: (1) seroprotection rate (proportion of participants with serum antibody titres ≥40 1/dil), (2) seroconversion/significant increase rate (proportion of participants with either seroconversion from a pre-vaccination titre <10 to a post-vaccination titre ≥40, and a ≥4-fold increase from pre- to post-vaccination titre), and (3) geometric mean of individual titre ratios between post- and pre-vaccination titres.²⁰ For each vaccine strain and each vaccine, the immunogenicity recommendations were to meet at least one of the following three criteria for participants aged 18–60 years: seroprotection rate >70%, seroconversion/significant increase rate >40%, and geometric mean titre ratio >2.5.

Safety and reactogenicity evaluation. After vaccination, participants were provided with diary cards and were asked to keep a daily record of any solicited injection site reactions or any solicited systemic reactions that occurred on the day of vaccination or on any of the following 7 days. The daily record included the size of measurable injection-site reactions, peak daily body temperature and the severity of other reactions, judged based on a set of pre-defined grading scales (see results for details). If the reaction lasted longer than 7 days the participants were asked to record the last day of the reaction. Participants also recorded any unsolicited adverse events (AEs) occurring within 21 days of vaccination. Participants were followed to six months after vaccination for the occurrence of serious adverse events (SAEs).

Statistical methods. Statistical data were analyzed by the biostatistics platform of Sanofi Pasteur, Marcy l'Etoile, France using SAS version 8.2 (SAS Institute, Cary, North Carolina, USA).

A total of 2,250 participants were to be enrolled: 600 participants in each of the three intradermal vaccine lot groups and 450 in the intramuscular control vaccine group. Only a subset of participants (450 per group) was considered for immunogenicity in each investigational vaccine lot as the sample size was shown to be sufficient to obtain a power of 90% for the statistical testing of both lot consistency and non-inferiority.

Immunogenicity. Statistical testing was performed with the per-protocol set and confirmed with the full analysis set. Reasons for exclusion from the per-protocol set were non-respect of the inclusion or exclusion criteria, receipt of the wrong vaccine or no vaccine, receipt of a contraindicated treatment, missing blood samples or samples obtained outside the required timeframe. The full analysis set included all participants having received the vaccination with a blood sampling drawn after vaccination. Participants were analyzed according to the vaccine they actually received in the per-protocol set, or according to the vaccine allocated in the full analysis set.

Lot-consistency. The statistical methodology was based on the use of a two-sided 90% confidence interval, calculated using normal approximation of log-transformed titres, of the differences of the means of the log₁₀-transformed post-vaccination titres between pairs of lots. Equivalence among the three lots was demonstrated if, for each pair of lots and for each strain, the two-sided 90% CI lay between -0.176 and 0.176, i.e., if the ratio of the GMTs between each pair of lots and for each strain lay between -1.5 and 1.5.

Non-inferiority of intradermal versus IM. Once lot-to-lot consistency had been established, the immunogenicity data from the three ID lot groups were pooled and compared with that of the intramuscular control group using a non-inferiority testing approach on each strain.

The statistical methodology was based on the use of the two-sided 95% CI, calculated using normal approximation of log-transformed titres, of the differences of the means of the log₁₀ post-vaccination titres. Non-inferiority was demonstrated if, for each strain, the two-sided 95% CI lay above -0.176.

Immunogenicity data were also summarized in terms of GMTs, the EMA criteria, and 95% CIs for each criterion calculated using the normal approximate method for GMTs and GMT ratios using the exact binomial distribution for percentages (Clopper-Pearson's method).^{20,28}

Safety. The safety analysis set included all vaccinated participants. Participants were analyzed according to the vaccine actually received and data from three intradermal vaccine groups were pooled. Safety data were described in terms of the proportion of participants reporting reactions: solicited injection site or systemic reactions occurring between day 0 and 7, spontaneously reported adverse events occurring up to day 21, and serious adverse events occurring up to month 6. Unsolicited adverse events were described using MedDRA coding.

Additionally, the number and percentage of participants experiencing the following reactions in the three days following

vaccination were presented, as defined in the EMEA criteria: injection site induration >5 cm observed for more than 3 days, injection site ecchymosis, fever (oral temperature >37.5°C or rectal equivalent temperature >38°C) for 24 hours or more, malaise or shivering.²⁰ The 95% CIs for percentages were calculated using the exact binomial distribution for percentages as described for immunogenicity.

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Conflicts of interest

A.A. has served as principal investigator on sanofi pasteur-sponsored clinical trials for which he has received funding, and travel and accommodation costs paid for by sanofi pasteur towards participation at the 3rd European Influenza Conference, 14–17 September 2008. F.W. and M.K. are employed by sanofi pasteur. All authors had access to the study data and take responsibility for the integrity of the data and the accuracy of the data analysis.

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