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Baloxavir Marboxil for Uncomplicated Influenza in Adults and Adolescents

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ABSTRACT

BACKGROUND

Baloxavir marboxil is a selective inhibitor of influenza cap-dependent endonuclease. It has shown therapeutic activity in preclinical models of influenza A and B virus infections, including strains resistant to current antiviral agents.

METHODS

We conducted two randomized, double-blind, controlled trials involving otherwise healthy outpatients with acute uncomplicated influenza. After a dose-ranging (10 to 40 mg) placebo-controlled trial, we undertook a placebo- and oseltamivir-controlled trial of single, weight-based doses of baloxavir (40 or 80 mg) in patients 12 to 64 years of age during the 2016–2017 season. The dose of oseltamivir was 75 mg twice daily for 5 days. The primary efficacy end point was the time to alleviation of influenza symptoms in the intention-to-treat infected population.

RESULTS

In the phase 2 trial, the median time to alleviation of influenza symptoms was 23.4 to 28.2 hours shorter in the baloxavir groups than in the placebo group ($P<0.05$). In the phase 3 trial, the intention-to-treat infected population included 1064 patients; 84.8 to 88.1% of patients in each group had influenza A(H3N2) infection. The median time to alleviation of symptoms was 53.7 hours (95% confidence interval [CI], 49.5 to 58.5) with baloxavir, as compared with 80.2 hours (95% CI, 72.6 to 87.1) with placebo ($P<0.001$). The time to alleviation of symptoms was similar with baloxavir and oseltamivir. Baloxavir was associated with greater reductions in viral load 1 day after initiation of the regimen than placebo or oseltamivir. Adverse events were reported in 20.7% of baloxavir recipients, 24.6% of placebo recipients, and 24.8% of oseltamivir recipients. The emergence of polymerase acidic protein variants with I38T/M/F substitutions conferring reduced susceptibility to baloxavir occurred in 2.2% and 9.7% of baloxavir recipients in the phase 2 trial and phase 3 trial, respectively.

CONCLUSIONS

Single-dose baloxavir was without evident safety concerns, was superior to placebo in alleviating influenza symptoms, and was superior to both oseltamivir and placebo in reducing the viral load 1 day after initiation of the trial regimen in patients with uncomplicated influenza. Evidence for the development of decreased susceptibility to baloxavir after treatment was also observed. (Funded by Shionogi; JapicCTI number, 153090, and CAPSTONE-1 ClinicalTrials.gov number, NCT02954354.)

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A Quick Take is available at NEJM.org

Additional effective antiviral agents are needed for the treatment and prevention of influenza virus infections. Two classes of agents, M2 ion-channel inhibitors and neuraminidase inhibitors, are widely available. However, circulating influenza viruses are now largely resistant to M2 ion-channel inhibitors, and the emergence of antiviral resistance to neuraminidase inhibitors remains a threat, as shown by the global circulation of oseltamivir-resistant seasonal influenza A(H1N1) viruses in 2008–2009[1] and by community clusters of oseltamivir-resistant influenza A(H1N1)pdm09 viruses.[2]

The influenza virus polymerase complex has received considerable attention as a target for developing antiviral agents.[3,4] The polymerase heterotrimer is composed of three protein subunits (polymerase basic protein 1 [PB1], polymerase basic protein 2 [PB2], and polymerase acidic protein [PA]) that are highly conserved and essential for efficient viral replication.[4-6] The PB2 subunit binds to the cap of host cellular pre–messenger RNA, which is subsequently cleaved by the cap-dependent endonuclease in the PA subunit. This "cap-snatching" process provides an RNA primer for transcription of viral messenger RNA by the RNA-dependent RNA polymerase function of PB1.[7]

Several new antiviral agents that target the polymerase complex have been studied in patients with naturally occurring influenza infections. These include the PB1 inhibitor favipiravir (Avigan), which was approved in Japan in 2014 for the treatment of novel influenza viruses unresponsive to other agents[8] but has shown inconsistent therapeutic efficacy in randomized, controlled trials of uncomplicated influenza.[9] Pimodivir (also known as JNJ-63623872 and VX-787), a PB2 inhibitor with selective activity against influenza A viruses,[10] has shown virologic efficacy alone and in combination with oseltamivir in patients with uncomplicated influenza[11] and recently in hospitalized patients[12] and is advancing in clinical development.

Baloxavir marboxil (S-033188, hereafter referred to as baloxavir) is the small-molecule prodrug of the selective PA inhibitor S-033447 that has shown nanomolar antiviral activity against influenza A and B viruses in vitro, including strains resistant to current antiviral agents.[13] In murine models of seasonal influenza and avian influenza A(H5N1) or A(H7N9), orally administered baloxavir was associated with rapid reductions in pulmonary viral loads and decreased mortality.[13] In an ascending single-dose study involving healthy volunteers, baloxavir was administered up to the highest dose tested (80 mg) without evident safety concerns, and it showed linear pharmacokinetic characteristics and a long plasma elimination half-life (range, 49 to 91 hours).[14] We now report the results of single-dose baloxavir treatment in otherwise healthy persons with acute influenza from phase 2 and 3 randomized, controlled trials.

METHODS

TRIAL DESIGN AND OVERSIGHT

The phase 2 trial was a double-blind, placebo-controlled, dose-ranging, randomized trial (randomization ratio, 1:1:1:1) of single doses of baloxavir (10, 20, or 40 mg) or placebo. The trial enrolled Japanese adults 20 to 64 years of age with acute influenza from December 2015 through March 2016.

The phase 3 trial (CAPSTONE-1) was a double-blind, placebo- and oseltamivir-controlled, randomized trial that enrolled outpatients 12 to 64 years of age with influenza-like illness in the United States and Japan from December 2016 through March 2017. Patients 20 to 64 years of age were randomly assigned, in a 2:2:1 ratio, to receive a single oral dose of baloxavir (40 mg for patients weighing <80 kg or 80 mg for those weighing ≥80 kg), oseltamivir at a dose of 75 mg twice daily for 5 days, or matching placebos. Patients in all three groups received a 5-day regimen (baloxavir and a placebo matching oseltamivir, oseltamivir and a placebo matching baloxavir, or placebos only). Patients 12 to 19 years of age were randomly assigned, in a 2:1 ratio, to receive either baloxavir or placebo (on day 1 only). For patients of all ages, the first dose of the trial regimen was administered under direct observation.

Both trials were conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonisation. All the patients provided written informed consent, or assent as appropriate for adolescents. The sponsor (Shionogi) designed the trials in collaboration with the first author. The authors' access to the data was not restricted by confidentiality agreements. Data were compiled by the sponsor and analyzed by a statistician employed by the sponsor. The sponsor and authors vouch for the completeness and accuracy of the data and analyses and for the adherence of the trials to the protocols

(available with the full text of this article at NEJM.org).

PATIENTS

Patients who were enrolled had fever (axillary temperature, ≥38.0°C), at least one systemic symptom and at least one respiratory symptom of at least moderate severity, and a symptom duration of no more than 48 hours (see the Supplementary Appendix, available at NEJM.org). A positive rapid antigen test was an entry criterion for the phase 2 trial but not the phase 3 trial. The trials excluded patients with underlying conditions, including pregnant women, those weighing less than 40 kg, and those with illness resulting in hospitalization. Acetaminophen was allowed, but no other symptomatic therapies, antiviral agents for the treatment of influenza, or antibiotic agents were allowed, except for the treatment of suspected bacterial infections that developed after enrollment. For full details of the design of the trials, see the protocols and statistical analysis plans (available with the protocols).

CLINICAL AND LABORATORY MONITORING

Patients assessed the severity of seven influenza-associated symptoms (cough, sore throat, headache, nasal congestion, feverishness or chills, muscle or joint pain, and fatigue) on a 4-point scale (with 0 indicating no symptoms, 1 mild symptoms, 2 moderate symptoms, and 3 severe symptoms) twice daily from enrollment (day 1) to day 9 and once daily on days 10 through 14. Body temperature was measured four times daily through day 3 and twice daily through day 14. In addition, patients assessed their overall health status on a scale of 0 (worst possible) to 10 (normal) each evening through day 14. On days 1, 5 or 6, 15, and 22, safety laboratory tests (hematologic tests, blood chemical tests, and urinalysis) were performed.

Serum for influenza neutralizing antibody testing was obtained on days 1 and 22. Nasopharyngeal swabs (or throat swabs, if nasopharyngeal swabbing was not feasible) were obtained up to day 8 (phase 2 trial) or 9 (phase 3 trial) for viral quantitation and susceptibility testing (see the Supplementary Appendix).

OUTCOMES MEASURED

In both trials, the primary efficacy end point was the time to alleviation of symptoms, defined as the time from the start of the trial regimen to the time when all seven influenza-related symptoms (described above) were rated by the patients as absent or mild for at least 21.5 hours. Secondary clinical end points included the time to resolution of fever, the time to a return to usual health, and newly occurring complications leading to antibiotic use.

Virologic end points included the changes from baseline in infectious virus and viral RNA titers, the duration of virus detection, and the frequency of the emergence of amino acid changes associated with reduced susceptibility to baloxavir. The safety end points included the frequencies and severity of adverse events.

STATISTICAL ANALYSIS

Assuming that 65% of the patients enrolled in the phase 3 trial would be positive for influenza, we calculated that a sample of 1494 patients would provide that trial with at least 90% power to detect a 28-hour difference in the median time to alleviation of symptoms between the baloxavir group and the placebo group at a two-sided significance level of 0.05. The intention-to-treat infected patients, defined according to antigen positivity in the phase 2 trial and positivity on a reverse-transcriptase–polymerase-chain-reaction assay in the phase 3 trial, comprised the primary efficacy analysis population. In the phase 3 trial, the time to alleviation of symptoms was compared between the baloxavir group and the placebo group with the use of a generalized Wilcoxon test, with stratification according to a composite symptom score at baseline and country (see the Supplementary Appendix). A similar approach was used in the secondary efficacy analysis that compared the time to alleviation of symptoms between the baloxavir group and the oseltamivir group and in analyses of subgroups of interest.

A generalized Wilcoxon test, an analysis of covariance, the van Elteren test, the Mantel–Haenszel test, and Fisher's exact test were used for the various secondary efficacy outcomes. The numbers of events and patients with adverse events were reported for each intervention group.

RESULTS

PHASE 2 TRIAL

Of the 400 patients who underwent randomization, 389 completed the trial (Fig. S1 in the Supplementary Appendix). A majority of the patients

who underwent randomization were infected with the influenza A(H1N1)pdm09 virus (61.0 to 71.0% of patients in the three baloxavir groups and the placebo group) (Table S1 in the Supplementary Appendix). The median time to alleviation of symptoms in each of the baloxavir dose groups (54.2 hours in the 10-mg group, 51.0 hours in the 20-mg group, and 49.5 hours in the 40-mg group) was significantly shorter than in the placebo group (77.7 hours) (P=0.009, P=0.02, and P=0.005, respectively) (Fig. S2 in the Supplementary Appendix).

All three baloxavir dose groups had significantly greater reductions in influenza virus titers on days 2 and 3 than the placebo group (Fig. S3 in the Supplementary Appendix). One day after administration of the trial regimen, the median reduction was 4.5 $\log_{10}$ 50% tissue-culture infective dose ($TCID_{50}$) per milliliter in the baloxavir 40-mg group, as compared with 1.6 $\log_{10}$ $TCID_{50}$ per milliliter in the placebo group (P<0.001). Four of 182 baloxavir recipients (2.2%) with paired sequencing had post-treatment viruses with PA amino acid substitutions (I38T/F) that confer reductions by a factor of more than 10 in susceptibility to baloxavir in influenza A(H1N1) viruses. All the recipients with these substitutions had influenza A(H1N1)pdm09 infection.

Adverse events were reported in 23.0 to 27.0% of patients in the three baloxavir dose groups and 29.0% of patients in the placebo group, with no important differences in rates of specific events between each baloxavir group and the placebo group (Table S4 in the Supplementary Appendix). There were no adverse events leading to withdrawal from the trial and no serious adverse events.

PHASE 3 TRIAL

Patient Population

Overall, 1436 patients underwent randomization, of whom 1366 completed the trial and 1064 were included in the intention-to-treat infected population (Fig. 1). No relevant differences in demographic or clinical characteristics were noted between those assigned to baloxavir and those assigned to placebo (Table 1) or between those assigned to baloxavir and those assigned to oseltamivir (Table S2 in the Supplementary Appendix). In the intention-to-treat infected population, 52.9% of the patients initiated the trial regimen within 24 hours after symptom onset, the influenza A(H3N2) virus accounted for 84.8 to 88.1% of infections in the three trial groups, and 77.2% of the patients were enrolled in Japan.

Clinical and Virologic Efficacy

The median time to alleviation of symptoms was shorter in the baloxavir group than in the placebo group in both the intention-to-treat infected population (53.7 hours vs. 80.2 hours, P<0.001) and intention-to-treat population (65.4 hours vs. 88.6 hours, P<0.001), corresponding to median differences of 26.5 hours (95% confidence interval [CI], 17.8 to 35.8) and 23.2 hours (95% CI, 34.2 to 14.0), respectively. More rapid alleviation of symptoms with baloxavir than with placebo was evident by day 2 (Fig. 2). A shorter time to alleviation of symptoms with baloxavir than with placebo was observed in both adolescents (median difference, 38.6 hours; P=0.006) and adults (median difference, 25.6 hours; P<0.001).

The difference in the time to alleviation of symptoms between the baloxavir group and the placebo group was greater in patients who initiated the trial regimen within 24 hours after symptom onset (median difference, 32.8 hours; P<0.001) than in those who initiated it later (median difference, 13.2 hours; P=0.008). The median time to alleviation of symptoms among placebo recipients was shorter in those enrolled in Japan (77.7 hours) than in those enrolled in the United States (117.9 hours), but the magnitude of the difference in the time to alleviation of symptoms between the baloxavir group and the placebo group was similar in the two countries (median difference, 31.3 hours in Japan and 30.6 hours in the United States). The median time to alleviation of symptoms was similar in the baloxavir group (53.5 hours) and the oseltamivir group (53.8 hours) (Fig. S4 in the Supplementary Appendix).

The median time to the resolution of fever was shorter with baloxavir than with placebo (24.5 hours vs. 42.0 hours, P<0.001). The median time to a return to usual health was 129.2 hours in the baloxavir group and 168.8 hours in the placebo group; the difference was not significant (P=0.06). No deaths occurred during the trial, and there was one hospitalization (in the oseltamivir group). The frequency of complications that resulted in antibiotic treatment was low (3.5% with baloxavir, 4.3% with placebo, and 2.4% with oseltamivir).

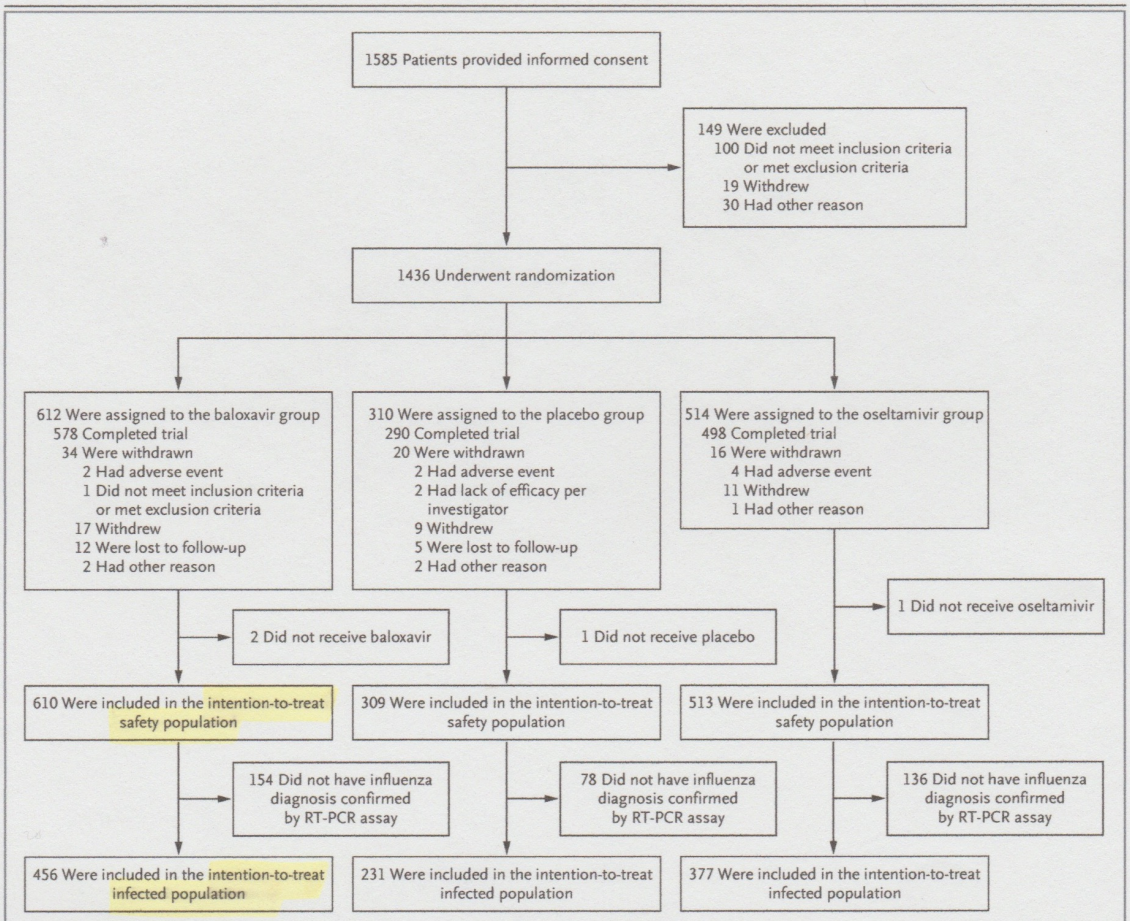

Figure 1. Randomization and Follow-up in the Phase 3 Trial.

The patients were otherwise healthy adolescents and adults with uncomplicated influenza. One patient who was enrolled twice was counted only once. The intention-to-treat safety population consisted of the patients who received the trial regimen. RT-PCR denotes reverse transcriptase–polymerase chain reaction.

Baloxavir was associated with significantly more rapid declines in infectious viral load than placebo or oseltamivir (Fig. 3A and 3B). By 1 day after initiation of the trial regimen, the median reductions from baseline were 4.8, 2.8, and 1.3 $\log_{10}$ $TCID_{50}$ per milliliter in the baloxavir, oseltamivir, and placebo groups, respectively. The reductions in viral RNA loads were also significantly greater with baloxavir than with placebo or oseltamivir (Fig. S5A and S5B in the Supplementary Appendix). The median duration of infectious virus detection was shorter in the baloxavir group (24.0 hours) than in the oseltamivir group (72.0 hours, $P<0.001$) and the placebo group (96.0 hours, $P<0.001$) (Fig. S6A and S6B in the Supplementary Appendix).

The frequencies of neutralizing antibody seroconversion (increase in the neutralizing antibody titer by a factor of ≥4) were similar among patients with influenza A(H1N1)pdm09, influenza A(H3N2), or influenza B infection, and the ratio of antibody titers in serum samples obtained in the convalescent phase and those obtained in the acute phase did not differ significantly among the groups. (For details, see Table S3A and S3B in the Supplementary Appendix.)

Table 1. Baseline Demographic and Clinical Characteristics of the Patients in the Phase 3 Trial.*

Characteristic	Influenza-Positive			Influenza-Negative		
	Baloxavir (N=456)	Placebo (N=231)	Oseltamivir (N=377)	Baloxavir (N=150)	Placebo (N=74)	Oseltamivir (N=132)
Age — yr						
Median	32.0	33.0	35.0	32.5	35.5	38.0
Range	12–64	12–64	20–64	12–61	12–63	20–64
Weight						
Mean — kg	65.4±15.1	67.9±15.6	68.5±16.3	76.3±17.5	78.3±20.0	79.9±17.8
<80 kg — no. (%)	377 (82.7)	190 (82.3)	306 (81.2)	92 (61.3)	45 (60.8)	73 (55.3)
Body-mass index†	23.6±4.6	24.3±5.1	24.4±5.0	27.2±5.7	28.2±5.8	28.4±5.4
Male sex — no. (%)	232 (50.9)	120 (51.9)	218 (57.8)	60 (40.0)	25 (33.8)	55 (41.7)
Country — no. (%)						
Japan	343 (75.2)	175 (75.8)	303 (80.4)	12 (8.0)	5 (6.8)	7 (5.3)
United States	113 (24.8)	56 (24.2)	74 (19.6)	138 (92.0)	69 (93.2)	125 (94.7)
Current smoker — no. (%)	94 (20.6)	56 (24.2)	103 (27.3)	28 (18.7)	11 (14.9)	34 (25.8)
Composite symptom score‡	13.2±3.2	13.5±3.3	13.2±3.1	15.0±3.1	14.5±2.8	15.2±3.3
Body temperature — °C	38.5±0.5	38.4±0.5	38.5±0.5	38.4±0.5	38.4±0.7	38.4±0.6
Time from symptom onset to initiation of the trial regimen — no. (%)§						
≥0 to ≤12 hr	60 (13.2)	34 (14.7)	41 (10.9)	17 (11.3)	13 (17.6)	12 (9.1)
>12 to ≤24 hr	178 (39.0)	87 (37.7)	163 (43.2)	52 (34.7)	34 (45.9)	51 (38.6)
>24 to ≤36 hr	139 (30.5)	67 (29.0)	94 (24.9)	49 (32.7)	20 (27.0)	36 (27.3)
>36 to ≤48 hr	79 (17.3)	43 (18.6)	79 (21.0)	32 (21.3)	7 (9.5)	33 (25.0)
Influenza virus type or subtype on RT-PCR assay at enrollment — no. (%)						
A(H1N1)pdm09	7 (1.5)	7 (3.0)	2 (0.5)	—	—	—
A(H3N2)	393 (86.2)	196 (84.8)	332 (88.1)	—	—	—
B	38 (8.3)	20 (8.7)	34 (9.0)	—	—	—
Mixed infection	8 (1.8)	3 (1.3)	6 (1.6)	—	—	—
A, uncertain subtype¶	10 (2.2)	5 (2.2)	3 (0.8)	—	—	—
Influenza viral load — $\log_{10}$ $TCID_{50}$/ml	5.79±1.87	5.56±1.89	5.94±1.69	—	—	—
Influenza vaccination — no. (%)	108 (23.7)	55 (23.8)	98 (26.0)	22 (14.7)	10 (13.5)	14 (10.6)

* Plus–minus values are means ±SD. Influenza status (positive or negative) was based on a reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay at enrollment (trial day 1). Influenza-positive patients made up the intention-to-treat infected population. $TCID_{50}$ denotes the 50% tissue-culture infective dose.
† The body-mass index is the weight in kilograms divided by the square of the height in meters.
‡ The composite symptom score ranged from 0 to 21, with higher scores indicating more severe illness. Patients assessed the severity of seven influenza-associated symptoms (cough, sore throat, headache, nasal congestion, feverishness or chills, muscle or joint pain, and fatigue) on a 4-point scale (with 0 indicating no symptoms, 1 mild symptoms, 2 moderate symptoms, and 3 severe symptoms).
§ A significantly higher percentage of patients initiated the trial regimen within 24 hours after illness onset in Japan (56%) than in the United States (41%) ($P<0.001$ by Fisher's exact test).
¶ The virus subtype could not be determined by RT-PCR assay.

In patients with paired sequenced samples, PA I38T/M amino acid substitutions were detected after initiation of the trial regimen in 9.7% of 370 baloxavir recipients (all the recipients with these substitutions had influenza A(H3N2) infection), typically at day 5 or later, but in none of 95 randomly selected placebo recipients. PA non-I38 substitutions (>25 different ones) were found in approximately 8% of both baloxavir recipients and placebo recipients (Table S5 in the Supplementary Appendix), although their effect on susceptibility to baloxavir has yet to be assessed. Infectious virus was detected on day 5 in 7% of baloxavir recipients shedding viruses without PA substitutions (22 of 295 patients), 91% of baloxavir recipients with I38T/M substitutions (29 of 32 patients), and 31% of placebo recipients (27 of 87 patients). On day 9, the percentages were 2% (5 of 288 patients), 17% (6 of 36), and 6% (5 of 90), respectively. The median time to alleviation of symptoms was longer in baloxavir recipients with I38T/M substitutions than in those without variants (63.1 hours vs. 49.6 hours); 36% (13 of 36 patients) and 31% (94 of 304 patients), respectively, had a time to alleviation of symptoms that exceeded the median time (80.2 hours) in the placebo group.

Safety and Side-Effect Profile

Adverse events were reported in 20.7% of baloxavir recipients, 24.6% of placebo recipients, and 24.8% of oseltamivir recipients (Table 2). Adverse events that were associated with cessation of the trial regimen occurred in 0.3 to 0.4% of patients across groups. Two serious adverse events were noted in baloxavir recipients (incarcerated inguinal hernia and aseptic meningitis), but neither was considered to be related to the trial regimen by investigators who were unaware of the trial-group assignments. Adverse events that were considered to be related to the trial regimen were more common in oseltamivir recipients (8.4%) than in baloxavir recipients (4.4%, P=0.009) or placebo recipients (3.9%).

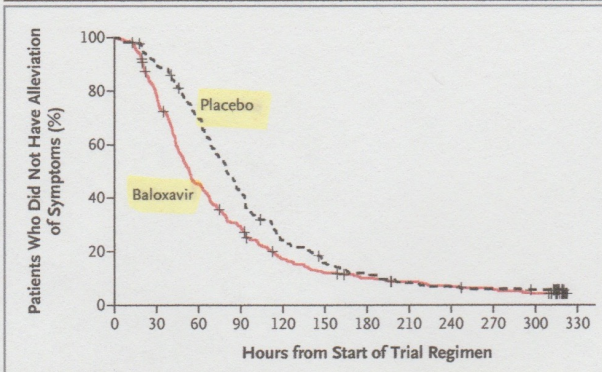

Figure 2. Kaplan–Meier Curves of the Time to Alleviation of Influenza Symptoms with Baloxavir versus Placebo in the Phase 3 Trial.

Shown are data for 455 patients assigned to baloxavir and 230 assigned to placebo (intention-to-treat infected population; 1 patient in each group did not have data that could be evaluated). The median time to alleviation of symptoms was 26.5 hours shorter in the baloxavir group (53.7 hours; 95% CI, 49.5 to 58.5) than in the placebo group (80.2 hours; 95% CI, 72.6 to 87.1) (P<0.001). Data from patients who did not have alleviation of symptoms were censored (tick marks) at the last observation time point.

DISCUSSION

These trials showed that single doses of the cap-dependent endonuclease inhibitor baloxavir were superior to placebo in alleviating influenza symptoms in patients with uncomplicated influenza, without clinically significant side effects. In the phase 3 trial, the difference in the time to alleviation of symptoms between the baloxavir group and the placebo group was greater in patients who initiated the trial regimen early (≤24 hours) after symptom onset than in those who initiated it later, a finding that is consistent with those observed in earlier studies of neuraminidase inhibitors.[15-17] Despite differences in the time to alleviation of symptoms between patients enrolled in Japan and those enrolled in the United States (perhaps related to differences in health care–seeking behavior or symptom reporting), baloxavir treatment was associated with similar clinical benefit in the two countries.

Baloxavir was superior to both placebo and oseltamivir in antiviral activity. The magnitude and rapidity of antiviral effects of single doses of baloxavir in these two trials were greater than those observed with systemic neuraminidase inhibitors in earlier studies involving adults with uncomplicated influenza.[16,17] The infectious virus titer after 1 day was lower by 3.5 $\log_{10}$ $TCID_{50}$ per milliliter with baloxavir than with placebo, whereas the difference between oseltamivir and placebo was 1.5 $\log_{10}$ $TCID_{50}$ per milliliter in the phase 3 trial and 0.5 to 1.0 $\log_{10}$ $TCID_{50}$ per milliliter in two previous randomized, controlled trials.[16,17] Similarly, the difference between intravenous peramivir and placebo was less than 1.0 $\log_{10}$ $TCID_{50}$ per

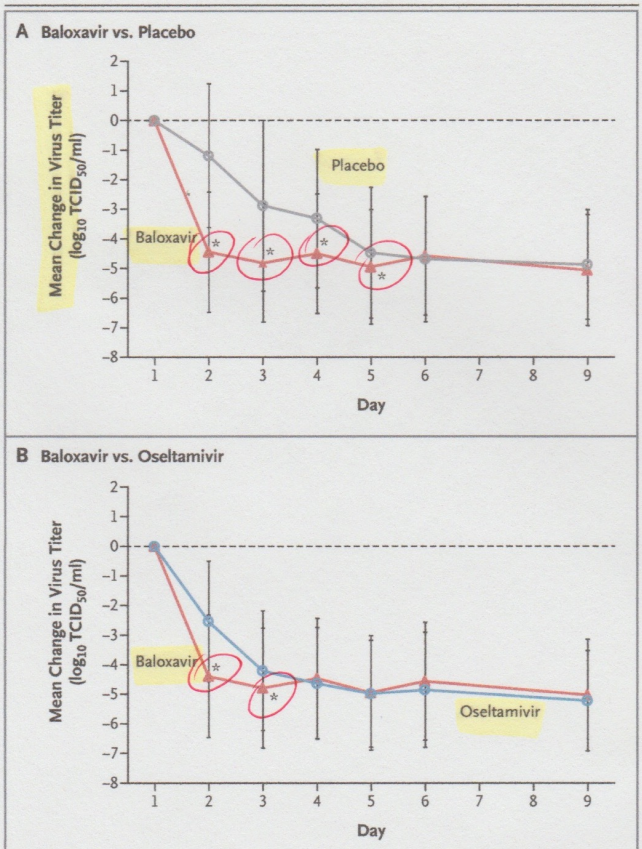

Figure 3. Change from Baseline in Influenza Infectious Viral Load over Time in the Phase 3 Trial.

Panel A shows the change from baseline (dashed line) in influenza infectious viral load over time in the baloxavir group (427 patients) and placebo group (210 patients). The mean (±SD) viral loads on day 1 (before the initiation of the trial regimen) were 5.79±1.87 and 5.56±1.89 log_{10} 50% tissue-culture infective dose ($TCID_{50}$) per milliliter in the baloxavir and placebo groups, respectively. Asterisks indicate a P value of less than 0.05 for the comparison with placebo. Panel B shows the change from baseline in influenza infectious viral load in adults 20 to 64 years of age in the baloxavir group (352 patients) and oseltamivir group (359 patients). The mean (±SD) viral loads on day 1 were 5.76±1.90 and 5.94±1.69 log_{10} $TCID_{50}$ per milliliter in the baloxavir and oseltamivir groups, respectively. Asterisks indicate a P value of less than 0.05 for the comparison with oseltamivir. In both panels, I bars indicate standard deviations.

milliliter after 1 day.[18] The possibility that this antiviral effect might be associated with a reduced risk of virus transmission requires further study.

It is unclear why the time to alleviation of symptoms was similar in the baloxavir group and the oseltamivir group even though baloxavir showed greater antiviral activity. The findings suggest that the symptom benefit of antiviral agents may have a ceiling in self-limited influenza illness in adults, perhaps because viral replication levels are decreasing by the time of presentation and illness pathogenesis is linked to host proinflammatory responses. However, the duration of influenza virus replication is longer in high-risk groups — such as infants, the elderly, hospitalized patients (including those with avian influenza), and immunocompromised hosts — than in otherwise healthy persons with uncomplicated infections.[19-26] Previous reports of oseltamivir treatment in seriously ill patients with influenza have shown prolonged virus replication, specifically protracted detection in the lower respiratory tract of critically ill patients with influenza A(H1N1)pdm09–associated viral pneumonia without the emergence of antiviral resistance.[27] A doubling of the oseltamivir dose[28] or the administration of intravenous peramivir or zanamivir[29,30] does not appear to enhance antiviral activity or clinical outcomes.

The antiviral effects that were observed with baloxavir in patients with uncomplicated influenza provide encouragement with respect to its potential value in treating complicated or severe influenza infections. Baloxavir has shown synergistic antiviral effects with neuraminidase inhibitors in vitro.[31] Such combinations could be studied clinically to determine whether they reduce the risk of resistance emergence and enhance clinical efficacy.

All influenza-specific antiviral agents lead to the emergence of resistant variants.[15] Specific amino acid substitutions in the active endonuclease site (I38T/F) reduce susceptibility to baloxavir by a factor of 11 to 57 for representative influenza A viruses in cell culture.[32] Variant viruses with I38T/M/F substitutions that confer reduced susceptibility to baloxavir were detected in 2.2% of baloxavir recipients in the phase 2 trial (all with influenza A(H1N1)pdm09 infection) and 9.7% of recipients in the phase 3 trial (all with influenza A(H3N2) infection), sometimes in association with rebounds in viral titers and possibly prolongation of symptoms. Although laboratory strains of influenza viruses with the I38T PA substitution showed reduced replication fitness in cell culture,[32,33] the substitution had no effect on polymerase activity in a reporter assay.[34] Therefore, ongoing characterization of the frequency, replication competence, and transmission fitness

Table 2. Adverse Events during the Phase 3 Trial (Safety Population).*

Event	Baloxavir (N=610)		Placebo (N=309)		Oseltamivir (N=513)	
	Any Grade	Grade 3 or 4	Any Grade	Grade 3 or 4	Any Grade	Grade 3 or 4
	number of patients (percent)					
Any adverse event	126 (20.7)	6 (1.0)	76 (24.6)	4 (1.3)	127 (24.8)	1 (0.2)
Adverse events reported in ≥1% of patients in any group						
Diarrhea	18 (3.0)	1 (0.2)	14 (4.5)	1 (0.3)	11 (2.1)	0
Bronchitis	16 (2.6)	0	17 (5.5)	1 (0.3)	18 (3.5)	0
Nasopharyngitis	9 (1.5)	0	2 (0.6)	0	4 (0.8)	0
Nausea	8 (1.3)	1 (0.2)	4 (1.3)	1 (0.3)	16 (3.1)	0
Sinusitis	7 (1.1)	0	8 (2.6)	1 (0.3)	5 (1.0)	0
Increase in ALT level	6 (1.0)	0	4 (1.3)	0	7 (1.4)	0
Headache	5 (0.8)	1 (0.2)	3 (1.0)	0	4 (0.8)	0
Vomiting	5 (0.8)	1 (0.2)	2 (0.6)	0	6 (1.2)	0
Dizziness	3 (0.5)	0	4 (1.3)	0	1 (0.2)	0
Leukopenia	0	0	3 (1.0)	0	1 (0.2)	0
Constipation	0	0	3 (1.0)	0	0	0
Adverse event considered to be related to the trial regimen	27 (4.4)	2 (0.3)	12 (3.9)	1 (0.3)	43 (8.4)†	0
Adverse events considered to be related to the trial regimen and reported in ≥1% of patients in any group						
Diarrhea	11 (1.8)	1 (0.2)	4 (1.3)	0	7 (1.4)	0
Nausea	2 (0.3)	1 (0.2)	2 (0.6)	1 (0.3)	8 (1.6)	0
Serious adverse event	2 (0.3)	2 (0.3)	0	0	0	0
Adverse event leading to discontinuation of the trial regimen‡	2 (0.3)	0	1 (0.3)	1 (0.3)	2 (0.4)	0

* The severity of an event was categorized by the investigators according to definitions based on the Common Terminology Criteria for Adverse Events, version 4.0. ALT denotes alanine aminotransferase.
† No significant differences were noted between the groups except for the prespecified comparison of adverse events that were considered to be related to the trial regimen, which were more common in the oseltamivir group than in the baloxavir group (P=0.009).
‡ Adverse events leading to discontinuation of the trial regimen occurred in two patients who received baloxavir (bronchitis and pneumonia in one patient and acute bronchitis in one patient), in one patient who received placebo (nausea, hip pain, low back pain, and jaw pain), and in two patients who received oseltamivir (nausea in one patient and pneumonia in one patient).

of clinical isolates with reduced susceptibility to baloxavir is needed.

In conclusion, single-dose oral baloxavir in these modest-size trials did not result in apparent safety concerns and was associated with clinical benefit and antiviral activity in patients with uncomplicated influenza. Because this treatment is inhibitory for influenza virus strains resistant to neuraminidase inhibitors or M2 ion-channel inhibitors, it could provide an option for patients with infections caused by such viruses. A randomized, controlled trial involving patients at high risk for influenza complications (ClinicalTrials.gov number, NCT02949011) is in progress.

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