Vaccine 29 (2011) 1921–1928

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

# A nasally administered trivalent inactivated influenza vaccine is well tolerated, stimulates both mucosal and systemic immunity, and potentially protects against influenza illness

J.M. Langley[a,g,*], F. Aoki[b], B.J. Ward[c], A. McGeer[d], J.B. Angel[e], G. Stiver[f], I. Gorfinkel[g], D. Shu[h], L. White[i], B. Lasko[j], P. Dzongowski[k], K. Papp[l], M. Alexander[m], G. Boivin[n], L. Fries[o]

[a] Canadian Centre for Vaccinology, Dalhousie University and IWK Health Centre, Halifax, Canada
[b] University of Manitoba, Winnipeg, Canada
[c] Vaccination Studies Center, Research Institute of the McGill University Health Centre, Montreal, Canada
[d] Mount Sinai Hospital, Toronto, Canada
[e] Ottawa Hospital and Ottawa Research Institute, Ottawa, Canada
[f] Vancouver General Hospital, Vancouver, Canada
[g] PrimeHealth Clinical Research, Toronto, Canada
[h] Gain Medical Center, Coquitlam, Canada
[i] University of Ottawa Health Services, Ottawa, Canada
[j] Manna Research, Toronto, Canada
[k] London East Medical Centre, London, Canada
[l] Probity Medical Research, Waterloo, Canada
[m] Alexander Medical Innovations, Niagara Falls, Canada
[n] Infectious Diseases Research Centre, Centre Hospitalier Universitaire de Québec, Ste-Foy, Canada
[o] GlaxoSmithKline Biologicals, Columbia, MD, United States

ARTICLE INFO

*Article history:*
Received 13 May 2010
Received in revised form
16 December 2010
Accepted 22 December 2010
Available online 8 January 2011

*Keywords:*
Influenza
Human
Intranasal vaccine
Randomized controlled trial

ABSTRACT

A randomized placebo-controlled double-blind trial of a nasally administered inactivated trivalent influenza vaccine formulated with partially purified meningococcal outer membrane proteins (OMP-TIV) was conducted in 1349 healthy adults aged 18–64 years. Subjects received either vaccine containing 15 μg of haemagglutinin (HA) of each of three influenza strains for the 2003–2004 season on days 0 and 14, or 30 μg on day 0 and saline placebo on day 14, or placebo on days 0 and 14. Vaccination was well tolerated, with similar reactogenicity as placebo. Compared to placebo, statistically significant increases in mean serum haemagglutinin inhibition reciprocal titers and salivary secretory IgA to all 3 antigens were seen on day 28 for both vaccine dose groups. The incidence of culture-positive influenza and fever >37.8 °C and cough and one or more of sore throat, runny nose or nasal congestion, muscle or joint ache, headache, fatigue, or chills or culture positive influenza and at least two of these symptoms was low (16/1349; 1.2%). In the intent-to-immunize population too few febrile culture-confirmed illness events ($n = 4$) occurred to perform analysis. Fever occurred infrequently, even in the presence of positive cultures and disabling multi-symptom disease. In participants receiving all doses of either vaccine regimen the incidence of culture-confirmed influenza with respiratory symptoms and with or without fever was 0.77% (7/904) vs. 2.03% (9/443) in placebo recipients ($p = 0.045$, Fisher's exact test; relative risk reduction 62%), despite circulation of a drift variant A/H3N2 that was poorly matched to vaccine. An OMP-TIV vaccine was well tolerated and reduced risk of symptomatic culture confirmed influenza. Vaccine efficacy will need to be validated in a season with a higher attack rate.



* Corresponding author at: Canadian Center for Vaccinology, IWK Health Centre, 5850 University Avenue, Halifax NSB3G 3G9, Canada. Tel.: +1 902 470 8141; fax: +1 902 470 7232.
*E-mail address:* joanne.langley@dal.ca (J.M. Langley).

0264-410X/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2010.12.100

## 1. Introduction

Annual vaccination is identified as the most important measure to prevent morbidity and mortality associated with seasonal influenza each winter [1]. Although currently available influenza vaccines provide substantial protection against infection in healthy persons, this protection is incomplete [2] and uptake of the vaccine, especially among persons at highest risk for complicated influenza and their caregivers, remains suboptimal [1].

Vaccines delivered to the nasal mucosae have the advantage of not requiring injection and thus being more acceptable to vaccinees. They may also stimulate both systemic and mucosal immune responses, thus enhancing personal protection and potentially reducing transmission to others [3]. A prototype monovalent, influenza A/H1N1 adjuvanted vaccine had an acceptable safety profile and was immunogenic when given nasally to 154 healthy adults in two phase 1 clinical trials and so subsequently a trivalent formulation was evaluated in a randomized, double-blind, placebo-controlled, dose-ranging clinical trial to assess the safety and immunogenicity [4], as well as in a human challenge study [5].

The adjuvant/delivery system for this vaccine consists of hydrophobic, proteinaceous, nanoparticles composed of purified *Neisseria meningitidis* outer membrane proteins (OMPs). These nanoparticles can non-covalently associate with amphiphilic antigens, such as the key antigens of inactivated influenza vaccines, the viral haemagglutinins and neuraminidase, by virtue of an antigen's hydrophobic anchor sequence.

We conducted a randomized, placebo-controlled trial to provide a preliminary assessment of the protective efficacy of a nasally administered meningococcal outer membrane protein adjuvanted trivalent influenza vaccine (hereinafter abbreviated OMP-TIV) against laboratory-confirmed influenza infection during the 2003–2004 influenza season in Canada.

## 2. Materials and methods

### 2.1. Vaccine

Formulation of OMP-TIV product has been previously described [4]. The vaccine contains equal parts of three monovalent egg-grown, formalin-inactivated influenza antigens formulated with OMPs of *N. meningitidis* serogroup B strain 8047 at an initial ratio of OMP to haemagglutinin (HA) of 4:1. After diafiltration to remove detergents necessary to keep the OMPs in stable solution in the absence of antigen, the overall total protein to HA ratio in the final vaccine product is 2.5 to 5:1. The trivalent vaccine stock for this study contained HA from each of A/New Caledonia/20/99 [H1N1], A/Panama/2007/99 [H3N2] and B/Shangdong/7/97 [H1N1], which were the influenza antigens recommended for inclusion for the 2003–2004 season [1,6]. There were two test articles studied: a lot with $75 \pm 15$ μg of iHA from each of the three influenza strains and a lot with $150 \pm 30$ μg HA from each of the three influenza strains per milliliter. Both lots are sterile, colorless to yellowish opalescent and preserved with 0.01% thimerosal. The placebo control was sterile phosphate-buffered isotonic saline with 0.01% thimerosal, and was colorless.

### 2.2. Study population

Adults aged 18–64 years inclusive, in good general health as determined by a screening evaluation (history, physical examination, selected clinical laboratory tests), who were willing to forgo the approved 2003–2004 intramuscular influenza vaccine and who gave informed consent, were eligible to participate. Exclusion included membership in groups for which annual influenza vaccination is recommended; presence of significant acute or chronic, uncontrolled medical or psychiatric illness; pregnancy; infection with Human Immunodeficiency Virus, Hepatitis B or Hepatitis C Virus; chronic use of any medication or product for symptoms of rhinitis or nasal congestion or any chronic nasopharyngeal complaint or use of such product within seven days prior to immunization; asthma; symptoms or diagnosis suggesting gag reflex impairment or predisposition to aspiration; use of systemic glucocorticosteroids or immunosuppressive medications; receipt of investigational drugs in the prior month, presence of febrile or upper respiratory tract illness on the day of immunization, and known hypersensitivity to mercurials or chicken eggs.

### 2.3. Study design

The study protocol was approved by the Research Ethics Board at each participating institution or by a central ethics board. Enrolment was conducted at 28 sites in Canada. Written informed consent was obtained from all participants.

The study was double-blind, randomized and placebo-controlled. Neither the subject nor the site study team (staff performing clinical safety or efficacy evaluations and investigators) were aware of patient assignment. One research nurse at each site was responsible for randomization, maintenance of the treatment log, test article preparation and administration. This staff member did not perform any safety or efficacy observations and could not reveal treatment assignment to participants or other study staff. Subjects were assigned centrally within blocks and stratified within each site by age $\leq$49 and >49 years, and history of prior influenza immunization within 2 years.

The primary outcome measure for efficacy was culture-confirmed influenza illness (CCI) defined as fever (oral temperature >37.8 °C) and cough and at least one of sore throat, runny nose or nasal congestion, muscle or joint ache, headache, fatigue, or chills (with symptoms sufficient to impede normal daily activities) and a positive nose and throat swab culture for influenza A or B virus (Table 1). A co-primary endpoint measure was a positive nose and throat swab culture for influenza A or B virus and at least two of the following 8 symptoms: fever, cough, sore throat, runny nose or nasal congestion, muscle or joint ache, headache, fatigue, or chills. The secondary outcome measure, influenza-like illness with evidence of influenza infection, required laboratory confirmation of influenza by either a positive culture for influenza A or B virus, or positive RT-PCR for influenza A or B virus or a 4-fold rise in reciprocal titer for a circulating influenza strain between days 28 and 180 and fever and cough and at least one of sore throat, runny nose or nasal congestion, muscle or joint ache, headache, fatigue, or chills.

### 2.4. Study procedures

Vaccine and placebo were delivered to the nasal mucosae using a VP3/100 nasal spray pump (Valois of America, Greenwich, CCCN) with the participant in a sitting position. The test article was administered on days 0 and 14 following one of the following regimens: (1) meningococcal OMP-adjuvanted trivalent influenza vaccine with 15 μg of each HA antigen on days 0 and 14, or (2) meningococcal OMP-adjuvanted trivalent influenza vaccine with 30 μg of each HA antigen on day 0 and saline placebo on day 14, or saline placebo on days 0 and 14. The volume administered intranasally was 0.20 mL (0.10 mL per nostril). The nose piece of the pump was placed in one nostril while the participant occluded the other nostril. While the participant gently inhaled through the nose, the actuator was fully depressed. The process was repeated for the other nostril.

A complete physical examination was performed and recorded at the screening visit. Symptom-focused or complete physical

**Table 1**
Efficacy outcome measures for influenza.

| Outcome measure | Type of endpoint | Laboratory method | | | Clinical symptoms | |
|---|---|---|---|---|---|---|
| | | Positive influenza culture | Influenza RT-PCR positive or 4-fold rise in reciprocal HAI titer for a circulating influenza strain between day 28 and end of study | Fever (oral temperature >37.8 °C) | Cough | ≥1 of: sore throat, runny nose/nasal congestion, muscle or joint ache, headache, fatigue, chills |
| Culture confirmed influenza (CCI) | Primary | √ | | √ | √ | √ |
| Laboratory confirmed influenza | Co-primary | √ | | At least 2 of these 8 clinical symptoms (e.g. cough and headache *or* fever and joint ache *or* sore throat and fatigue) | | |
| Influenza -like illness | Secondary | One of these 3 laboratory tests positive | √ | √ | √ | |

examinations were performed, as appropriate, for evaluation of participant complaints throughout the trial. On days 0 and 14 a directed physical examination of the ears, nose, throat and cervical and auricular lymph nodes was conducted. Participants were monitored for 30 min after the immunization on days 0 and 14 for any immediate adverse events, and then completed a questionnaire which graded selected complaints as 0 (none), Grade 1 (mild), Grade 2 (moderate) or Grade 3 (severe). From days 0 to 7 subjects self-monitored evening oral temperature and completed a written memory aid of reactogenicity. On days 3, 7, 17 and 21 participants reported the maximum oral temperature and severity score in the previous days via an interactive voice response system. A clinic visit for subject assessment was initiated if symptom complaints exceeded Grade 2. Prior to the day 14 dose subjects were questioned about interim adverse events, and a physical exam was performed. Coding for adverse events was according to Medical Dictionary for Regulatory Activities (MeDRA®, Chantilly, VA) version 6.1.

Blood and nasal mucous samples were collected on days 0 and 28 for haemagglutiin inhibition (HI) reciprocal titers and salivary secretory IgA (sIgA) measurement, respectively. Nasal mucous samples were obtained by instilling 5 mL of sterile phosphate-buffered saline through the nostril with the participant tilting his or her head back to a 45° angle and waiting 10 s. The participant then leaned forward to allow the saline to pour into a sterile sample container. This procedure was completed for each nostril.

Telephone contacts with subjects were made every two weeks to solicit adverse events and identify influenza-like illness. Spontaneous illness reports were received via toll-free telephone call center and reported to investigators. If the participant illness included at least two of the illness criteria, and was severe enough to impede normal daily activities then a nurse visit was initiated. The nurse verified symptoms, collected nose and throat swabs and recorded the participant's temperature.

The final clinical visit occurred at approximately 7 months after enrolment (April–May) to record changes in concomitant medication, adverse events, and for physical examination or laboratory testing if deemed appropriate by the site investigator.

### 2.5. Laboratory methods

Influenza viruses were cultured on MDCK cells in small 1 mL vials and cytopathic effects and/or positive hemadsorption tests were confirmed by monoclonal antibodies. A multiplex RT-PCR test was used to detect influenza A and B viruses as reported [7]. Influenza A viruses were subsequently subtyped by another RT-PCR assay [8]. Systemic immune responses were assessed by serum HI antibody titers specific for the three viruses included in the vaccine, prior to vaccination, just prior to the first dose and 28 days after the first dose. Sera were tested by standard microtitre haemagglutination-inhibition methods after treatment with receptor-destroying enzyme. Nasal washes were concentrated approximately 4-fold using Centricon™ centrifugal filter devices (50,000 molecular weight cut-off) as described by the manufacturer (Millipore Corporation, Billerica, MA). Strain-specific sIgA responses were measured by kinetic enzyme-linked immunosorbent assay (KELISA) as previously described [10]. With the following modification: optical density at 650 nm was recorded every 12 s for a total run time of 5 min at 25 ± 3 °C using a Benchmark Plus™ Microplate Spectrophotometer and Microplate Manager™ version 5.2 build 103 Software (Bio-Rad Laboratories, Mississauga, ON, Canada) to generate a KELISA rate. A mean rate was calculated from the triplicate for each sample. Repeated assays on standard samples yielded a day-to-day coefficient of variation of ≤20%. A small number of samples required assay at additional dilutions to obtain KELISA rates in the linear response range of the assay; results were corrected for the dilution factor. Total sIgA levels in nasal wash were quantified by radial immunodiffusion using BINDARID™ kits as described by the manufacturer (The Binding Site Ltd., Birmingham, UK). Virus-specific sIgA KELISA rates in each sample were then normalized to the arithmetic mean of total sIgA determinations for all specimens from the entire study population.

### 2.6. Data analysis and statistical considerations

The safety analysis included all enrolled subjects who received at least one dose of the test article. Subjects were analyzed according to the actual article received rather than the article to which they were randomized. A 1-sided hypothesis test at the overall 0.025 level of significance was used in primary analyses, or a 2-sided hypothesis test at the overall 0.05 level of significance in other analyses. The analysis of the primary efficacy endpoint, culture-confirmed influenza, was based on an intention-to-immunize sample (ITI) which included any subject who received at least one dose of test article. Subjects were analyzed under the treatment to which they were randomized. This method excluded persons who were randomized but did not receive test article. (A number of subjects were enrolled but not given the test article because enrollment closed when overall study subject accrual targets were met.) The additional primary efficacy endpoint, culture-positive influenza A or B in participants that met criteria for an illness visit, was based on the evaluable subjects (ES) i.e., those who had a complete regimen (i.e. one dose of placebo in the placebo group, at least one dose of 30 μg, 2 doses of 15 μg). The secondary analysis of participants with influenza-like illness and evidence of influenza infection (i.e.

**Table 2**
Demographics and baseline characteristics—intent to immunize population.

| | OMP-TIV 15 μg × 2 | OMP-TIV 30 μg × 1 | Placebo | Total |
|---|---|---|---|---|
| *N* | *N* = 455 | (*n* = 450) | (*n* = 443) | (*n* = 1348) |
| Age | | | | |
| Mean (SD) | 37.5 (11.9) | 36.9 (12.5) | 36.9 (12.5) | 37.1 (12.2) |
| Median | 38 | 37 | 36 | 37 |
| Age categories | | | | |
| ≤ 49 years | 372 (81%) | 369 (82%) | 365 (82.4%) | 1106 (82%) |
| ≥49 years | 83 | 81 | 78 | 242 |
| Gender | | | | |
| Male | 204 (44.8%) | 206 (45.8%) | 208 (47%) | 618 (45.8%) |
| Ethnicity | | | | |
| White | 424 (93.2%) | 415 (92.2%) | 420 (94.8%) | 1259 (93.4%) |
| Asian | 12 (2.6%) | 18 (4.0%) | 11 (2.5%) | 41 (3.0%) |
| African-Canadian | 11 (2.4%) | 7(1.6%) | 7 (2.5%) | 25 (1.9%) |
| Prior influenza immunization within 2 years | 133 (29.2%) | 128 (28.4%) | 127 (28.7%) | 388 (28.8%) |
| Any prior influenza immunization | 186 (40.9%) | 190 (42.2%) | 181 (40.9%) | 557 (41.3%) |

at least one of culture, PCR or 4-fold antibody rise) was conducted on the evaluable subjects.

For both the ITI and ES comparisons of 2 OMP-TIV doses with placebo, 1-sided categorical data analysis models were used that adjusted for stratification (age, gender and prior influenza immunization within 2 years) as well as interactions with the vaccination group. The final analysis included the vaccination group and those covariates which were significantly associated with outcome. Vaccine efficacy estimates are presented as relative risk, their lower 97.5% confidence bounds, and a *p* value for the test of zero efficacy. Continuous data were summarized using descriptive statistics (*n*, median, mean, range).

Serum HI antibody reciprocal titers were log transformed (log 2) and their means and 95% confidence intervals (CI) presented by vaccination group. Titers less than 1:10 or greater than 1:640 were treated as 1:5 or 1:1280, respectively, during log transformation. Proportions of subjects with HI antibody reciprocal titers of at least 40 or showing a 4-fold rise from baseline were calculated. Salivary sIgA kinetic enzyme-linked immunosorbent assay levels were normalized on a total sIgA content of the specimen. HI antibody reciprocal titers and salivary-specific IgA levels were compared between groups postimmunization by a linear model (SAS PROC GLM). The model adjusted for baseline HI reciprocal titer, age, gender, and prior influenza immunization within 2 years.

Proportions of subjects in each group having each reactogenicity complaint after each dosage administration and overall were calculated; the higher grade severity of immediate complaint and oral temperature for two doses was used in the overall summary and compared by a generalized Cochran–Mantel–Haenszel test adjusting for site, age and prior influenza immunization within 2 years. Maximum reaction rates complaints (Grade 2 or higher) or temperature ≥37.8 °C were also compared between each dosage using McNemar's Chi squared test. Adverse events were coded according to system organ class by the Medical Dictionary for Regulatory Activities (MeDRA®) version 6.1.

Safety analysis included all enrolled subjects who received at least one dose of test article; subjects were analyzed according to actual test article received.

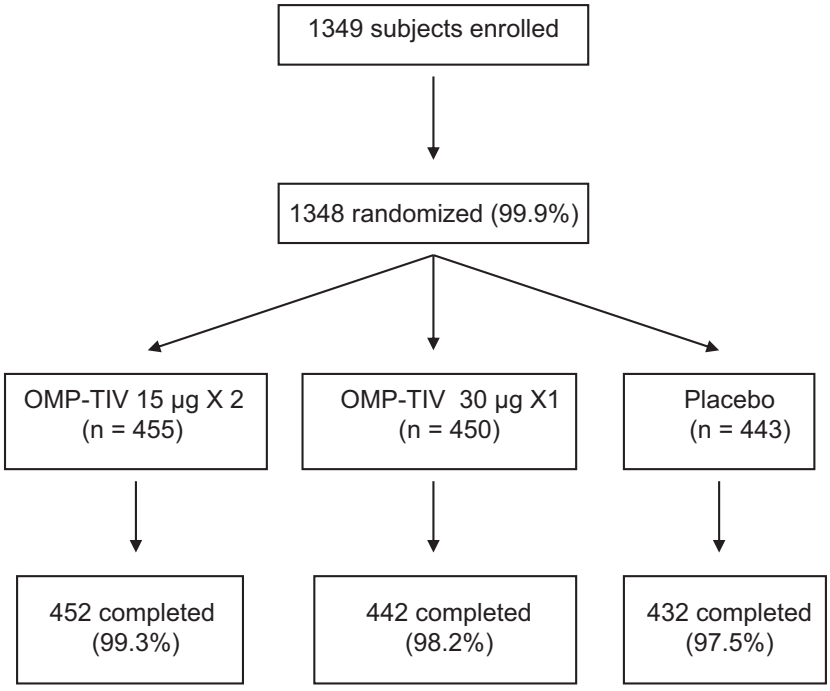


**Fig. 1.** Participant flow.

The software SAS® (SAS Institute, version 8.2 or later, Cary, NC) was used for all analyses.

## 3. Results

### 3.1. Demographics

Of 1349 participants enrolled, 1348 were randomized (99.9%). Completion rates in the three study arms are seen in Fig. 1. One subject was ineligible for the intent-to-immunize sample, and two were ineligible for the evaluable subject population. Thus the intent-to-immunize population was 1348, the safety population 1349 and the evaluable subject population 1347. The demographics of the intent-to-immunize population are seen in Table 2.

### 3.2. Reactogenicity and safety

There were no significant differences in the incidence of any immediate complaint or post-immunization temperature between the three study groups (Table 4). Less than 10% of participants reported any immediate complaint after either dose of vaccine or control. Among the Grade 1 complaints (mild), the most common were: burning or stinging in the nose (133/1349; 9.9%), lightheadedness or dizziness (107/1349; 7.9%), itching in the nose, throat or eyes (96/1349; 7.1%), and burning or stinging in the throat (39/1349; 2.9%). The incidence of these complaints decreased after exposure to the second dose of the test article. There were no Grade 3 complaints; two placebo recipients reported Grade 2 dizziness or lightheadedness (2/1349; 0.5%). Fever (37.8–38.2 °C) occurred in one placebo and one 30 μg dose recipient.

Considering reactogenicity complaints reported over 7 days after either dose, incidence rates were remarkably similar between the three treatment groups, and no significant differences between active and placebo treatment groups were noted. For each complaint solicited, >50% of subjects in all groups reported no symptom (Grade 0) at any time point, and <6% of subjects overall reported any complaint at Grade 2 or 3 severity. The most common complaints were all of Grade 1 severity and included runny nose (571/1349, 42.3%), stuffy nose (517/1349, 38.3%), headache (450/1349, 33.4%), sneezing (386/1349, 28.6%), sore throat (363/1349, 26.9%), tiredness/fatigue (338/1349, 25.1%), and cough (282/1349, 20.9%). In no case did the incidence in any treatment group deviate by more than 3% from the overall rate.

Spontaneously reported adverse events occurred in 66.9% of the trial population, including 305/455 recipients of two OMP-TIV doses at the 15 μg dose level (67.0%), 296/451 recipients of one OMP-TIV dose at the 30 μg dose level (65.6%), and 302/443 recipients of placebo (68.2%); there were no clear imbalances among MedDRA preferred terms across the treatment groups. There were 7 serious adverse events, 6 of which were assessed by the investigators as not related to the test article. An episode of hypersensitivity in a single participant (15 μg group) was deemed probably related to the test article by the local investigator. This event occurred in a 50-year-old woman who had never previously received influenza vaccine, after the second 15 μg dose of vaccine. About 8 min after vaccine receipt she developed rash, dyspnea, palpitations, dizziness, blurred vision, tachycardia and hypotension. She improved within 15 min of supine positioning and intramuscular diphenhydramine, and had no further sequelae.

### 3.3. Immunogenicity

Statistically significant increases in geometric mean serum HI titers and in the proportion of subjects with HI titers ≥1:40, a level suggested as correlating with protection in adults, were observed

**Table 3**
Serum haemagglutination-inhibition and specific salivary sIgA responses to study treatments.

| Serum HI antibody reciprocal titers | | OMP-TIV 15 μg × 2 | A/New Caledonia/20/99 (H1N1) | | A/Panama/2007/99 (H3N2) | | | B/Shandong/7/97 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | OMP-TIV 30 μg × 1 N = 447[a] | Placebo N = 443 | OMP-TIV 15 μg × 2 N = 439 | OMP-TIV 30 μg × 1 N = 447 | Placebo N = 443 | OMP-TIV 15 μg × 2 N = 439 | OMP-TIV 30 μg × 1 N = 447 | Placebo N = 443 | N = 439 |
| Pre | GMT (95% CI) | 17.6 (15.5–20.2) | 17.6 (15.2–20.4) | 17.1 (14.8–21.7) | 34.1 (29.8–38.6) | 34.3 (29.8–39.4) | 33.1 (29.0–37.8) | 7.8 (7.3–8.5) | 7.8 (7.2–8.4) | 7.4 (6.9–7.9) |
| | N, % with titer ≥40 (95%CI) | 149, 33.3% (29.0–37.9) | 139, 31.4% (27.1–35.9) | 138, 31.4% (27.3–35.9) | 244, 54.6% (49.8–59.3) | 242, 54.6% (49.9–59.3) | 238, 54.4% (49.4–59.0) | 39, 8.7% (6.3–11.7) | 41, 9.3% (6.7–12.4) | 37, 8.4% (6.0–11.4) |
| Day 28 | GMT (95% CI) | 39.1* (34.0–44.6) | 40.5* (35.2–46.8) | 16.9 (14.6–19.6) | 75.6* (68.6–83.8) | 83.8* (75.6–93.7) | 35.0 (30.7–39.9) | 9.0* (8.3–9.8) | 9.3* (8.5–10.1) | 7.5 (7.0–8.1) |
| | N, % with titer ≥40 (95% CI) | 268, 60.0%* (55.3–64.5) | 254, 57.3%* (52.6–62.0) | 135, 30.8% (26.6–35.2) | 377, 84.3%* (80.6–87.6) | 383, 86.5%* (82.9–89.5) | 245, 55.8% (51.0–60.5) | 61, 13.7%* (10.6–17.2) | 60, 13.5% (10.5–17.1) | 42, 9.6% (7.0–12.7) |
| | N, % with ≥4-fold rise (95%CI) | 145, 32.4%* (28.1–37.0) | 148, 33.4%* (29.0–38.0) | 7, 1.6% (0.6–3.3%) | 148, 33.1%* (28.8–37.7) | 150, 33.9%* (29.5–38.5) | 15, 3.4% (1.9–5.6) | 30, 6.7%* (4.6–9.4) | 36, 8.1%* (5.8–11.1) | 7, 1.6% (0.6–3.3) |
| Virus-specific salivary IgA levels[b] | | | | | | | | | | |
| Pre | GM (95% CI) | 6.1 (5.8–4.8) | 5.6 (5.2–5.9) | 5.9 (5.5–6.3) | 8.1 (7.6–8.6) | 8.1 (7.6–8.6) | 7.9 (7.5–8.5) | 6.2 (5.9–6.6) | 5.9 (5.5–6.2) | 6.0 (5.6–6.4) |
| Day 28 | GM (95% CI) | 9.3* (8.7–9.9) | 8.1* (7.6–8.6) | 6.5 (6.1–7.0) | 12.9* (12.2–13.7) | 11.5* (10.9–12.2) | 8.6 (8.1–9.2) | 9.3* (8.8–9.9) | 7.9* (7.4–8.4) | 6.5 (6.1–7.0) |

[a] N = subjects in ITI population with paired serum HI data. *p < 0.001 vs. placebo group at same time point.
[b] Virus-specific salivary IgA levels are expressed as the geometric means of absorbance units per minute in a specific kinetic ELISA as previously described (see Section 2).

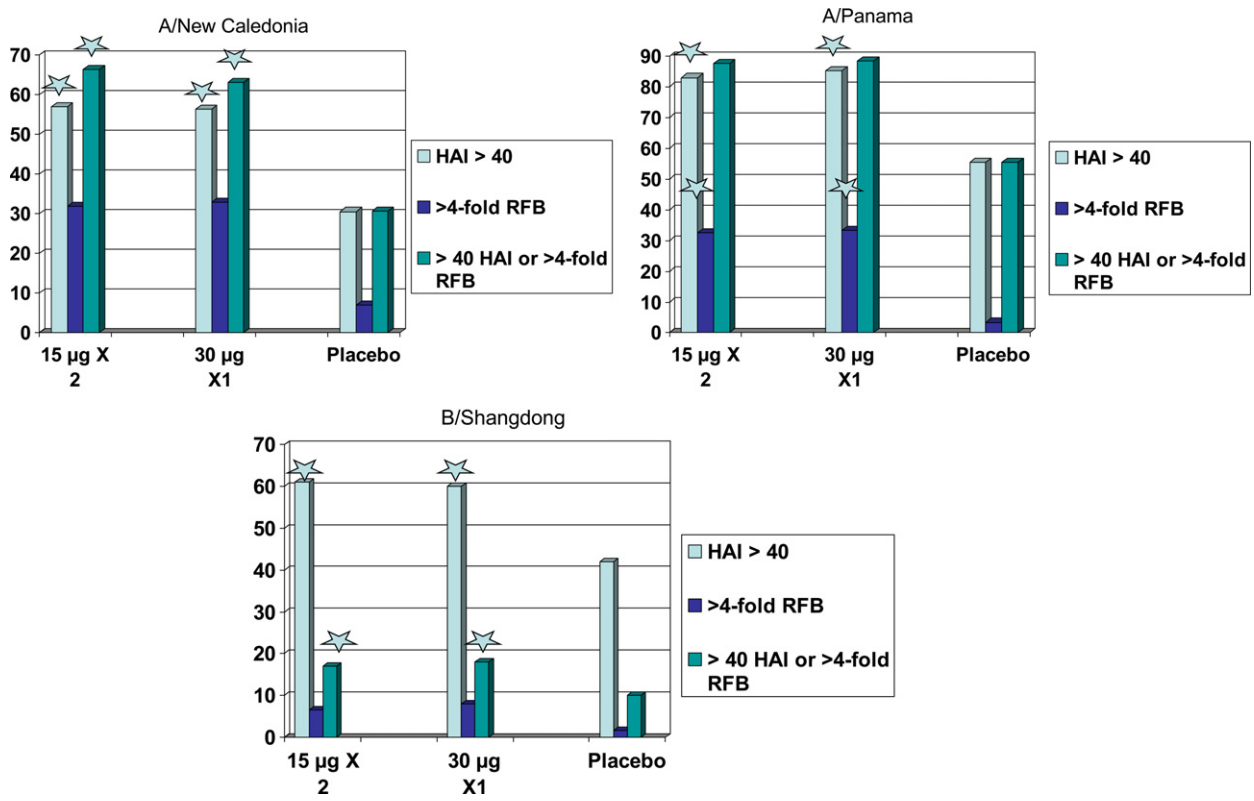


**Fig. 2.** Percentage of study population with serum antibody responses to vaccine-specific antigens, day 28, ITI population.

in both active treatment groups in contrast to placebo recipients (Table 3 and Fig. 2). Responses were more frequent, and of larger magnitude, for the two influenza A viruses than for the influenza B component. Rises in geometric mean salivary sIgA levels specific for all 3 antigens were also observed on day 28 for both active vaccine regimens compared to placebo (Table 3 and Fig. 3)

### 3.4. *Efficacy*

The incidence of laboratory confirmed influenza was low in the overall study population; with only 16/1347 participants overall having a positive culture (1.2%). The incidence of disease by outcome measure is seen in Table 5. In the intent-to-immunize population ($n = 1348$) too few cases of CCI (primary outcome measure) occurred to perform a meaningful analysis ($n = 4$). This result was conditioned primarily by the very low incidence of fever (either self-observed or nurse-confirmed) in these adult subjects, even in the presence of influenza culture positivity and 4–5 other symptoms which interfered with normal daily activities. In participants receiving either active vaccine regimen, and using a clinical outcome of without fever, the incidence of influenza was 0.77% (7/904) compared to 2.03% (9/433) in placebo recipients ($p = 0.045$, Fisher's exact test; relative risk reduction 62%). The addition of RT-PCR as a diagnostic tool increased the case rates only slightly and did not materially affect the apparent efficacy. Considering seroconversion to circulating strains as evidence of influenza infection (4-fold antibody rise) increased the number of cases while slightly decreasing apparent efficacy. The influenza season in Canada peaked in December and the predominant strain was A/Fujian/411/02-like [H3N2], a drift variant that was not well-matched to the vaccine.

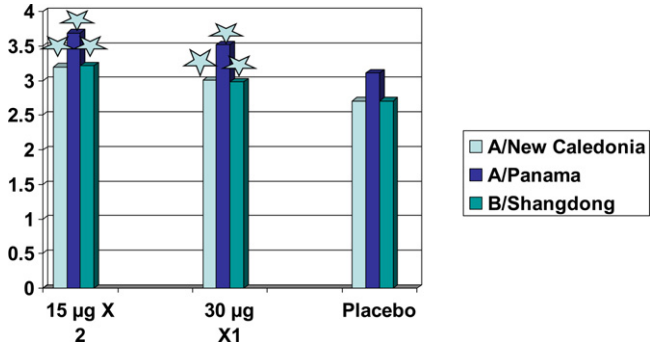


**Fig. 3.** Mean salivary IgA response to three influenza antigens, day 28, ITI population.

## 4. Discussion

In this multicenter phase 2 randomized placebo-controlled trial of a nasally administered inactivated influenza vaccine we have demonstrated that this novel adjuvanting method produces a vaccine that is minimally reactogenic and immunogenic. A relatively mild influenza season among the adult subjects enrolled in this study precluded evaluation of efficacy.

Although the incidence of influenza was too low in our study population to convincingly demonstrate vaccine efficacy, we did observe a relative risk reduction in symptomatic culture-confirmed influenza of 62% compared to placebo recipients despite the circulation of a drift variant. The 2003–2004 influenza season saw the emergence of the A/Fujian strain, accounting for 96.8% of H3N2 isolates in Canada that year [9], which was mismatched with A/Panama/2007/99 [H3N2] in the recommended vaccine. Although vaccines with strains well matched to circulating virus can result in 80% (95% CI 56–91%) efficacy in preventing influenza in healthy adults, the efficacy of mismatched vaccines is estimated at 50% (95% CI 27–65%) in systematic reviews [2]. National influenza surveillance for 2003–2004 indicated that the age groups most affected

**Table 4**
Immediate complaints and temperature in healthy adults randomixed to one of two dosing regimens of a nasally administered influenza vaccine or placebo—safety population.

| Overall[c] | OMP-TIV 15 μg × 2(*N* = 455) | OMP-TIV 30 μg × 1(*N* = 451) | Placebo(*N* = 443) | Total(*N* = 1349) |
|---|---|---|---|---|
| Burning or stinging in the nose[a] | | | | |
| Grade 0 | 410 (90.1%) | 400 (88.7%) | 406 (91.6%) | 1216 (90.1%) |
| Grade 1 | 45 (9.9%) | 51 (11.3%) | 37 (8.4%) | 133 (9.9%) |
| Grade 2, Grade 3, or no observation | 0 | 0 | 0 | 0 |
| *p* value[b] | 0.521 | 0.298 | | |
| Burning or stinging in the throat[a] | | | | |
| Grade 0 | 442 (97.1%) | 439 (97.3%) | 429 (96.8%) | 1310 (97.1%) |
| Grade 1 | 13 (2.9%) | 12 (2.7%) | 14 (3.2%) | 39 (2.9%) |
| Grade 2, Grade 3, or no observation | 0 | 0 | 0 | 0 |
| *p* value[b] | 0.777 | 0.943 | | |
| Itching in the nose, throat, or eyes[a] | | | | |
| Grade 0 | 423 (93.0%) | 413 (91.6%) | 417 (94.1%) | 1253 (99.5%) |
| Grade 1 | 32 (7.0%) | 38 (8.4%) | 26 (5.9%) | 96 (7.1%) |
| Grade 2, Grade 3, or no observation | 0 | 0 | 0 | 0 |
| *p* value[b] | 0.365 | 0.147 | | |
| Shortness of breath[a] | | | | |
| Grade 0 | 454 (99.8%) | 450 (99.8%) | 438 (98.9%) | 1342 (99.5%) |
| Grade 1 | 1 (0.2%) | 1 (0.2%) | 5 (1.1%) | 7 (0.5%) |
| Grade 2, Grade 3, or no observation | 0 | 0 | 0 | 0 |
| *p* value[b] | 0.058 | 0.061 | | |
| Lightheadedness or dizziness[a] | | | | |
| Grade 0 | 425 (93.4%) | 409 (90.7%) | 406 (91.6%) | 1240 (91.9%) |
| Grade 1 | 30 (6.6%) | 42 (9.3%) | 35 (7.9%) | 107 (7.9%) |
| Grade 2 | 0 | 0 | 2 (0.5%) | 2 (0.1%) |
| Grade 3 or no observation | 0 | 0 | 0 | 0 |
| *p* value[b] | 0.215 | 0.284 | | |
| A new rash or a rash that has become itchy[a] | | | | |
| Grade 0 | 455 (100%) | 449 (99.6%) | 443 (100%) | 1347 (99.9%) |
| Grade 1 | 0 | 2 (0.4%) | 0 | 2 (0.1%) |
| Grade 2, Grade 3, or no observation | 0 | 0 | 0 | 0 |
| *p* value[b] | – | 0.306 | | |
| Feverishness[a] | | | | |
| Grade 0 | 452 (99.3%) | 451 (100%) | 441 (99.5%) | 1344 (99.6%) |
| Grade 1 | 3 (0.7%) | 0 | 2 (0.5%) | 5 (0.4%) |
| Grade 2, Grade 3, or no observation | 0 | 0 | 0 | 0 |
| *p* value[b] | 0.646 | 0.150 | | |
| Temperature (°C) | | | | |
| <37.8 | 455 (100%) | 450 (99.8%) | 442 (99.8%) | 1347 (99.9%) |
| 37.8–38.2 | 0 | 1 (0.2%) | 1 (0.2%) | 2 (0.1%) |
| 38.3–38.9 | 0 | 0 | 0 | 0 |
| ≥39.0 | 0 | 0 | 0 | 0 |
| No observation | 0 | 0 | 0 | 0 |
| *p* value[b] | 0.317 | 0.977 | | |

[a] Grade 0 = none = I did not have it at all; Grade 1 = mild = just noticeable; Grade 2 = moderate = unpleasant/uncomfortable but not incapacitating; Grade 3 = severe = preventing resumption of normal activities.
[b] *p* values between OMP-TIV and placebo groups were based on generalized CMH test adjusting for stratification factors (site, age, and prior influenza immunization [within 2 years]).
[c] The higher grade of severity for all visits was reported. Percentages were based on the safety sample.

were children <5 years of age (33% of isolates) followed by persons over 65 years (25% of isolates) [9]. Adults 25–64 years of age contributed only 20% of confirmed cases. Convincing evidence of efficacy of the nasally administered meningococcal outer membrane protein adjuvanted trivalent influenza vaccine would require validation in much larger numbers of subjects, and preferably a season with a higher attack rate and better match between circulating strains and vaccine antigen. The vaccine was significantly immunogenic relative to placebo as measured by both rise in both serum HAI titers and salivary IgA levels on day 28, and the majority of recipients had a four-fold rise in antigen-specific antibody titers, or an HI titer >1:40, or both on day 28. The vaccine was significantly immunogenic relative to placebo as measured by both rise in both serum HAI titers and salivary IgA levels on day 28. We have previously shown that local immune responses occur by day 14 after immunization [4]. It is important to note that serum HAI titers, which are widely used for the evaluation of parenterally delivered influenza vaccines and serve as the basis for annual re-registration of these products, capture of only one aspect of the immune response to a mucosal vaccine. Past experience with this vaccine suggests we should not expect serum HAI responses of the same magnitude as those elicited by an intramuscular product. The

**Table 5**
Incidence of influenza according to outcome measure among three study arms.

| | OMP-TIV 15 μg × 2 *N* = 455 | OMP-TIV 30 μg × 1 (*n* = 450) | Placebo (*n* = 443) | Total (*n* = 1348) |
|---|---|---|---|---|
| 1° Outcome measure | | | | |
| Culture-confirmed influenza (CCI, intent-to-immunize population) | 1 | 0 | 3 | 4/1348 (0.3%) |
| 2° Outcome measure | | | | |
| Culture positive (CCI, but with no requirement for fever (evaluable subjects) | 4 | 3 | 9 | 16/1347 (1.2%) |
| Influenza-like illness | 2 | 2 | 4 | 8/1348 (0.59%) |

potential efficacy of a mucosal vaccine may depend to a substantial extent on secretory IgA or even cellular responses, for which there are no gold-standard assays at present or accepted standards for protective responses.

Fever and cough are considered important clinical hallmarks of influenza. Interestingly we found the majority of outpatient adults with laboratory-confirmed influenza and multi-symptom respiratory disease impeding normal daily activities and cough, could not provide confirmation of fever. It is possible that due to the intermittent nature of fever, we were unable to document this sign at the time of clinical assessment. However, no specific symptom or combination of symptoms is diagnostic of influenza [10]. The combination of fever and cough increases the likelihood ratio that a patient has influenza by only 1.9, and absence of these symptoms (negative likelihood ratio) performs even more poorly at 0.54 [10]. Future trials should include as an outcome measure laboratory-confirmed influenza associated with significant respiratory illness both with and without fever.

The larger sample size of this trial has permitted a more accurate estimate of reactogenicity than was possible in earlier and smaller phase 1 and 2 studies using a monovalent and trivalent product [4,11]. Of over 1300 participants, one vaccine recipient had a fever (37.8–38.2 °C) in the post-vaccinal period, and less than 10% had transient burning or stinging in the nose or itching in the nose, throat or eyes. These complaints decreased in frequency after the second dose of vaccine. There were no Grade 2 or 3 complaints. One subject had a hypersensitivity reaction deemed probably related to the test article.

Ease of administration is an important characteristic of vaccine delivery, for both the health care provider and the vaccine recipient. Nasally administered vaccines should be more acceptable than injectable ones for up to 10% of the population with a fear of needles [12,13]. Inactivated influenza vaccines based on nasal spray administration appear to be more acceptable than nasal drops to vaccinees [3]. Programs using nasally administered vaccines could also be simpler to implement since vaccine providers would not have to have the skill set required for injectable medications, and thus potentially less expensive. Vaccines administered by this route also have the potential advantage of enhanced production of nasal IgA [14].

## Acknowledgments

We would like to thank the participants, clinical research coordinators, and Janine Linden of IDBiomedical.

## References

[1] Fiore AE, Shay DK, Haber P, Iskander JK, Uyeki TM, Mootrey G, et al. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2007. MMWR Recomm Rep 2007;56(RR–6):1–54.
[2] Jefferson TO, Rivetti D, Di Pietrantonj C, Rivetti A, Demicheli V. Vaccines for preventing influenza in healthy adults. Cochrane Database Syst Rev 2007;(2):CD001269.
[3] Eyles J, Williamson D, Alpar H. Intranasal administration of influenza vaccines current status. BioDrugs 2000;13(1):35–59.
[4] Langley JM, Halperin SA, McNeil S, Smith B, Jones T, Burt D, et al. Safety and immunogenicity of a proteosome-trivalent inactivated influenza vaccine, given nasally to healthy adults. Vaccine 2006;24(10):1601–8.
[5] Fries L, Lambkin R, Gelder Cea. FluINsure and inactivated trivalent influenza vaccine for intranasal administration is protective in human challenge with A/Panama/2007/99 (H3N2). In: Options for the Control of Influenza V. 2003.
[6] An advisory committee statement (ACS). National Advisory Committee on Immunization (NACI). Statement on influenza vaccination for the 2003–2004 season. Can Commun Dis Rep 2003;29:1–20.
[7] Boivin G, Cote S, Dery P, De Serres G, Bergeron MG. Multiplex real-time PCR assay for detection of influenza and human respiratory syncytial viruses. J Clin Microbiol 2004;42(1):45–51.
[8] Ellis JS, Fleming DM, Zambon MC. Multiplex reverse transcription-PCR for surveillance of influenza A and B viruses in England and Wales in 1995 and 1996. J Clin Microbiol 1997;35(8):2076–82.
[9] Influenza in Canada: 2003–2004 season. Can Commun Dis Rep 2005;31(1):1–18.
[10] Call SA, Vollenweider MA, Hornung CA, Simel DL, McKinney WP. Does this patient have influenza? JAMA 2005;293(8):987–97.
[11] Treanor J, Nolan C, O'Brien D, Burt D, Lowell G, Linden J, et al. Intranasal administration of a proteosome-influenza vaccine is well-tolerated and induces serum and nasal secretion influenza antibodies in healthy human subjects. Vaccine 2006;24(3):254–62.
[12] Ribner BS, Hall C, Steinberg JP, Bernstein WA, Chakkalakal R, Emamifar A, et al. Use of a mandatory declination form in a program for influenza vaccination of healthcare workers. Infect Control Hosp Epidemiol 2008;29(4):302–8.
[13] Deacon B, Abramowitz J. Fear of needles and vasovagal reactions among phlebotomy patients. J Anxiety Disord 2006;20(7):946–60.
[14] Atmar RL, Keitel WA, Cate TR, Munoz FM, Ruben F, Couch RB. A dose–response evaluation of inactivated influenza vaccine given intranasally and intramuscularly to healthy young adults. Vaccine 2007;25(29):5367–73.