This text is carried over from the 2012 version of this review and is provided for record completeness

Methods

The hypotheses (expressed as null hypotheses) are listed below, in order of their generation (not necessarily of importance). Their rationale is explained further down the text.

Hypothesis 1. Incidence of certain harms is not associated with placebo content.
Hypothesis 2. Oseltamivir (or zanamivir) does not affect antibody production in treatment trials.
Hypothesis 3. Oseltamivir does not affect antibody production in post-exposure (or secondary prophylaxis) trials.
Hypothesis 4. The number of trial centres and centre withdrawals does not affect the proportion of placebo patients subsequently diagnosed with influenza infection (originally the outcome was effect size).
Hypothesis 5. In oseltamivir treatment trials there is no association between the order of randomisations and naso-pharyngeal swabbing (i.e. randomising participants first and then swabbing or swabbing first and then randomising) and the proportion of placebo patients subsequently diagnosed with influenza infection.

Hypothesis 1. Incidence of certain harms is not associated with placebo content.
Rationale. While reviewing the US Food and Drug Administration (FDA) critique of zanamivir, we noted the regulators' concern over the apparent drop in forced expiratory volume (FEV) following zanamivir inhalation (FDA 1999a), which appeared to be enhanced by the lactose powder excipient content of the active blister (FDA 1999b). The powder, which causes bronchospasm in susceptible individuals, was contained in both the active and the placebo blisters. This principle of using a matching placebo is of course correct but may have had the effect of increasing the incidence of bronchospasm (or asthma-related episodes) in both arms. This is clearly reported as a warning in the 1999 FDA label "Because the placebo consisted of inhaled lactose powder, which is also the vehicle for the active drug, some adverse events occurring at similar frequencies in different treatment groups could be related to lactose vehicle inhalation" (FDA 2000b p.10).

We reasoned by analogy and reviewed the medication content of the available clinical study reports of oseltamivir trials. The detailed information comparing content and physical characteristics and batch numbers is in Table 11. Roche's use of the word 'matching' is not strictly correct as two principles present in the placebo capsules (dehydrocholic acid and dibasic calcium phosphate dihydrate) are not listed as being present in the active oseltamivir capsules. We could not locate the reason for such a choice in the clinical study reports but both substances may have gastrointestinal action if consumed in large enough quantities.

On this basis we formulated two **hypotheses**:
1a. There is no association between incidence of gastrointestinal harms and a placebo containing dehydrocholic acid in oseltamivir trials.
1b. There is no association between incidence of asthma-related events and a placebo containing lactose powder in zanamivir trials.

To test hypothesis 1a we assessed the oseltamivir trials for which we had clinical study reports Module 1 (M76001; WV15670; WV15671; WV15707; WV15812/WV15872; WV15730; WV15819/WV15876/WV15978; WV15758; WV15799) for gastrointestinal tract (GIT) harms including nausea, vomiting and diarrhoea as well as participants withdrawing from the studies due to adverse events. We meta-analysed the results from these studies using the inverse variance random-effects method. We assessed heterogeneity using the Chi^2 test and used Tau^2 to estimate between-study variance. To investigate whether placebo containing dehydrocholic acid may be associated with gastrointestinal harms we compared adverse event rates in placebo groups from the oseltamivir trials (where placebo contained dehydrocholic acid) with adverse event rates in the placebo groups from the zanamivir trials (where placebo did not contain dehydrocholic acid). This comparison was done informally using 1) data obtained from the FDA labels of oseltamivir and zanamivir (FDA 2000b; FDA 2011a) as well as 2) the trials for which we have clinical study reports. As a sensitivity analysis we assumed a similar gastrointestinal adverse event rate in the placebo groups of the oseltamivir trials as was observed in the placebo groups of the zanamivir trials and then repeated the meta-analysis (as described above). We also speculated that withdrawals in the placebo groups due to gastrointestinal adverse events were possibly related to dehydrocholic acid and removed these for the sensitivity analysis.

For hypothesis 1b we assessed asthma-related events in nine zanamivir trials for which we had clinical study reports (NAIA3002; NAIB3002; NAIA2005; NAIB2005; NAIB2007; NAIB3001; NAIA3005; NAI30010; NAI30009). We meta-analysed the results from these studies using the inverse variance random-effects method. We assessed heterogeneity using the Chi^2 test and used Tau^2 to estimate between-study variance. To investigate whether placebo containing lactose powder may be associated with asthma-related events we informally compared event rates in placebo groups from the zanamivir trials (where placebo contained lactose powder) with event rates in the placebo groups from the oseltamivir trials (where placebo did not contain lactose powder). As a sensitivity analysis we assumed a similar asthma-related event rate in the placebo groups of the zanamivir trials as was observed in the placebo groups of the oseltamivir trials and then repeated the meta-analysis (as described above).

Hypothesis 2. Oseltamivir (or zanamivir) does not affect antibody production in treatment trials.
Rationale. All oseltamivir influenza treatment trials specify the primary efficacy analysis population as the influenza-infected population, not the randomised intention-to-treat (ITT) base population. The influenza-infected population (known as ITTI, or intention-to-treat-infected in clinical study reports) is determined post-randomisation based on the results of laboratory testing by culture and/or antibody rise (comparing paired sera from the same participant). The sample for culture and the first sample of sera are taken before commencement of trial product but the second or the third sera are taken after patients are treated with trial medication. It is vital that placebo and active groups of patients have the same odds of being classified as influenza-infected, otherwise any comparison between influenza-infected groups will be potentially affected by bias and will essentially be a non-randomised comparison. If trial medication affects the production of antibodies, the selection of the influenza-

infected population (which is partly based on antibody production) is confounded by taking the trial medication.

Roche have stated on multiple occasions (Smith 2006; Ward 2005; section 3.2.4.2 Serology WV15799) that ingestion of oseltamivir does not affect antibody production and the FDA supports this, stating that "In studies of naturally acquired and experimental influenza, treatment with TAMIFLU did not impair normal humoral antibody response to infection" (FDA 2011a).

However, we noticed unequal numbers of individuals in the influenza-infected population subgroup in numerous trials. In addition, Takahashi et al reported that oseltamivir significantly suppressed respiratory mucosal secretory immunoglobulin (Ig) A responses to antigen (Ag)-specific antibody (Ab) production and also the induction of Ag-specific IgA Ab-forming cells in an animal experiment (Takahashi 2010). If taking oseltamivir affects the production of IgG antibody as well, it may affect the selection of the influenza-infected population.

We are also unsure of the implication for immunisation with influenza vaccine. According to the FDA, no influenza vaccine interaction study has been conducted with oseltamivir (FDA 2011a).

To test the hypothesis we compared: (1) the odds of participants in the ITT population subsequently classified as influenza-infected; and (2) the odds of participants in the ITT population with a four-fold or more rise of antibody between the placebo and active arms of the trials. If ingestion of oseltamivir does not affect antibody production then we expect the odds of being classified as influenza-infected to be the same for the placebo and active arms. Therefore, we tested a null hypothesis that the odds of having a four-fold or more rise of antibody was the same for the placebo and active arms. We meta-analysed the results from these studies using the inverse variance random-effects method. We assessed heterogeneity using the Chi^2 test and used Tau^2 to estimate between-study variance. The trials included in this analysis were the 10 oseltamivir treatment trials analysed by Kaiser 2003 plus WV15758 for oseltamivir and NAIA3002, NAIB3002, NAIA2005, NAIB2005, NAIB2007, NAIB3001, NAI30009 for zanamivir. These are all the treatment trials for which we have clinical study reports Module 1. In an additional analysis we also assessed the oseltamivir trial conducted in China by Shanghai Roche Pharmaceutical Ltd for which we have a partial clinical study report (ML16369).

Hypothesis 3. Oseltamivir does not affect antibody production in post-exposure (or secondary prophylaxis) trials.
Rationale. According to the clinical study report of WV15799, the trial programme assessing the effects of oseltamivir in post-exposure prophylaxis (PEP) consisted of two trials: WV15799 and WV16139. The Module 1s of both trials together with copious FDA notes on trial WV15799 were available to us at 'time lock'. However the PEP trial WV16139 was not standard care or placebo-controlled and so we excluded it from the review.

WV15799 was a double-blind, cluster-randomised trial in which contact clusters of index cases were randomised to oseltamivir 75 mg a day or placebo for seven days. The trial formed an integral part of the "pivotal" trials package for the supplementary

application and review for prophylaxis use of oseltamivir 75 mg in people aged more than 13 years of age, submitted to the FDA on 22 May 2000, approved on 20 November 2000 (FDA 2000c). In the clinical study report Module 1 the manufacturer claimed that the trial provided evidence of the drug's capacity to prevent influenza in contacts by interrupting its transmission from index cases. Since all index cases were left untreated except for a paracetamol rescue pack, it is hard to see how such a claim can be made. The interruption of transmission claim has two components: reduction of viral spread from index cases (measured by nasal shedding of influenza viruses) and prevention of onset of influenza in contacts. This latter claim was based on the definition of (prevented) influenza cases: a mixture of symptoms signs and 'laboratory confirmation' (i.e. viral culture from the upper airways and/or at least a four-fold rise in antibody titres measured between baseline and two to three weeks later). The results of the trial later formed the basis for claims of the drug's effectiveness in interrupting transmission from person to person (WHO 2007) and allow time before the arrival of vaccines in the event of a pandemic. The interruption of transmission claim provided a powerful rationale for stockpiling oseltamivir (see for example vol 8, p.61-62 NICE 2000: "Ro 64-0796 successfully interrupts the transmission of influenza within households ... and suggests that Ro 64-0796 [oseltamivir] would control the spread of influenza in other closed communities associated with high risk of transmission, such as nursing homes" … "Ro 64-0796 also effectively interrupted virus transmission within households.")

The interruption of transmission indication was accepted by agencies such as the World Health Organization (WHO) and the US Centers for Disease Control and Prevention (CDC), but the US FDA refused to register and allow publicity based on any further indication beyond treatment and prophylactic effects on symptoms (FDA 2000f). Review of the evidence from the study protocol and Module 1 together with the FDA criticism explains the rationale for the FDA not supporting the manufacturers' claims. The design of the trial did not allow for comparison of the effects of treating index cases with oseltamivir versus placebo (as all index cases were not medicated) and a repeat viral culture was not performed for all participants. Viral culture was performed at baseline for all participants and thereafter only in participants with influenza-like illness symptoms (see Schedule of assessment for the contact case, WV15799, and the FDA Medical Officer report (FDA 1999c)). Any participants presenting at follow-up with symptoms of influenza had throat and nasal swabs taken in order to confirm the presence or absence of influenza infection (FDA 2000c), thereby missing out on potential asymptomatic infected people. However, a recent review of transmission studies has found no convincing evidence of spread from pre-symptomatic or asymptomatic subjects (Patrozou 2009), which might explain the FDA's caution in sanctioning any such claim for oseltamivir.

Our review of the clinical study report's Module 1 identified further problems with the conduct and reporting of the trial and discrepancies both within the clinical study reports and between the study and its protocol. In the protocol (version H) there is no mention of viral shedding measurement. This appears to be a post-protocol addition, which would explain the unsystematic nature of the viral excretion measurement remarked on by the FDA (i.e. taken from symptomatic contacts only). The primary population of analysis is the so called ITTIINAB population (contacts of ITT influenza-infected index cases who had negative virology at baseline). Although defined in the protocol, the selection and presentation of results for the intention-to-

treat contacts of the influenza-infected index case not infected at baseline (ITTIINAB) population has the effect of excluding 57% of the placebo (200/456) and 59% of the oseltamivir (205/497) participants. The effect of selection on the clustering was not formally tested in a sensitivity analysis. Nor is the potential weakness of such a choice discussed in the WV15799 clinical study report. We carried out an analysis using Fisher's exact test, which showed that there was no statistical evidence that the placebo and oseltamivir groups' cluster sizes were distributed differently based on households with an infected index case (P = 0.56) (Table 2). By analysing the population by influenza status of the index case, instead of unit of randomisations (all index cases), the beneficial effects of the cluster-randomisations are potentially lost, introducing unknown biases into the analysis. In addition, the generalisability of the conclusions may not be easily applied to clinical practice where testing of suspected influenza cases is often not practical. Cross-checking the definition of ITTIINAB with that reported in the protocol of the other PEP trial, WV16193 (excluded from this review) yields a different definition (PDF page 589) "The primary outcome in this study (WV15799) was the incidence of influenza occurring among contacts of influenza-infected index cases (the intent-to-treat-index-infected population)".

Throughout the clinical study report of trial WV15799 there are many other apparently contradictory statements on important aspects of the trial, for example, on how many viral swabs and paired sera tests were carried out. The text at page 50 of the Module 1 reports that "For 21 of the 26 contacts with laboratory-confirmed clinical influenza in the ITTIINAB population the diagnosis was confirmed by culture" but Table 19 shows the 26 contacts as shedding virus at days two to eight. The same table reports that 178 placebo contacts and 201 oseltamivir contacts were negative for virology (which suggests that they were tested) at days two and eight. However, viral testing only took place at baseline and thereafter only in symptomatic participants. The number of contacts in which influenza was diagnosed only by serology is unclear but it appears to be five (26 minus 21). These inconsistencies highlight one of the fundamental conceptual problems in understanding the whole oseltamivir prophylaxis trial programme: the mode of action of the drug. Our interpretation of the text suggests that oseltamivir does not prevent infection and does not affect influenza antibody response. As stated above, the claim that oseltamivir does not affect antibody responses has been made by the manufacturers. However, an antibody response is part of the definition of influenza. We are unsure how it is possible that oseltamivir could prevent influenza by stopping symptoms appearing and antibodies rising while at the same time leaving antibody production unaffected.

It is for this reason that we decided to test whether administration of oseltamivir for PEP affected the production of antibodies to influenza viruses. The distribution of change in antibodies from baseline to follow-up was compared between the arms of the trials for contacts of the index cases. Analysis was performed using Wilcoxon two-sample test separately for each type of antibody in each trial. An additional analysis of proportion of contacts having a four-fold or greater rise in influenza-specific antibody titre in antibodies was compared between groups using the Chi^2 test. Antibody data were not available for index cases, who were left untreated. In WV15799, antibody testing may have been undertaken at day 1, day 8 and at day 21 ± 4 days for all contacts. Day 8 blood samples for influenza antibody analysis were stored to measure influenza antibody levels only in those contacts who did not attend

the follow-up visit (day 17 to 25). Analysis was based on data from the ITTIINAB population at pages 59-60 and Appendix 60 of the clinical study report's Module 1.

Hypothesis 4. The number of trial centres and centre withdrawals does not affect the proportion of placebo patients subsequently diagnosed with influenza infection (originally the outcome was effect size) and **Hypothesis 5.** In oseltamivir treatment trials there is no association between the order of randomisations and naso-pharyngeal swabbing (i.e. randomising participants first and then swabbing or swabbing first and then randomising) and the proportion of placebo patients subsequently diagnosed with influenza infection (originally the outcome was effect size).
Rationale. The proportion of ITT population in the treatment trials of NIs that are subsequently diagnosed as infected with influenza is higher (~ 50% to 80%) than is usually seen in the course of the winter season in routine clinical care, although high peaks can occur for a very limited period. We know that in some treatment trials, such as WV15670 and WV15671, centres were activated to "recruit subjects during an influenza outbreak in the locality, detected using standardised surveillance techniques." We postulated that unreported procedures may also have been used in the trials to obtain these high proportions of influenza to ILI cases. Two procedures that may have been used are: 1) use of rapid influenza tests to screen out patients based on negative results; 2) dropping of centres that recruited low proportions of infected patients. The use of rapid testing of patients prior to randomisation has been reported in at least one of the zanamivir treatment trials (NAIB3001), in oseltamivir trial WV15670 as a means of excluding infection with H5N1 in the Hong Kong Centre, as a pilot surveillance in suburban London during the 1998 to 1999 winter (NICE 2000 vol.1) and in most oseltamivir paediatric trials to exclude respiratory syncytial virus (RSV) infection. In addition, the schedule of testing varies by trial for the oseltamivir trials with swabbing performed either before randomisation or after randomisation. In at least one oseltamivir treatment trial (WV15730) it was reported that no viral culture was performed at centres from South America (FDA 1999c). As a result of these observations we reformulated **Hypothesis 4** as follows: the number of centres and centre withdrawals does not affect the proportion of placebo patients subsequently diagnosed with influenza infection (originally the outcome was primary outcome effect size) in oseltamivir treatment trials and **Hypothesis 5** as in oseltamivir treatment trials there is no association between the order of randomisations and naso-pharyngeal swabbing (i.e. randomising participants first and then swabbing or swabbing first and then randomising) and the proportion of placebo patients subsequently diagnosed with influenza infection.

To test **hypothesis 4**, we used Spearman's rank method to estimate the correlation between average number of patients recruited per centre and the proportion of placebo patients subsequently diagnosed with influenza infection. The placebo patients were used for the proportion of patients subsequently diagnosed with influenza infection because, as we show later in the review, there is evidence that oseltamivir interferes with antibody production and antibody response was used to diagnose influenza infection. We did not analyse the number of centres dropped from studies because information on this variable was not available in Module 1s of the clinical study reports for the included trials (information on patients recruited to each centre is reported in Module 2 which we do not currently have access to).

Hypothesis 5 was generated to attempt to explain the seemingly high proportion of influenza-infected influenza-like illness cases in treatment trials. However, we did not formally test this hypothesis as there was only one clinical study report reporting randomisation first then swabbing second (WV15819/WV15876/WV15978) (see also Appendix 9).

Results

The results of our post-protocol analyses are also reported in Figure and/or Table format.

Hypothesis 1a tested in a sensitivity analysis whether the incidence of gastrointestinal harms may be associated with exposure of participants to a placebo containing dehydrocholic acid. The data obtained from the oseltamivir trials clinical study reports is shown in Table 15.

Overall, the crude adverse event incidence in the placebo groups of the oseltamivir trials was 5.5% for nausea, 3.6% for vomiting and 7.0% for diarrhoea. This compares with crude incidence in the nine zanamivir treatment trials' placebo groups of 4.1% for nausea and vomiting (reported as a combined outcome in the clinical study reports) and 2.8% for diarrhoea. Two studies (WV15670; WV15671) compared three treatment groups: oseltamivir 150 mg bid, oseltamivir 75 mg bid and placebo. To maintain the blinding in these trials, each participant took two pills twice daily. Therefore the participants in the oseltamivir 75 mg bid group took one placebo tablet twice daily. We note that in trial WV15671 there was evidence of a dose-response effect of placebo on incidence of diarrhoea: oseltamivir 150 mg bid (5.9%), oseltamivir 75 mg bid (8.7%) and placebo (11.8%) ($P = 0.036$). However, there was no evidence found of a similar trend in trial WV15670 ($P = 0.88$). We were unable to carry out a similar analysis for paediatric treatment trial WV15758 because a detailed content of the placebo preparations is not available (see Table 11).

Random-effects meta-analysis of the data in Table 15 provided the following results.

Nausea: increased odds of adverse events due to oseltamivir (OR 1.62, 95% CI 1.17 to 2.26, $P = 0.004$).
Vomiting: increased odds of adverse events due to oseltamivir (OR 2.32, 95% CI 1.62 to 3.31, $P < 0.001$).
Diarrhoea: decreased odds of adverse events due to oseltamivir (OR 0.72, 95% CI 0.53 to 0.97, $P = 0.03$).
Withdrawal from treatment due to adverse events: no evidence of a difference between treatment groups (OR 1.08, 95% CI 0.66 to 1.76, $P = 0.75$).

We carried out a sensitivity analysis by assuming placebo rates of gastrointestinal adverse events in oseltamivir trials based on those observed in placebo groups of similar zanamivir trials. Overall rates of nausea, vomiting and diarrhoea in placebo groups of zanamivir treatment trials for adults and adolescents were 3%, 2% and 4% compared to oseltamivir treatment trials for adults and adolescents where rates were 6%, 3% and 10% respectively based on FDA-reported data (FDA 2000b; FDA 2011a). Conversely, other common adverse events such as headaches, cough and dizziness had similar incidences of 2% to 3% in the placebo groups of zanamivir and

oseltamivir treatment trials (FDA 2000b; FDA 2011a). In the treatment trials of children the rates of nausea, vomiting and diarrhoea in placebo groups of zanamivir treatment trials were 2%, 3% and 2% compared to oseltamivir treatment trials of children where rates were 4%, 9% and 11% respectively. Our conservative estimate is that the oseltamivir placebo increased rates of nausea two-fold (risk ratio (RR) = 2), vomiting (RR 1.5) and diarrhoea (RR 2.5) compared to the placebo arms in zanamivir trials. Based on the adult and adolescent trials we could conservatively speculate that the substances in the oseltamivir trials placebo increase nausea, vomiting and diarrhoea by 100% (6%/3%), 50% (3%/2%) and 150% (10%/4%) respectively. This could also be considered a conservative assumption because it is plausible that the lactose powder used as the placebo in the zanamivir trials also induced gastrointestinal symptoms, especially in patients that were lactose intolerant. Adjusting the actual rates of these events in the oseltamivir trials placebo groups to be consistent with the zanamivir trials placebo group rates (as reported by the FDA (FDA 2000b; FDA 2011a) and re-running the random-effects meta-analysis we obtained the following results.

Nausea: increased odds of adverse events due to oseltamivir (OR 3.33, 95% CI 2.44 to 4.54, $P < 0.001$; test for heterogeneity $P = 0.33$).
Vomiting: increased odds of adverse events due to oseltamivir (OR 3.46, 95% CI 2.51 to 4.78, $P < 0.001$; test for heterogeneity $P = 0.37$).
Diarrhoea: increased odds of adverse events due to oseltamivir (OR 1.86, 95% CI 1.39 to 2.50, $P < 0.001$; test for heterogeneity $P = 0.50$).

The estimated effect sizes for nausea and vomiting have increased based on the sensitivity analysis. The effect on diarrhoea has reversed, indicating oseltamivir is possibly associated with increased odds of this adverse event. The results of our analysis support an alternative interpretation to that of the FDA.

Finally, we carried out a sensitivity analysis of withdrawal from treatment due to adverse events by assuming no withdrawals due to gastrointestinal events in the placebo group. In total there were nine patients in the oseltamivir trials' placebo groups that withdrew due to gastrointestinal events. When these withdrawals are not included the following result is obtained based on random-effects meta-analysis:

Withdrawal from treatment due to adverse events: no evidence of a difference between treatment groups (OR 1.48, 95% CI 0.87 to 2.51, $P = 0.15$; test for heterogeneity $P = 0.40$).

We conclude that participants in placebo arms of oseltamivir treatment trials experience a higher rate of gastrointestinal adverse events compared to their zanamivir counterparts. As the zanamivir trials' inclusion criteria were similar to the oseltamivir trials (fever and two additional symptoms of influenza-like illness (ILI)) this observation cannot plausibly be explained by an incremental role of influenza infection in the genesis of such heterogeneity. It is possible that the difference in reported gastrointestinal adverse events in the placebo groups of zanamivir and oseltamivir trials is due to differences in the collection of these events. However, other common adverse events such as headaches, cough and dizziness had very similar rates in the placebo groups of zanamivir and oseltamivir trials. Despite the results of this sensitivity analysis it is impossible without a clear statement of dosage

and rationale of use to assess the role of dehydrocholic acid and possibly calcium phosphate in the causation of such a high incidence of gastrointestinal adverse events.

For **hypothesis 1b** the data obtained from the zanamivir treatment trials clinical study reports are shown in Table 16.

Over all the nine zanamivir trials the incidence of asthma (including asthma exacerbation) in the placebo groups was 2.1% compared to 0.9% in the placebo groups of the oseltamivir trials. Random-effects meta-analysis of the data in Table 16 provided the following results for the combined outcome of any asthma event:

Asthma: decreased odds of adverse events due to zanamivir (OR 0.54, 95% CI 0.34 to 0.86, P = 0.01).

We carried out a sensitivity analysis by assuming placebo rates of asthma-related adverse events in zanamivir trials based on those observed in similar oseltamivir trials. If we assume a rate of asthma events in the placebo groups of the nine zanamivir trials similar to that observed in the oseltamivir trials we obtain the following result based on random-effects meta-analysis:

Asthma: no evidence of a difference between treatment groups (OR 1.27, 95% CI 0.71 to 2.26, P = 0.42; test for heterogeneity P = 0.68).

We conclude that zanamivir trial placebo recipients appear to have a higher incidence of asthma-related events than their oseltamivir counterparts. Again, as the inclusion criteria were similar for both trial programmes this finding is not likely to be due to severity of influenza infections but associated with exposure to lactose powder and possibly to the active principle. This is a point remarked on by the FDA.

For **hypothesis 2** (oseltamivir (or zanamivir) does not affect antibody production in treatment trials) the relevant trials showed strong and consistent evidence that patients randomised to active treatment had reduced odds of being classified as influenza-infected (OR 0.83, 95% CI 0.73 to 0.94, P = 0.003) with no evidence of heterogeneity (heterogeneity Chi^2 test = 2.80 (df = 7) P = 0.90; estimate of between-study variance Tau^2 = 0.00) (see Table 14). There was also strong evidence that patients randomised to active treatment had reduced odds of having four-fold or higher rise in antibody titres (OR 0.79, 95% CI 0.70 to 0.90, P < 0.001) with no evidence of heterogeneity (heterogeneity Chi^2 test = 4.61 (df = 7) P = 0.71; estimate of between-study variance Tau^2 = 0.00) (see Table 14).

In contrast, the zanamivir trials showed no evidence that patients randomised to active treatment had reduced odds of being classified as influenza-infected (OR 1.05, 95% CI 0.90 to 1.24, P = 0.52) with no evidence of heterogeneity (heterogeneity Chi^2 test = 3.03 (df = 6) P = 0.81; estimate of between-study variance Tau^2 = 0.00).

These results have important implications for the oseltamivir treatment trials programme and for all ongoing trials. All influenza-infected populations are selected post-randomisation and post-trial termination on the basis of laboratory findings (all ITT participants being symptomatic at entry, with aetiology unknown). However, as oseltamivir appears to affect antibody production (or perhaps testing, or both), there

may be some participants in the oseltamivir group who were infected with influenza but not diagnosed by the antibody rise and were therefore not counted in the influenza-infected population. These may have subsequently been excluded from the efficacy analysis. It is also possible that the strength of the antibody production limit to qualify for an influenza infection-induced antibody rise (four-four fold and above from baseline) had the effect of selecting the 'stronger' responders into the influenza-infected subgroup of the oseltamivir arm. This would mean that the best antibody producers were selected and this may have led to inflated treatment estimates of efficacy in influenza-infected populations.

To investigate this possibility we calculated the correlation between the odds of being classified as infected in the oseltamivir group compared to the placebo group and the size of the primary treatment effect (time to alleviation of symptoms in the ITTI population). In treatment trials all participants are recruited on the basis of symptoms of influenza-like illness. According to the mechanism of action proposed by the manufacturer, infected participants given oseltamivir up to 48 hours from symptom onset should have an antibody response which, given the effects of randomisation, should be similar to that of placebo recipients. Non-responders or weak responders should be spread evenly across the trial arms. All treatment trials of oseltamivir showing evidence of a treatment effect on the primary outcome of the study were included in the analysis. This included two trials for which we did not have clinical full study reports (ML16369; JV15823). We included these trials to increase variation in the two variables used for the analysis. In addition, two trials were excluded: WV15707 which had a total ITTI sample size of 12 participants; and WV15812/WV15872, which was a treatment trial in chronically ill adults that showed no evidence of a treatment effect. Results showed strong evidence of a correlation (Spearman rank correlation = -0.83, P = 0.01) (Table 19). The correlation was highly negative, indicating that lower odds of being classified as ITTI in the oseltamivir group compared to the placebo group is associated with larger treatment effects for the primary outcome of the studies. In contrast, there was no evidence of a correlation between the odds of being classified as infected in the oseltamivir group compared to the placebo group (Table 19) and the size of the treatment effect in the ITT population (Spearman rank correlation = -0.23, P = 0.66). A limitation of this analysis is that data for the ITT population for two trials were not available (WV15730; JV15823) (Table 19).

Thus, all influenza-infected comparisons are potentially confounded by the action of the drug (oseltamivir but probably not zanamivir) and are essentially non-randomised comparisons. Any analyses should be based on ITT populations in oseltamivir treatment trials. Analyses and data considered for inclusion in systematic reviews should be based on the ITT (or safety) populations only.

Our analysis of **Hypothesis 3** shows that the odds of having a four-fold rise in antibodies is 0.33 (95% CI 0.16 to 0.67) for the oseltamivir group compared to placebo (hence a much bigger effect compared to the treatment trials). Due to insufficient information provided in the clinical study report we were unable to take account of the clustering in this analysis, hence the confidence intervals are possibly under-estimated; however an analysis that takes into account clustering is unlikely to change the conclusions. These results show that oseltamivir prophylaxis is associated with lower odds of a four-fold rise in antibodies and this appears to be due to a

difference in the distribution of antibody rise in HIAAH3 antibodies but not HIAAH1 or HIB antibodies (see Table 14). In summary no conclusions can be drawn from the available evidence on the effects of the drug on viral transmission. The mode of action in prophylaxis appears mainly to be ascribed to symptom suppression or control. There is uncertainty around other possible effects of the drug especially given its interaction with the production of antibodies.

We rejected **Hypothesis 4** and are currently unable to test **Hypothesis 5**

We rejected **Hypothesis 4** as there was no evidence of correlation between average recruited subjects per centre and the proportion of placebo patients subsequently diagnosed with influenza infection (Spearman correlation = 0.26; P = 0.53). Two studies failed to reach their recruitment target (WV15707 and WV15730) and two clinical study reports were made up of multiple trials due to the original trial's poor recruitment (WV15819/WV15876/WV15978 and WV15812/WV15872). In addition the proportion of placebo patients subsequently diagnosed with influenza infection ranged from 63% to 75%, implying little between-trial variation.

We are currently unable to test **Hypothesis 5** as only one oseltamivir clinical study report (of three trials) reported randomisation first then swabbing second (WV15819/WV15876/WV15978). In this study the proportion of placebo patients that were confirmed as influenza-infected was 68.1%. This compares with the other seven clinical study reports where swabbing was carried out first and randomisation second and the proportion of placebo patients that were confirmed as influenza-infected ranged from 63.2% to 74.9% with mean 68.1%. Hence it seems that swabbing after randomisation made no difference in the treatment trial programme where this practice is reported. However, with only one clinical treatment study report randomising prior to swabbing available to us, the power to detect a difference in the proportion of placebo patients subsequently diagnosed with influenza infection is low. We hope to be able to retest this hypothesis as more data become available.