A phase 1, open-label, randomized study to compare the immunogenicity and safety of different administration routes and doses of virosomal influenza vaccine in elderly

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ARTICLE INFO

ABSTRACT

Background: Influenza remains a significant problem in elderly despite widespread vaccination coverage. This randomized, phase-I study in elderly compared different strategies of improving vaccine immunogenicity.

Methods: A total of 370 healthy participants (⩾65 years) were randomized equally 1:1:1:1:1:1 to six influenza vaccine treatments (approximately 60–63 participants per treatment arm) at day 1 that consisted of three investigational virosomal vaccine formulations at doses of 7.5, 15, and 45 µg HA antigen/strain administered intradermally (ID) by MicronJet600™ microneedle device (NanoPass Technologies) or intramuscularly (IM), and three comparator registered seasonal vaccines; Inflexal V™ (Janssen) and MF59 adjuvanted Fluad™ (Novartis) administered IM and Intanza™ (Sanofi Pasteur) administered ID via Soluvia™ prefilled microinjection system (BD). Serological evaluations were performed at days 22 and 90 and safety followed-up for 6 months.

Results: Intradermal delivery of virosomal vaccine using MicronJet600™ resulted in significantly higher immunogenicity than the equivalent dose of virosomal Inflexal V™ administered intramuscularly across most of the parameters and strains, as well as in some of the readouts and strains as compared with the 45 µg dose of virosomal vaccine formulation. Of 370 participants, 300 (81.1%) reported ⩾1 adverse event (AE); more participants reported solicited local AEs (72.2%) than solicited systemic AEs (12.2%).

Conclusions: Intradermal delivery significantly improved influenza vaccine immunogenicity compared with intramuscular delivery. Triple dose (45 µg) virosomal vaccine did not demonstrate any benefit on vaccine's immunogenicity over 15 µg commercial presentation. All treatments were generally safe and well-tolerated.

1. Introduction

Influenza results in about 3–5 million cases of severe illness and 250,000–500,000 deaths every year, globally [1]. Over 90% of these deaths and ∼50% of hospitalizations occur among individuals' ⩾65 years of age [2,3]. Regardless of the progressive increase in influenza vaccine coverage, the rates of hospitalization and deaths due to seasonal influenza in elderly individuals have continued to increase substantially in the past decades [4–6]. The elderly patients present a particular immunization challenge for influenza due to the unfortunate combination of reduced immunity (immunosenescence) and an increased vulnerability to morbidity and mortality [7,8].

Various strategies have been developed to improve the immunogenicity of influenza vaccine in this population, which includes adjuvantation, increasing antigen dose, and more recently, delivering the vaccines intradermally [6,9–16]. Modern adjuvant and carrier systems (e.g., virosomes) can increase the immunogenicity without compromising vaccine safety and tolerability, especially in populations with immunosenescence [17]. Intradermal (ID) administration of vaccines has demonstrated

improved immunogenicity compared with intramuscular (IM) route of administration in older adults [14,18]. Attempts to increase immunogenicity by increasing the antigen content demonstrated superior relative efficacy over standard dose [6,14,19,20].

Vaccination of the elderly presents a number of challenges including suboptimal immunogenicity and hence decreased vaccine efficacy [21]. There is an unmet medical need to evaluate whether the immune response after vaccination can be further improved through alternative vaccine delivery such as intradermal delivery, a higher intramuscular dose administration or through the use of adjuvants [22]. Moreover, to counteract the known phenomenon of immunosenescence in elderly, commonly used approach is to use a high IM dose or intradermal administration of a standard or lower vaccine dose [23]. In addition, the device used for intradermal administration may have an influence on the antigen delivery to the intradermal layer of the skin, and consequently the level of the immune response and should be taken into consideration.

The aim of this exploratory study was to perform immunogenicity and safety assessments of different administration routes and doses of influenza vaccine, across investigational virosomal vaccine formulations and registered vaccine comparators. We used the European Committee for Medicinal Products for Human Use (CHMP/EMA) criteria for re-licensure of influenza vaccine as a basis of analysis and comparison. This study was not designed to make statistical comparisons of equivalency or non-inferiority across different vaccines, rather, sample size was planned to meet the minimal requirements of influenza vaccine re-licensure (e.g. 50 per treatment arm). Several pairwise comparisons of immune responses of vaccines delivered ID versus IM, standard versus high dose formulation, and investigational (e.g. adjuvanted) versus comparator (same dose, IM) were statistically evaluated.

2. Materials and methods

2.1. Study population

Medically stable, healthy participants (⩾65 years) who were vaccinated against influenza in season 2011–2012 were enrolled in the study. Exclusion criteria included previous vaccination with an influenza vaccine for season 2012–2013, previous history of a serious adverse events [SAE] or allergic reaction to influenza vaccine, acute exacerbation of bronchopulmonary infection or other acute disease, acute febrile illness (temperature ⩾38 °C), and participation in another clinical trial.

2.2. Study design

This randomized, open-label, phase I study was conducted in 6 centers in Belgium and Germany between November 2012 and July 2013. Elderly participants received influenza vaccine with strain composition for season 2012–2013 either via IM or ID administration at baseline (day 1). Serological evaluations were performed at days 22 (within ±3 days) and 90 (±5 days), and safety was followed-up for 6 months (±7 days).

In total, 370 Participants were stratified by gender and study site and randomized approximately equally 1:1:1:1:1:1 by a web-based procedure to 1 of 6 vaccinations that consisted of three investigational virosomal influenza vaccine formulations and three comparator registered seasonal vaccines. Enrolled number of participants in each of the 6 treatment arms ranged from 60 to 63 (Table 1).

The study protocol and amendments were reviewed by an independent Ethics Committee or Institutional Review Board, as appropriate, for each site. All studies were conducted in compliance with Declaration of Helsinki consistent with Good Clinical Practices and applicable regulatory requirements. Written informed consent was obtained from all participants before enrollment.

2.3. Vaccines

All vaccines used in this study contained as active ingredient the following 3 influenza serotypes recommended for vaccine use during 2012–2013 season: A/California/7/2009 (H1N1), A/Victoria/361/2011 (H3N2), and B/Wisconsin/1/2010 like viruses.

Modern adjuvant and carrier systems (e.g., virosomes) can increase immunogenicity [24–26] especially in populations with reduced responsiveness to active immunization [21]. Virosomes (vir) were produced by inserting purified antigens from inactivated influenza viruses propagated in fertilized hens' eggs into a bilayer of phospholipid vesicles (approximately 150 nm in diameter) composed of predominantly of phosphatidylcholine in phosphate buffered saline [27].

Investigated virosomal influenza vaccines (surface antigen, inactivated, virosome) were formulated as virosomes containing influenza antigens from strains for 2012–2013. The formulations were presented as suspension for injection in a prefilled syringe (type I glass) fitted with a needle size of 25G and 5/8″ (0.5 mm × 16 mm) for intramuscular (IM) injection and with MicronJet600™ microneedle (MJ) device for intradermal (ID) injection, (a disposable 3-prong 0.6 mm hollow microneedle device that attaches to any standard luer lock or luer tip syringe [NanoPass

Table 1
Study influenza vaccines, type, route of administration, dose and volume.

Vaccine identification	Vaccine type/brand	Route of administration	Dose (μgHA/strain)	Volume (mL)
Investigational				
Inflexal-ID-MJ-7.5vir	Surface purified antigen, inactivated virosome[a]	ID (MJ600)	7.5 μg	0.085
Inflexal-ID-MJ-15vir	Surface purified antigen, inactivated, virosome[a]	ID (MJ600)	15 μg	0.17
Inflexal-IM-NS-45vir	Surface purified antigen, inactivated, virosome[a]	IM	45 μg	0.5
Comparators				
Inflexal-IM-NS-15vir	Inflexal V™ surface purified antigen, inactivated, virosome	IM	15 μg	0.5
Fluad-IM-NS-15adj	Fluad™ adjuvanted, surface antigen, inactivated	IM	15 μg	0.5
Intanza-ID-SO-15	Intanza™ split-viron, inactivated	ID	15 μg	0.1

Notes: study vaccines were identified by a naming convention: name-route of administration-delivery device-dose-adjuvant.
ID = intradermal, IM-intramuscular, MJ, MJ600 = MicronJet600™ microneedle (NanoPass Technologies), NS = needle-syringe, SO = Soluvia™ minineedle (Becton Dickinson), vir = virosomal, adj = adjuvant, HA = Hemagglutinin.
Inflexal V™ (Crucell Switzerland) is seasonal Virosomal Influenza Vaccine (surface antigen, inactivated).
Fluad™ (Novartis Vaccines and Diagnostics SRL, Italy) adjuvanted with MF-59™ (oil-in-water emulsion of squalene oil) is a seasonal adjuvanted, subunit (HA and neuraminidase) influenza vaccine.
Intanza™ (Sanofi Pasteur, Lyon, France) is an inactivated, split-virion influenza vaccine.
  [a] The purified antigens from inactivated influenza viruses (mainly HA antigens) are presented on a phospholipid bilayer vesicle called a virosome.

Technologies, Israel]) both in deltoid region. The virosomal vaccine formulations at doses of 7.5, 15 µg HA antigen/strain were administered as 0.085 mL and 0.17 mL respectively delivered with the MJ600 device into the dermis. The 45 µg HA antigen/strain formulation was delivered as standard 0.5 mL IM injection in the deltoid muscle.

Comparator registered influenza vaccines were as follows: Inflexal V™ (Crucell, Switzerland) is an inactivated, subunit (surface antigen) virosomal seasonal vaccine containing the standard dosage of 15 µg HA/strain in each 0.5 ml dose for IM injection; Fluad™ with MF-59™ adjuvant, an oil-in-water emulsion of squalene oil (Novartis Vaccines and Diagnostics SRL, Italy) is a seasonal, adjuvanted, subunit (HA and neuraminidase) influenza vaccine containing 15 µg HA/strain per 0.5 mL dose administered IM; and Intanza™ (Sanofi Pasteur, Lyon, France) is a seasonal, inactivated, split-virion influenza vaccine containing 15 µg HA/strain per 0.1-ml dose and was administered in the deltoid region using a prefilled intradermal microneedle injection system (Soluvia™ [SO], Becton Dickinson; Franklin Lakes, NJ, USA).

The treatment arms were abbreviated using a naming convention that provided vaccine trade name - route of administration (ID, IM) - delivery device (MJ, NS [needle-syringe] - antigen content - adjuvant, or virosome (vir). The three investigated virosomal influenza vaccines were abbreviated as Inflexal-ID-MJ-7.5vir, Inflexal-ID-MJ-15vir, and Inflexal-IM-NS-45vir respectively, and the three comparator vaccines were abbreviated using the same naming convention as Inflexal-IM-NS-15vir, Fluad-IM-NS-15adj, and Intanza-ID-SO-15.

2.4. Clinical evaluations

2.4.1. Sample collection

Blood samples (10 mL) were collected at baseline (day 1, predose) and on days 22 and 90 to assess the participants' immune response against homologous virus strains. Samples were aliquoted into small tubes and stored at $\leqslant -15\,°C$ until further analyses.

2.4.2. Safety assessments

Safety was evaluated based on solicited local, solicited systemic and unsolicited adverse events (AE). Tolerability and acceptance of study vaccine was also assessed. Solicited AEs were collected through a 'subject diary card' for 8 days after vaccination; unsolicited AEs (including SAEs) were captured by interviews on day 1 and during the subsequent visits on day 22, 90, and 180 or reported voluntarily. Solicited local AEs were pain at the injection site, erythema, induration, swelling, and ecchymosis; solicited systemic AEs included fever, malaise and shivering. This was an openlabel study, and because of the use of different administration routes and doses, blinding was not possible. Hence as per protocol, site investigators and their clinical collaborators who administered the study vaccines were not blinded to vaccine treatment arms and when reporting, or assessing adverse events and their relatedness to vaccination.

2.4.3. Immunogenicity assessments

The following immunogenicity parameters were assessed via Hemagglutination inhibition assay (HAI) as stipulated according to European Medicines Agency (EMA) guidelines [28]: Seroprotection rate (HAI antibody titer $\geqslant 1:40$ to be reached in >60% of participants); Seroconversion rate ($\geqslant 4$-fold increase in HAI antibody titer and a titer of $\geqslant 1:40$ to be reached in >30% of participants); Geometric mean titer (GMT) fold increase from baseline (>2.0-fold increase in GMT of HAI antibodies in participants) [28].

3. Statistical methods

3.1. Sample size determination

No formal sample size calculation was performed based on immunogenicity. Different parameters of interest explored were based on EMA requirements [28]. A limitation of this exploratory study was that it was not powered to compare the immunogenicity between the 6 vaccination treatment arms. For the HAI assay, exploratory pairwise treatment arm comparisons were made with no multiplicity adjustments for multiple analyses. The number of pairwise comparisons made was limited to 10 out of the 15 possible pairwise comparisons.

The sample size of 60 participants for each investigational treatment arm was adequate to detect a SAE rate of approximately 1 in 20. i.e.: if no SAEs were observed among 60 participants, the exact 95% upper confidence limit on SAE rate would be 4.9%.

3.2. Statistical analyses

Immunogenicity was evaluated according to the criteria described in EMA guideline [28]. Seroprotection rates, seroconversion rates, GMT, and fold-increase in GMT from baseline were calculated. Study was not powered to compare immunogenicity between all 6 vaccination treatment arms. For HAI assay, exploratory pairwise treatment arm comparisons were made, at each time point, with and without adjustment for preexisting immunity. Without adjustment, pairwise comparisons between vaccination treatment arms were made using logistic regression model on individual outcome for seroprotection (yes/no) and seroconversion (yes/no) with vaccination treatment arm as a factor and analysis of variance model on log-transformed titers for GMTs, and on log-transformed titer increases for GMT fold increases with vaccination treatment arm as a factor. With adjustment, pairwise comparisons were made using a logistic regression model on individual outcome for seroprotection (yes/no) and seroconversion (yes/no) with vaccination treatment arm as a factor and baseline logtransformed HAI antibody titer as a covariate, and using an analysis of variance model on log-transformed titers for GMTs and on log-transformed titer increases for GMT fold increases with vaccination treatment arm as a factor and baseline log-transformed HAI antibody titer as a covariate. For all these pairwise treatment arm comparisons of interest (Supplementary Table 1), 2-sided p-value <0.05 was considered as statistically significant. As this was an exploratory study, no adjustment for multiplicity was applied; number of comparisons made however was minimized. Analyses were interpreted descriptively. Due to high prevaccination titers (high seroprotection rates), the adjusted GMT analysis was conducted to crystalize differences between immune responses.

Solicited local and systemic AEs up to day 4 and 8 after vaccination and unsolicited AEs were summarized descriptively.

3.3. Analysis set

Intention-to-treat (ITT) population included all participants who received a study vaccine and for whom pre and postvaccination HAI antibody titers were available (day 1 and 22). Safety population included all participants who received a study vaccine and for whom safety data were available, irrespective of the occurrence of protocol deviations or violations. Overall, 9 participants were excluded from the ITT population as their blood samples were not taken within the time window defined by the protocol. These included, one participant each from Intanza-ID-SO-15 and Inflexal-ID-MJ-7.5vir treatment arms, 2 participants each from

Inflexal-ID-MJ-15vir, and Inflexal-IM-NS-45vir treatment arms, and 3 participants from Fluad-IM-NS-15adj treatment arm.

4. Results

Of the 370 participants randomized and vaccinated, 367 (99%) completed the study. The number of participants that completed each of the study arms included n = 60 (63 were randomized), in Fluad-IM-NS-15adj, n = 60 in Intanza-ID-SO-15, n = 61 in Inflexal-ID-MJ-7.5vir, and Inflexal-ID-MJ-15vir, n = 62 in Inflexal-ID-MJ-

45vir, and n = 63 in Inflexal-IM-NS-15vir arm. Demographics and baseline characteristics were well-balanced between the vaccination treatment arms. The study included 54% males and 46% females (Supplementary Table 2).

4.1. Safety

Total of 72.2% participants reported solicited local AEs, which were related to study vaccine administration. Unsolicited AEs were reported in 36.8% of participants. Eighteen participants (4.9%) had at least 1 SAE. Percentage of participants with at least

Table 2
Summary of study vaccine solicited (up to day 4) adverse events (safety analysis set).

	Intramuscular injections (IM)			Intradermal injections (ID)			
	Inflexal-IM-NS-15vir (N = 63)	Inflexal-IM-NS-45vir (N = 62)	Fluad-IM-NS-15adj (N = 63)	Intanza-ID-SO-15 (N = 60)	Inflexal-ID-MJ-15vir (N = 61)	Inflexal-ID-MJ-7.5vir (N = 61)	Total (N = 370)
Solicited local adverse events							
Solicited local AEs	29 (46.0%)	35 (56.5%)	36 (57.1%)	54 (90.0%)	57 (93.4%)	56 (91.8%)	267 (72.2%)
Mild	13 (20.6%)	22 (35.5%)	16 (25.4%)	7 (11.7%)	13 (21.3%)	11 (18.0%)	82 (22.2%)
Moderate	11 (17.5%)	8 (12.9%)	16 (25.4%)	24 (40.0%)	23 (37.7%)	30 (49.2%)	112 (30.3%)
Severe	5 (7.9%)	5 (8.1%)	4 (6.3%)	23 (38.3%)	21 (34.4%)	15 (24.6%)	73 (19.7%)
Erythema	19 (30.2%)	20 (32.3%)	15 (23.8%)	52 (86.7%)	56 (91.8%)	54 (88.5%)	216 (58.4%)
Mild	7 (11.1%)	10 (16.1%)	7 (11.1%)	6 (10.0%)	13 (21.3%)	10 (16.4%)	53 (14.3%)
Moderate	8 (12.7%)	6 (9.7%)	4 (6.3%)	24 (40.0%)	22 (36.1%)	29 (47.5%)	93 (25.1%)
Severe	4 (6.3%)	4 (6.5%)	4 (6.3%)	22 (36.7%)	21 (34.4%)	15 (24.6%)	70 (18.9%)
Ecchymosis	4 (6.3%)	3 (4.8%)	1 (1.6%)	0	1 (1.6%)	0	9 (2.4%)
Mild	2 (3.2%)	2 (3.2%)	1 (1.6%)	0	1 (1.6%)	0	6 (1.6%)
Moderate	2 (3.2%)	0	0	0	0	0	2 (0.5%)
Severe	0	1 (1.6%)	0	0	0	0	1 (0.3%)
Induration	12 (19.0%)	9 (14.5%)	11 (17.5%)	31 (51.7%)	34 (55.7%)	34 (55.7%)	131 (35.4%)
Mild	2 (3.2%)	2 (3.2%)	5 (7.9%)	16 (26.7%)	21 (34.4%)	26 (42.6%)	72 (19.5%)
Moderate	8 (12.7%)	6 (9.7%)	5 (7.9%)	11 (18.3%)	12 (19.7%)	8 (13.1%)	50 (13.5%)
Severe	2 (3.2%)	1 (1.6%)	1 (1.6%)	4 (6.7%)	1 (1.6%)	0	9 (2.4%)
Pain	21 (33.3%)	28 (45.2%)	28 (44.4%)	22 (36.7%)	11 (18.0%)	15 (24.6%)	125 (33.8%)
Mild	21 (33.3%)	24 (38.7%)	16 (25.4%)	19 (31.7%)	10 (16.4%)	11 (18.0%)	101 (27.3%)
Moderate	0	4 (6.5%)	12 (19.0%)	3 (5.0%)	1 (1.6%)	3 (4.9%)	23 (6.2%)
Unknown	0	0	0	0	0	1 (1.6%)	1 (0.3%)
Solicited systemic adverse events							
Solicited systemic AE (⩾1)	8 (12.7%)	5 (8.1%)	7 (11.1%)	8 (13.3%)	4 (6.6%)	5 (8.2%)	37 (10.0%)
Solicited systemic AE (⩾1) excluding fever[a]	8 (12.7%)	4 (6.5%)	5 (7.9%)	8 (13.3%)	4 (6.6%)	3 (4.9%)	32 (8.6%)
Mild	7 (11.1%)	4 (6.5%)	3 (4.8%)	6 (10.0%)	2 (3.3%)	2 (3.3%)	24 (6.5%)
Moderate	1 (1.6%)	0	1 (1.6%)	2 (3.3%)	1 (1.6%)	0	5 (1.4%)
Severe	0	0	1 (1.6%)	0	0	1 (1.6%)	2 (0.5%)
Unknown	0	0	0	0	1 (1.6%)	0	1 (0.3%)
Shivering	4 (6.3%)	1 (1.6%)	3 (4.8%)	2 (3.3%)	2 (3.3%)	2 (3.3%)	14 (3.8%)
Mild	3 (4.8%)	1 (1.6%)	3 (4.8%)	2 (3.3%)	2 (3.3%)	1 (1.6%)	12 (3.2%)
Moderate	1 (1.6%)	0	0	0	0	0	1 (0.3%)
Severe	0	0	0	0	0	1 (1.6%)	1 (0.3%)
Malaise	7 (11.1%)	3 (4.8%)	3 (4.8%)	7 (11.7%)	3 (4.9%)	2 (3.3%)	25 (6.8%)
Mild	6 (9.5%)	3 (4.8%)	1 (1.6%)	5 (8.3%)	1 (1.6%)	1 (1.6%)	17 (4.6%)
Moderate	1 (1.6%)	0	1 (1.6%)	2 (3.3%)	1 (1.6%)	0	5 (1.4%)
Severe	0	0	1 (1.6%)	0	0	1 (1.6%)	2 (0.5%)
Unknown	0	0	0	0	1 (1.6%)	0	1 (0.3%)
Fever (body temp. ⩾38 °C[b])	0	1 (1.6)	2 (3.2%)	1 (1.7)	0	3 (4.9%)	7 (1.9%)

AE: Adverse event.
See Table 1 footnotes for description of abbreviations used and vaccines studied.
  [a] Note that the severity of the solicited systemic AE fever was not graded.
  [b] Or body temperature reported as unknown (not measured or recorded).

1 AE that the investigator considered related to study vaccine was 8.9% for solicited systemic AEs and 15.7% for unsolicited AEs.

Incidence of solicited local AEs up to day 4 was higher in ID (Intanza-ID-SO-15, Inflexa-ID-lMJ-7.5vir and Inflexal-ID-MJ-15vir; 90.0–93.4%) compared with IM treatment arms (Inflexal-IM-NS-15vir, Fluad-IM-NS-15adj, Inflexal-IM-NS-45vir; 46.0–57.1%) due to higher incidence of erythema and induration in participants vaccinated intradermally vs. intramuscularly (erythema: 86.7–91.8% vs 23.8–32.3%, induration: 51.7–55.7% vs. 14.5–19.0%) (Table 2; Supplementary Table 3). Incidence of solicited systemic AEs up to day 4 was slightly lower in Inflexal-ID-MJ-7.5vir, Inflexal-ID-MJ-15vir and Inflexal-IM-NS-45vir (6.6–8.2%) compared with other treatment arms (11.1–13.3%). Most common solicited systemic AE was malaise in 25 (6.8%) of 370 vaccinated participants. Incidence of malaise was higher in participants in Inflexal-IM-NS-15vir treatment arm (11.1%) and Intanza-ID-SO-15-treatment arm (11.7%) than in the other treatment arms (3.3–4.9%). Incidences of solicited local and systemic AEs up to day 8 were very similar to those up to day 4.

A total of 136 participants (36.8%) had at least 1 unsolicited AE. Percentage of participants with at least 1 unsolicited AE was highest in Inflexal-ID-MJ-7.5vir (44.3%) and Inflexal-IM-NS-45vir treatment arm (38.7%) and with least reported in Fluad-IM-NS-15adj (31.7%) and Inflexal-ID-MJ-15vir treatment arm (32.8%). Most common unsolicited AEs were nasopharyngitis (8.9%), injection site pruritus (5.4%), and headache (4.6%).

At least 1 SAE was reported for 5 participants in Inflexal-ID-MJ-7.5vir, 5 participants in Inflexal-ID-MJ-15vir, 4 participants in Fluad-IM-NS-15adj, 2 participants in Inflexal-IM-NS-45vir, and 1 participant each in Inflexal-IM-NS-15vir and Intanza-ID-SO-15 treatment arm (Supplementary Table 4). All non-fatal SAEs were resolved by the end of study, except for 1 case of severe polymyalgia rheumatica (Inflexal-ID-MJ-15vir; 67 years old woman; onset: day 10). All other SAEs were considered not related to the study vaccination. There were 3 deaths (Fluad-IM-NS-15adj) reported during the follow-up phase because of reasons considered not related to the vaccine (due to postoperative complications following oesophagectomy for oesophageal adenocarcinoma (66 years, male; day 91); due to spinal epidural abscess (76 years, male;

day 133); due to natural causes (79 years, male; day 162). All other participants completed the study.

4.2. Immunogenicity

Immunogenicity parameters assessed based on results from the HAI assay against homologous strains are presented based on the analysis with adjustment for preexisting immunity. The baseline GMT titers and seroprotection rates were relatively high for strains A/California/7/2009 and A/Victoria/361/2011.

4.2.1. Seroconversion rate

Immunogenicity results for day 22 and day 90 were not statistically compared across the six vaccine treatment arms. Seroconversion rate was above the threshold (>30%) specified by the EMA [28] in all study arms except for Inflexal-IM-NS-15vir and Inflexal-IM-NS-45vir in B/Hubei-Wujiagang/158/2009 strain (Table 3). On day 22, seroconversion rate trended higher in Inflexal-ID-MJ-15vir (65.6%) for strain A/California/7/2009 whereas it was numerically higher in Fluad-IM-NS-15adj (69.8%) and Intanza-ID-SO-15 (46.7%) for strain A/Victoria/361/2011 and B/Hubei-Wujiagang/158/2009, respectively compared with other vaccines (Table 3). On day 90, seroconversion rates were numerically lower in all 6 vaccines compared to day 22.

Ten treatment arm-wise comparisons of percentage seroconversion rates per strain (on day 22 and day 90) between selected investigational and comparator vaccine arms (adjusted for preexisting immunity) were made and the pairwise difference in seroconversion rates with 2-sided 95% CI and p-values were determined. The data for these pairwise comparisons for day 22 is shown in Fig. 1.

Significantly higher seroconversion rates (p < 0.05) were observed for the following pairwise comparisons: Inflexal-ID-MJ-15vir vs. Inflexal-IM-NS-15vir (day 22 and 90), Inflexal-ID-MJ-15vir vs. Fluad-IM-NS-15adj (day 22), and Inflexal-ID-MJ-15vir vs. Inflexal-IM-NS-45vir (day 22) for strain A/California/7/2009; Inflexal-ID-MJ-15vir vs. Inflexal-IM-NS-15vir (day 22) and Inflexal-ID-MJ-7.5vir vs. Inflexal-IM-NS-15vir (day 90) for strain A/Victoria/361/2011; and Inflexal-ID-MJ-7.5vir vs. Inflexal-IM-NS-15vir (day 22) and Inflexal-ID-MJ-15vir vs. Inflexal-IM-NS-15vir (day 22) for B/Hubei-Wujiagang/158/2009 strain. The day

Table 3
Summary of main immunogenicity results for study vaccines (with adjustment for preexisting immunity, ITT population).

| Days | Intramuscular injections (IM) | | | | | | Intradermal injections (ID) | | | | | |
| | Inflexal-IM-NS-15vir (N = 63) | | Inflexal-IM-NS-45vir (N = 62) | | Fluad-IM-NS-15adj (N = 63) | | Intanza-ID-SO-15 (N = 60) | | Inflexal-ID-MJ-15vir (N = 61) | | Inflexal-ID-MJ-7.5vir (N = 61) | |
	22	90	22	90	22	90	22	90	22	90	22	90
Seroconversion[a] (%)												
A/California/7/2009	42.9	28.6	45.2	25.8	50.8	39.7	53.3	43.3	65.6	44.3	49.2	37.7
A/Victoria/361/2011	38.1	28.6	53.2	43.5	69.8	60.3	58.3	48.3	60.7	44.3	49.2	47.5
B/Hubei-Wujiagang/158/2009	19.0	14.3	25.8	16.1	31.7	27.0	46.7	25.0	34.4	16.4	36.1	26.2
Seroprotection[b] (%)												
A/California/7/2009	88.9	82.5	95.2	93.5	93.7	92.1	93.3	88.3	98.4	90.2	98.4	95.1
A/Victoria/361/2011	93.7	92.1	98.4	96.8	100	96.8	98.3	96.7	98.4	91.8	98.4	96.7
B/Hubei-Wujiagang/158/2009	27.0	20.6	32.3	22.6	38.1	31.7	58.3	36.7	39.3	23.0	44.3	39.3
GMT fold increase from baseline[c]												
A/California/7/2009	3.59	2.53	3.99	2.45	5.37	3.35	5.02	3.04	5.60	3.16	4.70	3.10
A/Victoria/361/2011	4.14	3.36	4.79	3.70	6.24	4.42	5.06	3.71	6.31	4.40	6.51	4.88
B/Hubei-Wujiagang/158/2009	2.25	1.74	2.79	2.10	3.58	2.49	4.61	2.77	3.46	2.26	2.89	2.21

Note: Percentages are based on number of available observations; p-value is based on a logistic regression model with vaccination treatment arm as an explanatory variable when adjusting for baseline log transformed HAI antibody titer.
See Table 1 footnotes for description of abbreviations and vaccines studied.
  [a] Seroconversion is defined as ⩾4-fold increase in HAI antibody titer compared to baseline and a titer of ⩾1:40.
  [b] Seroprotection is defined as a HAI antibody titer of ⩾1:40.
  [c] GMT (increase) ratio and p-value are based on an analysis of variance model on log transformed GMTs (GMRs) with vaccination treatment arm as a factor and log (baseline HAI antibody titer value) as a covariate; results are back-transformed to original scale.

90 data is not presented in the figure as the results were similar to that of day 22. Since Fluad-IM-NS-15adj and Intanza-ID-SO-15 treatment arm were only used as comparators for the investigational treatment arms, limited comparisons were made with them as follows: Fluad-IM-NS-15adj was only compared with Inflexal-ID-MJ-15vir and Inflexal-IM-NS-45vir and, Intanza-ID-SO-15 with Inflexal-ID-MJ-7.5vir and Inflexal-ID-MJ-15vir treatment arm; however the results were not statistically significant, except for strain A/California/7/2009.

4.2.2. Seroprotection rate

In all 6 treatment arms of all strains, seroprotection rate was above the threshold (>60%; on days 22 and 90) specified by the EMA [28], except for strain B/Hubei-Wujiagang/158/2009 (Table 3).

On day 22, the seroprotection rates trended higher in Inflexal-ID-MJ-7.5vir and Inflexal-ID-MJ-15vir (98.4%) for strain A/California/7/2009 and in Fluad-IM-NS-15vir (100%) for strain A/Victoria/361/2011. For strain B/Hubei-Wujiagang/158/2009, rates were numerically higher in Intanza-ID-SO-15 (58.3%) compared with other treatment arms.

Pairwise comparisons of investigational vs. comparator vaccines for seroprotection rates (day 22, per strain) with percentage differences, 95% CI and p-values are shown in Fig. 2 (data for day 90 not presented). No comparisons were statistically significant, neither for day 22 nor for day 90 for strains A/California/7/2009 and A/Victoria/361/2011 after adjustment for preexisting immunity. However for strain B/Hubei-Wujiagang/158/2009, significantly higher seroprotection rates (p < 0.05) were observed in

*** p-value < 0.001 ** p-value < 0.01 * p-value < 0.05 + p-value < 0.1

▢ Inflexal-IM-NS-15vir ▣ Inflexal-ID-MJ-7.5vir ▥ Intanza-ID-SO-15 ▨ Fluad-IM-NS-15adj ▤ Inflexal-ID-MJ-15vir ▬ Inflexal-IM-NS-45vir

ID, intradermal; MJ, MicronJet600™ microneedle; vir, virosomal; IM, intramuscular;
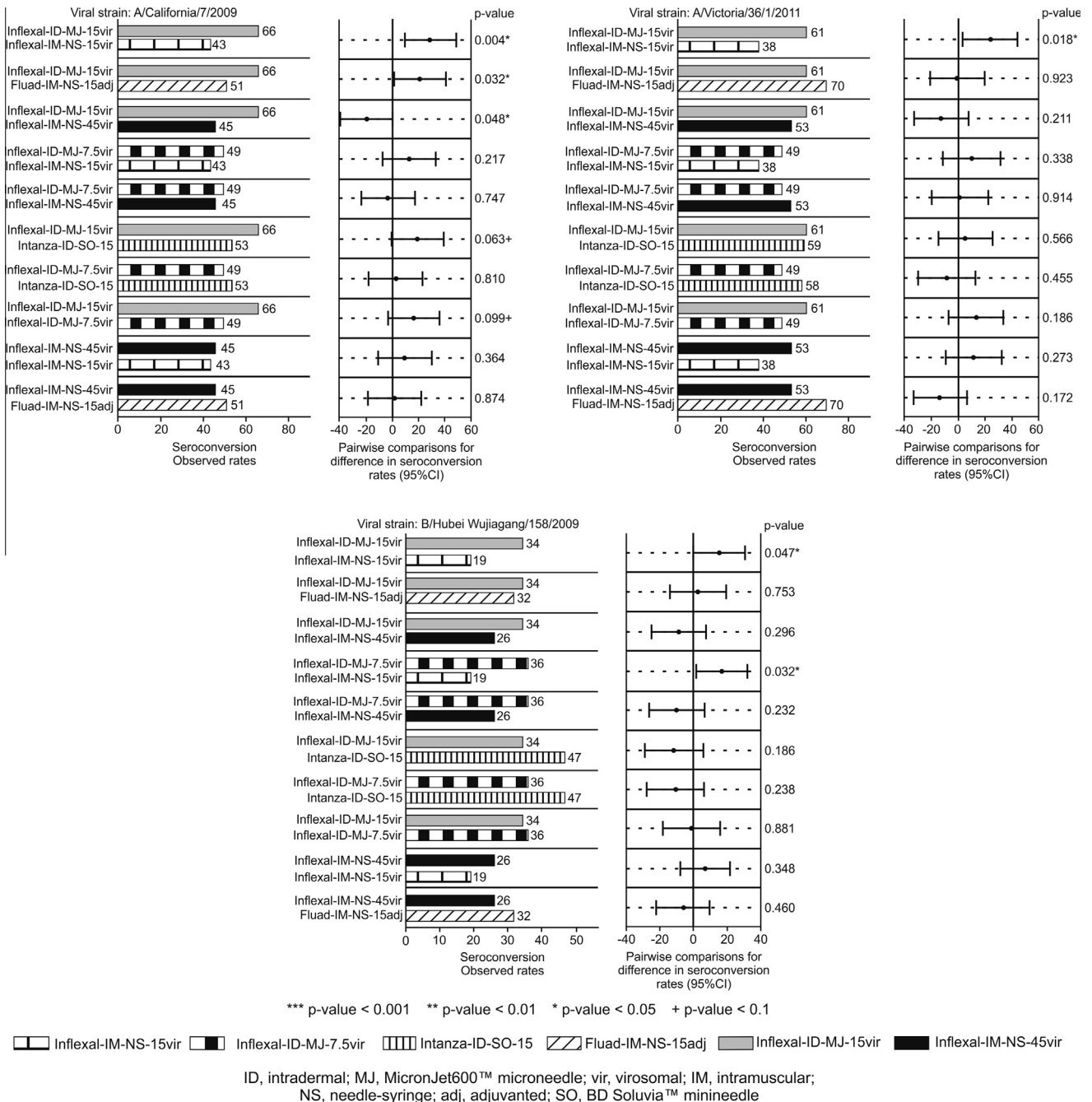NS, needle-syringe; adj, adjuvanted; SO, BD Soluvia™ minineedle

Fig. 1. Immunogenicity - seroconversion adjusted for pre-existing immunity - day 22 - ITT population.

Inflexal-ID-MJ-7.5vir vs. Inflexal-IM-NS-15vir on day 22 and 90 and in Inflexal-ID-MJ-15vir vs. Inflexal-IM-NS-15vir on day 22 (Table 3; Fig. 2).

4.2.3. GMT fold increase

In all vaccine treatment arms for all strains, GMT fold increase was above the threshold (>2.0) specified by the EMA, except in Inflexal-IM-NS-15vir for strain B/Hubei-Wujiagang/158/2009 on day 90 (Table 3). GMT values ranged between 35.66 and 53.28 at baseline, between 152.25 and 237.62 on day 22, and between 103.85 and 141.87 on day 90 for strain A/California/7/2009; between 42.41 and 72.20 at baseline, between 236.77 and 372.89 on day 22, and between 192.39 and 279.62 on day 90 for strain A/Victoria/361/2011; and between 6.91 and 8.88 at baseline,

between 17.47 and 35.78 on day 22, and between 13.50 and 21.52 on day 90 for strain B/Hubei-Wujiagang/158/2009.

Pairwise comparisons of investigational vs. comparator vaccines for GMT fold increases from baseline (day 22, per strain) with GMT fold increase ratios, 95% CI and p-values are shown in Fig. 3 (data for day 90 not presented). Significantly higher GMT fold increases from baseline (p < 0.05) were observed in Inflexal-ID-MJ-15vir vs. Inflexal-IM-NS-15vir on days 22 and Fluad-IM-NS-15adj vs. Inflexal-IM-NS-45vir on day 90 (strain A/California/7/2009), Inflexal-ID-MJ-7.5vir and Inflexal-ID-MJ-15vir vs. Inflexal-IM-NS-15vir on day 22 (strain A/Victoria/361/2011) and Inflexal-ID-MJ-15vir vs. Inflexal-IM-NS-15vir and Intanza-ID-SO-15 vs. Inflexal-ID-MJ-7.5vir on day 22 (strain B/Hubei-Wujiagang/158/2009) (Table 3; Fig. 3).
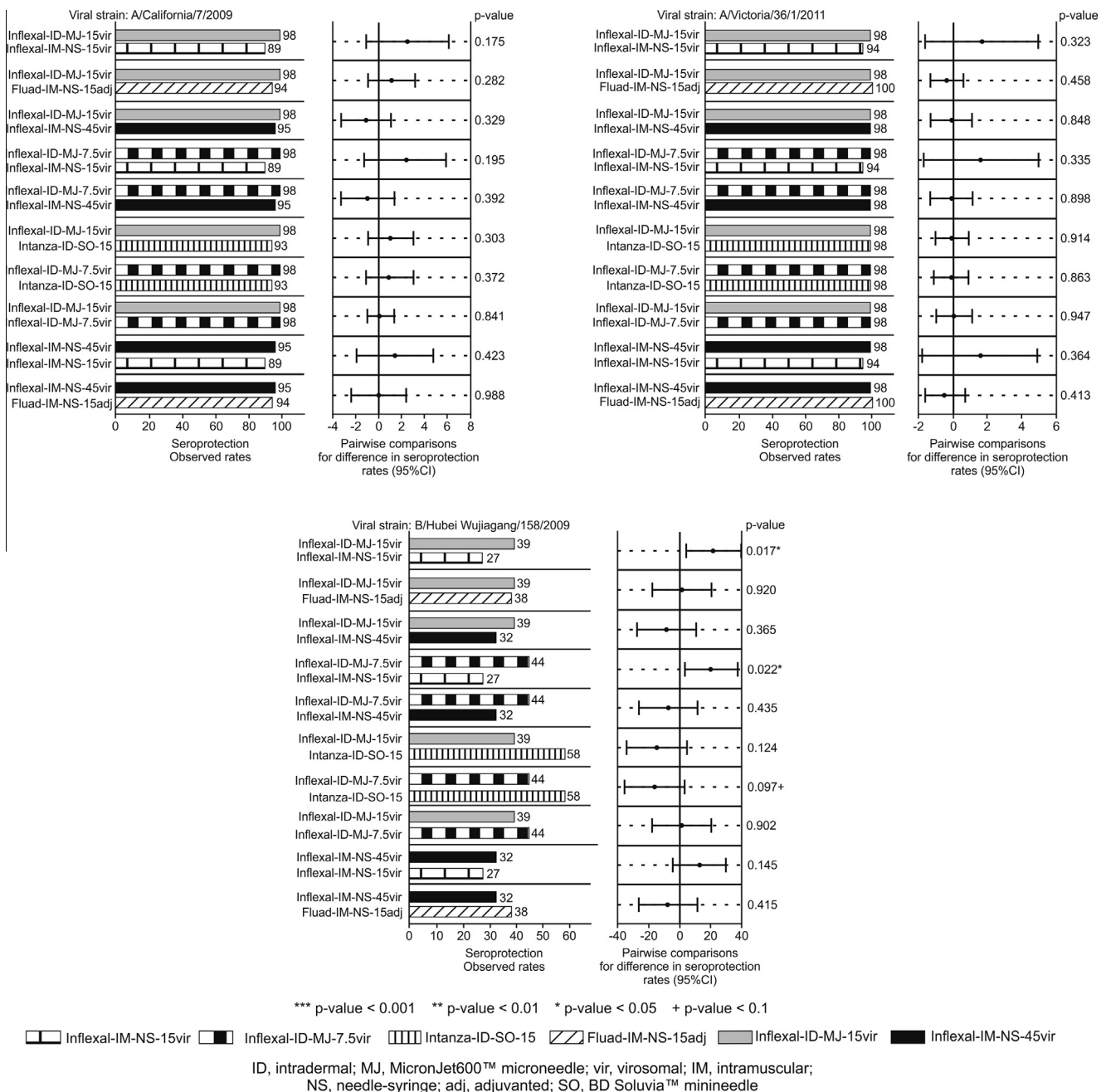

Fig. 2. Immunogenicity - seroprotection adjusted for pre-existing immunity - day 22 - ITT population.

5. Discussion

In this study we report one of the first clinical trials comparing two major approaches to improve influenza vaccine effectiveness: intradermal delivery, high-dose content with other approaches such as alternative ID devices and adjuvantation. The focus of this study was not to evaluate either accomplished strategies (Intanza-ID-SO-15 and Fluad-IM-NS-15adj) vs. commercial Inflexal-IM-NS-15vir presentation. Both approaches were evaluated separately or conjointly in previous clinical studies and have reported divergent effects [29–39]. Immunogenicity evaluations of study vaccines were exploratory in nature and the study was limited and not statistically powered to make comparisons of equivalency or non-inferiority across different vaccine modalities with no formal hypothesis testing. As this was an exploratory study, no adjustment for multiplicity was applied for pairwise comparisons of interest however; total number of comparisons was minimized.

The immune response to influenza vaccination can be influenced by previous influenza vaccinations due to concern with the ability of influenza vaccine to induce seroconversion. EMA guidance requirements for yearly clinical trials exclude recruiting healthy volunteers receiving influenza vaccine within the previous 6 months. In this study, vaccination against influenza in the 2011–2012 season was an inclusion criterion whereas previous vaccination with an influenza vaccine for season 2012–2013 were excluded from enrollment. Influenza-vaccine "naives" were not
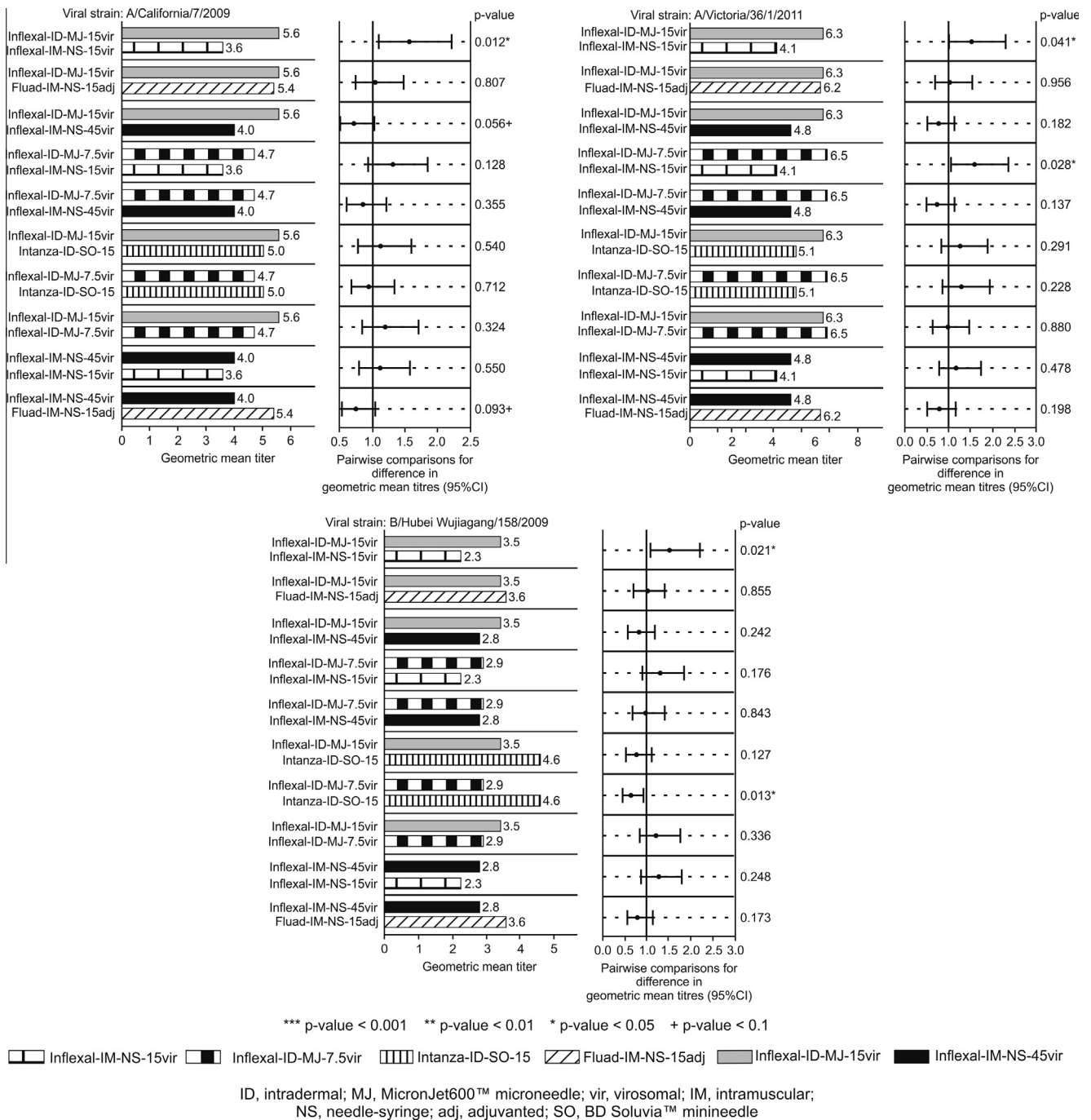

*** p-value < 0.001   ** p-value < 0.01   * p-value < 0.05   + p-value < 0.1

Inflexal-IM-NS-15vir   Inflexal-ID-MJ-7.5vir   Intanza-ID-SO-15   Fluad-IM-NS-15adj   Inflexal-ID-MJ-15vir   Inflexal-IM-NS-45vir

ID, intradermal; MJ, MicronJet600™ microneedle; vir, virosomal; IM, intramuscular;
NS, needle-syringe; adj, adjuvanted; SO, BD Soluvia™ minineedle

Fig. 3. Immunogenicity - geometric mean ratio (GMT fold increase) adjusted for pre-existing immunity - day 22 - ITT population.

included in the study population in order to obtain a more homogenous population and all immunogenicity results at each time point was adjusted for the baseline antibody level to account for preexisting immunity.

We used the CHMP/EMA guideline for re-licensure of influenza vaccine as a basis of exploratory immunogenicity analyses and comparison of investigational and licensed vaccines. To our knowledge at the time of this study no other guidelines for such assessment were available and the EMA regulatory guidelines are widely used in industry for yearly clinical trials and yearly licensing of influenza vaccines in Europe [40].

In our study, intradermal approach with MicronJet600™ device used in Inflexal-ID-MJ-7.5vir and Inflexal-ID-MJ-15vir suggested overall favourable immunogenicity as compared with Inflexal-IM-NS-15vir (standard dose) and Inflexal-IM-NS-45vir (high dose) in elderly population for 2 of the 3 strains. High dose (45 µg) investigational virosomal formulation did not demonstrate any benefit on vaccine's immunogenicity over 15 µg commercial IM presentation. In addition, Inflexal-IM-NS-45vir had numerically lower seroconversion rate for strain A/California compared with Inflexal-ID-MJ-15vir, lower seroprotection for strain B vs. Inflexal-ID-MJ-7.5vir, and lower in GMT fold increase vs. Inflexal-ID-MJ-15vir and Fluad-IM-NS-15adj. Fluad-IM-NS-15adj and Intanza-ID-SO-15 appeared to have good immunogenicity across all strains, although direct comparisons with Inflexal-IM-NS-15vir were not conducted in this study. The immunogenicity of B/Hubei-Wujiagang/158/2009 was consistently lower than that for the A-strains, both at baseline and days 22 and 90. The HAI assay, the standard assay for immunogenicity of influenza vaccines, is well known to have a relatively low specificity to influenza B-viruses in human sera [41].

Previous clinical studies have focused mostly on specific approaches vs. their standard dose (15 µg) unadjuvanted presentations, and have generally been able to demonstrate superior immune responses. Fluzone™ High-Dose vaccine, for example, demonstrated both improved immunogenicity[6,14,16] as well as a 24% additional vaccine efficacy in a phase IV study [19]. In another study [42], Fluad™ demonstrated improved immunogenicity when compared with its unadjuvanted pair Fluvirin™; by day 21, HAI and microneutralization (MN) antibody titers of about 1:40 were reported in 77–96% (HAI) and 92–100% (MN) of participants receiving MF59-adjuvanted vaccine, compared with 63–72% (HAI) and 67–76% (MN) of those who received non-adjuvanted vaccine.

Both the ID administered 7.5 µg and 15 µg dose and the 45 µg IM dose of virosomal influenza vaccine were generally safe and well tolerated. Incidence of solicited local AEs up to day 4 was higher in ID treatment arms (90.0–93.4%) compared with IM (46.0–57.1%) because of a higher incidence of erythema and induration in participants vaccinated intradermally vs intramuscularly (erythema: 86.7–91.8% vs 23.8–32.3%, induration: 51.7–55.7% vs 14.5–19.0%). Incidence of erythema and induration was slightly lower in participants who received 15 µg ID Intanza vs who received 7.5 µg ID or 15 µg ID virosomal influenza vaccine. A higher incidence of erythema after intradermal vaccination in elderly has been reported in other studies also [31,36,43,44]. As the vaccine is administered just below the skin surface, local reactions occur earlier and more frequently than after vaccination deeper into the muscle [29]. Importantly, the antigens are delivered into an immune rich environment, which is thought to enhance the immunogenicity of influenza vaccines. There appeared to be no correlation between injection volume or injection dose and incidence or severity of local AE's. It should be noted here that the current study being an open-label stud y site investigators and their clinical collaborators who administered the study vaccines were not blinded to vaccine treatment arms and when reporting, or assessing adverse events and their relatedness to vaccination.

Overall, 23 SAEs were reported for 18 study participants among all vaccine treatment arms. SAEs were reported more commonly in Inflexal-ID-MJ-7.5vir, Inflexal-ID-MJ-15vir and Fluad-IM-NS-15adj (6.3–8.2%) compared with other treatment arms (1.6–3.2%). All SAEs were considered not related to study vaccine and were resolved by study end except for 1 SAE of polymyalgia rheumatica in Inflexal-ID-MJ-15vir treatment arm. Three deaths (Fluad-IM-NS-15adj) reported during the study were considered not related to study vaccine.

6. Conclusions

This study was intended to compare the ID and high dose strategies for improving influenza vaccine immunogenicity in the elderly with an ID device and an adjuvanted vaccine. Safety results showed that all strategies were generally safe and well-tolerated. Incidence of solicited local AEs was higher after ID than IM administration. The ID delivery using microneedles improved immunogenicity to standard presentation in at least 2 of 3 strains as measured by HAI day 22 GMTs. Tripling the dose did not have any benefit on the vaccine's immunogenicity over 15 µg IM commercial presentation indicating that in this case a higher HA antigen load in the formulation is not a contributing factor in improving immune response. Since no direct comparisons between Fluad-IM-NS-15adj, Intanza-ID-SO-15 and Inflexal-IM-NS-15vir were conducted, limited conclusions can be made, although Fluad-IM-NS-15adj and Intanza-ID-SO-15 appeared to have good immunogenicity across all strains. However, further studies are required to evaluate direct vaccine efficacy among other effective parameters.

Registration

This trial was registered with EudraCT (EudraCT number is 2012-002195-14).

Study support

The study was sponsored by Crucell BV. NanoPass provided devices and support.

Previous presentation

None.

Conflicts of interest

Drs. Levin and Kochba hold executive positions at NanoPass Technologies Ltd., which provided support and devices for the study. Dr. Shukarev is an employee of Janssen Vaccines AG. Prof. Van Damme acts as chief and principal investigator for vaccine trials conducted on behalf of the University of Antwerp, for which the University obtains research grants from vaccine manufacturers; speaker's fees for presentations on vaccines are paid directly to an educational fund held by the University of Antwerp. He received no personal remuneration for this work. Ms. Rusch and Dr. Herrera-Taracena are employees of Janssen Pharmaceutical, a J&J company.

Author contributions

Dr. Levin was involved in study design, data analysis, writing interpretation and review of the manuscript. Dr. Kochba was

involved in training, data analysis, writing, interpretation and review of the manuscript. Dr. Shukarev was the study responsible physician and was involved in data analysis and interpretation and review of the manuscript. Dr. Herrera-Taracena was the medical lead for the program and was involved in the review of the manuscript. Prof. Van Damme participated in study design, data collection and conduct of the vaccine trial, data analysis and interpretation, and review of the manuscript. Ms. Rusch was the project statistician and was involved in study design, data analysis, and interpretation of the results. All authors meet ICMJE criteria and all those who fulfilled those criteria are listed as authors. All authors had access to the study data and made the final decision about where to publish these data.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.09.008.

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