Improving seasonal and pandemic influenza vaccines

Melanie Saville,[a] Grenville Marsh,[b] Agnes Hoffenbach[a]

[a]Research and Development Department, Sanofi Pasteur, Marcy L Etoile, France. [b]Global Scientific and Medical Affairs, Sanofi Pasteur, Lyon, France.
Correspondence: M. Saville, Sanofi Pasteur, 1541 Av Marcel Mérieux, 69280 Marcy L Etoile, France. E-mail: melanie.saville@sanofipasteur.com

Abstract Challenges facing seasonal and pandemic influenza vaccination include: increasing the immunogenicity of seasonal vaccines for the most vulnerable, increasing vaccination coverage against seasonal influenza, and developing vaccines against pandemic strains that are immunogenic with very low quantities of antigen to maximize the number of people who can be vaccinated with a finite production capacity. We review Sanofi Pasteur's epidemic and pandemic influenza research and development programmes with emphasis on two key projects: intradermal influenza vaccine for seasonal vaccination of both elderly and younger adults, and pandemic influenza vaccine.

Keywords Influenza vaccine, influenza, human, adjuvants, immunologic, intradermal injections.

Introduction

Since the initial development of inactivated influenza vaccines in the 1940s, vaccine manufacturers have continually sought to improve their products in response to three main drivers: increased safety and comfort of vaccinated individuals, improved manufacturing security and productivity, and greater vaccine efficacy. The most notable example of such improvements was the development in the 1970s of subvirion vaccines to replace whole virion inactivated vaccines to reduce vaccine reactogenicity, although these latter vaccines are still commercially available in some countries. The vast majority of influenza vaccines, both licensed and in development, are produced from virus propagated in embryonated hens' eggs. In recent years, considerable efforts have been made to develop alternative production systems, such as cell-culture vaccines, as a complementary source of influenza vaccine to further increase the global production capacity. Most vaccine manufacturers have cell-culture influenza vaccine development programmes and in 2007, a first cell-culture-based seasonal influenza vaccine received European licensure. Other recent developments include the reformulation of vaccine with novel adjuvants including virosomes, influenza virus-like particles and the production of recombinant influenza vaccines.[1,2]

The intense political and public interest surrounding avian and pandemic influenza in recent years has stimulated interest in influenza research by governments, non-governmental organizations, vaccine manufacturers and other researchers. The benefits of this renewed interest extend beyond our preparedness for the next influenza pandemic and into seasonal vaccination. There is increased awareness of the immediate need to increase seasonal influenza vaccination coverage and to optimize the immunogenicity of current influenza vaccines.[3]

Here, we review sanofi pasteur's epidemic and pandemic influenza research and development programme with emphasis on two key projects: intradermal influenza vaccine for seasonal vaccination and pandemic influenza vaccine development.

Intradermal vaccine against seasonal influenza

Elderly adults

The elderly are at high risk of serious disease from respiratory infections, particularly influenza, and this population accounts for the majority of the disease burden (for a review, see Ref. 4). Influenza vaccination is thus recommended for all individuals older than 65, 60 or even 50 years, depending on the country.[5] While many studies attest to the efficacy of influenza vaccination, the exact level of vaccine efficacy in the elderly remains a matter of debate.[6–14] In healthy adults aged <65 years, one dose of trivalent, inactivated vaccine is considered to be highly immunogenic.[15] With increasing age, however, changes in the immune system result in a lower

immunogenicity of influenza vaccines compared with that in younger adults.[16] Consequently, the efficacy of conventional intramuscular influenza vaccines is also lower among the elderly. This has led to calls to develop enhanced vaccines specifically for this population.[17]

One approach to increase influenza vaccine immunogenicity is the use of a vaccine adjuvant, such as MF59 in the licensed vaccine Fluad®.[18,19] A second approach is to formulate the vaccine with virosomes that mimic the structure of viral particles with the aim of improving antigen presentation, Invivac®.[20] A recent study comparing both of these vaccines, together with a licensed non-adjuvanted, subunit vaccine, found all three vaccines to have similar immunogenicity profiles.[2] Influenza vaccines change each year, so by definition, vaccination of individuals in at-risk groups is recommended before each influenza season, and thus individuals can be vaccinated every year for many years. The long-term safety of these repeated vaccinations is therefore an important issue. Although such data do not exist at an individual level, current formulations of conventional inactivated split-virion vaccines are well known and do have a good long-term safety record.[21–23] During its 40 years of existence, approximately 1 billion doses of the vaccine Vaxigrip® (Sanofi Pasteur, Lyon, France) have been distributed.

To further improve seasonal influenza vaccines, the approach adopted at Sanofi Pasteur has been to identify alternative methods to increase immunogenicity without using adjuvants or other additives. Intradermal vaccination is one such alternative. The immunological potential of the intradermal route for immunization has been known for a long time. Since the 1930s, studies with a variety of antigens have shown that vaccination via the dermis can induce comparable immune responses to intramuscular vaccination, but with a fraction of antigen dose (for a review, see Ref. 24). Intradermal vaccination against rabies is routinely used in some countries as a way of sparing antigen, and against hepatitis B, the intradermal route has also been shown to induce immune responses in individuals who were previously unresponsive to intramuscular vaccination.[25] Although the exact mechanism remains unclear, the efficiency of intradermal immunization is thought to be due to the capture and presentation of antigen by dendritic cells, predominantly dermal dendritic cells, which then drain through the extensive lymph network to the lymph nodes, as well as the direct migration to the nodes of free-antigen, resulting in the stimulation of resident lymph node dendritic cells. Together, these processes result in the activation of lymph node T cells and an efficient initiation of cellular arm immune responses.[26]

Although the potential of intradermal vaccination has been known for some time, it is only recently that advances in vaccine delivery systems have allowed this route to be considered for the large-scale production of vaccines such as influenza. Despite the successes attributable to the historical intradermal injection methods (such as the standard Mantoux intradermal injection technique that involves inserting a 27G, 3/8- inch, short- bevel needle attached to a plastic, 1-ml disposable syringe into the skin at a very slight angle, the bifurcated needle for smallpox vaccination, multipuncture devices for BCG and needle-free jet injectors), all have important drawbacks.[24] The development of an easy-to-use, disposable microdelivery system for intradermal vaccination (Soluvia™; Becton Dickinson, Franklin Lakes, NJ, USA) led to the reconsideration of the intradermal route for influenza vaccination. This system has been described in detail elsewhere.[27] Briefly, it consists of a pre-filled, ready-to-use syringe with an integral, narrow (30 gauge), short-bevel micro-needle that protrudes only 1·5 mm from the proximal end of a glass syringe. It was designed and engineered based on skin anatomical requirements to ensure a consistent and reliable injection of 0·1 ml of fluid into the papillary and reticular dermis.[27,28]

Sanofi Pasteur's intradermal influenza vaccine programme for the elderly is based on the hypothesis that the natural potential of the intradermal route can be exploited to increase immune responses to a vaccine produced using essentially the same process as that used to produce a licensed vaccine (Vaxigrip®). The physical properties of the skin limit the volume of fluid that can be injected into the dermal layer. The volume of a typical intradermal vaccination is 0·1 ml, five times less than most intramuscular vaccinations. Consequently, the challenges of developing an intradermal influenza vaccine have included not only the identification of the appropriate antigen dosage, but also the production of vaccine containing the appropriate dosage in one-fifth of the volume.

In a phase 2 study, the immunogenicity and safety of two different intradermal vaccine formulations containing 15 or 21 μg of haemagglutinin per strain were compared with that of Vaxigrip®, which contains 15 μg of haemagglutinin per strain.[29] The study was designed to test whether one or both of the investigational intradermal vaccines induced statistically superior immune responses compared with the control vaccine. Superiority was demonstrated in terms of the primary endpoint (geometric mean titres 21 days after vaccination) for all three strains, and higher responses were observed in all but one of the nine secondary analyses based on immunogenicity criteria defined by the European Committee for Medicinal Products for Human Use (CHMP) (day 21 seroprotection rates, day 21 seroconversion rates and day 0–21 mean titre increases for each of the three strains). Differences between responses in the two intradermal vaccine groups (15 or 21 μg haemagglutinin per strain) were not significant. Study subjects were aged 60–85 with a median age of

Table 1. Affect of age class on the difference in post-vaccination seroprotection rate after intradermal and intramuscular vaccination in a phase 2 study

| | Difference in seroprotection rate: 15 μg ID–15 μg IM | | |
	A/H1N1	A/H3N2	B
Aged 60–69 years	8·02 (−0·22; 16·25)	5·24 (1·33; 9·95)	8·73 (0·09; 17·31)
Aged 70–85 years	2·13 (−7·23; 11·41)	4·06 (−0·46; 8·85)	13·04 (5·10; 20·79)
All (60–85 years)	5·47 (−0·72; 11·65)	4·71 (1·81; 7·61)	11·10 (5·31; 16·89)
Heterogeneity test of inter-group differences between age class (P-value)	0·355	0·692	0·468

Results are expressed as the absolute difference in seroprotection rate and 95% confidence interval of the difference for each strain after with intradermal vaccine containing 15 μg haemagglutinin per strain or intramuscular vaccine containing 15 μg haemagglutinin per strain.

70 years. As could be expected, immune responses were lower among subjects aged 70–85 than among those aged 60–69. This was the case with both intradermal and intramuscular vaccines for each of the three strains and importantly, seroprotection rates remained higher after intradermal vaccination than after intramuscular vaccination in the older age group (Table 1). This study led to the continued development of an intradermal influenza vaccine for adults aged ≥60 years, containing 15 μg of haemagglutinin per strain, which is equivalent to the quantity of antigen contained in conventional intramuscular vaccines. The results of a subsequent phase 3 trial with this vaccine confirm the superiority observed in the phase 2 trial.[30]

Adults younger than 60 years

While the greatest challenge surrounding seasonal influenza vaccination for elderly adults is increasing vaccine immunogenicity and thus the level of protection, for younger adults the challenge is to increase the vaccination coverage. Surveys of vaccination uptake repeatedly show that coverage rates among young adults are low. In five western European countries in 2006–2007, coverage rates among adults in their twenties and thirties were around 10%.[21] In these countries, coverage rates increase with age to 15% of adults in their forties and 21% of adults in their fifties. Comparable coverage rates are reported in the USA and in countries in Asia, Latin America and Eastern Europe.[31,32] These numbers are considerably lower than the target coverage rates of between 50% and 90% set by National and International Health Organizations.[3,15,33] Even among healthcare professionals – a population assumed to be better informed of the risks of influenza for themselves and for their patients – influenza vaccination coverage remains low in many cases.[34]

The morbidity and mortality associated with influenza are lower among adults younger than 60 years than among the elderly. The vaccine-preventable disease burden in this former group is nevertheless substantial. Complications are less frequent in young adults than in the elderly, but do occur. For example, in a study of over 20 years of US National Hospital Discharge Survey data (1980s and 1990s), the average annual rate of primary pneumonia and influenza hospitalizations attributable to influenza was 6·8 per 1 00 000 person-years among persons aged 5–49 years and 37·9 among persons aged 50–64 years.[35] Typically, however, influenza infection of a healthy young adult results in uncomplicated illness with 3–7 days of high fever with other symptoms including cough, headache and myalgia. Bedrest is usually indicated for the duration of fever. The benefits of influenza vaccination in this age group are therefore often expressed in terms of cost benefits because of prevented work absenteeism. Studies among the general adult population or among working adults have shown that influenza vaccination can be cost-effective, and even cost saving.[36–38]

In addition to cost benefits, the benefits of vaccinating adults against influenza include reduced family and social disruption, and reduced transmission to others who may be at increased medical risk themselves. Indeed, while an individual's decision to get vaccinated is motivated predominantly to protect oneself,[34] it is the prevention of transmission to others who are at risk that motivates health authorities to specifically recommend influenza vaccination for healthcare workers and anyone with a household member who is in a high risk group.

Many studies have sought to understand the drivers and barriers to influenza vaccination uptake. Two recent surveys in Europe and the USA found that around 14–16% of individuals cited a dislike of needles and injections as one of the reasons for not getting vaccinated.[21,39] Other barriers included the common misperception that being healthy is sufficient protection against influenza, that the risk of

contracting the disease is low (most frequent responses in these two studies), or a lack of recommendation by an individual's family practitioner, or a lack of reimbursement. These findings suggest that, together with improved education about influenza disease and vaccination, and reimbursement programmes, alternative vaccination methods that avoid the need for a classic syringe and needle have the potential to contribute to increase vaccination uptake among such populations.

This is the rationale for the development of an intradermal influenza vaccine for young adults using the same microinjection system as described above. In contrast to the vaccine for elderly adults, the intradermal vaccine for young adults was developed with the aim of providing equivalent immunogenicity to current intramuscular vaccines. Phase 2 trials have demonstrated that a 9 μg intradermal dose of haemagglutinin per strain is sufficient to elicit an equivalent (statistically non-inferior) immune response to Vaxigrip® (J. Beran *et al.,* Vaccination and Travel Medicine Centre, Hradec Kralove, Unpublished results).[40]

Clinical trial results obtained to date reveal no safety issues with either the 9- or the 15-μg formulation of the intradermal vaccine.[29,40] The rates of solicited systemic reactions and unsolicited adverse events observed in each trial performed to date have been comparable between intradermal and intramuscular groups. Furthermore, a study conducted over 3 consecutive years, with (re-)randomization to intramuscular or intradermal vaccination each year revealed that the rates of reaction did not increase from year to year in any of the randomized subgroups, suggesting that intradermal vaccination could be safely administered repetitively or in alternation with intramuscular vaccine from year to year (J. Beran *et al.,* Vaccination and Travel Medicine Centre, Hradec Kralove, Unpublished results). Intradermal vaccination with the microinjection system is inherently safer than vaccination via the intramuscular route, since the 1·5 mm long microneedle limits the possibility of mechanical damage to nerves or blood vessels. Also inherent to intradermal vaccination is the higher frequency of minor visible injection site reactions, such as redness, swelling or induration, around the point of intradermal vaccination. Higher rates of these injection site reactions after intradermal vaccination have been observed in all the clinical trials performed to date, but importantly the incidence of injection site pain has been comparable between groups, and as with intramuscular vaccination, these reactions are short-lived and disappear spontaneously.

A marketing authorization dossier on both the 9-μg formulation for adults <60 years and the 15-μg formulation for adults ≥60 years was submitted to the European Medicines Agency and is currently (September 2008) under review.

Adjuvanted pandemic influenza vaccines

The challenges of developing a pandemic influenza vaccine candidate differ from those of developing improved vaccines against seasonal influenza in almost every respect. Although an influenza pandemic is expected, it is not known which strain of virus, or even which subtype, will cause it. The challenge is therefore to develop a vaccine against a disease that does not yet exist. Currently, the most widely accepted scenario is that the next pandemic strain will evolve out of one of the highly pathogenic avian influenza A (H5N1) strains that have been in circulation among both wild bird and poultry populations, and have caused almost 400 human cases over the past 5 years.[41] Seed strains used to produce most of the current pandemic vaccine candidates are derived by reverse genetics from one of these H5N1 avian strains.[42,43] Other possibilities include the emergence of an H7 or H9 strain, or the resurgence of a human H2N2 strain, which caused 1957 pandemic.

When a pandemic occurs, the priority will be to vaccinate as many people as possible, as quickly as possible. Among the many industrial, logistical and political challenges that this implies, the identification of a minimum dose of antigen needed to confer an acceptable level of protection against severe disease to optimize the manufacturing capacity is critical.

Sanofi Pasteur's strategy for pandemic influenza vaccine development has been to provide a first-generation vaccine as quickly as possible for stockpiling, whilst developing second-generation vaccines with improved immunogenicity profiles. Three egg-based pandemic vaccine projects have been conducted in parallel.

First, using a manufacturing process adapted from seasonal vaccine production (Fluzone® Sanofi Pasteur, Swiftwater, PA, USA), a non-adjuvanted H5N1 vaccine was produced in the USA under governmental contract and provided to the US National Institute of Allergy and Infectious Diseases (NIAID) for clinical evaluation. A dose ranging study, which tested doses of 7·5–90 μg of haemagglutinin of this non-adjuvanted vaccine, revealed that a two-dose regimen of 90 μg of haemagglutinin was needed to elicit a response expected to reduce the risk of getting influenza in 45% of subjects.[44] This led to the FDA's approval of this vaccine as a first measure to ensure the nation's readiness, pending the development of the next generation of vaccines,[45] and confirmed the need for formulations with improved immunogenicity. This vaccine has been further evaluated in a second study of the response to a third, booster vaccination 6 months later, which found that titres were higher after the booster injection than after the second injection.[46]

A second project with an adjuvanted vaccine has therefore been conducted with the dual objective of improving

the immune response to the vaccine whilst reducing the dose of antigen needed. Aluminium-based adjuvants are readily available, are widely used in licensed vaccines and have documented safety. The first clinical trial with an aluminium hydroxide adjuvant was performed in France.[47] This trial was also designed as a dose ranging and formulation finding trial and evaluated three antigen dosages (7·5, 15 and 30 μg of haemagglutinin), each with or without adjuvant. Two priming injections were given 21 days apart. The study findings were intriguing. Although the adjuvanted 30 μg formulation was the most immunogenic and 67% of subjects seroconverted after two vaccinations, there was no adjuvant effect at either of the two lower dosages. Furthermore, the lowest dose (7·5 μg) appeared to perform better without adjuvant than with adjuvant. These findings led us to select the adjuvanted 30 μg formulation and 7·5 μg non-adjuvanted formulation for further investigation. Results from a clinical trial of these two formulations in children in Thailand confirm the immunogenicity of the vaccine, with the adjuvant providing a clear immunogenic advantage.[48] Aluminium hydroxide adjuvanted H5N1 vaccines have been evaluated by others with similar findings.[49]

The MF59 oil-in-water emulsion adjuvant used in Novartis' licensed seasonal influenza vaccine for the elderly, Fluad®, has been shown to increase immune responses to H5N1 after both homologous vaccination as well as after heterologous vaccination with a non-pathogenic H5N3 virus-derived vaccine.[50,51] Another proprietary oil-in-water emulsion-based adjuvant has been developed by GlaxoSmithKline Biologicals (GSK) and has been tested in an H5N1 prototype vaccine formulation.[52] These studies have shown that these adjuvanted vaccine formulations are well tolerated and significantly dose-sparing compared with non-adjuvanted vaccine, and are able to induce cross-neutralizing antibody responses to various H5N1 clades and subclades.

Our third project, undertaken in parallel with the aluminium-based adjuvanted vaccine project, was the development of vaccine containing an oil-in-water emulsion of the same class as those described above. This proprietary adjuvant is a squalene-in-water emulsion with a very fine particle size and a narrow particle size distribution. The potential of this emulsion in a clade 1 influenza A/Vietnam/1194/2004 (H5N1) vaccine has been evaluated in preclinical studies in two species, as well as in a phase 1 clinical study. In a macaque viral challenge model, two injections of this emulsion-adjuvanted H5N1 vaccine with 30 μg of haemagglutinin were found to reduce the incidence and severity of interstitial pneumonia, and protect against infection in the lungs and upper respiratory tract after intratracheal challenge 3 months later with homologous wild-type virus.[53] Protection against disease and death was seen in a ferret model in which groups of animals received two injections of either 1·9, 3·8, 7·5 or 15 μg of haemagglutinin with emulsion

adjuvant or a saline control and were challenged 2 months later with the parental wild-type virus.[54] All vaccinated animals survived challenge, whereas five of the six controls died. Vaccinated animals also showed fewer and milder clinical signs of disease (temperature increase, body weight loss), shed less virus in nasal samples 2 and 4 days after challenge, and had fewer and milder lesions in the lungs upon histopathological examination. Both species mounted robust haemagglutination inhibition responses against the vaccine strain, as well as cross-reactive responses against the clade 2 Indonesia/5/05 strain. Strong homologous immunogenicity and cross-reactive responses were also seen in the first clinical trial with this vaccine.[55] This study in healthy 18- to 40-year olds, evaluated vaccine with antigen dosages ranging from 1·9 to 15 μg. Results showed that after two injections, even the lowest dosage elicited haemagglutination inhibiting antibody titres $\geq$32 (1/dil) in more than 70% of subject. Geometric mean titres were in the same range as 30 μg with aluminium hydroxide adjuvant and as 90 μg without adjuvant, i.e. a potential dose sparing of up to 48-fold (Figure 1).

None of the clinical or preclinical studies performed so far have revealed any clinically significant adverse events or safety signals after vaccination with any of the non-adjuvanted or adjuvanted formulations.[47,48,55] Clinical trials comparing adjuvanted and non-adjuvanted vaccine show that adjuvantation does increase the incidence of minor injection site reactions, such as redness, but that increasing the antigen dosage does not.
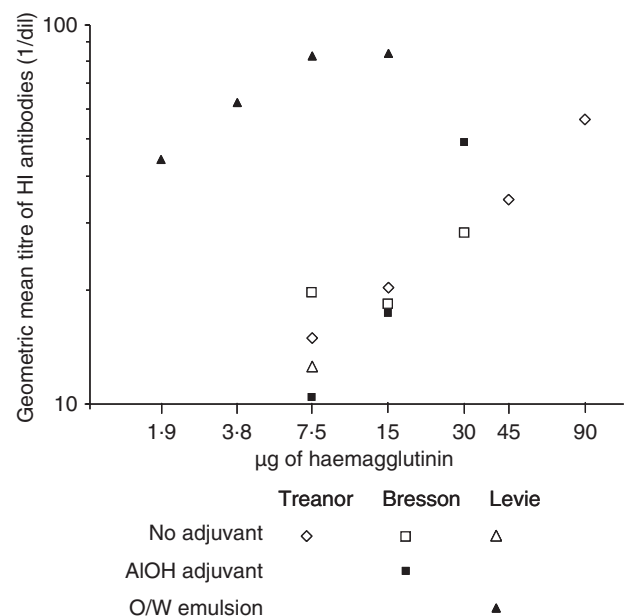

Figure 1. Geometric mean titres of haemagglutination inhibiting antibodies against clade 1 H5N1 influenza strains 21–28 days after two vaccinations with non-adjuvanted or adjuvanted vaccine in groups of healthy adults in three clinical trials. Adapted from data in Ref. 44,47,55.

Summary

The challenges facing seasonal and pandemic influenza vaccination are different. Sanofi Pasteur has therefore adopted different strategies to meet them. Recent advances in vaccine delivery systems have allowed the intradermal route of administration to be reconsidered for annual vaccination against seasonal influenza. This strategy avoids the unknowns associated with the repetitive annual administration of vaccine adjuvants and has led to the development of a new vaccine based on a current, well-known, trivalent, inactivated split-virion vaccine, with two dosage formulations designed specifically for younger and older adults. In contrast, adjuvanted vaccines will play a pivotal role in protection against the next human pandemic of influenza, helping to prime and immunize as many people as possible against a newly emerging strain of influenza with a finite vaccine production capacity. The ability of this new generation of vaccine to induce broadly cross-reactive antibodies opens up the possibility of priming with stockpiled vaccine to mitigate the impact of infection during the first few months of the next pandemic, pending the availability of vaccine against the actual pandemic strain. Defining the optimum vaccination strategy is just one of the remaining pandemic-preparedness challenges.

References

1 Tripp RA, Tompkins SM. Recombinant vaccines for influenza virus. Curr Opin Investig Drugs 2008;9:836–845.

2 de Bruijn I, Meyer I, Gerez L, Nauta J, Giezeman K, Palache B. Antibody induction by virosomal, MF59-adjuvanted, or conventional influenza vaccines in the elderly. Vaccine 2007;26:119–127.

3 World Health Organization. Fifty-Sixth World Health Assembly. Prevention and Control of Influenza Pandemics and Annual Epidemics, (Agenda Item 14.14). Geneva: WHA56.19, 2003.

4 Elliot AJ, Fleming DM. Influenza and respiratory syncytial virus in the elderly. Expert Rev Vaccines 2008;7:249–258.

5 The Macroepidemiology of Influenza Vaccination (MIV) Study Group. The macro-epidemiology of influenza vaccination in 56 countries, 1997–2003. Vaccine 2005;23:5133–5143.

6 Arnoux S, Weinberger C, Gessner BD. Vaccine-preventable influenza disease burden from clinical trials of Vaxigrip – an inactivated split virion influenza vaccine – supports wider vaccine use. Vaccine 2007;25:7720–7731.

7 Jansen AG, Sanders EA, Nichol KL, van Loon AM, Hoes AW, Hak E. Decline in influenza-associated mortality among Dutch elderly following the introduction of a nationwide vaccination program. Vaccine 2008;26:5567–5574.

8 Gross PA, Hermogenes AW, Sacks HS, Lau J, Levandowski RA. The efficacy of influenza vaccine in elderly persons. A meta-analysis and review of the literature. Ann Intern Med 1995;123:518–527.

9 Nichol KL, Wuorenma J, von Sternberg T. Benefits of influenza vaccination for low-, intermediate-, and high-risk senior citizens. Arch Intern Med 1998;158:1769–1776.

10 Nichol KL, Nordin J, Mullooly J, Lask R, Fillbrandt K, Iwane M. Influenza vaccination and reduction in hospitalizations for cardiac disease and stroke among the elderly. N Engl J Med 2003;348:1322–1332.

11 Rivetti D, Jefferson T, Thomas R et al. Vaccines for preventing influenza in the elderly. Cochrane Database Syst Rev 2006;3:CD004876.

12 Simonsen L, Viboud C, Taylor R. Influenza vaccination in elderly people. Lancet 2005;366:2086.

13 Simonsen L, Reichert TA, Viboud C, Blackwelder WC, Taylor RJ, Miller MA. Impact of influenza vaccination on seasonal mortality in the US elderly population. Arch Intern Med 2005;165:265–272.

14 Webster RG. Immunity to influenza in the elderly. Vaccine 2000;18:1686–1689.

15 Fiore AE, Shay DK, Broder K et al. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. MMWR Recomm Rep 2008;57:1–60.

16 Weinberger B, Herndler-Brandstetter D, Schwanninger A, Weiskopf D, Grubeck-Loebenstein B. Biology of immune responses to vaccines in elderly persons. Clin Infect Dis 2008;46:1078–1084.

17 McElhaney JE. The unmet need in the elderly: designing new influenza vaccines for older adults. Vaccine 2005;23(Suppl. 1):S10–S25.

18 O'Hagan DT. MF59 is a safe and potent vaccine adjuvant that enhances protection against influenza virus infection. Expert Rev Vaccines 2007;6:699–710.

19 Podda A. The adjuvanted influenza vaccines with novel adjuvants: experience with the MF59-adjuvanted vaccine. Vaccine 2001;19:2673–2680.

20 de Bruijn IA, Nauta J, Cramer WC, Gerez L, Palache AM. Clinical experience with inactivated, virosomal influenza vaccine. Vaccine 2005;23(Suppl 1):S39–S49.

21 Blank PR, Schwenkglenks M, Szucs TD. Influenza vaccination coverage rates in five European countries during season 2006/07 and trends over six consecutive seasons. BMC Public Health 2008;8:272.

22 Delore V, Salamand C, Marsh G, Arnoux S, Pepin S, Saliou P. Long-term clinical trial safety experience with the inactivated split influenza vaccine, Vaxigrip. Vaccine 2006;24:1586–1592.

23 El Sahly HM, Keitel WA. Clinical data on Fluarix: an inactivated split seasonal influenza vaccine. Expert Rev Vaccines 2008;7:713–719.

24 Lambert PH, Laurent PE. Intradermal vaccine delivery: will new delivery systems transform vaccine administration? Vaccine 2008;26:3197–3208.

25 Micozkadioglu H, Zumrutdal A, Torun D, Sezer S, Ozdemir FN, Haberal M. Low dose intradermal vaccination is superior to high dose intramuscular vaccination for hepatitis B in unresponsive hemodialysis patients. Ren Fail 2007;29:285–288.

26 Nicolas JF, Guy B. Intradermal, epidermal and transcutaneous vaccination: from immunology to clinical practice. Expert Rev Vaccines 2008;7:1201–1214.

27 Laurent PE, Bonnet S, Alchas P et al. Evaluation of the clinical performance of a new intradermal vaccine administration technique and associated delivery system. Vaccine 2007;25:8833–8842.

28 Laurent A, Mistretta F, Bottigioli D et al. Echographic measurement of skin thickness in adults by high frequency ultrasound to assess the appropriate microneedle length for intradermal delivery of vaccines. Vaccine 2007;25:6423–6430.

29 Holland D, Booy R, Looze FD et al. Intradermal influenza vaccine administered using a new microinjection system produces superior immunogenicity in elderly adults: a randomized controlled trial. J Infect Dis 2008;198:650–658.

30 Arnou R, Icardi G, De Decker M, Ambrozaitis A, Kazek M-P, Saville M. Intradermal influenza vaccine elicits superior immunogenicity in adults aged ≥60 years: a randomized controlled phase 3 trial. 13th International Congress on Infectious Diseases, Kuala Lumpur, Malaysia, International Society for Infectious Diseases, 19–22 June 2008.

31 Centers for Disease Control and Prevention (CDC). State-specific influenza vaccination coverage among adults aged > or =18 years–United States, 2003–04 and 2005–06 influenza seasons. MMWR Morb Mortal Wkly Rep 2007; 56:953–959.

32 De Lataillade C, Auvergne S, Rivas N, Delannoy I. 2005 and 2006 seasonal influenza vaccination coverage rates and drivers in ten countries from Africa, Asia Pacific, Europe, Latin America and the Middle East. J Public Health Policy 2008 (in press).

33 Orr P. An Advisory Committee Statement (ACS). National Advisory Committee on Immunization (NACI). Statement on influenza vaccination for the 2004-2005 season. Can Commun Dis Rep 2004;30:1–32.

34 Hofmann F, Ferracin C, Marsh G, Dumas R. Influenza vaccination of healthcare workers: a literature review of attitudes and beliefs. Infection 2006;34:142–147.

35 Thompson WW, Shay DK, Weintraub E et al. Influenza-associated hospitalizations in the United States. JAMA 2004;292:1333–1340.

36 Langley JM, Faughnan ME. Prevention of influenza in the general population. CMAJ 2004;171:1213–1222.

37 Postma MJ, Jansema P, van Genugten ML, Heijnen ML, Jager JC, de Jong-van den Berg LT. Pharmacoeconomics of influenza vaccination for healthy working adults: reviewing the available evidence. Drugs 2002;62:1013–1024.

38 Prosser LA, O'Brien MA, Molinari NA et al. Non-traditional settings for influenza vaccination of adults: costs and cost effectiveness. Pharmacoeconomics 2008;26:163–178.

39 Johnson DR, Nichol KL, Lipczynski K. Barriers to adult immunization. Am J Med 2008;121(7 Suppl. 2):S28–S35.

40 Leroux-Roels I, Vets E, Freese R et al. Seasonal influenza vaccine delivered by intradermal microinjection: a randomised controlled safety and immunogenicity trial in adults. Vaccine 2008 (in press).

41 Abdel-Ghafar AN, Chotpitayasunondh T, Gao Z et al. Update on avian influenza A (H5N1) virus infection in humans. N Engl J Med 2008;358:261–273.

42 Li S, Liu C, Klimov A et al. Recombinant influenza A virus vaccines for the pathogenic human A/Hong Kong/97 (H5N1) viruses. J Infect Dis 1999;179:1132–1138.

43 Nicolson C, Major D, Wood JM, Robertson JS. Generation of influenza vaccine viruses on Vero cells by reverse genetics: an H5N1 candidate vaccine strain produced under a quality system. Vaccine 2005;23:2943–2952.

44 Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. N Engl J Med 2006;354:1343–1351.

45 U.S. Food and Drug Administration. FDA Approves First U.S. Vaccine for Humans Against the Avian Influenza Virus H5N1. FDA News 2007. available at http://www.fda.gov/bbs/topics/NEWS/2007/NEW01611.html. accessed 29 September 2008.

46 Zangwill KM, Treanor JJ, Campbell JD, Noah DL, Ryea J. Evaluation of the safety and immunogenicity of a booster (third) dose of inactivated subvirion H5N1 influenza vaccine in humans. J Infect Dis 2008;197:580–583.

47 Bresson JL, Perronne C, Launay O et al. Safety and immunogenicity of an inactivated split-virion influenza A/Vietnam/1194/2004 (H5N1) vaccine: phase I randomised trial. Lancet 2006;367:1657–1664.

48 Chotpitayasunondh T, Thisyakorn U, Pancharoen C, Pepin S, Nougarede N. Safety and immunogenicity of candidate pandemic influenza A/H5N1 vaccine in children. Third European Influenza Conference, Vilamoura, Portugal, European Scientific Working Group on Influenza, 14–17 September 2008.

49 El Sahly HM, Keitel WA. Pandemic H5N1 influenza vaccine development: an update. Expert Rev Vaccines 2008;7:241–247.

50 Bernstein DI, Edwards KM, Dekker CL et al. Effects of adjuvants on the safety and immunogenicity of an avian influenza H5N1 vaccine in adults. J Infect Dis 2008;197:667–675.

51 Nicholson KG, Colegate AE, Podda A et al. Safety and antigenicity of non-adjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a randomised trial of two potential vaccines against H5N1 influenza. Lancet 2001;357:1937–1943.

52 Leroux-Roels I, Borkowski A, Vanwolleghem T et al. Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine: a randomised controlled trial. Lancet 2007;370:580–589.

53 Ruat C, Caillet C, Bidaut A, Simon J, Osterhaus AD. Vaccination of macaques with adjuvanted formalin-inactivated influenza A virus (H5N1) vaccines: protection against H5N1 challenge without disease enhancement. J Virol 2008;82:2565–2569.

54 Bigger J, Ruat C, Vasconcelos D, Legastelois I, Stark G, Caillet C. A new adjuvanted low-dose candidate pandemic influenza a(H5n1) vaccine protect ferrets against homologous clade 1 viral challenge and induces clade 2 cross-reactive antibodies. International Symposium on Avian Influenza: Integration from Control to Knowledge, Bangkok,Thailand, National Center for Genetic Engineering and Biotechnology, 23–25 January 2008.

55 Levie K, Leroux-Roels I, Hoppenbrouwers K et al. An adjuvanted, low-dose, pandemic influenza A (H5N1) vaccine candidate is safe, immunogenic, and induces cross-reactive immune responses in healthy adults. J Infect Dis 2008;198:642–649.