Oseltamivir, amantadine, and ribavirin combination antiviral therapy versus oseltamivir monotherapy for the treatment of influenza: a multicentre, double-blind, randomised phase 2 trial

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Summary

Background Influenza continues to have a substantial socioeconomic and health impact despite a long established vaccination programme and approved antivirals. Preclinical data suggest that combining antivirals might be more effective than administering oseltamivir alone in the treatment of influenza.

Methods We did a randomised, double-blind, multicentre phase 2 trial of a combination of oseltamivir, amantadine, and ribavirin versus oseltamivir monotherapy with matching placebo for the treatment of influenza in 50 sites, consisting of academic medical centre clinics, emergency rooms, and private physician offices in the USA, Thailand, Mexico, Argentina, and Australia. Participants who were aged at least 18 years with influenza and were at increased risk of complications were randomly assigned (1:1) by an online computer-generated randomisation system to receive either oseltamivir (75 mg), amantadine (100 mg), and ribavirin (600 mg) combination therapy or oseltamivir monotherapy twice daily for 5 days, given orally, and participants were followed up for 28 days. Blinded treatment kits were used to achieve masking of patients and staff. The primary endpoint was the percentage of participants with virus detectable by PCR in nasopharyngeal swab at day 3, and was assessed in participants who were randomised, had influenza infection confirmed by the central laboratory on a baseline nasopharyngeal sample, and had received at least one dose of study drug. Safety assessment was done in all patients in the intention-to-treat population. This trial is registered with ClinicalTrials.gov, number NCT01227967.

Findings Between March 1, 2011, and April 29, 2016, 633 participants were randomly assigned to receive combination antiviral therapy (n=316) or monotherapy (n=317). Seven participants were excluded from analysis: three were not properly randomised, three withdrew from the study, and one was lost to follow-up. The primary analysis included 394 participants, excluding 47 in the pilot phase, 172 without confirmed influenza, and 13 without an endpoint sample. 80 (40·0%) of 200 participants in the combination group had detectable virus at day 3 compared with 97 (50·0%) of 194 (mean difference 10·0, 95% CI 0·2–19·8, p=0·046) in the monotherapy group. The most common adverse events were gastrointestinal-related disorders, primarily nausea (65 [12%] of 556 reported adverse events in the combination group *vs* 63 [11%] of 585 reported adverse events in the monotherapy group), diarrhoea (56 [10%] of 556 *vs* 64 [11%] of 585), and vomiting (39 [7%] of 556 *vs* 23 [4%] of 585). There was no benefit in multiple clinical secondary endpoints, such as median duration of symptoms (4·5 days in the combination group *vs* 4·0 days in the monotherapy group; p=0·21). One death occurred in the study in an elderly participant in the monotherapy group who died of cardiovascular failure 13 days after randomisation, judged by the site investigator as not related to study intervention.

Interpretation Although combination treatment showed a significant decrease in viral shedding at day 3 relative to monotherapy, this difference was not associated with improved clinical benefit. More work is needed to understand why there was no clinical benefit when a difference in virological outcome was identified.

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Introduction

Influenza continues to have a substantial socioeconomic impact on health care despite a long-established vaccine programme and approved antivirals. The US Centers for Disease Control and Prevention (CDC) has defined the populations most at risk for adverse outcomes from influenza as being adults aged at least 65 years, those with some underlying medical conditions such as asthma, emphysema, heart failure, coronary artery disease, and diabetes, children younger than 2 years old, and pregnant women.[1] Additionally, for the 2009 H1N1 pandemic, morbid obesity was also suggested as an independent risk factor.[2]

The theory of using a combination of antivirals to more effectively control viral replication was explored in influenza

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See Online for appendix

Research in context

Evidence before this study

We searched PubMed from April 3 to 7, 2017, for studies using the search terms "combination", "antivirals", and "influenza", restricting to the article type of "clinical trials", and excluded studies that did not study the combination of more than one antiviral, studies that did not assess clinical or virological efficacy (ie, drug–drug interaction studies), and studies that used combinations targeting the same viral enzyme. Our search was restricted to English language publications, with no publication date restrictions. Three previous studies were identified. A pilot study assessed combination treatment in seven patients who were immunocompromised and had influenza A infection (three with randomised allocation and four without randomisation). The authors reported the general safety of combination antivirals in this population, but inference of efficacy could not be made. A second study was a retrospective review of 24 patients with influenza A H1N1 treated with combination antiviral therapy, and compared with 103 patients receiving oseltamivir monotherapy. The combination group, when compared with an oseltamivir monotherapy group, reported lower 14-day (17% *vs* 35%) and 90-day mortality (46% *vs* 59%), though the study was not randomised, did not have standard dosing of the combination antivirals, and did not have uniform inclusion criteria. A third study retrospectively reviewed six patients with severe H1N1 treated with combination antivirals, and were compared with eight patients treated with high-dose oseltamivir. The clinical outcomes and viral shedding were not significantly different between treatment groups.

Added value of this study

To our knowledge, this is the largest and most comprehensive study evaluating combination antivirals for the treatment of influenza. Our findings showed that participants with influenza treated with combination antivirals had less viral shedding on day 3, though there was no benefit in terms of resolution of symptoms or fever, or time to recovery after illness as measured by global assessment questions.

Implications of all the available evidence

The evidence from this study shows that the combination of oseltamivir, amantadine, and ribavirin had improved antiviral efficacy over oseltamivir alone, but there was no evidence of improved clinical outcomes in this population.

even before it became an established method in the treatment of HIV.[3] In-vitro studies of dual antivirals have assessed amantadine with oseltamivir,[4–6] amantadine with ribavirin,[5,7] rimantadine with neuraminidase inhibitors (ie, zanamivir, oseltamivir, or peramivir),[8] ribavirin with adamantanes (amantadine or rimantadine),[5–7] ribavirin with neuraminidase inhibitors (oseltamivir or peramivir),[5,6,9] dual neuraminidase inhibitor therapy,[6] and combinations involving novel agents such as favipiravir with oseltamivir.[10] Dual antiviral combinations have also been assessed in animal studies including amantadine with oseltamivir,[5,11] amantadine with ribavirin,[5] rimantadine with oseltamivir[12,13] and ribavirin with neuraminidase inhibitors (oseltamivir and peramivir).[4,5,14,15] Dual antiviral therapy has had mixed efficacy results in vitro and in vivo, although the combination of amantadine and oseltamivir might reduce the emergence of both oseltamivir and amantadine-resistant viruses in vivo.[16]

The combination of amantadine, oseltamivir, and ribavirin has been shown to increase the antiviral activity in vitro (reduction in the EC_{50}, or the half maximal effective drug concentration) of each drug compared with its activity in double combinations or as single agents.[17] Circulating strains of influenza A are highly resistant to amantadine,[18] though preclinical data suggest some activity of amantadine against amantadine-resistant viruses when used in combination with other drugs (oseltamivir and ribavirin).[19] The combination of amantadine, oseltamivir, and ribavirin was more effective at preventing death when compared with dual and single drug regimens in mice infected with either influenza A H5N1 or influenza A H1N1.[19] Furthermore, this combination provided survival benefit when treatment was delayed until 72 h after infection, whereas oseltamivir monotherapy was not protective after 24 h after infection.[19] Given the encouraging preclinical data, and the need for better therapeutics in high-risk populations, we did a double-blind, randomised trial to compare the combination of amantadine, oseltamivir, and ribavirin to oseltamivir alone in participants at risk for complications from influenza.

Methods

Study design and participants

The study was a double-blind, randomised, phase 2 study done in 50 sites consisting of academic medical centre clinics, emergency rooms, and private physician offices in the USA, Thailand, Mexico, Argentina, and Australia. The study protocol was approved by an institutional review board or ethics committee for each study site as well as by all local and/or country governing bodies as applicable. All study participants provided written informed consent.

Men and non-pregnant women aged at least 18 years who had the presence of one or more underlying medical conditions that might increase risk of complications from influenza, who had influenza A H1N1, A H3N2, or B virus infections diagnosed locally by rapid antigen or PCR, and who had the onset of respiratory symptoms no more than 96 h before screening were eligible for the study. The risk factors included: age 65 years or older, presence of one or more chronic medical conditions (appendix), or body-mass index of at least 40 kg/m². Participants were excluded if they had anaemia, leucopenia, neutropenia,

thrombocytopenia, genetic haemoglobinopathy, creatinine clearance less than indicated for clinical dosing with these antivirals (<50 mL/kg until Feb 1, 2016, then <60 mL/kg), history of autoimmune hepatitis, uncompensated or severe liver disease, or had received more than two doses of any influenza antiviral medication since the onset of influenza symptoms.

Randomisation and masking

Participants were randomly assigned to treatment by an online computer-generated randomisation system in a 1:1 ratio to receive either combination treatment of oseltamivir, amantadine, and ribavirin, or oseltamivir monotherapy. Randomisation was not stratified. The study treatment kits had open-label oseltamivir (75 mg), three capsules of over-encapsulated ribavirin (200 mg) or matching placebo, and one capsule of over-encapsulated amantadine (100 mg) or matching placebo. The study treatments were identical in appearance. The treatment of five capsules was given twice a day for 5 days. All participants, site staff, and the study team were masked to treatment allocation. A group who were not masked to treatment allocation managed the study kit supplies. Unmasking occurred after study base was locked. Masking success was not measured.

Procedures

Participants were assessed on day 0 (before the first dose was given), and on days 3, 7, and 28. The study was originally designed with visits on days 1, 2, 3, 5, 7, 10, 14, and 28, and was amended on July 31, 2013, after completion of a pilot study to simplify study visits and improve enrolment. All sites were supplied kits that contained standardised nasopharyngeal swabs and transport medium, which were used by the study team to test for influenza virus in the patients on days 0, 3, and 7, and blood samples were obtained on days 0, 3, 7, and 28. The study teams were trained by in-person training, training videos, and pre-study quality assurance test swabs (assaying for presence of housekeeping genes from nasal epithelium). Samples were tested at the Naval Health Research Center (NHRC) in San Diego, CA, USA. Participants received diary cards that were to be completed twice a day from day 0 to day 7, once a day for days 8–14, with a final entry on day 28.

Influenza type and subtype were identified by use of the CDC protocol of real-time reverse transcriptase PCR for influenza A and B, and done at the NHRC. Influenza viral load in swab samples was identified by qualitative PCR using the TaqMan method. See appendix for full virology methods.

Outcomes

The first 50 participants who were randomly assigned to treatment were enrolled in a pilot study to help determine the virological endpoint to be used in the primary efficacy analysis, which was based on virological and practical considerations (actual n=47). These participants had nasopharyngeal and oropharyngeal swabs done and all samples were tested by both PCR and tissue culture infectious dose ($TCID_{50}$) measurements. Additionally, these participants had duplicate virological samples obtained on day 0 to identify whether the virological assays were reproducible. After analysis of this pilot study without unblinding to treatment, the primary endpoint was selected to be the percentage of participants with virus detectable by PCR in nasopharyngeal swab at day 3.

Secondary clinical endpoints included time to alleviation of influenza clinical symptoms and fever (grade 1 or below); time to absence of fever; time to alleviation of influenza clinical symptoms; time to resumption of pre-influenza level of physical activity; proportion of participants who developed bronchitis, pneumonia, or other complications of influenza; proportion of participants that were admitted to hospital after randomisation; proportion of participants who required new or increased use of supplemental oxygen; safety and tolerability (adverse events and serious adverse events); and 28-day mortality.

At the time of study commencement, there was no standardised, validated patient-reported outcome method for influenza.[20] The diary cards asked participants to record maximum temperature and to rate 11 symptoms (derived from previous efficacy studies of oseltamivir): cough, fatigue, subjective fever (feverishness), diarrhoea, myalgia, vomiting, headache, nausea, sore throat, nasal obstruction, and nasal discharge to be graded from absent to severe (0–3).

Two global assessment questions were asked: "Are you feeling as good as you did before you had the flu?" and "Are you functioning as well as you were before you had the flu?" The diary card contained the ten-question physical domain of the 36-item short form health survey (SF-36), which was completed daily according to SF-36 instructions, and each functional characteristic was graded by participants as being limited by influenza a lot, a little, or not at all (1–3 points). These points were then transformed into a 100-point scale and averaged across the ten questions.[21] At baseline and at each subsequent visit, investigators asked participants about any signs or symptoms suggestive of otitis media, bronchitis or bronchiolitis, or pneumonia. The presence of these complications was identified by the site investigator on the basis of clinical data available at the time, because there was no protocol-defined assessment for these complications. The investigators were also asked to assess the use of antibiotics for reasons besides the complications noted above. A Data Safety Monitoring Board (DSMB) reviewed the safety data from the study.

Statistical analysis

We chose a sample size of 560 participants (including 50 in the pilot study), which we calculated would give

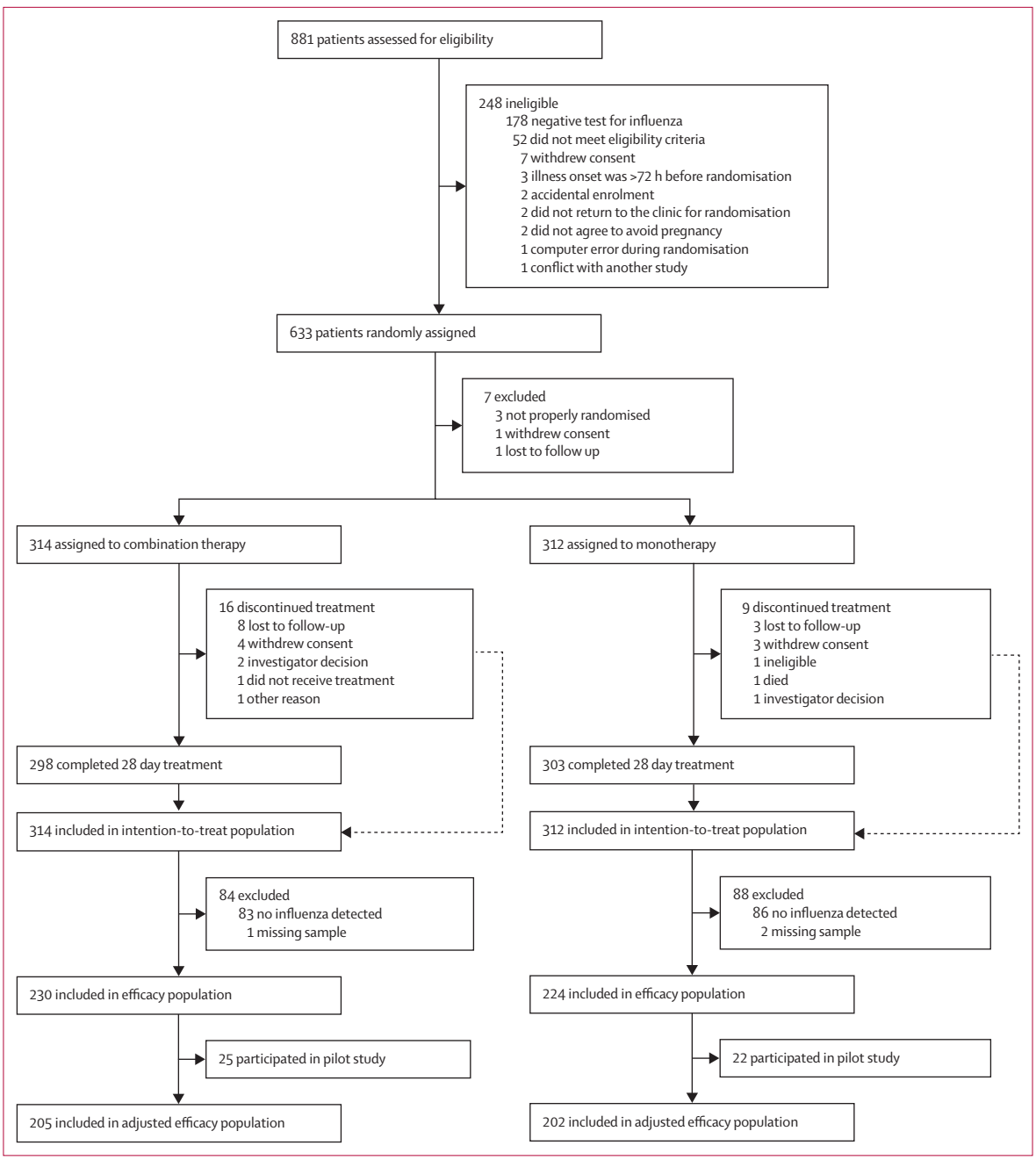

Figure 1: **Trial profile**

about 90% power to detect an absolute difference of 15% in the proportion of participants with undetectable virus shedding at day 3 in nasopharyngeal swabs (eg, a reduction of 15% from 57·5% in the combination therapy group to 42·5% in the monotherapy group) using a two-sided type I error rate of 0·05. We based this power calculation on the fact that about 50% of participants in the pilot study had undetectable virus in nasopharyngeal swabs at day 3 (pooled over both groups) and included an allowance for 10% of participants to have unavailable viral shedding results at day 3. Because of the larger than expected number of day 0 samples that did not have detectable virus, the protocol permitted randomisation until the end of the influenza season in 2016, up to a maximum of 720 participants.

The primary efficacy analyses are presented for all participants randomly assigned to treatment who had influenza infection confirmed by the central laboratory on a baseline nasopharyngeal sample and who had received at least one dose of study drug. Three participants

who were given study drug kits before randomisation were excluded. Additionally, because we chose the primary endpoint on the basis of data from the pilot study, the primary endpoint analysis was further restricted to exclude the 47 participants in the pilot study. The secondary efficacy results are presented for the intention-to-treat population, which consists of participants who were randomly assigned to treatment and who took at least one dose of the study medication. We used the Wald test, which assumes a normal approximation to the binomial distribution, to compare the primary endpoint between treatments. To assess whether the difference in viral shedding between treatments varied by subgroup, we used a logistic regression of treatment group by subgroup interaction test. We compared continuous viral load between groups at day 3 and day 7, which was based on the extension of Wilcoxon's test to handle censoring of measurements that were less than the assay lower limit of quantification. For secondary clinical endpoints such as time to alleviation of clinical symptoms, we used Kaplan-Meier plots and log-rank tests to compare the two groups. We also used proportional hazards models to assess whether the difference in time to events between groups varied by subgroup. We present adverse event data, coded using Medical Dictionary for Regulatory Activities (MedDRA), for all participants who were randomised to treatment and who had received at least one dose of study drug.

Role of the funding source

Employees of the sponsor of the study were involved with study design, analysis, and the writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between March 1, 2011, and April 29, 2016, 881 participants signed consent at 65 sites in the USA, Thailand, Mexico, Argentina, and Australia (figure 1). 248 participants were excluded during screening, resulting in 633 participants randomly assigned to treatment at 50 sites: 439 participants (69%) at 37 sites in USA, 131 participants (21%) at four sites in Thailand, 38 (6%) at three sites in Mexico, 23 participants (4%) at four sites in Argentina, and two participants (<1%) at two sites in Australia. 22 participants had minor enrolment deviations; all of these patients had influenza, took at least one dose of the study drug, and were included in the intention-to-treat population for analysis. Seven participants were excluded from all analyses (per protocol defined intention-to-treat population).

The median age of the randomised participants was 49·5 years (IQR 36·0–61·0); 128 (20%) were at least 65 years of age (table 1). The most common medical conditions that put the participants at risk for severe

	Total (n=626)	Combination therapy group (n=314)	Monotherapy group (n=312)
Median age, years	49·5 (36·0–61·0)	49·5 (37·0–61·0)	49·5 (35·5–61·0)
Sex			
Female	385 (62%)	187 (60%)	198 (63%)
Male	241 (38%)	127 (40%)	114 (37%)
Race			
White	392 (63%)	203 (65%)	189 (61%)
Asian	143 (23%)	70 (22%)	73 (23%)
Black or African American	43 (7%)	18 (6%)	25 (8%)
American Indian	3 (<1%)	1 (<1%)	2 (1%)
Race not available to clinic	45 (7%)	22 (7%)	23 (7%)
Ethnic origin			
Hispanic or Latino	113 (18%)	55 (18%)	58 (19%)
Country			
USA	436 (70%)	220 (70%)	216 (69%)
Thailand	131 (21%)	65 (21%)	66 (21%)
Mexico	35 (6%)	17 (5%)	18 (6%)
Argentina	22 (4%)	12 (4%)	10 (3%)
Australia	2 (<1%)	0	2 (1%)
Influenza type (by central lab)			
Influenza A H3N2	244 (39%)	126 (40%)	118 (38%)
Influenza A H1N1	104 (17%)	55 (18%)	49 (16%)
Influenza B	106 (17%)	49 (16%)	57 (18%)
Negative	169 (27%)	83 (26%)	86 (28%)
Number of missing samples	3 (<1%)	1 (<1%)	2 (1%)
Medical condition*			
Age 65 years or greater	128 (20%)	63 (20%)	65 (21%)
Asthma	199 (32%)	100 (32%)	99 (32%)
Endocrine	175 (28%)	87 (28%)	88 (28%)
Weakened immune system	90 (14%)	44 (14%)	46 (15%)
Heart disease	69 (11%)	30 (10%)	39 (13%)
Chronic lung disease	38 (6%)	17 (5%)	21 (7%)
Neurological condition	36 (6%)	17 (5%)	19 (6%)
Metabolic disease	20 (3%)	12 (4%)	8 (3%)
Kidney disease	14 (2%)	8 (3%)	6 (2%)
Blood disorder	13 (2%)	8 (3%)	5 (2%)
Liver disorder	10 (2%)	3 (1%)	7 (2%)
BMI ≥40 kg/m²	125 (20%)	74 (24%)	51 (16%)
Fever (≥38·0°C)	187 (30%)	94 (30%)	93 (30%)
Median time from onset of influenza-like illness to screening, hours	42 (27–54)	43 (28–55)	41 (25–52)
Median time from screening to randomisation, hours	1 (0–2)	1 (0–2)	1 (0–2)
Median time from randomisation to treatment initiation, hours	1 (0–2)	1 (0–2)	1 (0–2)
Proportion of participants who had ever been smokers	177 (28%)	88 (28%)	89 (29%)
Proportion of participants who had an influenza vaccination in the season of enrolment	193 (31%)	90 (29%)	103 (33%)

Data are median (IQR) or n (%). BMI=body-mass index.*Some participants had multiple conditions, so the sum of the percentages might exceed 100%.

Table 1: **Baseline characteristics of the intention-to-treat population**

	Total (n=454)	Combination group (n=230)	Monotherapy group (n=224)	p value
Day 0	454	230	224	..
Median viral count, log_{10} copies/mL	6·5 (5·4–7·4)	6·4 (5·6–7·2)	6·7 (5·1–7·7)	..
≥LLOQ	421 (93%)	221 (96%)	200 (89%)	..
≥LOD, < LLOQ	13 (3%)	4 (2%)	9 (4%)	..
<LOD	20 (4%)	5 (2%)	15 (7%)	..
Day 3	437	221	216	..
Median viral count, log_{10} copies/mL	3·4 (3·2–4·6)	3·4 (3·2–4·2)	3·9 (3·2–5·0)	0·004
≥LLOQ	152 (35%)	65 (29%)	87 (40%)	0·009
≥LOD, <LLOQ	47 (11%)	22 (10%)	25 (12%)	..
<LOD	238 (54%)	134 (61%)	104 (48%)	..
Day 7	431	216	215	..
Median viral count, log_{10} copies/mL	<3·2 (<3·2–3·4)	<3·2 (<3·2–3·4)	<3·2 (<3·2–3·4)	0·38
≥LLOQ	43 (10%)	19 (9%)	24 (11%)	0·24
≥LOD, <LLOQ	11 (3%)	4 (2%)	7 (3%)	..
<LOD	377 (87%)	193 (89%)	184 (86%)	..

Data are median (IQR) or n (%).Primary endpoint was the percentage of participants with virus detectable by PCR (ie, ≥LLOQ and ≥LOD, <LLOQ). LLOQ=lower limit of quantification of PCR assay. LOD=limit of detection of PCR assay.

Table 2: **Influenza virus over time in the efficacy population**

influenza were asthma and endocrine disorders such as diabetes, and were similar between groups. By local site testing, 440 (70%) had influenza A and 155 (25%) had influenza B, 15 (2%) not specified, and 16 (3%) reported more than one subtype, compared with that observed in central laboratory qualitative PCR testing (table 1). The primary efficacy analyses included the 454 participants with confirmed influenza infection by central laboratory testing.

At baseline, participants were moderately symptomatic (median 15 points on the 11-item symptom score graded 0–3, with 0 being scored if the patient was asymptomatic, and a score of 3 if symptoms were severe; appendix). Participants had moderate physical limitations as assessed on the SF-36 physical domain (median 35), and were almost universally not feeling as good (603 [97%] of 626 participants) or functioning as well as before they developed the influenza illness (576 [93%] of 626).

Of the 454 participants with confirmed influenza infection in central laboratory testing, 20 (4%) did not have virus detected on the quantitative PCR assay (five [2%] in the combination group *vs* 15 [7%] in oseltamivir group), and 13 (3%) had virus detected below the assay lower limit of quantification (four [2%] *vs* nine [4%]), though the presence of housekeeping genes *GAPDH* and *B2M* in most of these samples suggested that this was not due to poor technique when obtaining samples. The median viral load at baseline was 6·5 log copies/mL (IQR 5·4–7·4). These were similar in the two treatment groups: median 6·4 (5·6–7·2) in the combination group versus 6·7 (5·1–7·7) in the monotherapy group.

583 (93%) of 626 reported taking all study drugs on all 5 days and were similar in both groups (289 [92%] of 314 in the combination therapy group *vs* 294 [94%] of 312 in the monotherapy group); 36 (6%) of 626 participants (20 [6%] of 314 *vs* 16 [5%] of 312) had partly completed the treatment course, and six (1%) of 626 participants (five [2%] of 314 *vs* one [<1%] of 312) were lost to follow-up before treatment compliance was assessed.

Of 454 participants, 47 were excluded from the primary efficacy analysis because they were part of the pilot study. Of the remaining 407, 394 (97%) had a day 3 virological endpoint sample available for testing (200 [98%] of 205 in the combination group and 194 [96%] of 202 in the monotherapy group). 80 (40%) of 200 participants had virus detectable (ie, above the PCR limit of detection) in the combination group compared with 97 (50%) of 194 in the monotherapy group, a difference of 10·0% (95% CI 0·2–19·8, p=0·046). Similar results were observed when including the participants from the pilot study, 87 (39%) of 221 in the combination group versus 112 (52%) of 216 in the monotherapy group, a difference of 12·5% (95% CI 3·2–21·8, p=0·009; table 2). This difference between the two groups did not appear to vary by sex (interaction p=0·74), influenza type (interaction p=0·23), or country and race (interaction p=0·062). At day 3, the median viral shedding in the efficacy population was 3·4 (IQR 3·2–4·2) log_{10} copies/mL in the combination group compared with 3·9 (3·2–5·0) log_{10} copies/mL in the monotherapy group (p=0·004; table 2). By day 7, 54 participants (13%) had detectable virus (23 [11%] in the combination group *vs* 31 [14%] in the monotherapy group; p=0·24).

In the 454 participants in the efficacy population, the median duration of symptoms was 4·5 days (95% CI 4·0–5·0) in the combination group versus 4·0 days (3·5–4·5) in the monotherapy group (p=0·21). (figure 2A, table 3). When all 626 participants in the intention-to-treat population were assessed, the results were similar (table 3). When restricting the intention-to-treat population to those participants with more severe symptoms (at least one grade 2 or 3 symptom at randomisation), the medians were unchanged. In subgroup analyses for the intention-to-treat population, there was no significant difference in duration of symptoms by sex (interaction p=0·87), flu type (p=0·53), or country and race (p=0·55).

Patients treated with combination antivirals in the efficacy population took longer than patients who received monotherapy to feel as healthy as before the onset of influenza symptoms or until the participant functioned as well as before the onset of influenza illness (figure 2B, 2C; table 3). There was no difference in the physical function score on the SF-36 between groups (appendix). There were 74 complications noted in the 626 participants from randomisation until day 28: 28 participants had sinusitis (14 [4%] of 314 in the

combination group *vs* 14 [4%] of 312 in the monotherapy group), four had otitis media (one [<1%] *vs* three [1%]), 29 had bronchitis (18 [6%] *vs* 11 [4%]), and 13 had pneumonia (seven [2%] *vs* six [2%]). Additionally, antibiotics were given for reasons other than the complications noted above in 56 participants (27 [9%] *vs* 29 [9%]). Complications or antibiotic use occurred in a total of 100 participants (52 [17%] *vs* 48 [15%]; some participants had more than one complication or use of antibiotic), and was not different between treatment groups (p=0·69).

One death occurred in the study in an elderly participant who died of cardiovascular failure 13 days after being randomly assigned to oseltamivir monotherapy. The death was judged by the site investigator as not related to study intervention. 28-day mortality was a specified secondary endpoint, but given that there was only the one death during the study, no analysis was completed.

54 participants had detectable virus on day 7, and per protocol day 0 and 7 samples from these participants were sequenced for the presence of resistance to oseltamivir and amantadine. All successfully sequenced influenza samples contained the Ser31Asn substitution in the *M2* gene at baseline, known to be associated with resistance to amantadine. Five participants had virus with oseltamivir resistance in the *NA* gene at baseline: three H1N1 Ile117Thr, one H1N1 Ile117Met, and one H3N2 Lys150Ser (two in the combination group, and three in the monotherapy group). There were no participants with influenza B virus resistance at baseline. No day 7 samples showed a change in sequence at loci (compared with day 0) associated with oseltamivir resistance.

317 of the 626 participants randomly assigned to treatment reported a total of 1141 adverse events, between randomisation and day 28. The most common adverse events (numbers of events, not participants) were gastrointestinal-related disorders, primarily nausea (65 [12%] of 556 reported adverse events in the combination group *vs* 63 [11%] of 585 in the monotherapy group), diarrhoea (56 [10%] of 556 *vs* 64 [11%] of 585), and vomiting (39 [7%] of 556 *vs* 23 [4%] of 585). The most common adverse events in terms of number of patients were nausea and diarrhoea (appendix). All adverse events occurred in similar proportions in both groups. In the review of laboratory abnormalities, the median total bilirubin increased in the combination group from a median of 0·4 (IQR 0·3–0·6) mg/dL on day 0 to 0·5 (0·3–0·7) mg/dL on day 3 and to 0·6 (0·3–0·8) mg/dL on day 7; and returned to 0·4 (0·3–0·6) mg/dL on day 28, compared with a median of 0·4 mg/dL (0·3–0·6) on all study days in the monotherapy group. The median haemoglobin concentration was also not different between groups on day 0 (13·8 [IQR 12·8–14·9] in the combination group *vs* 13·7 [12·6–14·8] in the monotherapy group), day 3 (13·8 [12·9–14·8] *vs* 13·6 [12·6–14·6]), or day 7 (13·5 [12·5–14·5] *vs* 13·4 [12·5–14·6]).

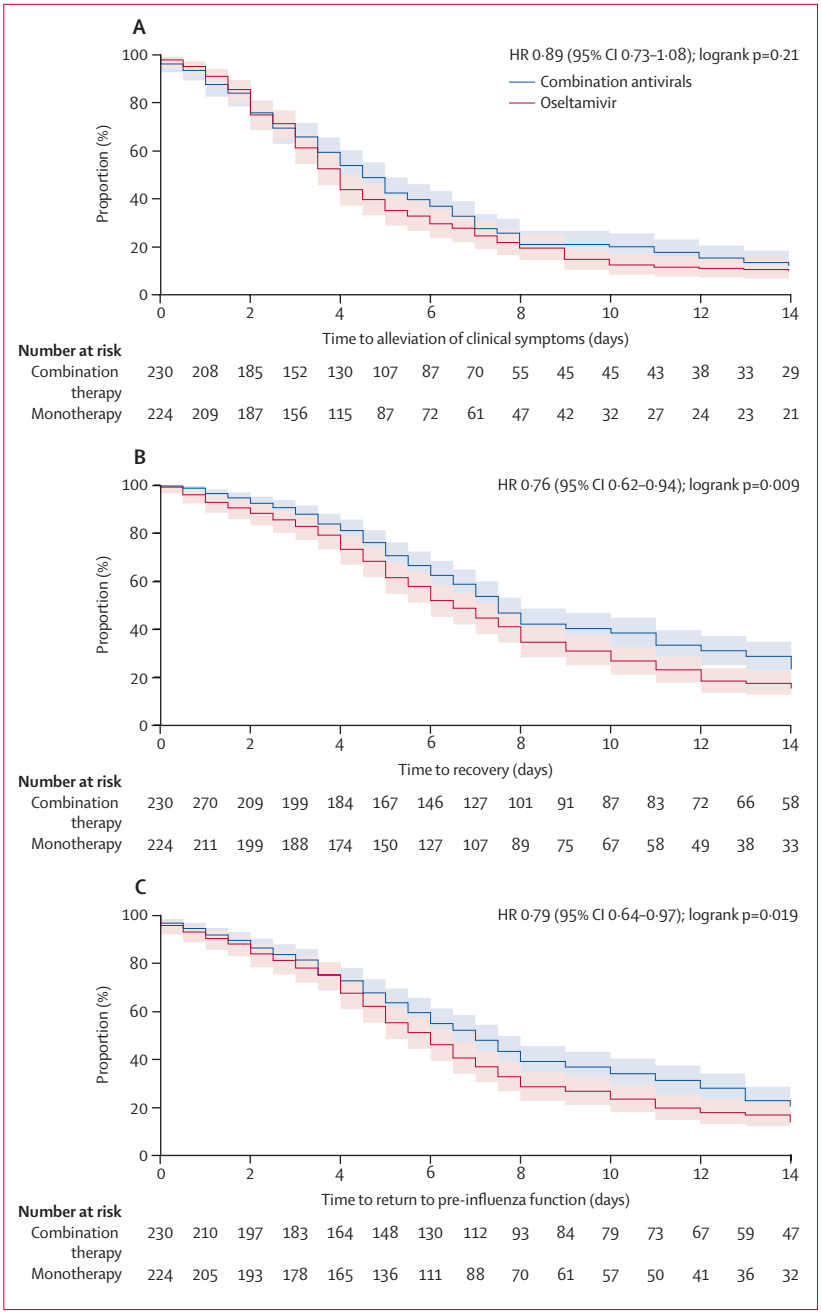

Figure 2: **Analysis of improvement of influenza symptoms and patient function**
(A) Proportion of participants with clinical symptoms (p=0·21), (B) extent of recovery after illness (p=0·009), and (C) function after illness (p=0·019) is shown in the efficacy population. Shaded areas denote 95% CI.

22 serious adverse events occurred in 20 participants: 16 events in 14 participants in the combination group and six events in six participants in the monotherapy group (table 4). Only asthma exacerbation, diarrhoea, and pneumonia occurred in more than one participant. There were four gastrointestinal serious adverse events in the combination group (three diarrhoea, one nausea) and none in the monotherapy group. Only two of the

	Combination group (n=230)	Monotherapy group (n=224)	p value
Efficacy population			
Duration of clinical symptoms	4·5 (4·0–5·0)	4·0 (3·5–4·5)	0·21
Duration of fever*	1·0 (1·0–1·5)	1·0 (0·5–1·5)	0·59
Duration of clinical symptoms or fever	5·0 (4·5–6·0)	4·0 (3·5–5·0)	0·10
Duration of time to feel as good as before the onset of influenza	7·5 (7·0–8·0)	6·5 (6·0–7·5)	0·0086
Duration of time to return to pre-influenza function	7·0 (6·0–7·5)	6·0 (5·0–6·5)	0·019
Intention-to-treat population			
Duration of clinical symptoms	4·5 (4·0–5·0)	4·0 (3·5–4·5)	0·44
Duration of fever*	1·0†	1·0 (0·5–1·0)	0·69
Duration of clinical symptoms and fever	4·5 (4·0–5·0)	4·5 (4·0–5·0)	0·30
Duration of time to feel as good as before the onset of influenza	7·5 (7·0–7·5)	6·5 (6·0–7·0)	0·0033
Duration of time to return to pre-influenza function	7·0 (6·0–7·5)	6·0 (5·0–6·5)	0·0086

Data are median duration in days (95% CI). *Data were restricted to patients who reported fever at randomisation. †95% CI was not estimable.

Table 3: **Clinical efficacy by treatment group**

serious adverse events (delirium and personality change) were judged to be related to study medication, and both of these occurred in the monotherapy group. One participant in the monotherapy group died from cardiovascular failure during the study observation period, which was judged by the site investigators to be unrelated to the study intervention. 13 participants in the combination group and three participants in the monotherapy group were admitted to hospital (p=0·011).

Discussion

To our knowledge, this is the first large randomised comparison of oseltamivir, amantadine, and ribavirin combination therapy versus oseltamivir monotherapy for the treatment of influenza. The study, done in an ambulatory population with risk factors for complications from influenza, showed a modest but significant 10% absolute reduction in the proportion of participants shedding virus on day 3. Together with the quantitative reduction in viral load at day 3, the combination treatment does appear to decrease viral shedding relative to conventional monotherapy. By day 7, however, a similarly high proportion of participants in both groups had stopped shedding influenza virus, and the difference between treatment groups was no longer significant.

This reduction in viral shedding by combination antivirals, however, did not translate into clinical benefit when assessed across multiple different parameters. When assessed by symptoms, fever, the physical domain of SF-36, and occurrence of complications, it also did not appear that participants felt better any more quickly after treatment with combination antivirals than with monotherapy. Indeed, by some metrics such as the global assessments, participants receiving the combination antivirals might have recovered slower.

	Total (n=626)	Combination group (n=314)	Monotherapy group (n=312)
Any hospital admission	16 (3%)	13 (4%)	3 (1%)
Patients with any serious adverse event*	20 (3%)	14 (4%)	6 (2%)
Respiratory, thoracic and mediastinal disorders	5 (1%)	4 (1%)	1 (<1%)
Asthma	2 (<1%)	2 (1%)	0
Pulmonary oedema	1 (<1%)	1 (<1%)	0
Respiratory distress	1 (<1%)	1 (<1%)	0
Bronchospasm	1 (<1%)	0	1 (<1%)
Gastrointestinal disorders	4 (1%)	4 (1%)	0
Diarrhoea	2 (<1%)	2 (1%)	0
Nausea	1 (<1%)	1 (<1%)	0
Haemorrhagic diarrhoea	1 (<1%)	1 (<1%)	0
Infections and infestations	4 (1%)	3 (1%)	1 (<1%)
Pneumonia	2 (<1%)	1 (<1%)	1 (<1%)
Gastroenteritis	1 (<1%)	1 (<1%)	0
Cellulitis	1 (<1%)	1 (<1%)	0
Cardiac disorders	3 (<1%)	2 (1%)	1 (<1%)
Atrial fibrillation	1 (<1%)	1 (<1%)	0
Cardiac failure	1 (<1%)	0	1 (<1%)
Atrial tachycardia	1 (<1%)	1 (<1%)	0
Psychiatric disorders	2 (<1%)	0	2 (1%)
Delirium	1 (<1%)	0	1 (<1%)
Personality change	1 (<1%)	0	1 (<1%)
Skin and subcutaneous tissue disorders	1 (<1%)	1 (<1%)	0
Diabetic foot	1 (<1%)	1 (<1%)	0
Metabolism and nutrition disorders	1 (<1%)	1 (<1%)	0
Dehydration	1 (<1%)	1 (<1%)	0
Injury, poisoning, and procedural complications	1 (<1%)	1 (<1%)	0
Spinal compression fracture	1 (<1%)	1 (<1%)	0
Blood and lymphatic system disorders	1 (<1%)	0	1 (<1%)
Febrile neutropenia	1 (<1%)	0	1 (<1%)

Data are n (%) and include all events reported after randomisation until day 28. *Some patients had more than one adverse event.

Table 4: **Number of participants with hospital admission or serious adverse events by treatment group**

This is the largest and most comprehensive study assessing combination antivirals for the treatment of influenza. A previous pilot study investigated the use of combination antivirals (amantadine 75 mg, oseltamivir 50 mg, and ribavirin 200 mg, each three times a day) in seven patients with influenza H1N1 or H3N2 who were immunocompromised (three with randomised allocation [one to oseltamivir monotherapy and two to combination therapy], and four were given combination antivirals without random allocation).[22] Seo and colleagues[22] report

the general safety of combination antivirals in this population, but made no inference of efficacy. A non-randomised retrospective review of patients with influenza H1N1 on mechanical ventilation compared the outcomes of 24 patients receiving combination antiviral therapy with 103 patients receiving oseltamivir monotherapy.[23] The dose and duration of all medications including oseltamivir was not standardised in this population. A lower 14-day and 90-day mortality was reported in the combination group than in the monotherapy group, though this observation was not significant and interpretation is difficult given differences in the populations and drug interventions. Another retrospective review of 14 patients with complicated H1N1 influenza compared the viral shedding seen in high-dose oseltamivir (150 mg twice a day) in eight patients with six patients treated with combination antiviral drugs (150 mg oseltamivir twice a day, 100 mg amantadine twice a day, and 300 mg ribavirin three times a day).[24] Kang and colleagues[24] concluded that combination antivirals could be useful to achieve virological clearance, though viral shedding after 5 days was seen in one of six participants treated with oseltamivir monotherapy, and two of six of those treated with combination antivirals.

In this study, although there were more serious adverse events in the combination antiviral group, the combination of oseltamivir, amantadine, and ribavirin appeared generally safe and well tolerated. There were significantly more hospital admissions in the combination group than in the monotherapy group. There were a greater number of respiratory serious adverse events, but these were unlikely to be due to the antiviral medications themselves given the known side-effects with these medications. There were more gastrointestinal serious adverse events in the combination arm, which could be due to either the antiviral medication or to the underlying influenza illness, but the rate of non-serious gastrointestinal adverse events was not notably different between the two treatment groups. Neurological adverse events, a particular concern of regimens using amantadine, were also not notably different between the treatment groups.

The scarcity of evident clinical benefit despite enhanced viral clearance is both intriguing and disappointing. In preclinical studies, viral titres measured by PCR and $TCID_{50}$ correlated with severity of disease across multiple animal models, and antiviral effects correlated with reduced morbidity.[25] In influenza challenge models, $TCID_{50}$, nasal cytokines and chemokines, and upper and lower respiratory symptoms were all strongly correlated with viral titres.[26] In observational studies, early oseltamivir treatment was associated with a decrease in viral RNA concentration, and viral RNA clearance on day 5 was associated with a shorter time to discharge from the hospital (median length of stay 6·0 days in patients with undetectable viral RNA *vs* 8·0 days in those with detectable RNA, on day 5; p=0·038).[27] For these reasons, it has been argued that virological primary endpoints should be used in antiviral studies in those at high risk of severe disease.[28] However, to date, the US Food and Drug Administration has not accepted this argument, noting "there is no established predictive relationship between magnitude and timing of viral reductions and extent of clinical benefit of how a patient 'feels, functions, or survives'".[29]

In this double-blind, multicentre, randomised trial in multiple countries, virological treatment benefit was observed without improved clinical outcomes. This calls into question whether changes in viral shedding from an antiviral can be used to predict changes in clinical outcomes. It is possible that assessing viral shedding by PCR at a fixed timepoint after influenza diagnosis is not sufficiently sensitive as an overall measure of antiviral efficacy; and other, more comprehensive or alternative measures might be required. Until the association between change in viral shedding and change in symptoms is better understood, the results of this study argue that the use of virological measures for clinical primary endpoints in future trials of promising antiviral treatments should be met with appropriate caution. There might be other benefits of reducing viral shedding, such as lower infectivity and decreased emergence of antiviral resistance, although this study was not designed to assess these important public health outcomes. In our population of patients with seasonal influenza, close to half of participants stopped shedding virus within 3 days with monotherapy. It is known that patients who are admitted to hospital with severe influenza or other influenza strains such as H5N1 and H7N9 shed higher amounts of virus for longer periods of time. Therefore, it is difficult to know if the absence of clinical benefit in our population would apply to those who are admitted to hospital with severe influenza or infected with other strains of influenza.

The main limitation in our study is the large percentage of participants (27%) without detectable virus at baseline by qualitative PCR testing at the central laboratory despite having had virus detectable at on-site testing. This was an unexpected finding because, on the basis of the pilot study, only about 5% of participants would have been expected to have a negative screening. With a median of 1 h between screening and randomisation, we do not believe this can be attributed to spontaneous resolution of viral shedding. Between the conclusion of the pilot study and the end of the full study, the study sites were given the option of using newer, more sensitive influenza diagnostic assays. In examining potential causes of the large number of baseline negative endpoint specimens, samples of local site influenza diagnostic assays (often processed in ≤200 µL) were tested and compared with the endpoint specimens (collected in 3 mL viral transport media). In a few site diagnostic assay samples tested by reverse transcriptase-PCR in the

central laboratory, many of the site assay samples were positive for influenza, although the virus could not be detected in the baseline central lab samples obtained. The unintended consequence of providing more sensitive influenza diagnostic testing to the sites is that, as a result of such factors as viral dilution in transport medium or possible attenuation during shipping, the ability to detect small amounts of virus in the larger volume of endpoint sample might have suffered.

Another limitation is the choice of medications used in the combination therapy group. At the time of protocol development, there were few new antivirals. Amantadine and ribavirin were chosen as they are generic, and if efficacy was shown, the combination could have been available at a low cost. However, both amantadine and ribavirin can cause adverse effects and intolerances, some of which might have contributed to persistent clinical symptoms. It is possible that newer, more effective, antivirals with fewer side-effects, when used in combination, could lead to a different clinical outcome.

We have shown that the combination of oseltamivir, amantadine, and ribavirin had improved antiviral efficacy over oseltamivir monotherapy in a population with influenza at risk for severe disease. However, no clinical symptomatic benefit could be shown with the use of combination antivirals; indeed, by some measures the oseltamivir monotherapy group was superior. More work is needed to explore and hopefully better understand this outcome and the similarity in clinical outcome between groups even when a difference in virological outcome was identified.

Contributors

JHB, YB, NM, JH, HCL, MDH, and RTD were responsible for initial study design, though all authors were involved with subsequent study amendments. JHB, JB, SP-P, GS, MHL, DRB, HPH Jr, JH, and RTD were responsible for study implementation and ongoing management. WM, AS, SMD, JP, and RLB enrolled most participants (all participating sites are noted in the appendix). JB was responsible for monitoring oversight, and HPH was responsible for safety oversight of the study. JH and CM were responsible for the virology testing. JHB, YB, MDH, and RTD analysed and interpreted the data and wrote the first draft of the report though all authors had opportunity to review the data and provided editing of the final report.

Declaration of interests

JH reports stock ownership in Adamas Pharmaceuticals. NM reports employment and stock ownership in in Adamas Pharmaceuticals. SP-P, MHL, JH, and MDH report receiving funding by subcontracts to National Institute of Allergy and Infectious Diseases for work done during the completion of this study. All other authors declare no competing interests.

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