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Original Article

# Low-dose oral interferon alpha as prophylaxis against viral respiratory illness: a double-blind, parallel controlled trial during an influenza pandemic year

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**Background and objective** Interferon alpha (IFNα) is a known antiviral agent. A double-blind, placebo-controlled clinical trial was conducted investigating the use of low-dose oral interferon alpha for preventing acute viral respiratory illnesses.

**Methods** Two hundred healthy adults aged 18–75 years were enrolled and completed weekly health data questionnaires to monitor for symptoms and impact of respiratory illness. Serum samples were tested for antibodies against influenza and other common respiratory viruses.

**Results** Low-dose oral IFNα prophylaxis did not reduce the incidence or impact of acute respiratory illness (ARI) or the impact of illness on daily activities. *Post hoc* analysis of participant subgroups, however, identified significant reductions in the incidence of ARI reported by males, those aged 50 years or more and those who received the 2009 seasonal influenza vaccine. Interferon alpha prophylaxis had a significant impact on the reporting of moderate-to-severe feverishness by the study population. Seropositive participants in the IFN group were more likely to report asymptomatic or mild symptoms compared with those in the placebo group who were more likely to report stronger symptoms.

**Conclusions** Low-dose oral IFNα prophylaxis was not effective in limiting the overall incidence of ARI in our study population. However, there was evidence that prophylaxis reduced the severity of symptoms and had a beneficial effect in some subpopulations, including those who received the 2009 seasonal trivalent influenza vaccination.

**Keywords** Influenza, interferon, low dose, oromucosal.

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## Introduction

Seasonal influenza causes up to 5 million cases of severe febrile illness and up to 500 000 deaths each year internationally[1] and is a major economic burden.[2] The highest disease burden occurs in older individuals, those with underlying chronic illnesses, the immunosuppressed, infants and pregnant women.[1,3]

Influenza vaccination is the primary intervention for reduction in the incidence and severity of influenza infection. However, many at-risk individuals do not get vaccinated,[4] and some vulnerable groups such as the very elderly and the immunosuppressed respond poorly to the vaccine. Furthermore, protection is reduced if there is a mismatch between the circulating strain and the vaccine, and in the event of a newly emerging influenza strain, such as the recent pandemic A (H1N1) 2009, vaccines may be unavailable for several months. Antiviral drugs are available; the M2 protein inhibitors (amantadine and rimantadine) and neuraminidase inhibitors (zanamivir and oseltamivir) shorten and reduce severity of illness and may reduce complications, but need to be commenced within 48 hours of onset.[5] Resistance has already emerged in some strains and remains a potential risk for the future.[6] Therefore, it is important that we continue to investigate new therapies that can be used independently or in conjunction with treatments currently available.

The production of type 1 IFN is a critical early host response to viral infections, inducing over 200 antiviral gene products and modulating downstream innate and adaptive immune responses.[7] Despite its broad therapeutic potential, current use of IFN is largely restricted to high-dose parenteral therapy for treatment of severe infections such

as hepatitis B and C. Oral IFN administration at very low doses is believed to mimic a natural mode of action and result in a concentration of type 1 IFN in local nasal mucosa similar to that induced by exposure to respiratory viruses. Investigation into the physiological levels of endogenous type 1 IFN during viral infections has reported levels between 116 IU/ml[8] and 138 IU/ml[9] in nasal secretions and only 10–30 IU/ml in serum.[9] This is approximately 10 000–100 000 times less than doses delivered parenterally for the treatment of hepatitis C. The use of IFN at such low doses means treatment is relatively inexpensive.

Experience with IFN for treatment of influenza has been inconsistent. Early reports from Russia using intranasal interferon indicated that it could be an effective prophylaxis and treatment.[10,11] Follow-up studies conducted elsewhere found minimal benefits and unacceptable side effects when high-dose intranasal IFN ($>10^6$ International Units, IU) was used.[12–15] Intranasal IFN prophylaxis has been shown to be effective in reducing the incidence of rhinovirus infection, demonstrating a dose–response relationship. Increasing doses were associated with increasing efficacy but also increased local adverse effects, mainly irritation of the nasal mucosa.[16–18]

Interestingly, data from murine studies suggest that low-dose IFN can be more effective than higher doses,[19–21] indicating that low-dose oral IFN may be effective despite the disappointing previous experience. While the mechanism underlying effective low-dose oral IFN therapy is unknown, murine studies have shown that IFN-α administered to the oral cavity is retained by tissues proximal to lymphoid regions, including the posterior nasal cavity, posterior tongue and small intestine,[22] and type I interferon receptors are also present at high concentrations in the oropharyngeal cavity and elsewhere in the gastrointestinal tract.[19] It is hypothesized that IFN delivered by the oral route binds to receptors on specialized cells in the oral and/or pharyngeal cavity and elicits a therapeutic immune response without the need for delivery of exogenous IFN into the systemic circulation.[7] Oral administration of low-dose recombinant IFN 1 has been shown to produce immunomodulatory effects in animal studies[19,20,23] and human clinical trials,[24–26] and to reduce influenza mortality in a mouse model.[21] It has been well tolerated in these trials with no evidence of significant adverse effects. Therefore, we undertook a clinical trial to examine the efficacy of low-dose oral IFN in the prevention of acute respiratory illness during the winter influenza season in Western Australia in 2009, which coincided with the emergence of the pandemic A/(H1N1) 2009 virus.

## Materials and methods

### Study design

This phase 2, randomized, double-blind, placebo-controlled clinical trial was conducted at a single site in Perth, Western Australia. The purpose of this study was to evaluate the efficacy of once-daily lozenges containing low-dose IFN-α in preventing acute respiratory viral illness in healthy adults between the ages of 18 and 75 years. All subjects provided written informed consent. The study was approved by Sir Charles Gardner Hospital Human Research Ethics Committee (Perth, Western Australia) and was conducted in accordance with the current revision of the Declaration of Helsinki (Revised South Africa 1996) and with local laws and regulations. The trial was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12609000976280) at http://www.anzctr.org.au/ and at http://clinicaltrials.gov/ (NCT00895947).

The primary endpoint for this study was the incidence of acute respiratory illness (ARI) as defined by the presence of one or more symptoms, with a severity rating of moderate or severe, during the 16-week treatment period.

### Placebo and interferon lozenges

All lozenges contained anhydrous crystalline maltose and magnesium state. Active lozenges contained 150 IU of natural human interferon alpha (3:1 ratio of IFNα 2b and IFNα 8) prepared by HBL (Hayabashi Biochemical Labs., Okayama, Japan)[27] and supplied by Amarillo Biosciences Inc. (Amarillo, TX, USA). The active lozenges retain full potency for at least 5 years when refrigerated at 4°C and for up to 2 years when stored at room temperature (25°C). The IFNα and placebo lozenges were identical in appearance, taste and odour. No formal test of blinding was performed for this trial; however, HBL IFN lozenge preparations have been used in 10 previous clinical trials and no bias of reporting has been noted. Participants were randomized into one of two treatments groups: 150 IU IFNα lozenges or placebo lozenges. During the 16-week treatment period, participants were asked to dissolve one lozenge in their mouth each morning. Compliance was monitored through the use of the weekly health data questionnaire. Participants were asked to report how many days they had taken the trial medication. These counts were confirmed at the end of the study when participants returned the trial medication, and the remaining lozenges were counted.

### Recruitment and screening

Healthy adults between the ages of 18 and 75 years were recruited from the Perth metropolitan area through email and newspaper advertisement. Medical and influenza vaccination histories, baseline full blood picture, renal functions tests, liver function tests, β-HCG (for females) and lipid profile were taken. Volunteers were excluded if they were found to be at increased risk of severe or complicated influenza;[3] had a history of other chronic respiratory conditions, chronic hepatitis B or hepatitis C infection, severe depression; were pregnant; or had any significant abnormal laboratory result (other than an abnormal lipid profile) as deemed by the trial

clinician. Subjects who had received the 2009 seasonal influenza vaccine were eligible for enrolment in the study. No participants received the pandemic influenza vaccine prior to or during the treatment period of the study.

### Randomization

Amarillo Biosciences Inc. carried out the study drug randomization and blinding. A total of 200 study ID numbers were allocated and used to label individual study drug containers from 001 to 200. These were held in the Pharmacy Department at Sir Charles Gairdner Hospital, where the drugs were dispensed to participants following randomization. Using randomly permuted blocks of four subjects, participants were assigned to one of two groups: (i) 150 IU IFNα or (ii) placebo. Recruitment and enrolment were progressive, with the first participant starting mid-May 2009 and the final participant completing the 16-week treatment period mid-November 2009.

### Determining episodes of acute respiratory illness (ARI)

An online weekly health data questionnaire, adapted from the Wisconsin Upper Respiratory Symptom-21 (WURSS-21) daily symptom report survey, was used to collect information on the incidence, severity and impact of ARI. Participants reported on 13 symptoms associated with acute respiratory illness: cough, sore throat, scratchy throat, hoarseness, runny nose, plugged nose, sneezing, headache, body aches, chills, fever, head congestion and chest congestion. A 7-point severity scale was used ranging from absent (0), very mild (1), mild (2–3), moderate (4–5) to severe (6–7). Participants were asked to rank the severity of their illness based on the following definitions. Mild symptoms cause minimal discomfort and do not interfere in a significant manner with normal activities; moderate symptoms are sufficiently uncomfortable to produce some impairment of normal activities; and severe symptoms are incapacitating and prevent participation in normal activities. Participants were asked to only rate symptoms they felt were due to colds or influenza, that is, if they believed a headache was due to a cause other than a cold or influenza, it did not need to be scored.

Participants were also asked to report on the impact of their illness on daily activities, the number of days they felt sick and/or missed work, whether they visited a medical practitioner and/or pharmacy, whether they took medications and whether they skipped any planned activities.

### Serology

Serum samples were collected from participants at the time of initial screening and again after the 16-week treatment period. Influenza A subtype-specific hemagglutination inhibition (HI) titres for antibody to pandemic influenza A/California/7/2009(H1N1), seasonal influenza A/Brisbane/59/2007 (H1N1) and seasonal influenza A/Brisbane/10/2007(H3N2) were performed at the WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia. Antibodies to influenza B, adenovirus, respiratory syncytial virus and parainfluenza viruses types 1, 2 and 3 were detected by complement fixation titres (CFT) at PathWest. A fourfold or greater increase in titre was taken to indicate infection with that virus during the trial period. For pre-trial influenza immunity, a HI titre $\geq 40$ against an influenza A subtype or a CFT $\geq$ 80 against the other viruses was regarded as significant. Participants who were vaccinated against seasonal influenza A were eligible for enrolment in the study but were excluded from the serology analysis. A monovalent pandemic (H1N1) 2009 vaccine was made available for adults on 30 September 2009, and participants reported whether they received this vaccine. None received the pandemic vaccine during the 16-week treatment period, but three participants received the vaccine after the 16-week treatment period and before collection of their post-treatment sera. These participants were excluded from the serological analysis.

### Statistical analysis

All efficacy analyses were carried out on the intent-to-treat (ITT) population, consisting of all patients who were randomized and took at least one dose of study medication. The incidence of ARI was calculated as the proportion of patients who experienced one or more episodes of ARI during the 16-week treatment period, and was analysed using binary logistic regression models comparing the two treatment groups and adjusting for seasonal influenza vaccination status, sex and age ($<50$ or $\geq 50$). The rate of ARI was defined as the number of episodes per participant experienced during the 16-week treatment period. Pearson chi-square test was used to determine significance, and omnibus test was used to identify subhypotheses (*post hoc*) investigations. Sex, age and seasonal influenza vaccination status interactions were explored by comparing adjusted odds ratios. Safety analysis included Pearson chi-square or Fisher's exact test to compare the treatment groups for the proportion of subjects reporting each adverse event, reporting severity level of each adverse event, reporting one or more serious adverse events, and failing to complete the 16 weeks of treatment. Treatment groups were also compared for changes in full blood counts and serum chemistry variables from the time of screening to the end of treatment by analysis of variance. All analyses were performed using SPSS Software (PASW Statistics 18, SPSS Inc., Chicago, IL, USA).

## Results

### Study population

One hundred and ninety-eight participants (Table 1) completed a total of 2963 weekly health data questionnaires,

**Table 1.** Demographic characteristics of randomized participant population at the time of screening

| Participant demographics | Placebo (*n*) | Interferon (*n*) | Total (*n*) |
|---|---|---|---|
| Randomized | 100 | 100 | 200 |
| Intent to treat (ITT) | 99 | 99 | 198 |
| Completed 16 weeks of treatment | 90 | 88 | 178 |
| Age distribution (years) | | | |
| 20–29 | 15 | 26 | 41 |
| 30–39 | 11 | 8 | 19 |
| 40–49 | 27 | 18 | 45 |
| 50–59 | 29 | 33 | 62 |
| 60–69 | 14 | 11 | 25 |
| 70–75 | 3 | 3 | 6 |
| Participant subgroups (ITT) | | | |
| Age | | | |
| Under 50 years old | 53 | 52 | – |
| 50 years old or more | 46 | 47 | 0·89 |
| Sex | | | |
| Females | 65 | 70 | – |
| Males | 34 | 29 | 0·45 |
| Seasonal influenza vaccination in 2009 | | | |
| No | 48 | 62 | – |
| Yes | 51 | 37 | 0·05* |

*Denotes a statistical difference between the groups. Fewer subjects in the IFN group received seasonal influenza vaccination.

representing 94% of the expected 3168 returns. A total of 178 participants (89%) completed the 16-week treatment course, and, within this population, study drug compliance averaged 96%. The median age of the study population was 49 years (range 20–74 years), of whom 68% were females.

## Incidence of ARI

A case of acute respiratory illness (ARI) was defined by the reporting of one or more symptoms, with a severity rating of moderate or severe, during the 16-week treatment period. During the study, participants reported 317 ARI, of which 163 occurred in the placebo group (mean 1·65 ± 0·20 per participant) and 154 occurred in the IFN group (mean 1·55 ± 0·21 per participant, $P = 0·752$). There was also no significant difference in the proportion of participants reporting ARI between the two treatment groups (Figure 1A). *Post hoc* analysis of demographic subgroups was performed, and the results are reported below.

## *Post hoc* analysis

*Post hoc* subgroup analysis based on sex (Figure 1B), age (Figure 1C) and seasonal influenza vaccination status (Figure 1D) was performed to further examine differences in the incidence of ARI between IFN and placebo groups. Prophylaxis with 150 IU IFNα significantly reduced the incidence in males, those aged 50 years and above and those who had received the 2009 southern hemisphere seasonal influenza vaccine. Sex, age, vaccination status and treatment group

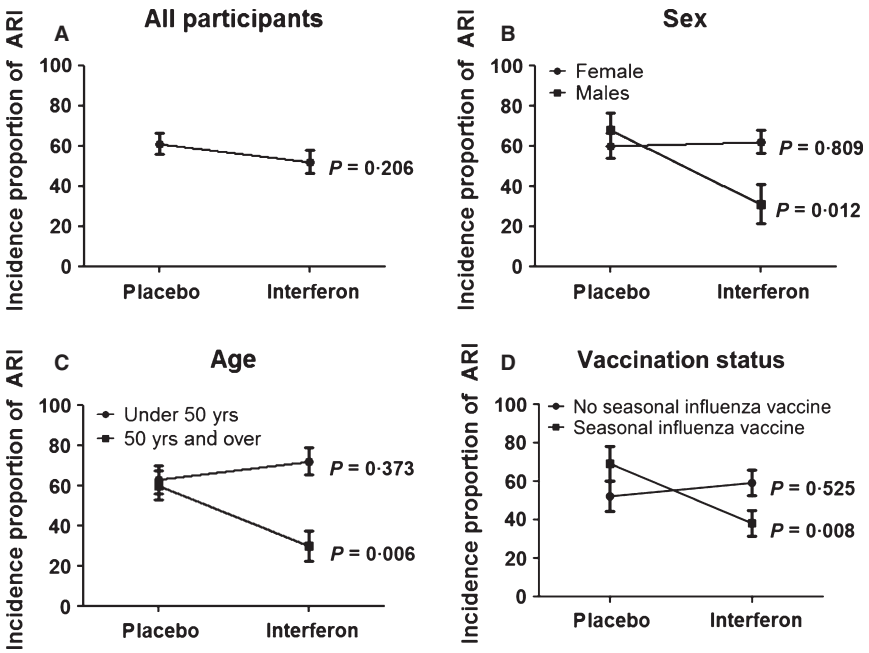


**Figure 1.** Proportion of participants from each treatment group reporting one or more episodes of acute respiratory illness (ARI) during the study period. An episode of ARI was defined as ≥1 respiratory symptoms reported within a given weekly health data report and rated moderate or severe. Error bars indicate 95% confidence intervals. Binary logistic regression models used adjusted for sex, age and seasonal influenza vaccination status.

were included in all regression models to correct for multiple comparisons.

## Incidence and severity of individual symptoms

Participants were asked to report on a total of 13 symptoms associated with ARI. To determine whether there was any overall effect on symptoms, we performed an area-under-the-curve (AUC) analysis of total weekly symptom scores for all participants, independent of whether they met the criteria for an ARI. A mean AUC value of 62·56 ± 6·75 was observed for the placebo group and 57·29 ± 6·65 for the interferon group, indicating no significant difference between the treatment groups ($P = 0·579$). The incidence and severity of the individual symptoms were also analysed. No significant difference was seen in the overall incidence of any of the 13 symptoms reported by the two treatment groups.

For moderate or severe symptoms only, feverishness was significantly reduced from an incidence proportion of 23·3 ± 4·4% of participants in the placebo group to only 11·7 ± 3·4% in the IFN group ($P = 0·036$). This reduction in reporting of feverishness was observed across all sex, age and vaccination status subgroups. No significant difference was observed in the reporting of moderate-to-severe symptoms in the other 12 variables measured. However, statistically non-significant trends in reduction were observed for two other symptoms: head congestion (reduced from 32·7 ± 5·0% to 20·5 ± 4·4%, $P = 0·053$) and sore throat (reduced from 35·9 ± 5·1% to 23·5 ± 4·6%, $P = 0·056$) in the IFN group. These reductions were only observed in those aged 50 years and above, in males and in those vaccinated against the seasonal influenza vaccine.

Sex, age, seasonal influenza vaccination status and treatment group interactions were included in all regression models to correct for multiple comparisons.

## Secondary outcomes

Secondary outcomes for the study were categorized into three groups: negative impacts due to colds and flu, serology and adverse events.

## Negative impacts due to colds and flu

Interferon prophylaxis had no impact on the number of days participants reported missing work, taking cold or flu medication, number of medical practitioner visits, pharmacy visits, days spent feeling sick or number of days a planned activity was skipped due to cold or influenza symptoms (Table 2).

**Table 2.** Effect of low-dose oral interferon treatment on the impact of acute respiratory infections. Participants reported the number of days they were negatively affected by acute respiratory illness (ARI) each week

| | Mean number of days | | |
|---|---|---|---|
| | **Placebo** | **IFN** | ***P* value** |
| Felt sick | 6·2 ± 8·4 | 5·9 ± 8·2 | 0·78 |
| Absent from work | 1·1 ± 2·0 | 1·1 ± 2·4 | 0·75 |
| Visited a medical practitioner | 0·29 ± 0·8 | 0·27 ± 0·77 | 0·96 |
| Visited a pharmacy | 0·79 ± 2·0 | 0·87 ± 1·9 | 0·52 |
| Took medication | 6·1 ± 9·9 | 5·3 ± 7·6 | 0·57 |
| Skipped a planned activity | 1·7 ± 2·8 | 2·4 ± 4·4 | 0·69 |

IFN, interferon.

## Serology

Analysis of pre-trial samples showed 124 participants to have evidence of pre-existing infection with one or more respiratory viruses: 70 in the placebo group and 54 in the IFN group. A total of 236 past infections were identified, including 148 influenza virus types and subtypes and 88 other respiratory viruses. Of these, 63 influenza (mean 0·64 per participant) and 43 other viruses (mean 0·44 per participant) occurred in the IFN group (mean 1·07 per participant), and 85 influenza (mean 0·86 per participant) and 45 other viruses (mean 0·45 per participant) occurred in the placebo group (mean 1·31 per participant).

A total of 179 paired participant samples were available for analysis, 89 from the IFN treatment group and 90 from the placebo group. Seven participant samples were excluded due to seroconversion as a results of seasonal ($n = 4$) or pandemic ($n = 3$) influenza vaccination. During the trial, 20 participants from the IFN treatment group were infected with 22 respiratory viruses, and 23 participants from the placebo group were infected with 23 respiratory viruses (Table 3). Overall, the incidence of influenza and non-influenza infections detected by serology was the same in the IFN and placebo groups (Figure 2A). The serology data were then correlated with reporting of moderate-to-severe symptoms (which defined an ARI) collected from the weekly health data questionnaires. The proportion of seropositive participants reporting moderate-to-severe symptoms was 76·2% placebo recipients compared with 23·8% IFN recipients ($P = 0·0082$) (Figure 2B). These results indicate that while IFN did not reduce the incidence of serologically proven infection, it did significantly reduce the incidence of moderate or severe illness.

## Adverse events

The analysis of early study terminations included all 200 randomized subjects. Otherwise, the safety analyses included all 198 subjects who took at least one dose of study drug. The treatment groups were compared for changes in full blood count and serum chemistry variables from screening to the end of treatment by analysis of variance. Chi-square or

**Table 3.** Number of serologically confirmed past and acute respiratory viral infections

| | Serological evidence of past exposure* | | Acute infection** | |
|---|---|---|---|---|
| **Number of participant samples tested** | **Placebo 100** | **IFN 100** | **Placebo 90** | **IFN 89** |
| Pandemic influenza A/California/7/2009(H1N1) | 13 | 6 | 16 | 13 |
| Seasonal influenza A/Brisbane/59/2007(H1N1) | 20 | 15 | 0 | 0 |
| Seasonal influenza A/Brisbane/10/2007(H3N2) | 38 | 23 | 2 | 3 |
| Influenza B | 14 | 19 | 0 | 0 |
| Adenovirus | 11 | 15 | 0 | 1 |
| Respiratory syncytial virus | 20 | 8 | 3 | 3 |
| Parainfluenza virus type 1 | 3 | 4 | 0 | 1 |
| Parainfluenza virus type 2 | 0 | 1 | 0 | 0 |
| Parainfluenza virus type 3 | 11 | 15 | 2 | 1 |
| Total | 130 | 106 | 23 | 22 |

*Significant antibody titre in the screening serum sample.
**Fourfold or greater rise in antibody titre between the screening and post-treatment serum samples.

Fisher's exact tests were used to compare the treatment groups for the proportion of subjects (i) reporting each adverse event, (ii) reporting each severity level (mild, moderate or severe) for each adverse event, (iii) reporting one or more serious adverse events, and (iv) failing to complete 16 weeks of treatment. The overall rate of adverse events was very low: an average of 1·44 adverse event reports per placebo-treated subject and 1·31 adverse event reports per IFN-treated subject in the 20 weeks of study treatment and follow-up. The same proportion of subjects in both groups (50·5%) reported one or more adverse event during treatment. There were no significant differences between the groups in the proportion of subjects reporting one or more mild, moderate or severe adverse events, either overall or for any particular adverse event. Adverse events reported with the greatest frequency were hay fever (27 participants), diarrhoea (24 participants) and headache (not associated with cold and flu symptoms) (24 participants). There was no significant difference in the proportion of IFN- or placebo-treated participants reporting these symptoms. A total of 89% of the randomized subjects completed all 16 weeks of treatment, and the treatment groups did not differ significantly in the rate of early termination overall or due to adverse events. A single serious adverse event was reported in each group. Both were the result of inpatient hospitalizations for events unrelated to respiratory illness, and neither was deemed by the investigators who were blind as being related to study drug. A subject in the placebo group underwent an emergency appendectomy, while a subject in the IFN group required an emergency haemorrhoidectomy. Both subjects were able to complete the study.

In addition, there were no clinically meaningful adverse laboratory findings. Of the 31 blood counts and serum chemistry variables assessed, the only variable that was significantly different between the treatment groups at week 16 was the mean creatinine level. The placebo group reported a mean level of 80·8 μM ± 13·2 while the IFN group reported a mean level of 76·2 μM ± 10·9 ($P = 0·02$). The reference range for this variable is 60–120 μM for males and 40–90 μM for females. Given the small absolute difference in mean creatinine levels between the groups and the fact that mean change in levels did not differ significantly between the groups, this finding is not believed to be clinically meaningful.

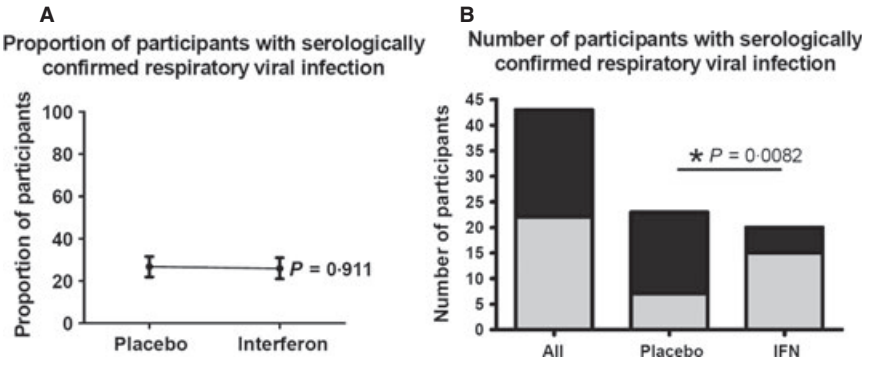


**Figure 2.** Participants with one or more serologically confirmed acute viral infection. (A) The proportion of participants who seroconverted during the treatment period. (B) The maximum severity of illness reported by participants with one or more serologically confirmed respiratory virus. Areas of light grey shading represent the number of participants who were asymptomatic or only reported mild symptoms; areas of dark grey shading represents the number of participants reporting moderate or severe symptoms. Fisher's exact test was used to compare the proportion of participants who reported moderate or severe illness in the two treatment groups (* denotes significance).

## Discussion

This study investigated the efficacy of low-dose oral IFNα (150 IU) for protection against acute viral respiratory illnesses. Oral IFNα prophylaxis did not significantly reduce the incidence of ARI (Figure 1A) in healthy adults aged 18–75 years old, nor did it reduce the weekly symptom scores for participants or reduce the overall severity of symptoms. However, individual symptom reports showed a ~50% reduction in the incidence of moderate-to-severe episodes of feverishness in the 150 IU IFNα population; trends were also observed in the reduction of head congestion ($P = 0{\cdot}053$) and sore throat ($P = 0{\cdot}056$).

This study also found that while the overall incidence of ARI was not reduced by IFN prophylaxis, *post hoc* analysis of the data demonstrated a protective effect in males, those aged 50 years and above and those who had received the 2009 southern hemisphere seasonal influenza vaccine (Figure 1B–D). These were all independent predictors of a beneficial IFNα effect. Also, the IFNα treatment group had significantly fewer vaccinated participants compared with the placebo group (Table 1) and may have biased against an IFNα effect in this study. For the age effects, we chose to analyse the effect of IFNα on participants aged 50 years and above separately because they represented approximately 50% of our study population and are also recognized to be at increased risk of severe or complicated influenza.[28] There were insufficient numbers to look in older age groups, such as those aged 65 years and above. Sex and age differences in the effects of type 1 IFN on hepatitis C[29,30] and in cytokine-based immune responses have previously been reported, but the mechanisms behind these observations are unknown.[31–33] The apparent effect of IFNα in participants who had received the 2009 seasonal vaccine is intriguing and suggests that the IFNα may have improved cross-protection against the pandemic influenza A (H1N1) 2009 strain following seasonal influenza vaccination. It has been reported that the 2009 seasonal trivalent vaccine also afforded cross-protection from confirmed pandemic H1N1 influenza illness in up to 34% of those vaccinated.[34] It has also been shown that prior exposure to pandemic H1N1 influenza strains that circulated until 1957 established immunological memory in the older population providing clinical protection from severe illness.[35,36] While the mechanism underlying these effects is unknown, animal studies suggest immunological priming of cross-reactive memory $CD4^+$ T cells and cytolytic $CD8^+$ T cells.[37–39] Others have proposed, and we agree, that IFN likely acts as an adjuvant further enhancing dendritic cell function to drive both humoral and cell-mediated cross-protection [40]. Further studies designed to confirm and further investigate these effects are required.

The weekly health questionnaires were also used to assess the impact of ARI on daily life, and we did not find any benefit from IFNα prophylaxis for any of the parameters assessed (Table 2).

There was no evidence that 150 IU IFNα prophylaxis reduced the incidence of serologically confirmed infection with influenza or other respiratory viruses. That contrasts with a recent study reporting that human IFNα-2b nose and throat spray prevented acute viral respiratory infections in military recruits.[35] This may be due to differences in the IFN dose and route of administration, study populations and study design (they conducted their study over a 5-day period) or limitations in their diagnostic testing. The study used IgM detection in single serum samples, which may have significantly underestimated infection rates in adults.[34,36,37]

We did, however, find that patients with serologically confirmed respiratory viral infection taking IFNα had a significantly lower incidence of moderate or severe symptoms (Figure 2B). Similar results have previously been reported following intranasal administration of IFNα ($1 \times 10^4$ IU/day) where there was no reduction in serological influenza infections, but clinical illness was milder in the interferon group.[38] These findings suggest that while low-dose oral IFNα prophylaxis does not reduce the risk of infection with influenza or other respiratory viruses, it does appear to ameliorate the symptoms. That is consistent with our other findings of a significant reduction in feverishness in those on IFNα prophylaxis.

Unfortunately, we were unable to carry out direct testing for viruses by PCR. Therefore, we were unable to assess the role of respiratory virus infections that could not be detected serologically, such as rhinoviruses, coronaviruses or human metapneumovirus. We also did not directly measure endogenous local or systemic IFN levels. A recent murine study found low-dose intranasal IFNα treatment to be protective against a lethal H5N1 influenza A challenge and also reported that low-dose IFN treatment had an additive effect on IFNα induction and enhanced the production of interferon-stimulated genes detected in the lungs.[39]

In summary, our results show that 150 IU IFNα administered via the oral route was not effective in limiting the overall incidence of ARI in the study population or in reducing the impact of respiratory virus infections on daily activities. However, we did find evidence that it reduces the severity of illness though; in this study, that effect was not sufficient to reduce the impact on daily activities. Furthermore, the benefits of IFNα prophylaxis may be more substantial in certain subpopulations, that is, males, those aged 50 years or more and those who had received seasonal influenza vaccination. Further studies would help clarify whether IFNα reduces the severity of illness and complications of influenza and other respiratory infections in the elderly and other high-risk groups. Additionally, they are needed to determine the potential for IFNα to enhance vaccine-induced protection against influenza.

## Acknowledgements

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## Conflict of interest

Dr J Cummins is the CEO and M. Cummins is the Vice President of Clinical and Regulatory Affairs at Amarillo Biosciences Inc. Amarillo Biosciences Inc. is a US biotechnology firm operating in partnership with the Hayashibara Group of Japan. The company's primary focus is extensive and ongoing research and development into the use of low-dose orally administered interferon as a treatment for a variety of conditions. Additional information is available on the Amarillo Biosciences Inc. website at http://www.amarbio.com/. Dr M. Beilharz of the University of Western Australia has acted as a consultant for Amarillo Biosciences Inc. (Amarillo, Texas, USA) and Pharma Pacific Management Pty. Ltd. (Brighton-Le-Sands, NSW, Australia). Amarillo Biosciences has supported some basic research in Dr M. Beilharz's laboratory, and Pharma Pacific Management Pty. Ltd has contracted work to his laboratory. Dr M. Beilharz is a member of the Scientific Advisory Board of Amarillo Biosciences Inc. and holds stock options in the company. Dr D. Smith is a director of two not-for-profit organizations that receive support from manufacturers of influenza vaccines and antiviral drugs. Dr P. Jacoby and A Bennett have no competing financial interests.

## Conference presentations

The results of the study have been presented at the 3rd World Summit of Antivirals, Busan, South Korea, on 31 July 2010, the 20th Combined Biological Sciences Meeting, Perth, Australia, on 27 August 2010, Options for the control of Influenza VII, Hong Kong, China (O-882), on 3 September 2010, and the 6th Annual Australian Influenza Symposium, Canberra, Australia, on 7 October 2010.

## References

1 World Health Organization. Fact Sheet No 211:Seasonal Influenza. 2009. Available at http://www.who.int/mediacentre/factsheets/fs211/en/index.html (Accessed 2 November 2010).
2 Molinari NA, Ortega-Sanchez IR, Messonnier ML *et al.* The annual impact of seasonal influenza in the US: measuring disease burden and costs. Vaccine 2007; 25:5086–5096.
3 National Health and Medical Research Council. The Australian Immunisation Handbook. 8th edn. Canberra: National Health and Medical Research Council, 2003.
4 Fedson DS. Meeting the challenge of influenza pandemic preparedness in developing countries. Emerg Infect Dis 2009; 15:365–371.
5 Fiore AE, Shay DK, Broder K *et al.* Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. MMWR Recomm Rep 2008; 57:1–60.
6 Hurt AC, Holien JK, Parker MW, Barr IG. Oseltamivir resistance and the H274Y neuraminidase mutation in seasonal, pandemic and highly pathogenic influenza viruses. Drugs 2009; 69:2523–2531.
7 Tompkins WA. Immunomodulation and therapeutic effects of the oral use of interferon-alpha: mechanism of action. J Interferon Cytokine Res 1999; 19:817–828.
8 Hall CB, Douglas RG Jr, Simons RL. Interferon production in adults with respiratory syncytial viral infection. Ann Intern Med 1981; 94:53–55.
9 McIntosh K. Interferon in nasal secretions from infants with viral respiratory tract infections. J Pediatr 1978; 93:33–36.
10 Soloviev V. The results of controlled observations on the prophylaxis of influenza with interferon. Bull World Health Organ 1969; 41:683–688.
11 Jordan W, Hopps H, Merigan T. Influenza and interferon research in the Soviet Union. J Infect Dis 1973; 128:261–264.
12 Merigan TC, Reed SE, Hall TS, Tyrrell DA. Inhibition of respiratory virus infection by locally applied interferon. Lancet 1973; 1:563–567.
13 Phillpots RJ, Higgins PG, Willman JS, Tyrrell DA, Freestone DS, Sheperd WM. Intranasal lymphoblastoid interferon ("Wellferon") prophylaxis against rhinovirus and influenza virus in volunteers. J Interferon Res 1984; 4:535–541.
14 Treanor JJ, Betts RF, Erb SM, Roth FK, Dolin R. Intranasally administered interferon as prophylaxis against experimentally induced influenza A virus infection in humans. J Infect Dis 1987; 156:379–383.
15 Hayden F, Mills S, Johns M. Human tolerance and histopathologic effects of long-term administration of intranasal interferon-alpha 2. J Infect Dis 1983; 148:914–921.
16 Farr BM, Gwaltney JM Jr, Adams KF, Hayden FG. Intranasal interferon-alpha 2 for prevention of natural rhinovirus colds. Antimicrob Agents Chemother 1984; 26:31–34.
17 Monto AS, Albrecht JK, Schwartz SA. Demonstration of dose-response relationship in seasonal prophylaxis of respiratory infections with alpha-2b interferon. Antimicrob Agents Chemother 1988; 32:47–50.
18 Sperber SJ, Hayden FG. Antiviral chemotherapy and prophylaxis of viral respiratory disease. Clin Lab Med 1987; 7:869–896.
19 Bosio E, Beilharz MW, Watson MW, Lawson CM. Efficacy of low-dose oral use of type I interferon in cytomegalovirus infections in vivo. J Interferon Cytokine Res 1999; 19:869–876.

**20** Bosio E, Cluning CL, Beilharz MW. Low-dose orally administered type I interferon reduces splenic B cell numbers in mice. J Interferon Cytokine Res 2001; 21:721–728.

**21** Beilharz MW, Cummins JM, Bennett AL. Protection from lethal influenza virus challenge by oral type 1 interferon. Biochem Biophys Res Commun 2007; 355:740–744.

**22** Beilharz MW, McDonald W, Watson MW, Heng J, McGeachie J, Lawson CM. Low-dose oral type I interferons reduce early virus replication of murine cytomegalovirus in vivo. J Interferon Cytokine Res 1997; 17:625–630.

**23** Satoh Y, Kasama K, Kuwabara M *et al.* Suppression of late asthmatic response by low-dose oral administration of interferon-beta in the guinea pig model of asthma. J Interferon Cytokine Res 1999; 19:887–894.

**24** Cummins MJ, Papas A, Kammer GM, Fox PC. Treatment of primary Sjogren's syndrome with low-dose human interferon alfa administered by the oromucosal route: combined phase III results. Arthritis Rheum 2003; 49:585–593.

**25** Brod SA, Nguyen M, Hood Z, Shipley GL. Ingested (oral) IFN-alpha represses TNF-alpha mRNA in relapsing-remitting multiple sclerosis. J Interferon Cytokine Res 2006; 26:150–155.

**26** Beilharz MW, Cummins M, Bennett A, Cummins J. Oromucosal administration of interferon to humans. Pharmaceuticals 2010; 3:323–344.

**27** Fukuda S, Ando S, Sanou O *et al.* Simultaneous production of natural human tumor necrosis factor-alpha, -beta and interferon-alpha from BALL-1 cells stimulated by HVJ. Lymphokine Res 1988; 7:175–185.

**28** Newall AT, Wood JG, Macintyre CR. Influenza-related hospitalisation and death in Australians aged 50 years and older. Vaccine 2008; 26:2135–2141.

**29** Sezaki H, Suzuki F, Kawamura Y *et al.* Poor response to pegylated interferon and ribavirin in older women infected with hepatitis C virus of genotype 1b in high viral loads. Dig Dis Sci 2009; 54:1317–1324.

**30** Hayashi J, Kishihara Y, Ueno K *et al.* Age-related response to interferon alfa treatment in women vs men with chronic hepatitis C virus infection. Arch Intern Med 1998; 158:177–181.

**31** Verthelyi D, Klinman DM. Sex hormone levels correlate with the activity of cytokine-secreting cells in vivo. Immunology 2000; 100:384–390.

**32** Pelfrey CM, Cotleur AC, Lee JC, Rudick RA. Sex differences in cytokine responses to myelin peptides in multiple sclerosis. J Neuroimmunol 2002; 130:211–223.

**33** Hewagama A, Patel D, Yarlagadda S, Strickland FM, Richardson BC. Stronger inflammatory/cytotoxic T-cell response in women identified by microarray analysis. Genes Immun 2009; 10:509–516.

**34** Vuorinen T, Meurman O. Enzyme immunoassays for detection of IgG and IgM antibodies to parainfluenza types 1, 2 and 3. J Virol Methods 1989; 23:63–70.

**35** Gao L, Yu S, Chen Q *et al.* A randomized controlled trial of low-dose recombinant human interferons alpha-2b nasal spray to prevent acute viral respiratory infections in military recruits. Vaccine 2010; 28:4445–4451.

**36** United States Centers for Disease Control and Prevention. Influenza symptoms and the role of laboratory diagnostics. 2011. Available at http://www.cdc.gov/flu/professionals/diagnosis/labrolesprocedures.htm (Accessed 18 January 2012).

**37** Falsey AR, Walsh EE. Respiratory syncytial virus infection in adults. Clin Microbiol Rev 2000; 13:371–384.

**38** Isomura S, Ichikawa T, Miyazu M *et al.* The preventive effect of human interferon-alpha on influenza infection; modification of clinical manifestations of influenza in children in a closed community. Biken J 1982; 25:131–137.

**39** Haasbach E, Droebner K, Vogel AB, Planz O. Low-dose interferon Type I treatment is effective against H5N1 and swine-origin H1N1 influenza A viruses in vitro and in vivo. J Interferon Cytokine Res 2011; 31:515–525.