Human Vaccines & Immunotherapeutics 8:1, 67–75; January 2012; © 2012 Landes Bioscience

Current evidence on intradermal influenza vaccines administered by Soluvia™ licensed micro injection system

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Keywords: intradermal, influenza vaccine, micro injection system, safety, tolerability, acceptability, immunogenicity

Abbreviations: TIV, trivalent inactivated influenza vaccine; MHC, major histocompatibility complex; BCG, Bacillus Calmette-Guérin; EMA, European Medicines Agency; CHMP, Committee for Medicinal Products for Human Use

Among the several strategies explored for (1) the enhancement of the immune response to influenza immunization, (2) the improvement of the vaccine acceptability and (3) the overcoming of the egg-dependency for vaccine production, intradermal administration of influenza vaccine emerges as a promising alternative to conventional intramuscular route, thanks to the recent availability of new delivery devices and the perception of advantages in terms of immunogenicity, safety, reduction of antigen content and acceptability.

Data from clinical trials performed in children, adults <60 y and elderly people and post-marketing surveillance demonstrate that actually, licensed intradermal influenza vaccines, Intanza™ 9 and 15 μg and Fluzone™ Intradermal, administered by the microinjection system Soluvia™, show an excellent acceptability, tolerability and safety profile. Formulations containing 9 and 15 μg per strain demonstrate, respectively, comparable and superior immunogenicity than conventional intramuscular vaccines. Licensed intradermal influenza vaccines can be considered a valid alternative to standard intramuscular vaccination offering significant advantages in low-responder populations and helping to increase influenza vaccination coverage rates especially in people with fear of needles or high apprehension associated with annual vaccination.

Introduction

Annual vaccination represents the main public health strategy for reducing outpatient visits, hospitalizations and deaths due to influenza viruses, especially in at-risk populations such as children, the elderly, pregnant women and people with chronic diseases.[1] Traditional targets of annual influenza vaccination are subjects classified as being at high risk, but recent recommendations broad vaccine administration to larger numbers of people: in the USA Centers for Disease Control and Prevention recommended seasonal influenza vaccination to all people aged ≥6 mo for the 2010–11 season.[2]

In Europe, the broadening of recommendation is object of active discussion by scientific community and policy makers. However, influenza vaccine and its production cycle present some important limitations. First, clinical efficacy of conventional influenza vaccines drops from 70–90% in young adults to 30–70% in patients with underlying conditions and to 17–53% in the elderly.[2,3] Furthermore, patients receiving immunosuppressive regimens experience impaired response to influenza vaccine, due to several mechanisms only partly known;[4,5] immune senescence, with reduction of Langerhans cells, decreased activity of antigen presenting cells, reduced expression of MHC class I and II molecules, decreased production of mature naïve T cells by the thymus, is the most important reason for the lack of efficacy of influenza vaccine in the elderly.[6-8] Moreover, antigenic mismatch between the vaccine and the circulating virus strains can negatively impact on vaccine effectiveness, resulting in a decrease in vaccine-induced immunogenicity and an inadequate protection of vaccinees.[9-14]

Second, a well-established barrier to influenza immunization is a general lack of patient acceptance of traditional intramuscular vaccination: results obtained from recent European surveys indicate that 20% of adults and 14% of elderly patients refuse vaccination because they dislike injections or needles, considering other ways of vaccine administration an encouraging option to implement vaccination against influenza.[15-18]

Third, the current manufacturing processes of conventional influenza vaccines are closely dependent on the production of large virus stocks from embryonated chicken eggs:[19] problems with surge capacity, egg-adaptation of viral strains, possible contaminations, can lead to vaccine shortage, as occurred in 1973 in the US or antigenic mismatching in 2004.[20,21]

Several strategies have been explored both to enhance immune response after influenza immunization, to improve vaccine acceptability and to overcome egg-dependency for vaccine production. Innovative approaches include the use of vaccines with an increased dosage, multiple dose vaccinations, nasal-spray live attenuated influenza vaccines, vaccines based on conserved antigens, adjuvanted vaccines, alternative cell-based substrates for

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Submitted: 08/18/11; Revised: 10/10/11; Accepted: 10/14/11
http://dx.doi.org/10.4161/8.1.18419

antigen production (mammalian cells or plants), new vaccine formulations (naked DNA immunization, viral vector based vaccines), alternative ways of administration, i.e., oral, intranasal, transcutaneous, intradermic, combined intradermic and intramuscular routes. However, most of these new approaches are still far from commercialization due to safety and immunogenicity issues, especially in children.

The recent availability of new delivery devices and the perception of advantages in terms of immunogenicity, safety, reduction of antigen content and acceptability make intradermal administration of influenza vaccine a promising alternative to conventional intramuscular route. Although different new tools for intradermal vaccination have shown promising results in preclinical and clinical trials, only the micro injection system Soluvia™ (Becton-Dickinson) has received marketing authorization in the European Union and other Countries for the intradermal administration of a trivalent inactivated influenza vaccine, due to its safety, acceptability, ease of use, and reliability.[22,23]

To meet the needs to improve (1) the acceptability of vaccination in young adults, by administering a low dose of antigen, and (2) the immunogenicity in the elderly, two intradermal vaccine formulations have been marketed: Intanza™ 9 μg and Fluzone™ Intradermal, approved for adults 18 through 59 y in Europe and 18 through 64 y in the USA, respectively, and Intanza™ 15 μg approved for elderly in Europe and Canada. With regards to people < 18 y, one recent study evaluated the intradermal administration of TIV using Soluvia™ microinjection system in children aged 3 or more years, showing the greater immunogenicity of ID administration and the possibility of dose-sparing in comparison with conventional TIV.[24]

This review examines immunogenicity and acceptability, tolerability and safety data from clinical trials in healthy volunteers and at-risk populations, during which influenza vaccines were administered intradermally, with particular attention to studies using the innovative injection system Soluvia™, currently the only intradermal device licensed for influenza vaccines.

Immune Mechanisms Elicited After Intradermal Vaccination

Skin is an efficient and attractive site for vaccination due to its unique immunological and micro-vascular properties and the extreme richness in specific resident and recruited antigen-presenting cells, capable of eliciting both innate and adaptive immune responses. In particular, the principal immune target of intradermal vaccination is the dermal population of specialized dendritic cells, expressing high levels of class II MHC and CD1 molecules, such as Langerhans cells and macrophages infiltrating dermis tissue after recruitment from circulating blood. These specialized dendritic cells are extremely efficient in antigen presentation.[25-27] Immature resident dendritic cells capture and process the antigens released in the dermis, re-express part of them as peptide-MHC complexes on the surface, then undergo functional maturation, acquiring immune stimulatory ability, and migrate to the paracortical area of the regional draining lymph nodes, where the peptide-MHC complexes are specifically recognized by $CD8^+$ and $CD4^+$ T cells.[28,29] This mechanism is activated and promoted by several signaling pathways, including increased expression of MHC antigens, co-stimulatory molecules and pro-inflammatory cytokines such as IL-1 β, IL-6, IL-12 and TNFα.[30] Intradermal antigen delivery also increases the recruitment of dendritic cell precursors from the blood stream to the dermis and facilitates the lymphatic drainage of free antigen and the subsequent capture by lymph node-resident dendritic cells and/or the direct activation of specific B-cell precursors through interaction with the B-cell receptor (BCR).[28,31,32] The elicited lymphocyte T $CD8^+$ population clonally expand and diffuse to the blood stream, acquiring skin-specific homing antigens (CLA and CCR4) and becoming effector and memory T cells, while $CD4^+$ T cells promote the differentiation of B cells into antibody-producing plasma cells.[29] Compared with intramuscular vaccination that activates T-cell response through transient antigen-presenting cells or free antigen migration to the regional draining lymph nodes, due to the lack of resident antigen-presenting cells in muscles, the intradermal route offers a potentially greater immunogenicity, justified by the direct delivery of antigen to the skin immune system: results obtained with BCG, rabies, smallpox and hepatitis B vaccines confirmed this theory.[33-36]

New Devices for Intradermal Vaccine Administration and Overview of the Market

After the introduction of the intradermal injection technique by Mantoux in 1908[37] and the experience with intradermally administered typhoid fever vaccine reported by Tuft in 1931,[38] the intradermal route of vaccine delivery has been extensively studied in order to evaluate immunogenicity, safety and tolerability of different commercially available vaccines licensed for intramuscular way but administered intradermally. These experiences included vaccination against measles,[39,40] cholera,[41] rabies,[42,43] hepatitis B[44-47] and poliomyelitis.[48-51] During the last century several clinical trials have been performed to evaluate the safety, tolerability and immunogenicity of intradermal administered influenza vaccines, but the absence of a consistent clinical design and standardized investigational method have not allowed efficient and significant comparisons and meta-analyses.[52-63] Moreover, the lack of an appropriate, reproducible, easy-to-use and safe technique has limited the use of intradermal immunization, mainly because the Mantoux technique requires specific skills, special training and experience and the use of conventional needles do not ensure a precise quantity of antigens and the appropriate depth of the puncture. To overcome these problems, in recent years new devices for intradermal vaccination have been developed and extensively tested. These new delivery systems provide benefits in terms of ease of use, consistency of administered volume, accuracy of injection depth and safety of vaccination, reducing the risk of accidental puncture of health workers and preventing syringe reuse. Regarding intradermal influenza vaccination, the most clinically investigated medical devices are the MicronJet™ microneedle device (Nanopass) and the intradermal microinjection system (Soluvia™). The first device consists of an array of four silicon microneedles, each

0.45 mm in length, which is fixed to an adaptor that could be mounted on a standard syringe.[64] The second system is a platform employing a pre-filled, ready to use syringe, the volume of which ranges from 100 to 200 μl, and a tiny hollow mini needle approximately 1.5 mm in length, able to ensure proper vaccine delivery in the dermis.[22,23] Due to the very small size of both devices, vaccine delivery is nearly imperceptible for the patient and, unlike deep intramuscular injection, the potential risk of injury to nerves and blood vessels is close to zero, offering a further advantage in terms of reduction in cross-contamination risk.[65,66] Other microneedle-based influenza vaccines have been recently evaluated in a number of preclinical studies, showing promising results in terms of immunogenicity and dose-sparing effects.[67-72] Currently, only the micro injection system Soluvia™ has received marketing authorization in the European Union, the USA and other Countries for the intradermal administration of influenza vaccine.

The first licensed intradermal trivalent inactivated split-virion influenza vaccines administered using BD's Soluvia™ device are Intanza™ 9 μg and Intanza™ 15 μg (also known in some countries as IDflu™ 9 μg and IDflu™ 15 μg, sanofi pasteur), respectively approved for human use in adults aged <60 y and in elderly aged >60 y, licensed in the European Union in February 2009 and in Canada in September 2010. On May 10th 2011, the US. Food and Drug Administration approved the company's supplemental biologics license application (sBLA) for licensure of Fluzone™ Intradermal (influenza virus vaccine, sanofi pasteur), a vaccine identical to Intanza™ 9 μg for antigen content, way of administration and injection system, indicated for active immunization of adults aging between 18–64 y.

Immunogenicity

In the present review, immunogenicity results are reported according to EMA/CHMP criteria, as: mean-fold increase or geometric mean titer ratio (MFI or GMTR; ratio of post- to pre-vaccination titer), seroconversion rate (percentage of subjects with a four-fold increase in HI antibody titer, providing a minimal post-vaccination titer of 1:40) and seroprotection rate (the percentage of subjects achieving an HI titer ≥40). To meet the CHMP requirements for immunogenicity, at least one of following criteria must be met for all the three virus strains included in the vaccine composition[73,74]: for adults, the seroprotection rate must exceed 70% of immunized subjects, the seroconversion rate 40% and the mean fold increase 2.5; the respective limits for the elderly are 60%, 30% and 2.0.

Children. Different studies evaluated immunogenicity of intradermal influenza vaccination in children and infants, but TIV were always administered using the Mantoux technique and no firm statements can be made on the basis of obtained data, due to discrepant immunogenicity results and well-known limitations of the administration method.[75-78] On August 2011, the results of a Phase III clinical trial of two different intradermal doses of a split-virion TIV administered by Soluvia™ microinjection system, involving 112 previously primed healthy children aged ≥ 3 y, were disseminated. Collected data suggested that the 15 μg intradermal formulation induced a similar or a significant better immune response against type A and B strains, respectively, compared with an intramuscular virosome-adjuvanted influenza vaccine. Moreover, the 9 μg intradermal formulation showed similar seroconversion and seroprotection rates and GMT compared with those obtained with the highest dose of the same vaccine and with the intramuscular virosome-adjuvanted vaccine for A/H1N1 and A/H3N2 strains, while for the B strain GMT was higher than that obtained with virosome-adjuvanted vaccine and lower than that induced by the highest dose.[24]

Healthy volunteers younger than 64 y. We identified four clinical trials that evaluated the intradermal delivery of influenza vaccine using the microinjection system Soluvia™ in healthy volunteers younger than 64 y, all performed in the past 10 y[79-82]; another study used a tuberculin syringe fitted with a plastic disc to limit skin penetration and ensure intradermal administration of the vaccine, thus identifying a forerunner of the Soluvia™ device.[83] This last study was performed by Belshe et al. during the 2001–02 season and can be considered the first clinical trial to evaluate an innovative technique for intradermal vaccination. In this study, an intradermal injection of a reduced dose of a trivalent inactivated influenza vaccine (6 μg for each strain) resulted in similarly vigorous antibody responses in the 18–60 y group, compared with an intramuscular injection of a full-dose vaccine (15 μg for each strain): all subjects were seroprotected after vaccination for the three strains in the vaccine, although a slightly lower fold increase to the strain B antigens and a lower seroconversion rate were observed in the intradermal recipients, probably due to higher pre-vaccination titers in this group.[83] The ability of a 6 μg intradermal vaccine formulation to show similarly immunogenicity profiles, as compared with an intramuscular injection of full-dose influenza vaccine among healthy volunteers younger than 64 y, was not confirmed by all Authors. In a three-year randomized controlled trial by Beran et al., a 3 μg and a 6 μg intradermal formulation of a trivalent inactivated influenza vaccine induced immune responses inferior to those elicited by a 15 μg intramuscular vaccine and failed to reach CHMP criteria for B strain, not confirming the results obtained by Belshe et al.[80] In a large multicenter study, Frenck and colleagues have recently compared the performance of a 6 μg and a 9 μg intradermal formulation with a standard full-dose intramuscular influenza vaccine, demonstrating the non-inferiority of the two intradermal vaccines as regards to post-vaccination Geometric Mean Titers (GMTs) for all the three strains, except for the subgroup of subjects aged between 50–64 y, in which the 6 μg dose given intradermally induced lower GMTs compared with standard intramuscularly TIV for the A/H1N1 and B strains.[82]

Four studies, performed by Beran et al., Leroux-Roels et al., Arnou et al. and Frenck et al., demonstrated the ability of the intradermal vaccine, containing 9 μg of HA for each strain, to elicit equal and sometimes better antibody responses than an intramuscular TIV influenza vaccine. In these published studies, including a population of almost 3,000 adults aged <65 y receiving at least one dose of intradermal vaccines containing 9 μg HA per strain administered using BD's Soluvia™ micro injection

Table 1. Immunogenicity profile of intradermal vaccines in healthy volunteers <65 y

Authors	No. of subjects (intradermal)	Age range	Study year	Intradermal antigen dose	Immunogenicity (CHMP criteria)								
					Seroprotection rate (%)			Seroconversion rate (%)			GMT ratio		
					H1N1	H3N2	B	H1N1	H3N2	B	H1N1	H3N2	B
Belshe	123 (60)	18–60 y	2001–02	6 μg	100.0	100.0	100.0	31.7	35.0	25.0	4.0	4.0	2.4
Leroux-Roels	766 (382)	18–57 y	2005–06	9 μg	92.4	99.7	90.6	74.3	85.1	76.4	16.2	28.2	12.1
Beran	762 (382)	18–57 y	2003–04	3 μg	72.7	88.5	28.5	53.1	35.4	21.0	7.32	3.48	2.38
	761 (381)	18–57 y	2003–04	6 μg	71.3	88.2	32.9	55.1	43.0	27.3	8.38	4.19	2.73
	1086 (541)	19–58 y	2004–05	9 μg	90.0	97.2	73.0	43.0	53.1	63.4	4.3	4.4	7.8
	826 (417)	20–59 y	2005–06	9 μg	90.7	100.0	83.3	14.8	60.2	24.1	2.0	4.6	2.3
Arnou	1744 (1308)	18–60 y	2006	9 μg	87.2	93.5	72.9	57.5	66.5	56.7	9.17	11.5	6.39
Frenck Jr	1591 (399)	18–64 y	2005–06	6 μg	76.5	99.7	75.0	-	-	-	3.62	3.59	4.78
	1591 (394)	18–64 y	2005–06	9 μg	81.0	99.5	76.2	-	-	-	3.92	4.03	5.59

system—intradermal vaccine fulfilled all three the CHMP criteria for all the three vaccine strains with few exceptions.[79-82] In the study performed by Leroux-Roels et al. subjects vaccinated with intradermal vaccine showed superior humoral immune responses against both A strain (H1N1, H3N2) compared with the intramuscular group, while Beran and colleagues observed GMTRs < 2.5 and seroconversion rates $< 40\%$ against A(H1N1) and B strains in both intradermal and intramuscular groups, showing a very similar immunogenicity profile of the two formulations.[79,80]

As observed by many Authors for intramuscular vaccines, Frenck et al. demonstrated an inverse correlation between immune response and age also in intradermal vaccine recipients: the percentage of younger adults (18–49 y of age) who achieved a post-vaccination HAI ≥ 40 and an higher GMT responses were significantly greater than in subjects of 50–64 y of age for each vaccine strain ($p \leq 0.01$).[82] In **Table 1** the principal characteristics of the cited studies and the immunogenicity results in healthy volunteers younger than 64 y are reported.

Elderly and immunocompromised patients. Four studies evaluated the immunogenicity profile provided by an intradermal influenza vaccine administered using BD's Soluvia™ device in elderly subjects aged >60 y and results obtained by using 15 μg per strain intradermal formulations showed a clear superiority compared with the intramuscular split vaccine and a non-inferiority respect to the intramuscular MF59-adiuvanted vaccine.[83-86] In the already-cited study by Belshe et al., intradermal vaccination with 6 μg HA per strain elicited a vigorous immune response in subjects >60 y, comparable to that provided by the conventional 15 μg intramuscular vaccine, with the only exception for H3N2 antigen, for which a significantly higher response was observed in the intramuscular group.[83] Holland and colleagues assessed the performance rates of two intradermal formulations, containing 15 and 21 μg HA per strain, in subjects >60 y, 40% of which presenting at least one medical condition that placed the subject at risk for influenza-related complications: both intradermally delivered vaccines were significantly superior to intramuscular control vaccine for each strain in terms of post-vaccination GMT, GMTR, seroprotection and seroconversion rates, except for the rate of seroprotection against H1N1 antigens in the 15 μg intradermal group, which did not reach significance.[84] In adults aged ≥60 y, most of whom (66%) with risk conditions for complicated influenza, one dose of a 15 μg influenza vaccine given intradermally showed superior antibody responses against each vaccine antigen compared with the intramuscular control vaccine also in a 3-y, phase III, multicenter study performed by Arnou et al. Moreover, intradermal vaccine

Table 2. Immunogenicity profile of intradermal vaccines in elderly

Authors	No. of subjects (intradermal)	Age range	Study year	Intradermal antigen dose	Immunogenicity (CHMP criteria)								
					Seroprotection rate (%)			Seroconversion rate (%)			GMT ratio		
					H1N1	H3N2	B	H1N1	H3N2	B	H1N1	H3N2	B
Belshe	102 (56)	>60 y	2001–02	6 μg	100.0	92.9	100.0	17.9	16.1	17.9	1.9	2.1	2.0
Holland	1101 (366)	>60 y	2006	15 μg	>70	>90	>80	>40	>40	>40	>3	>4	>3
	1101 (369)	>60 y	2006	21 μg	>80	>90	>80	>40	>40	>40	>4	>5	>4
Arnou	3685 (2604)	>60 y	2006	15 μg	>70	>90	>50	>30	>60	>30	>3	>8	>3
	407 (133)	>60 y	2007	15 μg	95.5	98.5	55.6	74.2	36.6	14.3	9.64	2.92	1.77
	468 (121)	>60 y	2008	15 μg	81.8	92.6	70.2	37.2	73.6	47.1	2.88	8.45	3.76
Van Damme	790 (395)	>65 y	2007	15 μg	81.3	>90	>50	>60	>40	>20	>8	>4	>2

met not only the European CHMP requirements defined for adults aged >60 y for almost all the vaccine strain, but also the more rigorous criteria established for younger adults for H1N1 and H3N2 seroprotection rates, H3N2 seroconversion rate and GMTRs for all strains.[85] Van Damme et al. demonstrated the non-inferiority of a 15 μg intradermal vaccine compared with a licensed MF59™-adjuvanted vaccine in elderly subjects aged ≥65 y: in the immunogenicity assessment by the HI and the Single Radial Hemolysis (SRH) methods, the results for all three strains satisfied all the CHMP immunogenicity criteria, with the exception of seroprotection and seroconversion rates for strain B antigens by using the HI assay.[86] The global population included in the studies conducted on adults aged ≥60 y using an intradermal vaccine containing 15 μg HA per strain and delivered by micro injection system BD's Soluvia™ was about 3,400 subjects. In **Table 2**, the principal characteristics of the cited studies and the immunogenicity results in elderly subjects aged >60 y are reported.

Only one study performed by Morelon et al. assessed the immunogenicity of a 15 μg intradermally administered influenza vaccine in immunocompromised patients, namely a renal transplant population previously identified as non-responder to a plain intramuscular vaccine. In this randomized, open-label, phase II descriptive study, enrolling 62 subjects, intradermal formulation elicited HI antibody responses higher than conventionally administered TIV, and CHMP criteria were met for H1N1 and H3N2 GMTRs and for H1N1 and B seroprotection rates in the intradermal group, while none of the CHMP requirements were met in the control group.[87]

Safety, Tolerability and Acceptability

In terms of safety and reactogenicity, CHMP guidelines state that within 3 d of vaccination the following reactions need to be assessed: injection site duration >5 cm observed for >3 d, injection site ecchymosis, body temperature of >38.0°C for ≥24 h, malaise, and shivering. It is noteworthy that CHMP reactions were specifically designed to determine the reactogenicity of intramuscular vaccine and may not be fully appropriate to assess the tolerability profile of intradermal vaccination.[73,74]

BD's Soluvia™ microinjection system. The intradermal microinjection system BD's Soluvia™ has been evaluated for performance and safety in two sponsored pivotal clinical trials (BDPS 05–01 and BDPS 05–02), conducted on 645 adults (age range 18–80 y) receiving saline solution without antigen. Assessments of skin reactivity at the injection site, local and systemic adverse events, the intensity of perceived pain during needle insertion, and fluid infiltration were performed. The observed local adverse events associated with the new injection technique did not significantly differ from reactions in subjects injected with standard Mantoux method. They spontaneously reversed within 20–30 min without sequel or requirement of any medical intervention and no serious adverse events were reported. Importantly, pain after the micro injection procedure was significantly lower than with standard Mantoux injection technique: it was usually reported as a faint burning perception and its intensity was a third compared with that observed in subjects injected using the standard Mantoux technique ($p < 0.0001$) according to the visual analog scale (VAS). Thus, BD's Micro Injection System Soluvia™ met the safety criteria required to successfully administer vaccines in a clinical setting representing a viable alternative to imtramuscular immunization.[22]

Children. Esposito et al. reported also safety and reactogenicity assessments of the two different formulations of intradermal vaccine administered by Soluvia™ microinjection system in a population of previously primed healthy children aged ≥3 y. Local adverse events, in particular swelling/induration and pain, were significantly more frequent among the children receiving both 9 and 15 μg intradermal formulation than among those receiving an intramuscular virosome-adjuvanted influenza vaccine, but they lasted no more than two days and did not require any medical intervention. The incidence of systemic reactions was low and comparable between the vaccine groups and no serious adverse events were reported. No differences between the two intradermal formulations were observed in terms of recorded adverse events.[24]

Healthy volunteers younger than 64 y. In all published studies evaluating safety profile of influenza vaccines given intradermally using BD's micro injection system Soluvia™ in healthy adults younger than 64 y, reactogenicity of the intradermal formulations was comparable to that of control intramuscular vaccines in terms of both CHMP reactions, solicited systemic reactions and spontaneously reported adverse events.[79-83] In fact, in all studies, no significant differences in the incidence of reactions included in the CHMP guideline between intradermally BD's Soluvia and intramuscularly administered influenza vaccines were observed. Principal characteristics of the cited studies and percentage of subjects with CHMP reactions after intradermal influenza vaccination in this age group are reported in **Table 3**. Leroux-Roels et al. and Arnou et al. reported a higher incidence of malaise and injection site ecchymosis in intradermal recipients than other studies, but a similar proportion of participants in the respective intramuscular control groups also experienced these reactions.[79,81]

Among solicited systemic reactions not included in the CHMP criteria, headache, myalgia, and fever of any temperature occurred at similar rates in intradermal and intramuscular groups, with the exception of myalgia which was more frequent after intramuscular vaccination, according to Leroux-Roels et al. and Arnou et al.[79,81] As regards solicited injection site reactions within 7 d of vaccination, significant local inflammatory signs occurred mostly in those receiving intradermal vaccinations as compared with intramuscular injection groups. Injection site erythema, swelling, mild induration, pruritus (itching) and redness were the more frequently reported local reactions, affecting 29–88% of intradermal recipients vs. 6–26% of intramuscular vaccinated subjects. However, these local reactions were not associated with an increased incidence of injection site pain: notably, a trend for fewer reports of pain after intradermal vaccination has been reported by different Authors.[81-83] Inflammatory local symptoms were mainly of mild or moderate severity, lasted on average two or three days and all resolved spontaneously without sequelae and/or medical interventions. Interestingly, Beran and colleagues

Table 3. Reactogenicity profile in healthy volunteers <65 y: Difference in incidence of adverse events in subjects immunized with intradermal and intramuscular vaccines, respectively

Authors	No. of subjects (intradermal)	Age range	Study year	Intradermal antigen dose	Reactogenicity (CHMP criteria) (difference intradermal-intramuscular: % of subjects)				
					Injection site induration	Injection site ecchymosis	Fever > 38.0°	Malaise	Shivering
Leroux-Roels	978 (588)	18–57 y	2005–06	9 μg	+0.2	-0.8	+0.7	-2.8	-1.4
Beran	766 (384)	18–57 y	2003–04	3 μg	-0.3	-1.3	-0.6	-1.9	+1.3
	765 (383)	18–57 y	2003–04	6 μg	-0.3	-1.5	-0.3	-0.6	+1.3
	1091 (544)	19–58 y	2004–05	9 μg	+0.9	-0.2	+0.6	-1.1	-0.5
	828 (418)	20–59 y	2005–06	9 μg	0	-0.3	+1.9	-1.1	+0.8
Arnou	1744 (1308)	18–60 y	2006	9 μg	+0.1	-2.5	+1.4	-2.4	+1.0

demonstrated that prior intradermal vaccination did not adversely affect the safety profile of subsequent intramuscular or intradermal injections, suggesting that influenza intradermal vaccination can be repeated annually without increasing reactogenicity.[80]

Both in terms of unsolicited adverse reactions occurring within 21 d after vaccination and serious adverse events reported up to six months after vaccination, intradermal and intramuscular vaccine groups were comparable and no safety concerns were raised when using intradermal influenza vaccines delivered by BD's Soluvia™ device, from available data in healthy adults <64 y.[79-83]

Elderly and immunocompromised patients. The analysis of safety and tolerability data from the three available studies reporting CHMP local reactions performed in older adults aged >60 y highlighted a comparable incidence in intradermal and intramuscular vaccine groups.[84-86] Holland et al. observed a higher incidence of injection site ecchymosis in patients receiving a 15 μg dose of intradermal vaccine than that observed in the intramuscular group.[84] With regard to solicited reactions occurring within 7 d after vaccination, the incidence of systemic reactions, mostly of mild intensity and lasting ≤3 d, was comparable between the vaccine groups, while intradermal recipients reported higher rates of injection site reactions, namely erythema (63–79% of patients), induration (33–67%), swelling (34–62%) and pruritus (28–30%).[83-86] In contrast, injection site pain occurred at similar rates in both intradermal and intramuscular groups.[83,84,86] More than 95% of the solicited injection site reactions were transient, lasted 3 d or less and were considered of mild severity.[84-86] Unsolicited adverse events reported within 21 d after vaccination occurred at a comparable frequency in both vaccine groups; Holland and colleagues reported a slightly higher incidence of injection site warmth in patients receiving 15 or 21 μg intradermal influenza vaccine.[84] Similar percentages of subjects in both intradermal and intramuscular vaccine groups reported at least one serious adverse event, most of which were unrelated to vaccination, and revealed no safety issue.[83-86] Principal characteristics of the cited studies and percentage of subjects with CHMP reactions after intradermal influenza vaccination in adults >60 y are reported in **Table 4**.

Morelon et al. reported safety evaluations of an intradermal vaccine containing 15 μg HA per strain in a population of renal transplant recipients on chronic immunosuppressive therapy. As described for healthy adults and elderly, injection site reactions (erythema, swelling, induration and pruritus) were more frequent in the intradermal vaccine group than in the intramuscular control group; incidence of injection pain was comparable in the two study groups; no unsolicited adverse events and no clinical signs of transplant rejection were reported.[87]

Intradermal vaccine acceptability. Two recent studies estimated perception, acceptance and willingness to get vaccinated with an intradermal influenza vaccine administered by BD's micro injection system, in European adults and elderly enrolled in two phase III clinical trials and recruited from a consumer panel, respectively.[88,89] Reygrobellet et al. administered a validated questionnaire to 1,679 adults <60 y and 2,262 elderly >60 y respectively receiving one dose of a 9 μg or 15 μg intradermal influenza vaccine, enrolled in five European countries during two large comparative clinical trials. Answers about anxiety before and after vaccination, bother from pain during injection, acceptability

Table 4. Reactogenicity profile in elderly: Difference in incidence of adverse events in subjects immunized with intradermal and intramuscular vaccines, respectively

Authors	No. of subjects (intradermal)	Age range	Study year	Intradermal antigen dose	Reactogenicity (CHMP criteria) (difference intradermal-intramuscular: % of subjects)				
					Injection site induration	Injection site ecchymosis	Fever > 38.0°	Malaise	Shivering
Holland	1101 (366)	>60 y	2006	15 μg	0	+3.6	-0.5	+2.6	0
	1101 (369)	>60 y	2006	21 μg	0	-1.1	0	+0.3	+1.3
Arnou	3695 (2606)	>60 y	2006	15 μg	+0.1	-0.2	-0.3	+0.8	-0.7
Van Damme	790 (395)	>65 y	2007	15 μg	0	+0.3	-1.5	-0.2	+0.2

of local reactions, satisfaction with the injection system and willingness to be re-vaccinated were recorded and compared with the control intramuscular population, revealing no marked differences between vaccine groups. Levels of injection site reactions acceptability, satisfaction and willingness to be re-vaccinated were high in both intradermal and intramuscular groups: more than 96% of participants rated local reactions after vaccination as either "totally acceptable" or "very acceptable," more than 92% were "very satisfied" or "satisfied," more than 81% answered "yes, definitely" or "yes, probably" about willingness to be re-vaccinated. The perception of the injection site reactions, although markedly more frequent in intradermal vaccine recipients, was not a cause for concern and did not negatively affect acceptability, satisfaction and willingness to get vaccinated the following year.[88] Arnou et al. investigated whether the availability of Intanza™ 9 μg and Intanza™ 15 μg (sanofi pasteur) might influence physicians and general public likelihood of recommending or seeking seasonal influenza vaccination. Although participants based their responses only on investigational information about intradermal vaccine, rather than experience of using or receiving it, making these surveys theoretical, results indicated that physicians would prefer Intanza™ to conventional intramuscular vaccination, perceiving its benefits in terms of shorter needles and immunogenicity. Moreover, the general public would be encouraged to get vaccinated, perceiving that intradermal route of administration reduces apprehension and fear associated with annual influenza vaccination.[89]

Conclusions

Data from clinical trials performed on children, adults aged <60 y and in elderly people and post-marketing surveillance demonstrated that actually, licensed intradermal vaccines showed an excellent acceptability, safety and tolerability profile. Immunogenicity of 9 μg and 15 μg formulations was comparable and superior to conventional intramuscular vaccines, respectively. Higher immunogenicity offered by Intanza™ 15 μg was essentially demonstrated in elderly adults, 21 d after immunization and against homologous egg-grown vaccine strains. Several unknown aspects deserve more in-depth studies: immune response and safety and tolerability profile in younger unprimed children, antibody persistence 3–5 mo after vaccination, cross-reactive immune response against drifted strains or heterologous viruses, cellular immune response, priming-effect against potentially pandemic deserve further research to better define the potential of intradermal vaccine. Data on the higher ability of intradermal vaccine to elicit cross-reactive antibodies against heterologous circulating viruses than intramuscular formulation were recently reported by Ansaldi et al. (The Fourth ESWI Influenza Conference, Malta, September 2011).

Moreover, clinical trials are needed to evaluate the advantages offered by use of Intanza™ 15 μg, currently only approved for the elderly population, or Intanza™ 9 μg in low responders aged <60 y, such as immunocompromised subjects or patients with chronic diseases. Furthermore, although greater immunogenicity induced by Intanza™ 15 μg has been observed in a number of studies, it is not yet clear how this translates into protection against influenza and influenza-related complications and mortality. Comparisons between the effectiveness of the intradermal formulation and that of plain intramuscular vaccine may provide evidence on the advantages offered by "implemented" vaccine in terms of clinical effects.

Disclosure of Potential Conflicts of Interest

G.I. has previously participated at speaker's bureaus and advisory board meetings sponsored by GSK, Pfizer and Sanofi Pasteur and has received research funding as principal investigator from Crucell Berna, GSK, Pfizer and sanofi pasteur. A.O. has not conflict of interest. A.C. has not conflict of interest. F.A. has previously participated at speaker's bureaus and advisory board meetings sponsored by Novartis Vaccines, GSK and sanofi pasteur and has received research funding as principal investigator or co-investigator from Pfizer, Novartis Vaccines and sanofi pasteur. No other relationships/conditions/circumstances that present a potential conflict of interest exist.

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