

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

Review

Randomized controlled trials for influenza drugs and vaccines: a review of controlled human infection studies



Shobana Balasingam*, Annelies Wilder-Smith

Lee Kong Chian School of Medicine, Nanyang Technology University, Singapore 308232, Singapore

ARTICLE INFO

Article history:

Received 15 March 2016

Received in revised form 5 May 2016

Accepted 12 May 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:

Influenza

Randomized controlled trials

Challenge studies

Controlled human infection

Influenza vaccine

Influenza therapeutics

SUMMARY

Objectives: Controlled human infection, the intentional infection of healthy volunteers, allows disease pathogenesis to be studied and vaccines and therapeutic interventions to be evaluated in a controlled setting. A systematic review of randomized controlled trials of countermeasures for influenza that used the experimental human infection platform was performed. The primary objective was to document the scope of trials performed to date and the main efficacy outcome in the trials. The secondary objective was to assess safety and identify serious adverse events.

Methods: The PubMed database was searched for randomized controlled influenza human challenge studies with predetermined search terms. Review papers, papers without outcomes, community-acquired infections, duplicated data, pathogenesis studies, and observational studies were excluded.

Results: Twenty-six randomized controlled trials published between 1947 and 2014 fit the study inclusion criteria. Two-thirds of these trials investigated antivirals and one-third investigated influenza vaccines. Among 2462 subjects inoculated with influenza virus, the incidence of serious adverse events was low (0.04%). These challenge studies helped to down-select three antivirals and one vaccine that were subsequently approved by the US Food and Drug Administration (FDA).

Conclusions: Controlled human infection studies are an important research tool in assessing promising influenza vaccines and antivirals. These studies are performed quickly and are cost-effective and safe, with a low incidence of serious adverse events.

© 2016 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Controlled human infection (CHI) studies, in which volunteers are intentionally infected with a pathogen, have historically been used to advance understanding of the pathogenesis, prevention, and treatment of a variety of infectious diseases.¹ CHI, also called human challenge studies, go back several centuries. Scientific status was achieved in 1776 when Edward Jenner demonstrated protection against smallpox by deliberately infecting a young boy first with cowpox virus and then smallpox.² The advantages of CHI approaches are that baseline status, host factors, timing, and the inoculation dose of infection are known and the pathogen is well-characterized. This allows for detailed studies on pathogenesis,

incubation time, time-course of disease progression, attack rates, and correlates of protection, in addition to efficacy studies of therapeutic and prophylactic interventions. Furthermore, enhanced by carefully timed sample taking, a whole range of basic science questions can be addressed.³

Indeed, CHI studies have rapidly become a core platform for performing proof-of-concept (POC) studies of potential vaccines and antivirals. In comparison to phase 2 and 3 trials, trials using the CHI platform require a smaller number of subjects, can be conducted in a shorter time frame, provide an early signal of efficacy that may allow for further product optimization, and can be a gatekeeper to discontinue or justify further investment. Challenge studies can also be done independently of seasons or outbreaks. This is especially relevant for diseases such as influenza, where variations in seasonal attack rates by geography and age can lead to delays in the commencement of trials, or there is a risk of not achieving recruitment of the calculated sample size when trials

* Corresponding author.

E-mail address: sbalasingam@ntu.edu.sg (S. Balasingam).

are conducted during the low season. Thus CHI offers a cost-effective research design to down-select potential vaccine or drug candidates, enabling only the most promising agents to move forward to a larger trial and thereby reducing the duration of the process from drug/vaccine development to introduction to the market.

CHI POC studies evaluating potential vaccines and antivirals follow extensive pre-clinical and pre-clinical animal testing. Currently, there are three animal models used for influenza research, namely the mouse, guinea pig, and ferret models; however, all three have limitations. Of the three models, the ferret model exhibits clinical symptoms that most closely mimic human symptoms of influenza, such as fever, sneezing, nasal discharge, lethargy, and anorexia. However, ferrets are usually low in supply and expensive; there is also a lack of ferret-specific immunological reagents available, which limits the immunological analysis following challenge.⁴ Mice, when challenged with influenza, exhibit symptoms such as anorexia, hunching, laboured breathing, and lack of grooming, whilst the guinea pig does not display any clinical symptoms of infection. Both of these models are readily available, are easy to handle, and are relatively inexpensive compared to the ferret model. However, unlike the other two animal models, the mouse model requires a mouse-adapted strain of influenza and exhibits a lower attack rate of respiratory infection following challenge. As for ferrets, there is a lack of guinea pig-specific immunological reagents available, which again make assessment of the immune response difficult.⁵ Whilst these animal models are an important step in evaluating potential vaccines and antivirals for influenza, the limitations of each make CHI studies an invaluable and integral step of the pathway.

Because of the global spread of influenza affecting billions of people over the past decades, influenza viruses have been a frequently used pathogen for CHI. Although human influenza virus was not isolated until 1933, deliberate exposure of volunteers to respiratory secretions and other biological fluids was undertaken during the 1918 pandemic to attempt to determine the causative pathogen. Human challenge studies with respiratory viruses such as those causing common colds and influenza were frequently performed in the latter part of the 20th century. The earliest challenge study where volunteers were successfully inoculated with influenza was performed in Russia by Smorodintseff et al. in 1937 using aerosolized influenza H1N1 strains.⁶ This challenge study demonstrated that the volunteers who had a high level of baseline neutralizing antibody were protected from infection.⁷ Following the study of Smorodintseff et al., advances were made in developing influenza vaccines. The human challenge platform was used in three studies to determine efficacy of the first generation of influenza vaccines, against both type A and type B strains, produced by chemically inactivating virus-infected allantoic fluid using formalin.^{8–10} All three studies showed that vaccination conferred some protection to the vaccinees against the influenza challenge virus. These studies led to the challenge platform being used to test the efficacy of the next generation of influenza vaccines, i.e., the live attenuated vaccines.^{11–14}

The first pivotal study that showed the usefulness of the challenge platform in evaluating antiviral efficacy against influenza was performed by Jackson et al. and was published in 1963.¹⁵ Challenge studies have subsequently been performed successfully at many institutions/centres, particularly in the USA and UK.

Randomized controlled trials (RCTs) are considered the gold standard for clinical trials and are used as the basis for many CHI studies to evaluate prophylactic and therapeutic measures against a range of organisms. To this end, a systematic review of published human challenge studies that employed influenza virus challenge in RCTs testing either vaccines or antivirals was conducted. An overview of all such trials that were conducted between 1947 and

2014, published in the English language literature, is provided. The objectives were to determine the extent to which CHI-based approaches have been used to study antivirals and vaccines for influenza, the main findings of such studies, the safety of CHI with influenza virus, and the performance characteristics of the various models used.

2. Methods

The United States National Library of Medicine and the National Institutes of Health Medical Database (PubMed) were searched from 1947 until December 2014 using the following search terms: “influenza” (all fields) AND “human challenge study” (all fields) OR “experimental study” (all fields) OR “controlled human infection” (all fields) AND “randomized controlled trials” (all fields).

There were 520 hits with the use of these search terms. All 520 abstracts were reviewed carefully by the two authors and judged for suitability for inclusion in the analysis based on the following inclusion criteria: purposeful infection of volunteers; randomized design and comparison of a test drug or vaccine versus a placebo control. Review papers, papers without outcomes, trials of community-acquired infections (rather than intentional infections), duplicated data, non-randomized and observational trials, pathogenesis studies, animal model studies, and studies using pathogens other than influenza virus for the challenge were excluded. Applying these inclusion and exclusion criteria resulted in 26 papers for inclusion in the analysis (Figure 1).

The following data were extracted systematically from the text, tables, and/or figures of the final 26 papers by applying a standardized format: research question; type of study (e.g. vaccine efficacy/antiviral efficacy); country of study; characteristics of the challenge virus; reported serious adverse events (SAEs); occurrence of acute respiratory illness as defined in each trial; laboratory-confirmed influenza infection; main findings.

The Jadad score was calculated to determine the quality of the reporting.¹⁹ The Jadad score measures the quality of the methods used and reported in a clinical trial and takes into account five parameters: randomization and the method of randomization, blinding and the method of blinding, and withdrawals or dropouts. Based on these parameters, a clinical trial could receive a score of 0 to 5, with 5 being optimal.

3. Results

The final analysis included 26 eligible articles published between 1963 and 2014. All of these trials were conducted in the USA, UK, New Zealand, and Russia. All subjects were healthy adults aged between 18 and 65 years. All subjects were screened for serum haemagglutination inhibition (HAI) antibody titres to assess eligibility for participation in the trials, with 81% of the trials requiring volunteers to have a haemagglutination inhibition unit (HAIU) titre of ≤ 8 HAIU.

3.1. Jadad score

Each trial was reviewed against each parameter and given a Jadad score (see Table 1). Of the 26 papers presented in this systematic review, only one received a score of 5; the median score was found to be 3.²⁰

3.2. Challenge virus

Three influenza A subtypes (A/H3N2, A/H1N1, A/H2N2) and/or influenza B were the strains of influenza virus used in the trials (Table 2). Twenty of the 26 studies were conducted with an influenza A virus, three with an influenza B virus, and three with

Potential relevant citations identified by the search of databases = 520 (including citations)

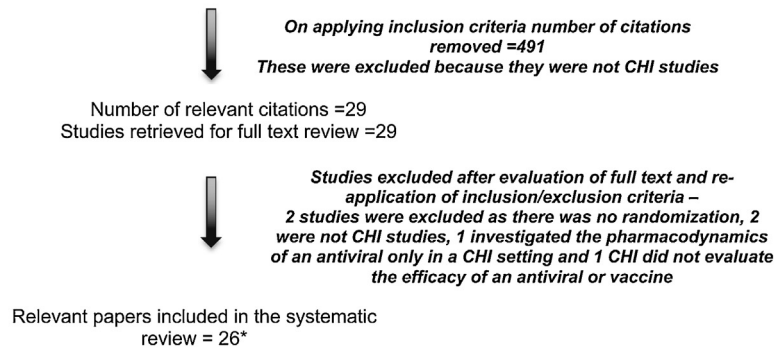


Figure 1. Pathway for determining the inclusion of papers in this review. (*Three additional papers by Hayden et al. (1982), Jackson et al. (1963), and Merigan et al. (1973), which did not appear as a result of the PubMed search with the search terms used, were included for full-text review; this resulted in the inclusion of a total of 26 papers in this systematic review.^{15–17} Jackson et al. (1963) and Merigan et al. (1973) were sourced from a review paper by Hayden (2012), and Hayden et al. (1982) was provided by the author for inclusion.^{15–18}).

both influenza A and B viruses (in different cohorts). The most common influenza A virus strain used was A/Texas/39/91 (H1N1) when an influenza A strain was used (30% of the studies); B/Yamagata/16/88 was the most common influenza B virus strain when a B strain was used (50% of studies).

The inoculum titre of the challenge virus used in these studies varied from a dose of $10^{3.9}$ 50% tissue culture infective dose (TCID₅₀)/ml to $10^{7.2}$ TCID₅₀/ml, whilst Merigan et al. reported using $10^{5.8}$ 50% egg infective dose (EID₅₀).¹⁷ The majority of studies inoculated volunteers with a total of 500 μ l (250 μ l per nostril). Two studies inoculated volunteers with 1 ml in total.^{21,22} All 26 studies reported inoculating volunteers via the intranasal route, usually through nasal drops, except for one study where subjects were inoculated using an intranasal spray.¹⁶ Jackson et al. did not report any details of the challenge virus inoculum except to state that the volunteers were inoculated with an Asian influenza A virus (A/H2N2).¹⁵

The challenge virus used in 13 of these papers was specifically reported to have been safety tested prior to inoculating volunteers and produced according to good manufacturing practice (GMP) standards, either by the National Institute of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID) or a commercial company (Table 2). Three studies reported that the challenge virus had been passaged in specific pathogen-free eggs prior to use only. Although not specifically stated at all times, all challenge strains produced by NIH/NIAID were safety tested (personal communication with Fred Hayden). The remaining studies did not report specific information on safety testing for the challenge virus.

3.3. Procedures performed in the challenge studies

In general, the studies followed similar procedures. Healthy adults with HAI titres below a defined titre to the challenge virus were selected. Following informed consent, subjects underwent a complete physical examination and had blood samples taken for standard biochemistry and haematology testing and urine/serum taken for pregnancy testing; subjects were often screened for chronic blood-borne infections (e.g. hepatitis B virus (HBV), hepatitis C virus (HCV), and HIV). Depending on the type of study, volunteers were immunized (single or two sequential doses; in one study up to 3–4 months prior to challenge), or received chemoprophylaxis prior to being inoculated. Treatment studies typically involved the initiation of dosing within 24 h after virus inoculation, whilst studies on chemoprophylaxis involved initiating the dosing of volunteers 24–72 h prior to virus inoculation.

On inoculation with the challenge virus, volunteers were quarantined for 7 to 10 days. During the quarantine period volunteers filled in a daily diary card to record symptoms (e.g., sore throat, cough, runny nose, headache, fatigue, earache, etc.). Volunteers also had their oral temperature taken up to six times a day, although one trial had axillary temperatures taken.¹⁶ All studies except for one that used nasal swabs, reported nasal washing as a means to determine virus shedding.²³ These were performed the day after challenge, in some cases on a daily basis, or on alternative days in others until the day of discharge. Some studies also did nasal washing on subjects the day before virus challenge. Two studies reported the use of oseltamivir treatment prior to the release of volunteers from quarantine with the aim of ceasing virus shedding for public health reasons.^{22,23}

3.4. Sample size calculations

The sample size used in challenge studies evaluating prophylactics or therapeutics need to account for the attack rate of the challenge virus ascertained in a preceding dose-ranging study. The sample size should be calculated taking into account the anticipated attack rate in the control group (e.g., from dose-ranging studies) and the expected effect size of the intervention. The sample size of volunteers used in all of these challenge studies was relatively low in comparison to field trials: the maximum number of subjects in the studies reported in this systematic review was 287 and the lowest 15.^{24,25} The larger trials involved enrolling serial cohorts of subjects. The dropout/withdrawal rates were very low to absent for the total number of subjects taking part in these trials. Sixteen studies did not report any dropouts/withdrawals; 10 studies reported dropouts/withdrawals but these were low.^{26–28} The largest number of subject dropouts was reported by Barroso et al., with 20 subjects withdrawing prior to virus inoculation or drug dosing and two being withdrawn due to adverse events (AEs) being experienced with the study drug; still, 288 subjects underwent the challenge study.²⁵ Some trials excluded data obtained from subjects for a number of reasons: due to retrospective HAI testing showing high pre-inoculation HAI titres, lack of documented infection after virus inoculation, and proven existence of an infection with a non-challenge virus.^{20,24,29–33} Some studies also withdrew subjects based on AEs being experienced with the study drugs.^{21,33} Jones et al. also withdrew a subject from the study from entering quarantine, as the subject was deemed unsuitable for the challenge study.²²

Table 1

Summary of each study (all studies randomized, double-blinded, and with an untreated challenged control group, unless stated otherwise)

First author and year	Country of study	Jadad Score	Purpose	Study design	Main conclusions
Jackson ¹⁵	USA	1	Evaluation of adamantine hydrochloride against influenza A infection	Volunteers received adamantine hydrochloride either 18 h prior to challenge or 4 h post challenge	Treatment 4 h post challenge resulted in a 72% reduction in infection in the <10 HAIU group but only a 10% reduction in the ≥20 HAIU group. Therefore there is no therapeutic effect with a high antibody level. Volunteers in the <10 HAIU group receiving prophylaxis had an infection rate of 37%, a statistically significant reduction when compared to the placebo control group. Adamantine hydrochloride when given 18 h prior to challenge resulted in a 46% protective efficacy against infection as determined by a rise in antibody levels in seronegative individuals.
Bearé ⁵¹	UK	3	To evaluate the efficacy of an adamantane compound	Volunteers administered either adamantane capsules or placebo capsules twice daily on day 2 for 7 days and challenged on day 4	There was a reduction in clinical symptoms (20%), virus shedding (30%), and seroconversion frequency (23%) in the adamantane treated group when compared to the placebo group.
Merigan ¹⁷	UK	2	Evaluation of prophylactic interferon against B/Hannover/1/70	Subjects administered 800 000 units of interferon in 16 doses at 4 time-points (–24 h, –5 h, –3 h, and –1 h) prior to challenge with B/Hannover/1/70	There was no reduction in the frequency or severity of influenza infection in terms of clinical signs, seroconversion, and virus shedding. Interferon treatment was found to delay the onset of infection (as evidenced by the low symptom scores) by 2 days when compared to the control group.
Douglas ³⁵	USA	3	Evaluation of a topical interferon inducer, CP-20.961, against challenge with A/England/42/72 (H3N2)	Subjects administered CP-20.961 or placebo on days 1–7, 4 times a day, and inoculated with the challenge virus on day 3	No efficacy determined for CP-20.961
Betts ³²	USA	4	Evaluation of a live attenuated influenza virus vaccine (A/Scotland/74 (H3N2)) against challenge with A/Victoria/3/75 (H3N2)	Subjects administered vaccine or placebo intranasally and then challenged 37 days later with A/Victoria/3/75 (H3N2)	The live attenuated vaccine exhibited significant efficacy against illness following challenge with A/Victoria/3/75. Only 52.4% of vaccine recipients showed clinical symptoms compared to 85.7% of the placebo recipients.
Hayden ¹⁶	Russia	1	To evaluate the therapeutic effect of aerosolized and oral rimantadine against challenge with A/Khabarovsk (H1N1)	Subjects inoculated with influenza virus and started on either aerosol treatment (rimantadine 25 mg or saline, 10 min daily) or oral treatment (rimantadine 50 mg or placebo, 3 times daily)	Low doses of aerosolized rimantadine had a therapeutic effect comparable to that with the larger dose of oral rimantadine and both were effective when compared to the placebo. Neither drug group showed a peak in illness score as observed in the placebo group, and the drug groups showed reduced mean illness scores of >4.4 when compared to the placebo group.
Clements ¹⁴	USA	3	Evaluation of a live attenuated, cold-adapted influenza vaccine vs. an inactivated vaccine against A/Washington/897/80 (H3N2)	Subjects received either 1 dose of attenuated cold-adapted vaccine intranasally (3 titres were evaluated in total) or the inactivated vaccine intramuscularly and were challenged 5–8 weeks after	The highest dose of the live attenuated cold-adapted virus vaccination ($10^{7.5}$ TCID ₅₀) completely protected subjects from illness by A/Washington/897/80 and reduced virus shedding by 1000 times compared to the inactivated vaccinees.
Al-Nakib ²¹	UK	3	Evaluation of an antiviral ICI 130,685 (prophylactic and therapeutic) against influenza A A/England/40/83 (H3N2)	Prophylactic study Study 1: 2 tablets of 200 mg ICI 130,685 or 2 placebo tablets once a day for 7 days; Study 2: 1 tablet of 100 mg ICI 130,685 or 1 placebo tablet a day for 7 days; 3 doses prior to viral inoculation Therapeutic study Volunteers exhibiting symptoms for 6–15 h received 200 mg ICI 130,685 or placebo for 4 days	Prophylactic study 100 mg/day was 72% effective at reducing illness. Therapeutic study 200 mg/day, reduction in virus shedding observed. Significant reductions seen from day 3 only.
Treanor ⁵²	USA	3	Evaluation of interferon prophylaxis against influenza A, A/California/78 (H1N1)	Subjects were given either IFN-α ₂ or a placebo spray 48 h prior to challenge and then 5 days post challenge (after the 5 th dose); the dosage was 5×10^6 IU twice per day	IFN-α ₂ reduced illness, resulted in lower mean virus shedding titres and reduced the duration of virus shedding (23%).

Table 1 (Continued)

First author and year	Country of study	Jadad Score	Purpose	Study design	Main conclusions
Reuman ²⁶	USA	3	Evaluation of the efficacy and safety of low dosage amantadine as prophylaxis against influenza A, A/Bethesda/1/85 (H3N2)	Subjects administered placebo, amantadine 50 mg/day, amantadine 100 mg/day, or amantadine 200 mg/day for 8 days (3 prior to challenge and for 5 days post challenge)	200 mg/day was 32% effective at reducing infection. 100 mg/day was 40% effective at reducing infection with fewer side effects than 200 mg/day.
Keital ³⁸	USA	2	Evaluation of a cold recombinant influenza vaccine virus (CRB 87) against a homotypic challenge B/Texas/1/84	Following a dose-ranging study, the highest dose of CRB 87 vaccine was rechallenged with CRB 87 (issues with B/Texas/1/84), 3–4 months after the first inoculation with the control group	The CRB 87 vaccine was 54% effective at reducing the infection rate on rechallenge with CRB 87.
Fries ³⁶	USA	3	Evaluation of the safety, immunogenicity, and protective efficacy of a recombinant protein influenza A vaccine against A/Kawasaki/86 (H1N1)	Subjects were vaccinated twice 22 days apart and challenged along with the unimmunized control group 58–61 days after the first vaccination	The recombinant protein influenza A vaccine was 33.3% effective at reducing clinical illness when compared to the control.
Hayden ³³	USA	2	Efficacy of an oral antiviral, LY217896, for the prevention of experimental influenza A virus A/Kawasaki/86 infection and illness	Subjects received either LY217896 (75 mg dose) or placebo once a day for 7 days; subjects were challenged with A/Kawasaki/86 after the 2 nd dose	LY217896 did not reduce the rate of influenza infection or the clinical symptoms of treated subjects.
Youngner ³⁷	USA	2	Efficacy of simultaneous administration of cold-adapted and wild-type influenza A virus against challenge with A/Kawasaki/86	The study consisted of 3 groups, (1) mixed cold-adapted and wild-type virus, (2) the cold-adapted virus alone, and (3) wild-type virus (A/Kawasaki/86) alone	There was some evidence of reduced pathogenicity in the mixed virus group when compared to the wild-type group alone. The cold-adapted virus when compared against the wild-type alone had reduced illness (14.3% vs. 42.9%) and a 100% reduction in fever.
Hayden ²⁹	USA	3	Safety and efficacy of the neuraminidase inhibitor GG167 in experimental human influenza A/Texas/91 (H1N1)	Four studies; prophylaxis where dosing (3 groups with intranasal drops, 1 group with a spray) was performed 4 h prior to viral challenge, early treatment (intranasal drops) where dosing occurred 26–32 h after inoculation, or delayed treatment (intranasal drops) where dosing occurred 50 h after inoculation; dosing continued for 4 days in the treatment study and for 5 days in the prophylaxis study	Prophylaxis activity: GG167 as drops was 96% effective at reducing viral shedding and 82% effective at reducing infection. GG167 as a spray was 83% effective at reducing virus shedding and 60% effective for reducing infection. Therapeutic activity: Early treatment was 84% effective at reducing fever and 40–65% effective at reducing clinical symptoms. Late treatment resulted in a reduction in virus titre on shedding and a 1-day reduction in shedding.
Walker ³⁴	USA	3	Evaluation of the effects of the neuraminidase inhibitor zanamivir on otological manifestations on experimental influenza A/Texas/36/91 and B/Yamagata/88)	Two arms for the influenza A study; prophylaxis with drug administration 4 h before challenge virus inoculation and early treatment starting 1 day after challenge virus inoculation. Zanamivir administered as either a spray or drops. The influenza B study involved prophylactic treatment only using 3 doses of zanamivir	Influenza A/Texas/36/91 Prophylaxis: zanamivir was effective at reducing infection 13% vs. 73% (placebo), fever 2% vs. 36% (placebo), upper respiratory illness 26% vs. 61% (placebo), and MEP abnormalities 15% vs. 61% (placebo). Early treatment: zanamivir was effective in reducing fever 6% vs. 38% (placebo), upper respiratory illness 52% vs. 81% (placebo), and MEP abnormalities under-pressure 32% vs. 65% (placebo) and over-pressure 6% vs. 27% (placebo). Influenza B/Yamagata/88 Zanamivir was effective at reducing infection and upper respiratory illness 52% vs. 100% (placebo) and MEP abnormalities 16% vs. 44% (placebo).
Doyle ²⁸	USA	4	Evaluation of rimantadine treatment on clinical manifestations and otological complications against influenza A/Kawasaki/86	Subjects challenged with virus on day 0 and then treated with either rimantadine (100 mg) or placebo 48 h after challenge; dosing occurred at 12-h intervals for 8 days (5 days in quarantine and 3 outpatient visits)	Rimantadine significantly reduced total symptoms on days 4 to 6.
Calfee ³¹	USA	4	Evaluation of IV zanamivir in preventing experimental influenza A/Texas/36/91	Subjects given zanamivir (IV) 4 h before virus inoculation at a dose of 600 mg; subjects were dosed over 30 min every 12 h for 5 days	Zanamivir significantly reduced fever 14.3% vs. 87.5% (placebo), and reduced both upper respiratory symptoms and myalgia by 100%.
Fritz ²⁴	USA	2	Evaluation of nasal cytokine and chemokine responses with zanamivir on infection with influenza A/Texas/36/91 (H1N1)	Subjects received zanamivir (600 mg) IV twice a day or placebo starting 4 h before virus inoculation	Zanamivir significantly reduced infection and illness in the recipients against influenza A infection. Zanamivir also reduced the increase in cytokines and chemokines.

Table 1 (Continued)

First author and year	Country of study	Jadad Score	Purpose	Study design	Main conclusions
Hayden ²⁰	USA	5	Evaluation of oseltamivir, an oral neuraminidase inhibitor against influenza A A/Texas/36/91 infection	Two studies Prophylactic study: oseltamivir (100 mg, once daily and twice daily) administered 26 h prior to virus inoculation; therapeutic study: oseltamivir at 20 mg, 100 mg, 200 mg twice daily, 200 mg once daily, or placebo, all given for 5 days	Prophylaxis (26 h prior to virus administration) and early treatment (28 h post virus administration) with oseltamivir significantly reduced infection and the severity and duration of clinical symptoms on challenge with A/Texas/36/91.
Skoner ⁴⁷	USA	2	Evaluation of cytokine mediation of disease expression in adults challenged with influenza A A/Kawasaki/9/86 (H1N1)	Subjects challenged with A/Kawasaki/9/86; 48 h after virus inoculation, administered with rimantadine (100 mg) or placebo, with dosing at 12-h intervals over 8 days	The rimantadine treated group had lower virus shedding, systemic symptom scores, and IL-8 secretion levels.
Hayden ³⁰	USA/UK/ New Zealand	3	Evaluation of oral oseltamivir in adults experimentally infected with influenza B/Yamagata/16/88	Three studies performed; studies A and B evaluating tolerability and efficacy of early treatment oseltamivir (75 mg or 150 mg twice daily for 5 days, 24 h after virus inoculation) and study C evaluating oseltamivir prophylaxis (75 mg once or twice daily for 7 days starting 24 h prior to virus inoculation)	Given as prophylactic, oseltamivir was effective at reducing infection by 61% and reduced virus shedding and illness by 100%. As treatment, oseltamivir reduced virus shedding and symptom scores.
Treanor ²⁷	USA	4	Evaluation of trivalent, live, cold-adapted (CAIV-T) and inactivated (TIV) influenza vaccines against challenge with influenza A (H1N1), A (H3N2), and B viruses	Subjects underwent vaccination 28 days prior to virus inoculation; subjects were given (1) CAIV-T by intranasal spray with IM saline placebo, (2) TIV IM with intranasal placebo spray, or (3) IM and intranasal placebos	CAIV-T was just as effective (69%, 93%) at protecting against influenza infection and illness as the TIV (84%, 87%), compared to control (45%, 55%). CAIV-T was effective in preventing respiratory illness with either isolation of influenza virus from the nasal washes or at least a 4-fold increase in HAI antibody after wild-type challenge. The level of efficacy against this endpoint of preventing respiratory illness was slightly higher with CAIV-T than with TIV (85% compared to 71%) Peramivir demonstrated significant antiviral effects as treatment against both viruses on a once-daily basis following inoculation with virus. There were no significant reductions in any of the parameters tested when peramivir was used as prophylaxis. This study shows that a trivalent DNA vaccine has efficacy against infection with A/Panama/2007/99. The group vaccinated with 4 µg trivalent DNA vaccine when compared to the placebo exhibited lower rates of illness (37% vs. 63%) and a lower rate of laboratory-confirmed influenza illness (33.3% vs. 61.5%). TCN-032 was found to be safe and well-tolerated. The number of AEs reported was similar in the treatment and placebo arms. The primary efficacy parameter was to evaluate the proportion of grade ≥ 2 influenza symptoms after challenge and it was found that 48% of the placebo group had symptoms of grade ≥ 2 compared to 34.5% of the TCN-032 treated group. It was also determined that the TCN-032 group had lower total symptom scores and shortened duration of symptoms.
Barroso ²⁵	USA	4	Evaluation of the oral neuraminidase inhibitor peramivir in experimental human influenza (A/Texas/36/91 (H1N1) and B/Yamagata/16/88)	Four studies performed; prophylaxis (administration 24 h prior to virus inoculation for 4 days with doses ranging from 50 to 800 mg/day) and treatment (administration 24 h after virus inoculation for 5 days with doses ranging from 100 to 800 mg/day)	Peramivir demonstrated significant antiviral effects as treatment against both viruses on a once-daily basis following inoculation with virus. There were no significant reductions in any of the parameters tested when peramivir was used as prophylaxis. This study shows that a trivalent DNA vaccine has efficacy against infection with A/Panama/2007/99. The group vaccinated with 4 µg trivalent DNA vaccine when compared to the placebo exhibited lower rates of illness (37% vs. 63%) and a lower rate of laboratory-confirmed influenza illness (33.3% vs. 61.5%). TCN-032 was found to be safe and well-tolerated. The number of AEs reported was similar in the treatment and placebo arms. The primary efficacy parameter was to evaluate the proportion of grade ≥ 2 influenza symptoms after challenge and it was found that 48% of the placebo group had symptoms of grade ≥ 2 compared to 34.5% of the TCN-032 treated group. It was also determined that the TCN-032 group had lower total symptom scores and shortened duration of symptoms.
Jones ²²	UK	4	Evaluation of a trivalent (A/New Caledonia/20/99, A/Panama/2007/99, and B/Jiangsu/10/2003) DNA vaccine against influenza A A/Panama/2007/99 (H3N2) using a novel approach	Subjects were vaccinated with either 4 µg trivalent DNA vaccine, 2 µg trivalent DNA vaccine, or an adjuvant known as DNA encoded immunostimulator-labile toxin (DEI-LT) or placebo 56 days prior to virus inoculation; the vaccine was administered via the epidermis on microscopic gold beads	Peramivir demonstrated significant antiviral effects as treatment against both viruses on a once-daily basis following inoculation with virus. There were no significant reductions in any of the parameters tested when peramivir was used as prophylaxis. This study shows that a trivalent DNA vaccine has efficacy against infection with A/Panama/2007/99. The group vaccinated with 4 µg trivalent DNA vaccine when compared to the placebo exhibited lower rates of illness (37% vs. 63%) and a lower rate of laboratory-confirmed influenza illness (33.3% vs. 61.5%). TCN-032 was found to be safe and well-tolerated. The number of AEs reported was similar in the treatment and placebo arms. The primary efficacy parameter was to evaluate the proportion of grade ≥ 2 influenza symptoms after challenge and it was found that 48% of the placebo group had symptoms of grade ≥ 2 compared to 34.5% of the TCN-032 treated group. It was also determined that the TCN-032 group had lower total symptom scores and shortened duration of symptoms.
Ramos ²³	UK	3	Evaluation and safety of treatment with an anti-M2e monoclonal antibody in experimental human influenza	Volunteers were inoculated intranasally with influenza A/Wisconsin/67/2005 (H3N2) and received 1 dose of the study drug, TCN-032, or placebo 24 h later	Peramivir demonstrated significant antiviral effects as treatment against both viruses on a once-daily basis following inoculation with virus. There were no significant reductions in any of the parameters tested when peramivir was used as prophylaxis. This study shows that a trivalent DNA vaccine has efficacy against infection with A/Panama/2007/99. The group vaccinated with 4 µg trivalent DNA vaccine when compared to the placebo exhibited lower rates of illness (37% vs. 63%) and a lower rate of laboratory-confirmed influenza illness (33.3% vs. 61.5%). TCN-032 was found to be safe and well-tolerated. The number of AEs reported was similar in the treatment and placebo arms. The primary efficacy parameter was to evaluate the proportion of grade ≥ 2 influenza symptoms after challenge and it was found that 48% of the placebo group had symptoms of grade ≥ 2 compared to 34.5% of the TCN-032 treated group. It was also determined that the TCN-032 group had lower total symptom scores and shortened duration of symptoms.

HAIU, haemagglutination inhibition unit; TCID₅₀, 50% tissue culture infective dose; IFN, interferon; MEP, middle ear pressure; IV, intravenous; IL, interleukin; IM, intramuscular; HAI, haemagglutination inhibition; AE, adverse event.

Table 2
Challenge virus details

First author and year	Challenge virus strain	Characterization of challenge virus	Dose (TCID ₅₀ /ml/ EID ₅₀)	Intranasal inoculation method	Volume (μl) of virus given (total)	Source of challenge virus
Jackson ¹⁵	Asian influenza virus (H2N2)	NR	NR	NR	NR	NR
Beare ⁵¹	A/Hong Kong/68 (H3N2)	NR	10 ^{5.0} to 10 ^{6.5}	NR	NR	NR
Merigan ¹⁷	B/Hannover/1/70	NR	10 ^{5.8} EID ₅₀	Nasal drops	NR	Dr A.S. Beare
Douglas ³⁵	A/England/42/72 (H3N2)	Safety tested	NR	Nasal drops	500	NR
Betts ³²	A/Victoria/3/75 (H3N2)	Safety tested	10 ^{6.1}	NR	NR	NR
Hayden ¹⁶	A/Khabarovsk/77 (H1N1)	NR	10 ^{7.2}	NR	NR	NR
Clements ¹⁴	A/Washington/897/80 (H3N2) lot E174	Safety tested	10 ^{6.0}	NR	NR	Children's Hospital National Medical Center of Washington, DC
Al-Nakib ²¹	A/England/40/83 (H3N2)	Volunteers infected with virus passaged in embryonated eggs; nasal washes obtained then passaged in SPF eggs for further use	10 ^{4.1}	Nasal drops	1000	Central Public Health Laboratory, Colindale, UK
Treanor ⁵²	A/California/78 (H1N1)	Plaque purified and passaged in SPF eggs	10 ^{4.5}	Nasal drops	500	NIAID
Reuman ²⁶	A/Bethesda/1/85 (H3N2)	NR	10 ^{7.15}	NR	500	NIH
Keital ³⁸	B/Texas/1/84 clone 6, lot E-229	NR	10 ^{3.9} , 10 ^{4.9} , 10 ^{6.1} , 10 ^{7.1}	Nasal drops	500	NIAID ^a
Fries ³⁶	A/Kawasaki/8/86 (H1N1)	Safety tested	10 ⁷	NR	NR	NIH ^a
Hayden ³³	A/Kawasaki/8/86 (H1N1)	Safety tested	10 ⁷	NR	500	PRI/DynCorp, USA
Youngner ³⁷	wt A/Kawasaki/9/86 (H1N1) (CI 2-1, lot E-262) and a mixture of the wt A/Kawasaki/9/86 and cold adapted A/Kawasaki/86 virus (CR 125, lot E-271)	NR	10 ⁷	NR	500	PRI/DynCorp, USA
Hayden ²⁹	A/Texas/91 (H1N1)	Safety tested	10 ⁵	Nasal drops	500	NIAID ^a
Walker ³⁴	A/Texas/36/91 (H1N1) and B/Yamagata/88	Safety tested	10 ⁷	NR	500	NIH ^a
Doyle ²⁸	A/Kawasaki/9/86 (H1N1) lot E-262	Safety tested	10 ⁷	Nasal drops	500	NIH ^a
Calfee ³¹	A/Texas/36/91 (H1N1)	Safety tested	10 ⁵	Nasal drops	500	NIAID ^a
Fritz ²⁴	A/Texas/36/91 (H1N1)	Safety tested	10 ⁵	NR	500	NIAID, NIH ^a
Hayden ²⁰	A/Texas/36/91 (H1N1)	Safety tested	10 ⁶	Nasal drops	500	NIAID ^a
Skoner ⁴⁷	A/Kawasaki/9/86 (H1N1) wt, lot E-262	NR	10 ⁷	NR	500	NIAID, NIH ^a
Hayden ³⁰	B/Yamagata/16/88	Safety tested	10 ⁷	Nasal drops	500	NIH ^a
Treanor ²⁷	A/Texas/36/91 (lot E-349) (H1N1), A/Shangdong/9/93 (H3N2) (lot E-337) B/Panama/45/90 (lot E-352)	NR	10 ⁷	Nasal drops	500	DynCorp, USA
Barroso ²⁵	A/Texas/36/91 (H1N1) and B/Yamagata/16/88	Safety tested	A/Texas 10 ⁶ B/Yamagata 10 ⁷	Nasal drops	500	NIAID ^a
Jones ²²	A/Panama/2007/99 (H3N2)	NR	10 ⁶	NR	1000	NR
Ramos ²³	A/Wisconsin/67/2005 (H3N2)	NR	10 ^{5.0-5.5}	NR	NR	NR

TCID, 50% tissue culture infective dose; EID, egg infective dose; SPF, specific pathogen-free; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institute of Health; wt, wild-type.

^a Although not reported, NIH/NIAID will have carried out safety testing on these challenge strains (personal communication with Professor Fred Hayden).

Table 2 summarizes the inoculum doses used in past studies. Such data are beneficial for future challenge studies to narrow down the inoculum titres required in dose-ranging studies.

3.5. Infection rate and virus shedding

In controls, the challenge virus infection rates (defined either by isolation of virus or antibody response) varied from 26%¹⁵ (note these volunteers had a pre-challenge HAI titre of ≥ 20) to 100% (Table 3). The virus shedding data reported a rate of 74% for A/H3N2, 80% for A/H1N1, and 67.1% for B/influenza. Where peak nasal virus titres were reported, those for upper respiratory symptoms (URI) and virus shedding were on day 2 and/or day 3 post virus inoculation for both A strain subtypes and for type B influenza. Virus shedding was determined via titration on canine

kidney cells in 81% of the studies. Jones et al. and Ramos et al. both reported using PCR to evaluate virus shedding from the nasal washes obtained from volunteers, whilst Merigan et al. reported inoculating embryonated eggs followed by the HAI assay to evaluate virus shedding.^{17,22,23}

3.6. Clinical illness

Clinical illness rates and virus shedding rates varied from trial to trial. Where URI symptoms such as runny nose, nasal stuffiness, sore throat, and sneezing were reported, the proportion of URI symptoms for each strain in the untreated challenged individuals was 54% for A/H3N2, 68% for A/H1N1, and 53% for B/influenza. The definition of fever also varied from >37.7 °C to >38.0 °C across studies. A low incidence of fever was reported in three studies

Table 3
Infection rate of untreated challenged individuals and documented adverse events

First author and year	Number of subjects: untreated and challenged (total number challenged)	Infection rate, clinical illness, and laboratory data (%)						AEs in relation to the challenge virus
		Illness ^a	Fever ^b	Laboratory-confirmed illness ^c	Virus shedding	Sero-conversion (≥ 4 -fold)	Infection rate ^d	
Jackson ¹⁵	(21, ≥ 20 HAIU; 90, < 10 HAIU) 111 total (238)	NR	NR	NR	NR	NR	26% ≥ 20 HAIU 73% < 10 HAIU	NR
Bearé ⁵¹	29 (57)	34	NR	NR	66	62	NR	NR
Merigan ¹⁷	11 (22)	73	45	NR	73	80	NR	NR
Douglas ³⁵	10 (20)	70	30	NR	50	40	50	NR
Betts ³²	21 (42)	76	24	NR	95	81	100	NR
Hayden ¹⁶	12 (36)	75	58	NR	58	58	NR	None in relation to the virus
Clements ¹⁴	24 (81)	46	38 (febrile or systemic/ both)	NR	83	NR	96	NR
Al-Nakib ²¹	40 (prophylactic 200 mg)	33	NR	85	85	78	93	AEs reported, but no details provided
	28 (prophylactic 100 mg) (227)	50	NR	89	89	75	93	
Treanor ⁵²	9 (25)	56	NR	NR	81	56	NR	NR
Reuman ²⁶	19 (78)	58	NR	NR	95	68	95	Severe headache reported as a severe AE
Keital ³⁸	16 (98)	31 ^c	NR	NR	100	44 ^c	100	Transient asymptomatic elevation in serum transaminase levels in 1 subject resulting in challenge virus not used
Fries ³⁶	16 (31)	80	40	NR	81	94	94	NR
Hayden ³³	16 (34)	69	6	NR	100	81	NR	None in relation to the challenge virus
Youngner ³⁷	14 (27)	43	21	NR	71	100	36	NR
Hayden ²⁹	33 (prophylactic 26 ^e)	61	36	NR	70	70	73	None in relation to the challenge virus
	26 ^e early treatment	81	38	NR	92	96	NR	
	26 ^e delayed treatment (166)	NR	NR	NR	92	96	NR	
Walker ³⁴	33 H1N1 prophylactic	61	36	NR	NR	NR	73	NR
	26 ^e H1N1 treatment	81	38	NR	NR	NR	NR	
	9 (B) prophylactic (185)	100	0	NR	NR	NR	100	
Doyle ²⁸	53 (103)	53	0	NR	79	87	92	None in relation to the challenge virus
Calfee ³¹	8 (16)	100	88	NR	100	100	100	Two severe AEs; 1 severe 'overall discomfort' 2 days after dosing, which resolved in 3 days; 1 severe headache that resolved within 3 h
Fritz ²⁴	8 (15)	100	88	NR	100	NR	NR	NR
Hayden ^{20,g}	13 (prophylactic)	33	25	67	50	NR	NR	None in relation to the challenge virus
	16 ^e (therapeutic) (117)	NR	13	81	100	NR	81	
Skoner ⁴⁷	38 (72)	NR	NR	NR	84	NR	NR	NR
Hayden ³⁰	13 ^e treatment	69	0	100	85	77	80	None in relation to the challenge virus
	29 ^e treatment	24	3	100	90	76	77	
	19 prophylactic (235)	21	10	NR	74	63	84	
Treanor ²⁷	12 (H1N1)	NR	NR	50	50	50	58	None in relation to the challenge virus
	8 (H3N2)	NR	NR	50	25	50	50	
	11 (B) (103)	NR	NR	36	18	36	36	

Table 3 (Continued)

First author and year	Number of subjects: untreated and challenged (total number challenged)	Infection rate, clinical illness, and laboratory data (%)						AEs in relation to the challenge virus
		Illness ^a	Fever ^b	Laboratory-confirmed illness ^c	Virus shedding	Sero-conversion (≥4-fold)	Infection rate ^d	
Barroso ²⁵	37 ^e (H1N1) treatment	NR	24 (H1N1)	94 (H1N1)	94 (H1N1)	NR	94 (H1N1)	One serious AE was reported during the study when a subject developed a dilated cardiomyopathy following challenge. At the last follow-up visit 5 years post study, the serious AE had resolved
	19 ^e (B) treatment	NR	0 (B)	42 (B)	42 (B)	NR	95	
	19 (H1N1) prophylactic	NR	NR	NR	58	NR	74	
	20 (B) prophylactic (287)	NR	NR	NR	55	NR	90	
Jones ²²	27 (86)	63	33	61.5	NR	NR	NR	None in relation to the challenge virus
Ramos ²³	31 (61)	NR	61	NR	NR	92	NR	93% had mild or moderate AEs such as abnormal spirometry results, epistaxis, and increased levels of ALT, CRP, and AST

AEs, adverse events; HAIU, haemagglutination inhibition unit; NR, not reported; ALT, alanine aminotransferase; CRP, C-reactive protein; AST, aspartate aminotransferase.

^a Definition of illness varied for each trial.

^b Fever (>37.7 to >38.0 °C), or symptoms after virus inoculation (exact definition varied by trial).

^c Subjects with respiratory illness and laboratory evidence of wild-type virus infection.

^d Respiratory illness with positive viral culture and/or a 4-fold increase in HAI titre.

^e Only infected subjects treated with test antiviral.

using a B strain, two reporting no fever being documented and one with just 4% of the placebo group demonstrating fever.^{25,30,34} Of studies with the A/H3N2 subtype, five reported low numbers in the control group showing fever.^{14,22,23,32,35} With the A/H1N1 subtype, 10 studies reported fever in 6% to 88% of the subjects.^{16,20,24,25,29,31,33,34,36,37}

3.7. Reporting of SAEs related to the challenge virus in the placebo group

Of 14 trials (11 were antiviral studies), eight stated that there were no AEs reported in the placebo group in relation to the challenge virus and two documented AEs that included a severe headache and overall discomfort. The remaining reported moderate AEs. Table 3 documents the AEs described for each trial in relation to the challenge virus itself. Twelve of the 26 trials (the majority were vaccine studies) did not report whether AEs occurred.

Of the studies that did state AEs, one reported a subject having an increased serum transaminase level upon challenge with B/Texas/1/84 in the dose-ranging study.³⁸ As a result of this AE, the challenge virus in the study was not used to challenge the vaccinated group of volunteers; the volunteers were instead re-challenged with the cold-adapted reassortant virus, CR influenza B/Texas/1/84.

One study documented a SAE in which a male subject with no history of cardiac problems developed a dilated cardiomyopathy following a prophylactic study with the neuraminidase inhibitor, peramivir, after challenge with influenza B/Yamagata/88 virus.²⁵ This SAE was reported to the US Food and Drug Administration (FDA). The dilated cardiomyopathy resolved with angiotensin-converting enzyme inhibitor treatment. The subject was followed up for 5 years after the study and remained clinically well.²⁵ The cause of the dilated cardiomyopathy was not ascertained but deemed unlikely to be due to the study drug and also deemed unlikely due to be due to the challenge virus.^{25,39}

4. Discussion

Twenty-six RCTs published between 1963 and 2014 were identified, with 19 studies being performed in the USA, five in the UK, and one in Russia; one study was multi-centre, with subjects recruited in the USA, UK, and New Zealand. These studies involved 2462 subjects in total undergoing challenge. About two-thirds of these trials were conducted to evaluate different influenza antivirals and one-third to evaluate the efficacy of a vaccine against influenza. Fewer studies were identified compared to a previous systematic review on influenza challenge studies by Carrat et al., as only RCTs were included in the present review.⁴⁰ However, a higher number of subjects who had undergone challenge studies was identified in the present review ($n = 2462$) compared to the review by Carrat et al. ($n = 1280$), indicating that since the period of the previous review (2008), many more trials using the CHI study approach have been conducted.⁴⁰ More than 2000 subjects having undergone challenge studies is a sizable population to assess safety issues and the value of such challenge studies. Given the ethical issues of intentionally infecting healthy volunteers, it is important to periodically review and revisit the value and safety of challenge studies.

The present findings on the clinical symptoms reported by inoculated subjects and virus recovery were compared to the data presented by Carrat et al.⁴⁰ These findings are consistent with those reported by Carrat et al. showing that the titre of influenza virus peaked on day 2 in the nasal washing of infected subjects (placebo/untreated groups), although in one study the peak was on day 3.^{35,40} The peak in symptom score followed a day after the peak in virus recovery on day 3. In the present review, it was found that the incidence of fever was not documented in all of the papers. The incidence of fever in the A/H3N2 challenge studies ranged from 24% to 61% in the control groups; in the A/H1N1 challenge studies, this ranged from 0 to 88% and in the B virus challenge study from 0 to 45%.^{17,25,30} There is agreement with the statement by Carrat et al. that the incidence of fever with influenza A is more common and frequent when compared to influenza B.⁴⁰ In terms of infection

rates, i.e., clinical illness with laboratory-confirmed infection, variation was found in infection rates, from 26% to 100%. One hundred percent infection rates were achieved in the placebo group with each strain of influenza virus H3N2, A/H1N1, and B.^{31,32,34,38} In general, the clinical symptoms observed in the present review were mostly confined to the upper respiratory tract and were less severe than would be observed in a naturally acquired influenza infection.³⁰ Also in agreement with Carrat et al., it was found that the majority of studies used A/H1N1 influenza virus strains (45%) rather than A/H3N2 (32%) influenza strains.⁴⁰ The variation in infection rates is likely due to studies using different influenza strains and doses, different challenge lots, varying handling and monitoring procedures, and different assaying methods, as well as the quality of the assays used – both the screening assays and assays such as the titration assay for determining the inoculum titre. In addition there are other immune parameters, for example pre-existing influenza-specific CD4 T-cell levels, which may contribute to protection against influenza infection and are not part of the screening process.⁴¹

As the challenged but untreated control groups had substantial infection rates and often also showed clinical symptoms and signs of mild disease, CHI studies with influenza virus provide a good platform for studying prophylactic and therapeutic measures for influenza. The first pivotal study that showed the usefulness of the challenge platform in evaluating antiviral efficacy was performed by Jackson et al. in 1963.¹⁵ That study demonstrated the antiviral effect of amantadine for the first time using experimental infection in volunteers with an attenuated influenza A virus strain. Jackson et al. determined that by administering amantadine, the clinical symptoms and viral shedding were reduced.¹⁵ They also showed that there was a 46% protective efficacy against infection, as determined by a rise in antibody levels in seronegative individuals.¹⁵ These findings led to amantadine being studied in further CHI studies and field studies, which supported the data demonstrated by Jackson et al. in their challenge studies.^{6,15,42–45} Smorodintseff et al. showed that amantadine was able to prevent infection in 51% of infected volunteers when given prophylactically and that those who did develop influenza had a mild form.⁶

When tested using the human challenge platform, rimantadine, an analogue of amantadine, was found to reduce the proportion and severity of Asian influenza.⁴⁶ Subsequently, both amantadine and rimantadine were approved for use as treatment against influenza by the FDA (1966 and 1994, respectively). Whilst efficacy was confirmed in follow-up CHI studies utilizing drug-susceptible virus,^{26,28,47} there is now widespread resistance among circulating seasonal influenza A strains, such that neither is recommended for routine use anymore.⁴⁸

Based in part on the data generated by challenge studies over the past decades, several other antivirals, such as zanamivir, oseltamivir, and peramivir, progressed into further clinical testing, which ultimately resulted in FDA approvals.^{16,20,24,25,29–31,34} Oseltamivir has become the drug of choice in a potential pandemic situation and has been stockpiled by countries such as the UK and the USA in amounts costing 0.5 billion USD and 1.5 billion USD, respectively.⁴⁹ Resistance to antivirals is increasingly a problem.⁵⁰ Gubareva et al.,⁵⁰ in a follow on study from a study by Hayden et al.,²⁰ used the nasal wash samples from the last day of shedding from all infected individuals (treated with oseltamivir and placebo) to determine the frequency of resistance to oseltamivir in challenged subjects with increasing concentrations of oseltamivir due to mutations arising in the N-acetylneuraminic acid receptor binding site.^{20,50} The study found an incidence of 4% neuraminidase resistance as determined by the neuraminidase inhibition assay.

Five other antiviral trials evaluated four additional antivirals: ICI 130,685 (a cyclo-nonane similar to amantadine), an adamantane

compound, interferon (IFN) $\alpha 2$, and a monoclonal antibody (TCN-032). ICI 130,685 was tested for prophylaxis and treatment against influenza A/England/40/83 (H3N2), the adamantane compound was tested for prophylactic activity only against A/Hong Kong/68 (H3N2), IFN was tested for prophylactic activity against A/California/78 and B/Hannover/1/70, and TCN-032 was evaluated for its therapeutic activity against A/Wisconsin/67/2005.^{17,21,23,51,52} These showed variable efficacy in reducing viral replication and symptoms of influenza-like illness. No evidence could be found that two of these compounds (the ICI 130,685 antiviral and the adamantane compound) were developed further after these papers were published. Treanor et al. demonstrated that IFN- $\alpha 2$ reduced clinical symptoms and virus shedding in volunteers challenged with influenza A/California/78 (A/H1N1).⁵² Merigan et al. found that prophylaxis with IFN conferred no protection against influenza B/Hannover/1/80 infection.¹⁷ Contrasting data for the efficacy of IFN against influenza has, however, been found in field studies where IFN- $\alpha 2$ was found to have no clinical benefit in those self-administering.^{18,53} The antiviral TCN-032 was found to reduce the proportion of grade ≥ 2 influenza symptoms to 34.5% in the treated group compared to 48% in the placebo control group.²³

Of the RCTs investigating the efficacy of vaccines, four vaccines showed efficacy: a live attenuated vaccine, a recombinant or recombinant protein vaccine, and a DNA vaccine.^{14,22,32,36–38} These RCTs reported efficacy against challenge with influenza virus either as efficacy on reducing the incidence of infection or reducing the severity of clinical symptoms (Table 1). The live attenuated vaccine tested by Clements et al. was subsequently approved by the FDA.¹⁴

Three of the RCTs showed that the test antivirals or vaccine were ineffective at reducing laboratory-confirmed infection or clinical symptoms when compared to the control or comparator control, and hence these antivirals were not brought forward for further development, thus saving resources and time.^{27,33,35} A good example of this is the oral antiviral LY217896 tested for its efficacy in volunteers challenged with A/Kawasaki/86 (A/H1N1). This trial showed no difference in clinical symptoms or virus shedding between the LY217896 (1,3,4-thiadiazol-2-ylcyanamide) treated group and the placebo group, demonstrating it to be an ineffective antiviral against influenza.³³

This review also provided information on the strain and inoculum dose of the challenge virus and the attack rates these challenge viruses achieved in healthy volunteers. This information is important for investigators designing new human challenge studies. Currently, the regulatory requirements for the virus inoculum, although different in different countries, require that the influenza virus inoculum be manufactured under GMP.³ This can be a costly and time-consuming procedure; however, it ensures that the virus inoculum is sterile, pure, and potent and elicits the expected symptoms (FDA, 21 CFR 600.3 sections (p), (r), (s)).⁵⁴

The priority of any clinical trial is the safety of the subjects and study personnel involved. In challenge studies, this involves another layer of safety procedures relative to clinical trials of influenza-infected outpatients in addition to ensuring that GMP inoculum is used: the isolation of inoculated subjects is required, along with appropriate infection control procedures, vaccination of staff (where appropriate), and the use of personal protective equipment.¹⁸ Human challenge studies have come under scrutiny for ethical reasons and this has been the subject of a number of reviews.^{55–58} The two regulatory bodies (European Medicines Agency and the FDA) of the two countries where the majority of human challenge studies are currently performed (USA and UK) have the same stance as Lynch.⁵⁷ Their conclusion is that

“exposure to toxicity versus infection is not itself a morally relevant difference” and so there should not be, in principle, an objection to human challenge studies. Lynch stated that the risks to the safety of participants in these studies should not be more than is acceptable in other forms of research.⁵⁷ Ultimately influenza challenge studies have a good safety record, as found in the present review, due to the strict inclusion/exclusion criteria of subjects enrolled, the use of an attenuated influenza virus challenge strain with lower pathogenicity than a naturally circulating strain, and strict regulatory and ethical guidelines in place.⁵⁵

This review found that while the majority of studies did report some mild AEs, no medical emergencies or AEs surfaced that required urgent medical intervention by emergency services. Furthermore, all AEs were transient. Foremost, no fatalities related to any influenza virus challenge studies have ever been reported. Only one SAE was reported in the 2462 subjects challenged in these studies (0.04%); this one subject in the USA developed a dilated cardiomyopathy following infection with an influenza B challenge virus.²⁵ Although myocarditis after natural influenza B infection is recognized rarely, the cause in this case was thought unlikely to be due to the challenge virus.⁵⁹ This incident did result in human challenge studies being discontinued in the USA for a number of years until they were restarted with the dose-ranging study for GMP influenza A (H1N1)pdm09 by Memoli et al. in 2015.⁶⁰

Various shortcomings were found in the reporting of the trials, as evidenced by an overall low Jadad score. Few studies reported the methods of randomization, methods of blinding, or reasons for withdrawal/loss-to-follow-up. It is agreed with Kalil et al. that the reporting of the study design or set-up of CHI studies is not consistent or comprehensive enough for future researchers to be able to replicate the study conditions.⁶¹ As many as 48% of the trials in this review did not document whether and how AEs were recorded. Only two studies outlined the administration of oseltamivir to all challenged subjects prior to their subsequent release from quarantine to prevent the spread of virus to the community.^{22,23} Kalil et al. reviewed 181 papers on human challenge studies and found that only 41% reported the source of the challenge agent.⁶¹ In contrast, in the present review it was found that 73% reported the source of the influenza virus inoculum. The findings of this systematic review underline the need for better reporting of human challenge studies and a set of guidelines specifically tailored for human challenge studies to ensure consistency.

Human challenge studies have significant limitations. One of the main limitations is the recruitment of volunteers who have variable immunity to the influenza strains utilized. While subjects are usually selected on the basis of sero-susceptibility with low or non-detectable antibodies to the challenge organism, specific pre-existing cellular immune responses can also affect outcomes in experimentally inoculated subjects.⁴¹ Another is that the pathogenesis of illness differs from natural influenza, including key sites of viral replication, such that the routes of drug delivery cannot be extrapolated to field conditions. For example, intranasal recombinant IFN or zanamivir were protective in experimentally infected subjects but failed to prevent influenza illness and infection under field conditions.^{29,31,52,53,62} In contrast, orally inhaled zanamivir was shown to be an effective prophylactic and therapeutic agent in naturally occurring influenza and was approved for these indications.^{63,64} Hence, challenge studies cannot replace efficacy trials.

In summary, these findings highlight the importance of the CHI model to down-select potentially effective influenza vaccines and antivirals in a timely and cost-effective manner, with small sample sizes.²⁴ This systematic review also confirms that the incidence of SAEs in relation to the challenge virus is extremely low. Challenge

studies can be safe, ethical, extremely informative, and an efficient use of resources during the clinical development of vaccines or therapeutics, but they cannot replace phase 3 trials.

Acknowledgement

We thank Professor Frederick Hayden, University of Virginia School of Medicine, Charlottesville, Virginia for his helpful editorial comments.

Funding: Funding by Prof. Annelies Wilder-Smith's Start Up Grant under the Lee Kong Chian School of Medicine.

Conflict of interest: None.

References

- Pollard AJ, Savulescu J, Oxford J, Hill AV, Levine MM, Lewis DJ, et al. Human microbial challenge: the ultimate animal model. *Lancet Infect Dis* 2012;**12**:903–5. [http://dx.doi.org/10.1016/S1473-3099\(12\)70292-X](http://dx.doi.org/10.1016/S1473-3099(12)70292-X)
- Riedel S. Edward Jenner and the history of smallpox and vaccination. *Proc (Bayl Univ Med Cent)* 2005;**18**:21–5.
- Balasingam S, Horby P, Wilder-Smith A. The potential for a controlled human infection platform in Singapore. *Singapore Med J* 2014;**55**:456–61.
- Belser JA, Katz JM, Tumpey TM. The ferret as a model organism to study influenza A virus infection. *Dis Model Mech* 2011;**4**:575–9. <http://dx.doi.org/10.1242/dmm.007823>
- Thangavel RR, Bouvier NM. Animal models for influenza virus pathogenesis, transmission, and immunology. *J Immunol Methods* 2014;**410**:60–79. <http://dx.doi.org/10.1016/j.jim.2014.03.023>
- Smorodintseff AA, Tushinsky MD, Drobyshevskaya I, Karolin AA, Osetroff AI. Investigation of volunteers infected with the influenza virus. *Am J Med Sci* 1937;**194**:159–70.
- Treanor JJ. Volunteer challenge studies. In: Nicolson KG WR, Hay AJ, editors. *Textbook of influenza*. Oxford: Blackwell Science; 1998, p. 517–37.
- Francis T, Salk JE, Pearson HE, Brown PN. Protective effect of vaccination against induced influenza A. *J Clin Invest* 1945;**24**:536–46. <http://dx.doi.org/10.1172/jci101633>
- Salk JE, Pearson HE, Brown PN, Francis T. Protective effect of vaccination against induced influenza B. *J Clin Invest* 1945;**24**:547–53. <http://dx.doi.org/10.1172/jci101634>
- Henle W, Henle G, Stokes Jr J. Demonstration of the efficacy of vaccination against influenza type A by experimental infection of human beings. *J Immunol* 1943;**46**:163–75.
- Burnet FM, Foley M. The results of intranasal inoculation of modified and unmodified influenza virus strains in human volunteers. *Med J Aust* 1940;**2**: 655–9.
- Burnet FM. Influenza virus B: II. Immunization of human volunteers with living attenuated virus. *Med J Aust* 1942;**1**:673–4.
- Burnet FM. Immunization against epidemic influenza with living and attenuated virus. *Med J Aust* 1943;**1**:385–9.
- Clements ML, Betts RF, Murphy BR. Advantage of live attenuated cold-adapted influenza A virus over inactivated vaccine for A/Washington/80 (H3N2) wild-type virus infection. *Lancet* 1984;**1**:705–8.
- Jackson GG, Muldoon RL, Akers LW. Serological evidence for prevention of influenza infection in volunteers by an anti-influenzal drug adamantanamine hydrochloride. *Antimicrob Agents Chemother* 1963;**161**:703–7.
- Hayden FG, Zylidnikov DM, Ilijenko VI, Padolka YV. Comparative therapeutic effect of aerosolized and oral rimantadine HCl in experimental human influenza A virus infection. *Antiviral Res* 1982;**2**:147–53.
- Merigan TC, Reed SE, Hall TS, Tyrrell DA. Inhibition of respiratory virus infection by locally applied interferon. *Lancet* 1973;**1**:563–7.
- Hayden FG. Experimental human influenza: observations from studies of influenza antivirals. *Antivir Ther* 2012;**17**(1 Pt B):133–41. <http://dx.doi.org/10.3851/IMP2062>
- Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996;**17**:1–12.
- Hayden FG, Treanor JJ, Fritz RS, Lobo M, Betts RF, Miller M, et al. Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza: randomized controlled trials for prevention and treatment. *JAMA* 1999;**282**: 1240–6.
- Al-Nakib W, Higgins PG, Willman J, Tyrrell DA, Swallow DL, Hurst BC, et al. Prevention and treatment of experimental influenza A virus infection in volunteers with a new antiviral ICI 130,685. *J Antimicrob Chemother* 1986;**18**:119–29.
- Jones S, Evans K, McElwaine-John H, Sharpe M, Oxford J, Lambkin-Williams R, et al. DNA vaccination protects against an influenza challenge in a double-blind randomised placebo-controlled phase 1b clinical trial. *Vaccine* 2009;**27**:2506–12. <http://dx.doi.org/10.1016/j.vaccine.2009.02.061>
- Ramos EL, Mitcham JL, Koller TD, Bonavia A, Usner DW, Balaratnam G, et al. Efficacy and safety of treatment with an anti-m2e monoclonal antibody in experimental human influenza. *J Infect Dis* 2015;**211**:1038–44. <http://dx.doi.org/10.1093/infdis/jiu539>

24. Fritz RS, Hayden FG, Calfee DP, Cass LM, Peng AW, Alvorð WC, et al. Nasal cytokine and chemokine responses in experimental influenza A virus infection: results of a placebo-controlled trial of intravenous zanamivir treatment. *J Infect Dis* 1999;**180**:586–93. <http://dx.doi.org/10.1086/314938>
25. Barroso L, Treanor J, Gubareva L, Hayden FG. Efficacy and tolerability of the oral neuraminidase inhibitor peramivir in experimental human influenza: randomized, controlled trials for prophylaxis and treatment. *Antivir Ther* 2005;**10**:901–10.
26. Reuman PD, Bernstein DI, Keefer MC, Young EC, Sherwood JR, Schiff GM. Efficacy and safety of low dosage amantadine hydrochloride as prophylaxis for influenza A. *Antiviral Res* 1989;**11**:27–40.
27. Treanor JJ, Kotloff K, Betts RF, Belshe R, Newman F, Iacuzio D, et al. Evaluation of trivalent, live, cold-adapted (CAIV-T) and inactivated (TIV) influenza vaccines in prevention of virus infection and illness following challenge of adults with wild-type influenza A (H1N1), A (H3N2), and B viruses. *Vaccine* 1999;**18**:899–906.
28. Doyle WJ, Skoner DP, Alper CM, Allen G, Moody SA, Seroky JT, et al. Effect of rimantadine treatment on clinical manifestations and otologic complications in adults experimentally infected with influenza A (H1N1) virus. *J Infect Dis* 1998;**177**:1260–5.
29. Hayden FG, Treanor JJ, Betts RF, Lobo M, Esinhart JD, Hussey EK. Safety and efficacy of the neuraminidase inhibitor GG167 in experimental human influenza. *JAMA* 1996;**275**:295–9.
30. Hayden FG, Jennings L, Robson R, Schiff G, Jackson H, Rana B, et al. Oral oseltamivir in human experimental influenza B infection. *Antivir Ther* 2000;**5**:205–13.
31. Calfee DP, Peng AW, Cass LM, Lobo M, Hayden FG. Safety and efficacy of intravenous zanamivir in preventing experimental human influenza A virus infection. *Antimicrob Agents Chemother* 1999;**43**:1616–20.
32. Betts RF, Douglas Jr RG, Roth FK, Little 3rd JW. Efficacy of live attenuated influenza A/Scotland/74 (H3N2) virus vaccine against challenge with influenza A/Victoria/3/75 (H3N2) virus. *J Infect Dis* 1977;**136**:746–53.
33. Hayden FG, Tunkel AR, Treanor JJ, Betts RF, Allerheiligen S, Harris J. Oral LY217896 (1,3,4-thiadiazol-2-ylcyanamide) for prevention of experimental influenza A virus infection and illness in humans. *Antimicrob Agents Chemother* 1994;**38**:1178–81.
34. Walker JB, Hussey EK, Treanor JJ, Montalvo Jr A, Hayden FG. Effects of the neuraminidase inhibitor zanamivir on otologic manifestations of experimental human influenza. *J Infect Dis* 1997;**176**:1417–22.
35. Douglas Jr RG, Betts RF, Simons RL, Hogan PW, Roth FK. Evaluation of a topical interferon inducer in experimental influenza infection in volunteers. *Antimicrob Agents Chemother* 1975;**8**:684–7.
36. Fries LF, Dillon SB, Hildreth JE, Karron RA, Funkhouser AW, Friedman CJ, et al. Safety and immunogenicity of a recombinant protein influenza A vaccine in adult human volunteers and protective efficacy against wild-type H1N1 virus challenge. *J Infect Dis* 1993;**167**:593–601.
37. Youngner JS, Treanor JJ, Betts RF, Whitaker-Dowling P. Effect of simultaneous administration of cold-adapted and wild-type influenza A viruses on experimental wild-type influenza infection in humans. *J Clin Microbiol* 1994;**32**:750–4.
38. Keitel WA, Couch RB, Cate TR, Six HR, Baxter BD. Cold recombinant influenza B/Texas/1/84 vaccine virus (CRB87): attenuation, immunogenicity and efficacy and homotypic challenge. *J Infect Dis* 1990;**161**:22–6.
39. Ison MG, Campbell V, Rembold C, Dent J, Hayden FG. Cardiac findings during uncomplicated acute influenza in ambulatory adults. *Clin Infect Dis* 2005;**40**:415–22. <http://dx.doi.org/10.1086/427282>
40. Carrat F, Vergu E, Ferguson NM, Lemaître M, Cauchemez S, Leach S, et al. Time lines of infection and disease in human influenza: a review of volunteer challenge studies. *Am J Epidemiol* 2008;**167**:775–85. <http://dx.doi.org/10.1093/aje/kwm375>
41. Wilkinson TM, Li CK, Chui CS, Huang AK, Perkins M, Liebner JC, et al. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. *Nat Med* 2012;**18**:274–80. <http://dx.doi.org/10.1038/nm.2612>
42. Halonen P, Mujunen R, Mantylarvi R. On the preventive effect of amantadine hydrochloride in influenza and other respiratory viral infections. *Sotilaslaak Aikak* 1965;**40**:130–7.
43. Stanley ED, Muldoon RE, Akers LW, Jackson GG. Evaluation of antiviral drugs: the effect of amantadine on influenza in volunteers. *Ann N Y Acad Sci* 1965;**130**:44–51.
44. Lee SH, Dobson PR, van Rooyen CE. Antiviral substances: 6-aminonicotinamide and 1-adamantanamine hydrochloride. *Chemotherapy* 1966;**11**:163–77.
45. Oker-Blom N, Hovi T, Leinikki P, Palosuo T, Pettersson R, Suni J. Protection of man from natural infection with influenza A2 Hong Kong virus by amantadine: a controlled field trial. *Br Med J* 1970;**3**:676–8.
46. Dawkins Jr AT, Gallager LR, Togo Y, Hornick RB, Harris BA. Studies on induced influenza in man: II. double-blind study designed to assess the prophylactic efficacy of an analogue of amantadine hydrochloride. *JAMA* 1968;**203**:1095–9.
47. Skoner DP, Gentile DA, Patel A, Doyle WJ. Evidence for cytokine mediation of disease expression in adults experimentally infected with influenza A virus. *J Infect Dis* 1999;**180**:10–4. <http://dx.doi.org/10.1086/314823>
48. Li F, Ma C, Wang J. Inhibitors targeting the influenza virus hemagglutinin. *Curr Med Chem* 2015;**22**:1361–82.
49. Van Noorden R. Flu Drug Stockpiling Reported to Be a Waste of Money. <http://www.scientificamerican.com/article/flu-drug-stockpiling-reported-to-be-a-waste-of-money/>. Nature Pharmaceuticals. April 12 2014.
50. Gubareva LV, Kaiser L, Matrosovich MN, Soo-Hoo Y, Hayden FG. Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. *J Infect Dis* 2001;**183**:523–31. <http://dx.doi.org/10.1086/318537>
51. Beare AS, Hall TS, Tyrrell DA. Protection of volunteers against challenge with A-Hong Kong-68 influenza virus by a new adamantane compound. *Lancet* 1972;**1**:1039–40.
52. 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**:379–83.
53. Tannock GA, Gillett SM, Gillett RS, Barry RD, Hensley MJ, Herd R, et al. A study of intranasally administered interferon A (rIFN-alpha 2A) for the seasonal prophylaxis of natural viral infections of the upper respiratory tract in healthy volunteers. *Epidemiol Infect* 1988;**101**:611–21.
54. Title 21—Food and drugs, Chapter I—Food and Drug Administration, Department Of Health And Human Services, Subchapter F—Biologics 21CFR600.3. FDA 2015. Available at: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=600.3>. (accessed March 29, 2015).
55. Darton TC, Blohmke CJ, Moorthy VS, Altmann DM, Hayden FG, Clutterbuck EA, et al. Design, recruitment, and microbiological considerations in human challenge studies. *Lancet Infect Dis* 2015;**15**:840–51. <http://dx.doi.org/10.1016/S1473-309900068-7>
56. Miller FG, Grady C. The ethical challenge of infection-inducing challenge experiments. *Clin Infect Dis* 2001;**33**:1028–33. <http://dx.doi.org/10.1086/322664>
57. Lynch HF. The rights and wrongs of intentional exposure research: contextualising the Guatemala STD inoculation study. *J Med Ethics* 2012;**38**:513–5. <http://dx.doi.org/10.1136/medethics-2011-100379>
58. Hope T, McMillan J. Challenge studies of human volunteers: ethical issues. *J Med Ethics* 2004;**30**:110–6.
59. Chang HL, Hsu JF, Tsai YM, Lin SY, Kuo HF, Yang CJ. Acute respiratory distress syndrome and acute myocarditis developed in a previously healthy adult with influenza B. *BMC Pulm Med* 2016;**16**:1. <http://dx.doi.org/10.1186/s12890-015-0163-3>
60. Memoli MJ, Czajkowski L, Reed S, Athota R, Bristol T, Proudfoot K, et al. Validation of the wild-type influenza A human challenge model H1N1pdMIST: an A(H1N1)pdm09 dose-finding investigational new drug study. *Clin Infect Dis* 2015;**60**:693–702. <http://dx.doi.org/10.1093/cid/ciu924>
61. Kalil JA, Halperin SA, Langley JM. Human challenge studies: a review of adequacy of reporting methods and results. *Future Microbiol* 2012;**7**:481–95. <http://dx.doi.org/10.2217/fmb.12.15>
62. Phillipotts RJ, Higgins PG, Tillman JS, Tyrrell DA, Freestone DS, Shepherd WM. Intranasal lymphoblastoid interferon ('Wellferon') prophylaxis against rhinovirus and influenza virus in volunteers. *J Interferon Res* 1984;**4**:535–41.
63. Kaiser L, Henry D, Flack NP, Keene O, Hayden FG. Short-term treatment with zanamivir to prevent influenza: results of a placebo-controlled study. *Clin Infect Dis* 2000;**30**:587–9. <http://dx.doi.org/10.1086/313696>
64. Monto AS, Robinson DP, Herlocher ML, Hinson Jr JM, Elliott MJ, Crisp A. Zanamivir in the prevention of influenza among healthy adults: a randomized controlled trial. *JAMA* 1999;**282**:31–5.