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Phase 4 randomized trial of intradermal low-antigen-content inactivated influenza vaccine vs. standard-dose intramuscular vaccine in HIV-1-infected adults

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Abbreviations: CHMP, committee for medicinal products for human use; HI, hemagglutination-inhibition; ID, intradermal; IM, intramuscular; MFI, mean-fold increases

This study evaluated safety, tolerability and immunogenicity of intradermal (ID) trivalent inactivated split influenza vaccine, with a lower antigen content (9 μg HA per strain) than the conventional intramuscular one (15 μg), in HIV-1-infected adults younger than 60 years. A total of 54 HIV-1-positive participants were enrolled and randomly assigned to receive a single dose of either ID-administered low-antigen-content split inactivated vaccine or intramuscularly-administered (IM) standard-dose inactivated split vaccine. Subjects were provided with a diary to monitor any local and/or systemic reactions to the vaccine for 7 days following vaccination. Serum samples were collected before, 28 and 90 days after immunization. The plasma HIV-RNA and $CD4^+$ T-lymphocyte count were checked at day 0 and day 90. Serum hemagglutination-inhibition (HI) activity for the three influenza strains included in the vaccine composition was measured to assess the antibody response at one month and 3 months after vaccination. Both vaccines showed optimal safety and tolerability profiles. All the three Committee for Medicinal Products for Human Use immunogenicity criteria for vaccine approval in adults younger than 60 were met by both vaccines against A(H1N1) and A(H3N2) viruses. Both vaccines met mean-fold-increase and seroprotection criteria but failed seroconversion criteria against B virus. No difference in terms of post-vaccination geometric mean titers, mean fold increase, seroprotection and seroconversion rates were found comparing ID and IM vaccines. In conclusion, the recently available low-antigen-content ID vaccine is safe, well-tolerated and as immunogenic as IM standard-dose influenza vaccine.

Introduction

CDC and many EU Member States recommend yearly influenza vaccination for HIV-infected adults, although this recommendation has not received universal support and coverage is suboptimal.[1,2]

Multiple studies have shown lower antibody responses in HIV-infected patients compared with uninfected individuals. In particular, individuals with advanced HIV disease may have a poor immune response to vaccination: $CD4^+$ cell count, HIV-RNA and age have been recognized as determinants of immune response by the majority but not all studies.[3,4]

Among strategies to improve immunogenicity, increased antigen dose or booster dosing of seasonal vaccine showed no significant effect and alternative influenza vaccines are required for this hyporesponsive population.[5]

Recently, more efficient routes of vaccine delivery, such as intradermal administration have been explored to augment immune response. Intradermal administration offers a number of advantages compared with intramuscular or subcutaneous routes regarding acceptability, immunological response and logistical aspects. The potential advantages offered by intradermally administered influenza vaccine, regarding both the improved immunogenicity in high-risk and low-responder groups and the antigen dose reduction, are mainly due to the extreme richness in various resident and recruited types of dendritic cells, the professional antigen-presenting cell capable of stimulating both innate and adaptive immune responses in this district.[6,7]

On February 2009 and September 2010, Intanza® 9 μg, the intradermal (ID) vaccine with a lower antigen content (9 μg HA per strain) than the conventional intramuscular (IM) vaccine (15 μg) was licensed for use in adults aged between 18–59 y in

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Table 1. Demographic, anamnestic and clinical characteristics of the participants receiving ID or IM vaccine

	Vaccine route of administration and antigen dose		p-value
	Intradermal 9 μg	Intramuscular 15 μg	
Median age (IQR, years)	47 (35.7–52.2)	48.7 (37–53.9)	0.345
Male sex, n (%)	20 (71.5%)	17 (65.4%)	0.771
Pandemic influenza vaccination during the 2009/10 season, n (%)	10 (35.7%)	10 (38.5%)	1.000
Seasonal influenza vaccination during the 2009/10 season, n (%)	13 (46.4%)	10 (38.5%)	0.593
Subjects on HAART, n (%)	27 (96.4%)	24 (92.3%)	0.604
Previous AIDS, n (%)	0*	4* (16%)	0.11
Nadir CD4⁺ T cell count, median (IQR) (cells/μl)	329 (193–475)	207 (95–434)	0.116
CD4⁺ cell count at baseline median (IQR) (cells/μl)	573 (431–726)	427 (334–594)	**0.038**
CD4⁺ cell count 3 mo post-vaccination, median (IQR) (cells/μl)	567 (405–805)	442 (351–760)	0.23
HIV VL at baseline, median (IQR) (log10/ml)	1 (1–1.48)	1 (1–1.29)	0.442
HIV VL 3 mo post-vaccination, median (IQR) (log10/ml)	1 (1–1)	1 (1–1.38)	**0.044**
HIV VL at baseline < L.D.L., n (%)	20 (71.4%)	14 (53.9%)	0.26
HIV VL 3 mo post-vaccination < L.D.L., n (%)	24 (88.9%)	15 (62.5%)	**0.046**

*data from 5 patients (4 and 1 in ID and IM group, respectively) are not available.

EU and Canada, respectively. Fluzone® Intradermal, the intradermal vaccine with identical characteristics, was approved on May 2011 by the US FDA for immunization of adults 18 through 64 y of age.

The aim of the study was to explore the safety, tolerability and immunogenicity profiles and the persistence of antibody responses of Intanza® 9 μg, in HIV-positive adults and to compare its performance with that of standard-dose intramuscularly-administered influenza vaccine.

Results

A total of 54 HIV-infected subjects were enrolled; 28 and 26 were randomly assigned to receive one dose of ID or IM influenza vaccine. Two enrolled subjects belonging to IM group were lost at follow-up.

For clinical data assessment, 99.1% and 96.2% of the samples under protocol were available for viral load and CD4⁺ cell counts, respectively. The reasons of drop-out were unwillingness to undergo additional blood draws. **Table 1** summarizes the demographic and clinical characteristics of the participants according vaccine. They were comparable in terms of age, gender, proportion of subjects who had received pandemic and seasonal influenza vaccines during the previous season, proportion of subjects on HAART and with previous AIDS-defining illnesses,† viral load at baseline, CD4⁺ cell count at nadir, while CD4⁺ cell count at baseline was significantly higher in ID compared with the IM group.

No serious adverse events related to the vaccines were reported during the study. Local adverse events were significantly more common after ID injection than after IM injection (64% vs. 19%): pruritus (29% vs. 4%), redness (46% vs. 8%), swelling (43% vs. 12%) induration (46% vs. 4%), as assessed on diary cards, were significantly more frequent among subjects immunized with the ID than among those with the IM vaccine. All local events were mild and moderate and resolved without sequelae. The incidence of solicited systemic reactions was comparable between groups and not significant difference emerged (fever 7% vs. 13%, headache 19% vs. 4%, shivering 21% vs. 12% in ID and IM groups, respectively).

No statistically significant pre-post vaccination differences in CD4⁺ cell count were recorded in both vaccine groups and across groups, whereas HIV-RNA‡ significantly decreased in the 3 mo after vaccination in the ID group (p = 0.019), but not in the IM groups (p = 0.233). Post-vaccination viral load and the proportion of individuals with detectable HIV-RNA were significantly higher in the IM than in the ID group (p = 0.044 and 0.046, respectively).

Immunogenicity results are summarized in **Table 2**. Pre-vaccination HI titers against all the 3 vaccine strains were similar in the ID and IM groups (data not shown). A significant increase of pre-vaccination titers was observed at 1 and 3 mo after vaccination in both vaccine groups and against all the vaccine strains (p < 0.001 for all combinations). HI titers at 3 mo significantly decreased compared with titers at 1 mo against all the 3 strains included in the vaccine composition (p = 0.013, 0.001 and 0.002 for A(H1N1), A(H3N2) and B, respectively) with a difference of about one third of HI dilution (difference in titers transformed into base 2 logarithms: -0.392, -0.333 and -0.29 for A(H1N1), A(H3N2) and B, respectively). A 2-fold titer increase for H1N1 was observed at 3 mo after vaccination in a non-responder immunized with IM vaccine, probably due to virus contact, although

†Proportion of subjects with detectable viral load.
‡In those individuals with detectable viral load.

Table 2. Antibody response determined using HI assays after vaccination with an ID and IM vaccine

	Vaccine route of administration and antigen dose		p-value
	Intradermal 9 μg	Intramuscular 15 μg	
Geometric mean titer 1 mo post-vaccination (95% C.I.)			
A(H1N1)	80 (49–130)	97 (52–181)	0.601
A(H3N2)	57 (34–95)	61 (37–99)	0.912
B	55 (36–85)	59 (37–95)	0.717
Mean fold Increase 1 mo post-vaccination (95% C.I.)			
A(H1N1)	7.1 (3.8–13.2)	10 (5.5–19)	0.387
A(H3N2)	5.4 (3.3–8.8)	5.7 (3.1–10.7)	1.000
B	2.6 (1.9–3.6)	2.5 (1.8–3.4)	0.861
Seroconversion rate 1 mo post-vaccination (%, (95% C.I.)			
A(H1N1)	50 (33–67)	64 (45–80)	0.407
A(H3N2)	54 (36–71)	60 (41–77)	0.783
B	36 (21–54)	32 (21–54)	1.000
Seroprotection rate 1 mo post-vaccination (%, (95% C.I.)			
A(H1N1)	79 (61–90)	80 (61–91)	1.000
A(H3N2)	82 (64–92)	80 (61–91)	1.000
B	75 (57–87)	76 (57–89)	1.000
Geometric mean titer 3 mo post-vaccination (95% C.I.)			
A(H1N1)	61 (38–97)	75 (45–126)	0.501
A(H3N2)	46 (29–75)	53 (35–82)	0.663
B	49 (32–76)	46 (30–71)	0.808
Seroprotection rate 3 mo post-vaccination (%, (95% C.I.)			
A(H1N1)	71 (53–85)	83 (64–93)	0.346
A(H3N2)	71 (53–85)	83 (64–93)	0.346
B	64 (46–79)	75 (55–88)	0.549

no respiratory symptoms or fever were reported by vaccinees and caregivers. No significant difference in titer decrease across vaccine groups was observed.

No difference in terms of post-vaccination GMTs, mean fold increase, seroprotection and seroconversion rates at 1 mo after vaccination were found comparing ID and IM vaccines. GMTs and seroprotection rates were similar in the two vaccine groups also at 3 mo after vaccination. All three CHMP immunogenicity criteria for vaccine approval were met by both vaccines against A(H1N1) and A(H3N2) viruses. Both vaccines met MFI and seroprotection CHMP criteria but failed seroconversion criteria against B virus.

Discussion

Newly licensed influenza vaccine using the Soluvia™ system for intradermal delivery and containing a reduced dose of antigen represents a promising tool to increase the coverage of influenza vaccination in the light of optimal acceptability and good safety and immunogenicity profile demonstrated in healthy young adults in recent clinical trials.[8-12] The level of satisfaction with the intradermal microinjection system was high, as was the willingness to be vaccinated the following year.[13,14] In this study, the performance of Intanza® 9 μg in terms of safety, tolerability and immunogenicity and the persistence of antibody responses were evaluated for the first time in HIV-positive subjects. We observed a similar response in antibody levels in HIV-infected individuals immunized with ID and IM vaccines and, more importantly, both vaccines met all the three CHMP immunogenicity criteria for vaccine approval in adults younger than 60 against A(H1N1) and A(H3N2) viruses and two criteria against B virus. The decrease of antibody titers at 3 mo after vaccination compared with titers at 1 mo was small for both vaccines and 71% and 64% of subjects vaccinated with ID formulation showed protective titers at 3 mo against type A and B viruses, respectively, during the circulation peak of influenza. Although the study protocol did not include a healthy-adult control group, the high MFI, seroconversion and seroprotection rates, and compliance with CHMP criteria showed that the response we observed in HIV-infected subjects in both vaccine groups was similar to the one expected in healthy individuals. This was in line with what other Authors reported when they evaluated the immune response in the HIV-positive population with a high proportion of treated subjects (> 90%), with undetectable HIV-RNA (> 60%) and with a high $CD4^+$ cell count (median > 350/mL).[15-17] We would expect a lower vaccine response among HIV-infected individuals with low $CD4^+$

cell counts and active HIV replication.[18] For this highly-depleted population, the use of ID vaccine containing a standard dose of antigen, actually recommended for ≥ 60 y adults, should be carefully evaluated and further studies are needed to assess the safety, tolerability and immunogenicity profile in HIV-infected individuals with advanced disease.

In HIV individuals, Intanza® 9 μg was safe and well tolerated; although significant injection site pruritus, redness, swelling and induration occurred more commonly in subjects receiving intradermal vaccination as compared with intramuscular injection. The injection site adverse events were mild and very well accepted and did not cause concern, as reported in healthy subjects.[13,14] Concerns were raised during the early 1990s that influenza vaccination may result in elevation of HIV load and in subsequent progression of disease.[19] Conflicting data on this point have emerged subsequently,[20-22] but the preponderance of information suggests that influenza vaccination does not have a clinically meaningful. In our study, ID group had a significantly lower viral load and lower proportion of patients with undetectable HIV-RNA compared with the IM group at 3 mo after vaccination. Additional data from larger studies would be needed to confirm this data. and many immunological factors may play a role in determining this result.†

This study presented some limitations: first, the relatively small sample size, due to the complexity of enrollment, reduced the study power. The not-significant lower GMT, MFI and seroconversion rate ($p > 0.3$ for all parameters) observed in ID group at 1 mo against A(H1N1) vaccine strain deserves further study with larger population. Second, although the study population was the result of randomization, subjects immunized with ID vaccine showed higher median $CD4^+$ cell compared with IM group. Baseline $CD4^+$ cell count was an important significant predictor of a positive vaccine response, but the immune response to influenza vaccine was heavily impaired in patient with <200/μl $CD4^+$ cell count and, to lesser extent, with 200–350/μl $CD4^+$ cell count.[15-17,23] In our study population, < 300/μl $CD4^+$ cell count population was not represented and one patient showed 334/μl $CD4^+$ cell count at baseline. The other 53 enrolled patients had > 350/μl $CD4^+$ cell count. Third, the lack of double blindness might have influenced tolerability data reporting.

Although additional data from larger studies would be needed to confirm our results, this trial showed that the recently available low-antigen-content ID vaccine was safe and as immunogenic as IM standard-dose influenza vaccine, offering a new tool for influenza vaccination in HIV-positive subjects.

Methods

This independent, prospective, open-label study was performed in HIV-infected adults (18–60 y) at the "San Martino" University Hospital, Genoa, Italy. Protocol was approved by "San Martino" University Hospital/University of Genoa Ethic Committee. Participants were recruited in December 2010 during routine outpatient HIV care. Informed consent was obtained on all patients.

They were consecutively randomly assigned in blocks of 4 to receive one dose of either a reduced-content ID (Intanza® 9 μg, Sanofi Pasteur, France) or a standard-dose IM trivalent inactivated influenza vaccine (Vaxigrip®, sanofipasteur, France). Intanza® 9 μg was administered using Soluvia™ device, the only intradermal device licensed for influenza vaccines. It has a microneedle approximately 1.5 mm in length, integrated with a ready-to-use pre-filled syringe, with a system specifically designed to limit the depth of penetration and ensure proper perpendicular needle insertion.

Both vaccines contained an A/California/7/2009(H1N1)-like, an A/Perth/16/2009(H3N2)-like and a B/Brisbane/60/2008-like virus, as recommended by the WHO. Exclusion criteria included any contraindication to vaccination, acute febrile illness, treatment with immunosuppressive therapy in the previous 6 mo, receipt of blood or blood-derived products in the previous 3 mo, influenza vaccination in the previous 6 mo.

Serum samples were collected before, 28 ± 2 and 90 ± 3 d after immunization. All sera were stored at -20°C before testing. Antibody responses were evaluated against the A(H1N1), A(H3N2) and B homologous vaccine strains and were measured using hemagglutination inhibition (HI) assays, following the WHO guidelines.[24,25] Guinea pig red blood cells were used in the HI assay. All samples were assayed blind and twice.

Antibody titer were expressed as the reciprocal of the last serum dilution showing hemagglutination. Immunogenicity results from the HI assay were reported as: geometric mean titers (GMT), mean-fold increases (MFI; ratios of post- to pre-vaccination titer), seroconversion rates (percentage of subjects with a 4-fold increase in HI antibody titer, providing a minimal post-vaccination titer of 40) and seroprotection rates (the percentage of subjects achieving a titer ≥ 40). The results from the HI assay were evaluated against the Committee for Medicinal Products for Human Use (CHMP) criteria for approval of influenza vaccines in adults younger than 60, requiring that at least one of the following be met: MFI ≥ 2.5, seroprotection rate ≥ 70% or seroconversion rate ≥ 40%.

$CD4^+$ cell counts before and at 3 mo after vaccination were determined by using a specific monoclonal antibodies and fluorescence-activated cellsorter analysis. Quantitative detection of HIV-1 RNA at baseline and at 3 mo was performed by using Versant HIV-1 RNA 1.0 assay (Siemens Healthcare Diagnostics), based on automated sample preparation and real-time PCR.

Subjects were given diary cards to record any of the following immunization reactions should they occur on the day of vaccination or during the six following days: pain, swelling, redness, induration or ecchymosis at the injection site; fever (a temperature of at least 38°C); headache; malaise; shivering; myalgia and arthralgia. The same diary was used for registration of unsolicited adverse events up to 21 d post-immunization and any serious adverse event appeared in the 6 mo after vaccination.

†It seems save to affirm that Intanza can exert no majer impact on HIV-RNA and $CD4^+$cell count than conventional vaccines.

The minimal sample size (n = 52) was estimated on the ability to determine a difference of 0.2 base 2 log in GMT between groups, considering type I error rate of $\alpha = 0.05$, type II error of $\beta = 0$. Two (power 80%) and standard error of standard deviation equal to 0.25 log.

Comparisons of qualitative and quantitative variables between IM and ID vaccine groups were analyzed using Fischer's exact and Wilcoxon tests, respectively. Antibody titers and viral load were transformed into base 2 and base 10 logarithms, respectively, and compared using the Wilcoxon test or Wilcoxon signed-rank test for paired samples.

Disclosure of Potential Conflicts of Interest

F. Ansaldi, P. Durando and G. Icardi A. have previously participated at speaker's bureaus and advisory board meetings sponsored by GSK, Novartis, Pfizer and Sanofi Pasteur and has recieved research funding as investigator or principal investigator from Crucell Berna, GSK, Novartis, Pfizer and Sanofi Pasteur. L.Valle, D. de Florentiis, V. Parodi, G. Murdaca, B. Bruzzone and M. Setti have no conflict of interest. No other relationships/conditions/circumstances that present a potential conflict of interest exist.

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