

# Expert Opinion

## 1. Commentary

# Strategies for preventing influenza: future perspectives in influenza vaccine technology

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Prevention of influenza transmission and containment of epidemics and pandemics require effective strategies that can be efficiently and easily addressed to the whole population. Annual vaccination is undoubtedly the most effective way to provide protection against influenza infection. Numbers of vaccines are actually available for yearly immunisation. However, the continuous increasing demand for rapidly available vaccine doses for immunisation of a larger proportion of population represents the stimulus for study and development of more efficient vaccine production technologies, which can guarantee reduction of vaccine manufacture times and better compliance by availability of easier routes of administration. New perspectives in influenza vaccination technology are making their way in the future panorama of influenza prevention strategies.

**Keywords:** administration route, influenza, vaccine technology

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## 1. Commentary

Effective prevention of infectious diseases transmission has always represented a challenge for scientists and public health authorities. Recently, the worldwide spread of the novel swine influenza H1N1 pandemic in 2009 highlighted the urgent need for rapid development of infectious diseases containment strategies for protecting population and preventing increasing morbidity and mortality.

Influenza is a highly infectious respiratory disease for which the World Health Organisation (WHO) has reported annually from three to five million cases [1]. Although generally benign, influenza is considered responsible for a number of complications in patients with chronic underlying diseases which result in increasing direct and indirect costs for the affected subjects and, globally, for the whole society.

Strategies for mitigation and containment of influenza epidemics can rely on well-known effective non-pharmaceutical and pharmaceutical interventions, such as individual hygiene, wearing face masks, quarantine, social distancing and administration of antiviral drugs and vaccines. Influenza vaccination represents actually the most effective strategy for preventing the disease and its eventually associated complications. For the 2010 – 2011 influenza season, the centers for Disease Control (CDC) suggested universal vaccination for all healthy and at risk subjects from 6 months of age onwards. Traditional trivalent inactivated influenza vaccines are largely available and, recently, a live-attenuated intranasally administered influenza vaccine has been licensed in the USA for healthy non-pregnant subjects from 2 to 49 years of age. Recent studies demonstrated a better immunogenicity of this vaccine particularly in children; however, safety data in the paediatric population are already contrasting [2].

Numbers of new influenza vaccines are being studied and developed by exploring either new production technologies or new, easier routes of administration.

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## Strategies for preventing influenza

The first challenge is the one associated with circumventing the problems of egg-based vaccine manufacture, characterised by poor flexibility and long-lasting production times, which cannot effectively respond to the eventually increasing demand for rapidly available vaccine doses in case of a new pandemic.

Encouraging results are being observed with studies on cell-based vaccines. The use of mammalian or insect cell-culture lines has numerous advantages: shortening of production times, easier production of avian strain influenza vaccines which generally poorly grow in egg substrates, absence of sterility issues and improved immunogenicity because of better antigen presentation. Madin Darbin Canine Kidney (MDCK) cells, VERO cells, from African green monkey kidney cells and PER C.6 from fetal retinal human cells are mammalian cell lines used in new influenza vaccination technologies. The MDCK cell line has been extensively studied and a numbers of experimental MDCK-grown influenza inactivated vaccines have been reported to have a good immunogenicity, at least comparable to the traditional vaccines, and a reassuring safety profile, with similar tolerability to the egg-grown vaccines [3,4]. A MDCK live cold-adapted attenuated influenza vaccine has been recently evaluated, too [5].

VERO-cell-based influenza vaccines were demonstrated to be highly immunogenic, with good humoral and cellular immune responses, with the latter being even more efficient than that of traditional inactivated vaccines [6].

As regards the PER C.6 cell-line, a Phase III study on adult subjects is being performed by Crucell and an application for license of the new vaccine is expected in 2014 [7].

Another promising alternative egg-independent vaccine production strategy is the one of viral vectors. Viral vectors (adenovirus, poxvirus, alphavirus) have been extensively studied for being used in influenza antigen presentation; however, they have not been initially considered for vaccine production because of safety concerns. Subsequent preclinical and clinical trials have later demonstrated that genetically modified viral vectors cannot multiply but continue presenting antigens effectively, so that they can be safely used for vaccine production. A preclinical study on animal models showed that an adenoviral-vector-based, adjuvant- and egg-independent pandemic influenza vaccine (HAd) is able to efficiently present influenza virus antigens and elicit good and long-lasting humoral and cellular responses against H5N1 strains [8]. Moreover, the adenovirus-vectored strategy, as intranasal or epicutaneous administered vaccine, has been evaluated in 24 healthy adult subjects, proving to be both immunogenic and safe [9].

Non-replicating virus-like particles (VLPs), resulting from a self-assembly process in a viral life cycle, are other candidates for new influenza vaccines. VLPs can be produced both from enveloped and non-enveloped viruses. Because of their non-infectious nature, VLP-based vaccines can be safely administered even in high-risk populations. A good

immunogenicity of this vaccine candidate has been observed in animal models; in humans, preliminary results from a running clinical trial showed that the vaccine could elicit a good immune response against H5N1 strains without safety concerns [10].

DNA technology was first used in influenza vaccine manufacture in 1993. A number of subsequent influenza vaccine preclinical trials in animal models suggested the possibility of eliciting both humoral and T cell-based immune responses. However, despite these encouraging preliminary results, the first human DNA-based intramuscularly administered influenza vaccine study showed no protective immune response. Subsequent human studies demonstrated positive results and a very recent Phase I clinical trial in 103 healthy adult subjects immunised with an adjuvanted DNA-based H5N1 intradermally administered influenza vaccine showed a good immunogenicity and safety [11].

Antigenic drift of viral membrane proteins haemagglutinin (HA) and neuroaminidase (NA) is the reason for the annual update of influenza vaccine formulations. Highly conserved viral proteins, such as membrane protein M2e and nucleoprotein (NP), represent new molecular targets for influenza vaccine production which have been evaluated both in preclinical and clinical trials with promising initial results. A good immunogenicity against highly pathogenic viruses has been observed with the combination of the two viral proteins [12].

Several studies of new vaccine administration routes have been recently published. Making vaccine administration easier is an important goal in a successful influenza prevention strategy. Other administration routes apart from intramuscular and deep-subcutaneous ones are intranasal, pulmonary, epidermal and oral. New anatomical administration sites offer in fact some important advantages if compared with the traditional ones: easier conservation and distribution of vaccines, reduction of adverse effect rates, in particular those associated with needle use, a better mucosal immune response through a local IgA-mediated stimulus, increasing patient compliance and cost savings.

A liquid intranasally administered influenza vaccine has been already licensed in the USA however a new dry-powder intranasal formulation is being evaluated. Powder formulation vaccines show better stability and sterility and can be stored at room or even somewhat higher temperatures, without necessity for cold chain facilities and so much easier conservation. Moreover, the stability of dry-powder formulations results in an increase of vaccine shelf-life, facilitating stockpiling of readily available vaccine doses in case of new pandemics. All these features make the new powder intranasally administered vaccine one of the best candidates for mass influenza vaccination. A preclinical study on rats demonstrated the generation of strong nasal mucosal and systemic immune response by intranasal delivery of a dry-powder-formulated influenza vaccine [13].

Pulmonary delivery of powder-formulated influenza vaccines can represent an alternative to intranasal administration. Lungs have a much larger highly vascularized absorptive surface area, contain mucosa-associated lymphoid tissue and an abundance of macrophages and dendritic cells which act as local antigen-presenting cells. Dry-powder formulations for pulmonary delivery are designed as micrometer size particles which can reach the lower airways, smaller bronchioles and alveoli, and induce both a local and systemic immunity. A pulmonary-delivered spray-freeze-dried influenza subunit vaccine has been administered in mice demonstrating a good mucosal, humoral and cell-mediated immune response which proved to be superior both to conventional intramuscular and liquid aerosolised administration routes [14].

The epidermis represent another promising anatomical target for influenza vaccine delivery. A great portion of the immune system has its localisation in the cutis: Langerhans cells, local antigen-presenting cells, can effectively stimulate a CD4 and CD8 T-cell-mediated immune response and favour antigen presentation by production of local stimulating cytokines. A number of epidermal delivery methods are being evaluated. Jet injectors for powder formulations have been developed to obtain high speed acceleration of 20 – 70  $\mu\text{m}$  particles which can penetrate the stratum corneum and effectively reach the epidermis. In a Phase I clinical trial, epidermal powder influenza immunisation in humans using a jet injector device resulted in humoral immune response with a good safety profile [15].

The ultimate nanotechnologies have been recently applied to manufacture of microscopic and minimally invasive devices for epidermal delivery of influenza vaccines. Microneedle devices are being developed for effective and pain-free administration of vaccines across the skin barrier layer.

Microneedles allowed delivery of vaccines in the underlying skin compartments inducing local and, through cutaneous circulation, systemic effects. Recent studies demonstrated that the delivery of vaccine by microneedle provides immunological responses at least equal if not superior to the intramuscular injection [16,17].

Functional miniaturisation of vaccine delivery devices has been recently achieved with development of a densely packed dissolving microprojection array, Nanopatch. This consists of highly dense silicon projections which are coated with vaccines in dry form and applied to the skin: stratum corneum is crossed and vaccine is directly delivered to the immunologically sensitive cells in the skin [18].

Oral administration is another stimulating possibility for influenza immunisation. It is a simple, safe, pain-free, non-invasive and cheap administration route. Oral vaccination can elicit an IgA-mediated mucosal immune response in the respiratory tract which protects the subject at the port of entry of infection and might offer broader protection against antigenically drifted influenza strains, too [19]. Unfortunately oral immunisation resulted in scarce IgG responses and studies are needed to evaluate if an IgA immune response could alone provide adequate protection against influenza infection.

Lastly, eye mucosa represent a new anatomical target and a fascinating alternative vaccine administration route. A first study in animal models demonstrated effective virus-specific humoral mucosal and systemic responses after eyedrop influenza vaccination [20].

### Declaration of interest

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