ORIGINAL ARTICLE

Activity of Oral ALS-008176 in a Respiratory Syncytial Virus Challenge Study

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ABSTRACT

BACKGROUND

Respiratory syncytial virus (RSV) infection is a cause of substantial morbidity and mortality. There is no known effective therapy.

METHODS

We conducted a randomized, double-blind, clinical trial in healthy adults inoculated with RSV. Participants received the oral nucleoside analogue ALS-008176 or placebo 12 hours after confirmation of RSV infection or 6 days after inoculation. Treatment was administered every 12 hours for 5 days. Viral load, disease severity, resistance, and safety were measured throughout the 28-day study period, with measurement beginning before inoculation. The primary end point was the area under the curve (AUC) for viral load, which was assessed immediately before administration of the first dose through the 12th day after inoculation in participants infected with RSV.

RESULTS

A total of 62 participants received placebo or one of three ALS-008176 dosing regimens: 1 loading dose of 750 mg followed by 9 maintenance doses of 500 mg (group 1), 1 loading dose of 750 mg followed by 9 maintenance doses of 150 mg (group 2), or 10 doses of 375 mg (group 3). In the 35 infected participants (23 of whom were treated with ALS-008176), the AUCs for viral load for groups 1, 2, and 3 and the placebo group were 59.9, 73.7, 133.4, and 500.9 $\log_{10}$ plaque-forming-unit equivalents × hours per milliliter, respectively ($P \leq 0.001$). The time to nondetectability on polymerase-chain-reaction assay ($P < 0.001$), the peak viral load ($P \leq 0.001$), the AUC for symptom score ($P < 0.05$), and the AUC for mucus weight were lower in all groups receiving ALS-008176 than in the placebo group. Antiviral activity was greatest in the two groups that received a loading dose — viral clearance was accelerated ($P \leq 0.05$), and the AUC for viral load decreased by 85 to 88% as compared with the placebo group. Within this small trial, no viral rebound or resistance was identified. There were no serious adverse events, and there was no need for premature discontinuation of the study drug.

CONCLUSIONS

In this RSV challenge study, more rapid RSV clearance and a greater reduction of viral load, with accompanying improvements in the severity of clinical disease, were observed in the groups treated with ALS-008176 than in the placebo group. (Funded by Alios BioPharma; ClinicalTrials.gov number, NCT02094365.)

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Respiratory syncytial virus (RSV) infections are a cause of substantial morbidity and mortality in various patient populations worldwide, including children. Globally, RSV infections were estimated to cause 3.4 million hospitalizations and 66,000 to 199,000 deaths in 2005 in children younger than 5 years of age, primarily in the developing world.[1] In U.S. infants, RSV infection causes substantial outpatient disease[2] and is a common cause of hospitalization.[3] The risk of death from respiratory causes is nine times as high among U.S. infants with RSV infection as the risk among infants with influenza.[4] Immunocompromised patients and elderly patients, especially those with coexisting conditions, are also at a higher risk for severe RSV infections.[5-7]

Currently, the standard of care for the management of RSV infection is limited to supportive care.[8] There are no licensed RSV vaccines, and palivizumab, an RSV-specific monoclonal antibody, is recommended only for the prevention of RSV infection in the small percentage of infants who are born prematurely or who have serious underlying conditions.[9,10]

Viral load appears to drive the clinical manifestations of RSV disease. Higher viral loads have been associated with more severe disease in infants and children,[11,12] faster rates of RSV clearance are associated with more rapid improvements in hospitalized children,[13] and the onset, peak, and resolution of disease parallel the viral dynamics in adults.[14]

ALS-008176 is an orally bioavailable prodrug of the novel RSV replication inhibitor ALS-008112, a cytidine nucleoside analogue (the molecular structure of ALS-008176 is shown in Fig. S4 in the Supplementary Appendix, available with the full text of this article at NEJM.org). In nonclinical studies, ALS-008112 enters various types of epithelial cells in the respiratory tract and is subsequently phosphorylated to form an intracellular nucleoside triphosphate with a half-life of approximately 29 hours. The nucleoside triphosphate analogue inhibits RSV replication by means of chain termination.

A phase 1 study of oral ALS-008176 identified no safety signals in a group of 76 healthy adults who received dosing regimens containing up to 2 loading doses of 750 mg followed by up to 26 maintenance doses of 500 mg that were administered every 12 hours over 14 days (see the description of safety data in Section 3.0 in the Supplementary Appendix). The current study, which used an established experimental viral challenge model, was conducted to evaluate proof of concept for the antiviral activity of ALS-008176 in healthy adults infected with a clinical strain of RSV.

Methods

Study Participants

Participants were 18 to 45 years of age and had a median serum neutralizing antibody titer for the Memphis-37b challenge virus of 973 Mu (range, 187 to 2023 Mu), which represented the lowest quartile of the screened population.[14,15] The selection criteria are available in the Supplementary Appendix and in the protocol, which is available at NEJM.org.

Study Design

We conducted this randomized, double-blind, placebo-controlled study over three separate study periods. Up to 22 eligible participants per period were confined to a specialized quarantine unit for 14 days (starting 2 days before inoculation and ending 12 days after inoculation [study days –2 to 12]), and outpatient assessments were conducted on study days 16 and 28 (Fig. S1 in the Supplementary Appendix). On study day 0, participants were inoculated intranasally with 4 $\log_{10}$ plaque-forming-units (PFUs) of the RSV-A Memphis 37b challenge virus.[16] From study day 2 until randomization, we monitored participants twice daily for RSV infection, using a Simplexa (Focus Diagnostics) qualitative reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay. The samples tested were fresh, nonfrozen, nasal washings. Participants underwent randomization and received the first dose of ALS-008176 or placebo approximately 12 hours after the qualitative detection of RSV or on the morning of day 6, whichever occurred first.

The number of participants receiving each dose and the dosing regimens for the first study period were predefined. The protocol allowed for the modification of these specifications in subsequent study periods, by a monitoring group whose members were aware of the study-group assignments, on the basis of emerging data on the numbers of participants who became infected after inoculation, the timing of that infection

relative to randomization, safety, and pharmacokinetic and viral kinetic data (see Section 4.0 and Fig. S1 in the Supplementary Appendix, and the protocol).

In each of the three study periods, ALS-008176 or matching placebo was administered orally every 12 hours over 5 days (10 total doses). Three ALS-008176 dosing regimens were evaluated over the three study periods: a single loading dose of 750 mg followed by 9 maintenance doses of 500 mg (group 1), a single loading dose of 750 mg followed by 9 maintenance doses of 150 mg (group 2), and 10 doses of 375 mg (group 3). The study medication was prepared by a pharmacist who was aware of the treatment assignments. The placebo consisted of methylcellulose and water, which was also used to administer ALS-008176. The investigators and all other members of the study team were unaware of the treatment assignments.

STUDY OVERSIGHT

The study was conducted in accordance with the Declaration of Helsinki (1996 version), the International Conference on Harmonisation Good Clinical Practice guidelines, applicable regional and local regulations, and the study protocol. The protocol was approved by the Medicines and Healthcare Products Regulatory Agency and the Cambridge East Regional Ethics Committee of the National Research Ethics Service, all in the United Kingdom. All participants provided written informed consent. The study was sponsored by Alios BioPharma and was designed and managed by the sponsor and the first author; the sponsor and the first author were also responsible for the data analysis. All authors were involved in the collection, analysis, or interpretation of the data. The authors vouch for the accuracy and completeness of the data and analyses and for the fidelity of the study to the protocol.

END POINTS AND ASSESSMENTS

The prespecified primary end point was the area under the curve (AUC) for viral load in nasal washes, as determined by the measurement of RSV RNA with the use of a quantitative RT-PCR assay.[14,15,17,18] Measurements were obtained immediately before administration of the first dose of the study drug or placebo through day 12. Secondary end points included time to nondetectability of the virus, the slope of the curve for viral load during the first 24 to 48 hours after the start of dosing, peak viral load, scores for symptoms related to RSV, and the AUC for mucus weight. All efficacy data were collected and analyzed by study team members and technicians who were unaware of the treatment assignments.

After the inoculation of participants on study day 0, the RSV load in nasal-wash samples was measured twice daily from study days 2 through 12 and once on study days 16 and 28.[18] Specimens were stored at a temperature of −80°C from the time of sample collection until the time of analysis. Results were reported as $\log_{10}$ PFU equivalents (PFUe) per milliliter. In aliquots of nasal washes, we also amplified and sequenced the RSV *L* gene region that encodes the polymerase domain (amino acids 550 to 1100) to evaluate for mutations developing during treatment that conferred resistance to the study drug. This region includes the sites of all amino acid substitutions previously shown to confer in vitro resistance to ALS-008112 (M628L, A789V, L795I, I796V). There are no other known sites of ALS-008112 resistance. Amino acid sequences were compared with a participant's first available RSV-positive sample and with the reference RSV Memphis 37b sequence.[16] The criteria for selecting participants for the evaluation of resistance are defined in Section 5.0 in the Supplementary Appendix. Although none of the participants met the prespecified criteria for the evaluation of resistance, 29 participants (17 receiving ALS-008176 and 12 receiving placebo) were selected for evaluation on the basis of less stringent, post hoc criteria (defined in Section 5.0 of the Supplementary Appendix).

Symptoms of RSV infection were assessed three times daily during confinement and once on study day 16 with the use of an established participant-reported symptom score.[15] Mucus weight was determined every 24 hours by measuring the net weight of secretions within facial tissues.

Safety data were collected through study day 28 (±3 days). The principal investigator, who was unaware of the treatment assignments, regularly assessed adverse events for the possibility of a relationship between an event and the study medication. Symptoms recorded on diary cards by participants were documented as adverse events only if the investigator considered them to be substantial. Plasma samples were collected

and analyzed with the use of high-performance liquid chromatography and tandem mass spectrometry to characterize the pharmacokinetic assessments of ALS-008176, ALS-008112, and other metabolites after each dose of ALS-008176.

STATISTICAL ANALYSIS

All participants who were inoculated with the challenge virus and received at least one dose of study drug were included in the safety (intention-to-treat [ITT]) population (Fig. S2 in the Supplementary Appendix). Participants who were RSV-positive according to the results of quantitative RT-PCR assay immediately before dosing (baseline) or who subsequently became positive on at least two occasions after baseline were defined as infected and were included in the primary analysis population for efficacy evaluation (ITT-infected population). Pharmacokinetic analysis was performed on all participants in the ITT population from whom at least one blood specimen had been obtained for the purpose of pharmacokinetic measurement.

On the basis of an enrollment of 20 participants per study period and three study periods, the study had a power of approximately 80% to detect a 50% reduction in the AUC for viral load for the comparison of active, drug-containing regimens with placebo, assuming a 45% coefficient of variation in the AUC for viral load. Up to 22 participants per study period were enrolled to allow for dropouts. Randomization was performed with the use of a permuted-block algorithm.

The primary end point was assessed for each of the three regimens for ALS-008176 as compared with placebo with the use of a mixed-effects model that included a repeated-measures approach to allow for unequal variances, with baseline viral load as a covariate. The errors were assumed to be normally distributed, and a standard covariance structure was assumed. The null hypothesis was that there was no difference between each of the three active treatment regimens and placebo. This same model was used to assess the following secondary end points: the slope of the viral load during the 24-hour and 48-hour periods after first dose, peak viral load, and total RSV symptom score for the AUC (from the time just before administration of the first dose to study day 16).

Between-group differences were evaluated with the use of an analysis-of-variance model, with treatment as the dependent variable for the following secondary end points: time to the point at which RSV was not detectable, time to symptom resolution, and the AUC for mucus weight (from the time just before administration of the first dose to study day 12). ALS-008176 dosing regimens were individually compared with the pooled placebo group, and all comparisons were two-sided, with the level of significance set at 0.05. The results for participants receiving the same dosing regimen in different study periods were pooled and analyzed as a single treatment regimen.

RESULTS

PARTICIPANT DISPOSITION AND PRETREATMENT CHARACTERISTICS

Among 302 persons screened for participation in the study (Fig. S2 in the Supplementary Appendix), 64 met the criteria for eligibility and were inoculated during one of the three study periods. Two participants did not receive a dose; 1 withdrew consent because of a family emergency, and the other had an elevated baseline level of serum alanine aminotransferase that was cause for exclusion. Of the remaining 62 participants (ITT population), 35 (56%) met the definition for RSV infection and were included in the ITT-infected population.

The pretreatment characteristics of the participants were similar across treatment regimens in both the ITT and ITT-infected populations (Table 1). The mean baseline viral load was higher in ITT-infected participants assigned to receive ALS-008176 than in the placebo group.

EFFICACY

A statistically significant reduction ($P \leq 0.001$) in the primary end point, the AUC for viral load, was observed in the ITT-infected population for each ALS-008176 dosing regimen as compared with placebo, with values of 59.9, 73.7, 133.4, and 500.9 $\log_{10}$ PFUe × hours per milliliter in group 1, group 2, group 3, and the placebo group, respectively (Table 2). These values represented respective reductions of 88.0%, 85.3%, and 73.4% of the AUC for viral load relative to placebo.

Viral load increased logarithmically in the placebo group, peaking approximately 3.5 days after randomization. As compared with placebo, the absolute reductions in viral load in the ALS-

Table 1. Baseline Characteristics of the ITT and ITT Infected Populations.*

Characteristic	ITT Population					ITT Infected Population				
	Group 1 ALS-008176 750 mg LD + 500 mg MD (N=14)	Group 2 ALS-008176 750 mg LD + 150 mg MD (N=19)	Group 3 ALS-008176 375 mg (N=11)	All ALS-008176 Groups (N=44)	Placebo (N=18)	Group 1 ALS-008176 750 mg LD + 500 mg MD (N=8)	Group 2 ALS-008176 750 mg LD + 150 mg MD (N=7)	Group 3 ALS-008176 375 mg (N=8)	All ALS-008176 Groups (N=23)	Placebo (N=12)
Age — yr	22.4±3.0	24.7±6.2	23.4±2.4	23.7±4.6	24.6±4.3	22.8±3.4	25.1±8.2	23.6±2.6	23.8±5.0	24.4±5.1
Body-mass index	25.4±2.3	23.8±3.0	23.9±2.4	24.4±2.7	22.4±1.6	25.4±2.0	22.7±2.2	23.5±2.6	23.9±2.5	22.7±1.7
Male sex — no. (%)	10 (71)	12 (63)	9 (82)	31 (71)	14 (78)	6 (75)	5 (71)	7 (88)	18 (78)	9 (75)
Race — no. (%)†										
White	13 (93)	16 (84)	10 (91)	39 (89)	13 (72)	7 (88)	5 (71)	8 (100)	20 (87)	8 (67)
Asian	0	1 (5)	1 (9)	2 (5)	1 (6)	0	1 (14)	0	1 (4)	1 (8)
Black	0	2 (11)	0	2 (5)	1 (6)	0	1 (14)	0	1 (4)	0
Other	1 (7)	0	0	1 (2)	3 (17)	1 (13)	0	0	1 (4)	3 (25)
Viral load (log_{10} PFUe/ml)‡	1.2±1.4	1.1±1.6	1.7±1.6	1.3±1.5	1.1±1.4	2.1±1.2	2.9±1.3	2.4±1.4	2.4±1.3	1.6±1.4

* Plus–minus values are means ±SD. The body-mass index is the weight in kilograms divided by the square of the height in meters. ITT denotes intention to treat, LD loading dose, MD maintenance dose, and PFUe plaque-forming-unit equivalents.

† Race was self-reported.

‡ Baseline samples were obtained from the last nasal wash collected before treatment was initiated; the samples were frozen and shipped to a central laboratory. Viral load was assessed with the use of a quantitative reverse-transcriptase–polymerase-chain-reaction assay.

Table 2. Efficacy Results for the ITT-Infected Population.*

End Point	Group 1 (N=8) ALS-008176 750 mg LD + 500 mg MD	Group 2 (N=7) ALS-008176 750 mg LD + 150 mg MD	Group 3 (N=8) ALS-008176 375 mg	Placebo (N=12)
Primary				
AUC viral load from baseline through day 12 (log_{10} PFUe × hr/ml)	59.9±69.5	73.7±48.3	133.4±118.4	500.9±219.9
P value	P<0.001	P<0.001	P<0.001	
Reduction relative to placebo (%)	88.0	85.4	73.4	
Secondary				
Time to nondetectability of RSV RNA (days)	1.3±1.3	1.4±0.5	2.3±1.5	7.2±3.1
P value	P<0.001	P<0.001	P<0.001	
Viral load slope: baseline to 24 hr after first dose (log_{10} PFUe/ml/24 hr)	−1.7±1.1	−1.7±1.2	−0.2±1.0	0.6±1.8
P value	P<0.001	P>0.02	P=0.66	
Baseline to 48 hours post 1st dose (log_{10} PFUe/ml/48 hr)	−1.0±0.5	−1.1±0.9	−0.6±0.5	1.1±1.0
P value	P<0.001	P<0.001	P<0.001	
Peak viral load (log_{10} PFUe/ml)	2.3±1.3	3.1±1.1	3.1±1.3	5.3±1.2
P value	P<0.001	P<0.001	P<0.001	
Viral load at 3.5 days after dose 1 (log_{10} PFUe/ml)†	0.6±1.1	0.0±0.1	0.8±1.4	4.3±2.1
P value	P<0.001	P<0.001	P<0.001	
AUC of symptom score from baseline through day 16 (change in score × hr)	111.7±94.0	73.2±63.4	113.1±110.3	606.9±564.8
P value	P=0.12	P<0.01	P>=0.01	
AUC of mucus weight from baseline through day 12 (g × no. days)	3.0±2.0*	5.9±6.3	5.4±7.6	18.6±18.4
P value	P>0.02	P=0.09	P=0.06	

* Plus–minus values are means ±SD. The area under the curve (AUC) was calculated with the use of log_{10} values for viral load. Baseline values were obtained immediately before the first dose of the study drug. P values were calculated for the comparison with placebo. RSV denotes respiratory syncytial virus.

† The viral load at 3.5 days after administration of the first dose represents the time at which the peak mean viral load occurred in the placebo group.

008176 groups were observed within the first 12 hours after the initiation of treatment (i.e., the time between administration of the study drug and the first post-treatment nasal wash) (Fig. 1). The clearance rate of RSV RNA (the slope of the viral load) was most rapid in the two regimens that incorporated a loading dose, but the clearance rates for all three regimens were significantly more rapid during the first 48 hours than the rates for placebo (Table 2 and Fig. 1). There was no evidence of rebound of viral load in any of the treatment regimens after the completion of dosing.

The mean time to nondetectability of RSV RNA ranged from 1.3 to 2.3 days for the three ALS-008176 regimens as compared with 7.2 days for placebo (P <0.001 for all comparisons). RSV RNA became undetectable in all participants in the ALS-008176 regimens within 4.5 days after the initiation of ALS-008176, except in one participant who received the 375-mg regimen, in whom levels of RSV RNA did not become undetectable until 7 days after the initiation of dosing. In contrast, most participants in the placebo group still had detectable levels of RSV RNA at the end of the 5-day dosing period, and it was not until 11 days after the initiation of dosing that RSV RNA levels became undetectable in all

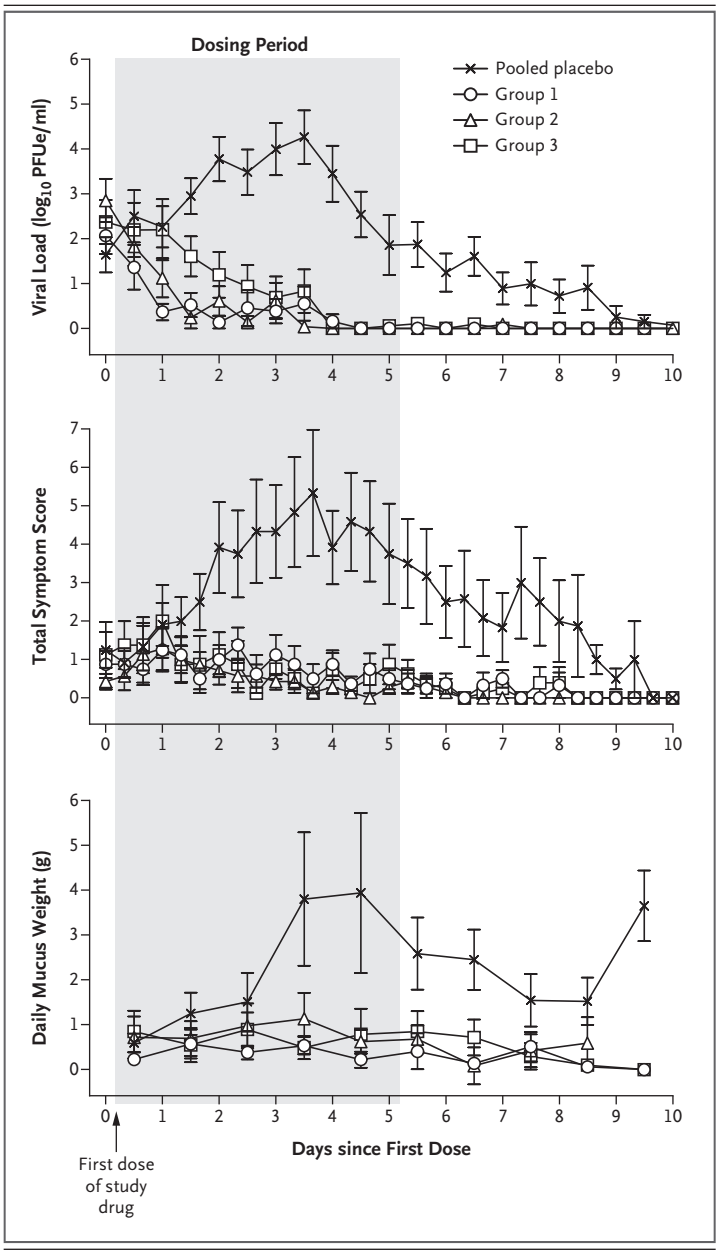

Figure 1. Viral Load, Symptom Score, and Daily Mucus Weight over Time.

Mean viral loads, symptom scores, and mucus weights are shown from the time immediately before administration of the first dose until 10 days afterward (i.e., study day ≥12) in the intention-to-treat infected population. Respiratory syncytial virus (RSV) RNA could not be detected in any participant 16 or 28 days after inoculation. Although participants were inoculated with RSV on the same day, they became infected on different days; consequently, they also began treatment on different days. Viral loads were determined with the use of a quantitative reverse-transcriptase–polymerase-chain-reaction assay of nasal-wash samples, which were collected twice daily and analyzed in a central laboratory. Participants recorded symptoms in diaries three times daily. Mucus weights were measured daily and plotted at the time corresponding to the midpoint of the mucus-collection period each day (i.e., weights plotted midway between the first and second days of treatment are from facial tissues collected between the morning of the first day of study treatment and the morning of the second day). After the loading doses were administered in participants in groups 1 and 2, they received maintenance doses every 12 hours until the conclusion of treatment. Treatment group 1 received a loading dose of 750 mg followed by nine maintenance doses of 500 mg, and treatment group 2 received a loading dose of 750 mg followed by nine maintenance doses of 150 mg. Treatment group 3 received 375 mg every 12 hours for 5 days. Plus–minus values are means ±SE. PFUe denotes plaque-forming-unit equivalents.

participants who received placebo. Mean peak viral loads were lower in each ALS-008176 treatment group than in the placebo group ($P \leq 0.001$ for all comparisons). At the time that the peak viral load occurred in the placebo group, the mean viral load in each of the three ALS-008176 treatment groups was more than 1000 times as low (Fig. 1).

The severity of RSV disease in the three ALS-008176 treatment groups was lower than that in the placebo group as assessed by means of symptom scores and quantity of nasal mucus produced (Fig. 1). The AUC for the total score for RSV symptoms was significantly reduced in each of the three ALS-008176 treatment groups as compared with placebo ($P \leq 0.05$ for all comparisons), and symptom scores and mucus weights in the treatment groups declined in tandem with the decline in viral load.

Sequence analysis of the RSV *L* gene region encoding the polymerase domain did not detect any of the amino acid mutations associated with in vitro resistance to ALS-008112 (M628L, A789V, L795I, and I796V). In addition, no new mutations associated with the potential emergence of resistance to ALS-008112 were identified.

SAFETY

No serious adverse events, premature discontinuations of the study drug, or clinically significant, treatment-related adverse events were observed in any participants in the ITT population. Adverse events were generally balanced in terms of frequency and intensity across recipients of ALS-008176 and placebo, and all but seven ad-

Table 3. Adverse Events.*

Event or Abnormality	Group 1 (N=14) ALS-008176 750 mg LD + 500 mg MD	Group 2 (N=19) ALS-008176 750 mg LD + 150 mg MD	Group 3 (N=11) ALS-008176 375 mg	All ALS-008176 Groups (N=44)	Placebo (N=18)
Adverse event (%)					
Epistaxis†	4 (29)	5 (26)	1 (9)	10 (23)	2 (11)
Upper respiratory tract infection	1 (7)	2 (11)	0	3 (7)	2 (11)
Cough	2 (14)	0	0	2 (5)	1 (6)
Laboratory abnormality (%)‡					
Elevated alanine aminotransferase level§	3 (21)	2 (11)	2 (18)	7 (16)	3 (17)
Elevated aspartate aminotransferase level	2 (14)	0	0	2 (5)	2 (11)
Low platelet count†¶	1 (7)	0	2 (18)	3 (7)	0
Elevated creatine kinase level‖	0	2 (11)	0	2 (5)	0

* Safety analyses were based on the primary safety analysis (ITT) population, which was defined as those participants who were inoculated with the challenge virus and received at least one dose of the study drug. An adverse event was listed in this table if it occurred after the start of the study medication in two or more participants receiving a treatment regimen or placebo. Participants were assessed from the time of RSV inoculation through day 28 (±3). See Section 3.0 in the Supplementary Appendix for further details on evaluations of safety.

† Epistaxis is a recognized complication of upper respiratory tract infection.[17] Epistaxis did not occur in participants whose platelet count was low enough to qualify as an adverse event. All episodes of epistaxis resolved without the need for treatment.

‡ When a laboratory abnormality was considered to be an adverse event, the Division of Acquired Immunodeficiency Syndrome (DAIDS) toxicity grading system was used to define the severity of the event except for cases in which there was no DAIDS criterion for the particular abnormality.

§ A single asymptomatic grade 3 event occurred in treatment group 1 in which the alanine aminotransferase level peaked at 3.6 times the upper limit of the normal range. This peak occurred on day 5 of dosing, and the principal investigator indicated that it was unlikely to be related to the study drug. No potentially hepatotoxic concomitant medications or other causes were identified. Overall, elevations of grades 1–2 (mild to moderate) were balanced across the treatment groups.

¶ The lowest platelet count (116,000 per cubic millimeter) was observed in a participant in whom thrombophlebitis developed at an intravenous cannula site on the same day as the day of sample collection. The lowest values observed in the two remaining participants for whom a low platelet count was defined as an adverse event were 129,000 per cubic millimeter and 134,000 per cubic millimeter. (The lower limit of a normal platelet count was defined as 150,000 per cubic millimeter.) All three events resolved without the need for treatment. The principal investigator at the quarantine unit considered it possible that the count of 116,000 per cubic millimeter was related to the study drug and unlikely that the other two events were related to the study drug.

‖ Elevated levels of creatine kinase were reported 9 to 10 days after the completion of dosing with ALS-008176. Both events occurred after the participants were discharged from the quarantine unit (on study day 16), and each was preceded by strenuous exercise. None of these participants had an elevation of grade 1 or higher during dosing or at any time before discharge from quarantine on study day 12. The principal investigator considered it unlikely that either event was related to the study drug.

verse events were mild in severity. Among these seven events, five events — one instance of low platelet levels (in treatment group 1), three instances of elevated alanine aminotransferase levels (in one participant each in treatment group 1, treatment group 2, and the placebo group), and one instance of elevated creatine kinase levels (in treatment group 2) — were moderate. Two events — one instance of elevated alanine aminotransferase levels in group 1 and one instance of elevated creatine kinase levels in group 2 — were severe in intensity. Neither severe adverse event was considered by the investigator to be related to the study drug. The most commonly reported adverse events (more than two participants reporting an event in any one treatment group) are listed in Table 3. (See Table S4 in the Supplementary Appendix for a comprehensive listing of all adverse events reported after the initiation of dosing; see Section 3.0 in the Supplementary Appendix for further descriptions of safety data in a phase 1 study of ALS-008176.) No clinically relevant findings were identified from laboratory analyses, electrocardiography, or physical examination, including vital signs.

PHARMACOKINETICS

As would be expected for a prodrug that is rapidly metabolized to its parent compound, ALS-008176 concentrations were below the limit of

Table 4. Pharmacokinetic Measures of ALS-008112 after Oral Dosing for ALS-008176.*

Measure	Group 1 (N=14)		Group 2 (N=19)		Group 3 (N=11)	
	ALS-008176 750 mg LD + 500 mg MD		ALS-008176 750 mg LD + 150 mg MD		ALS-008176 375 mg	
	Dose 1	Dose 9 or 10	Dose 1	Dose 9 or 10	Dose 1	Dose 9 or 10
C_{max} (ng/ml)	1740±655	1670±528	1730±750	601±210	1160±555	1580±485
T_{max} (hr)	1.1±0.6	1.1±0.6	1.5±1.4	0.8±0.6	1.0±0.7	0.7±0.3
$AUC_{0–12\ hr}$ (ng × hr/ml)	5660±1060	5400±881	5960±1620	1780±263	2710±584	4300±569
C_{min} (ng/ml)	NA	151±48	NA	65±16	NA	114±23

* Levels of ALS-008112 in plasma samples were determined with the use of high-performance liquid chromatography and tandem mass spectrometry. ALS-008176 is a oral prodrug and therefore was rapidly absorbed and efficiently converted to ALS-008112. The active antiviral is formed intracellularly after the triphosphorylation of ALS-008112. Antiviral effects are related to the intracellular concentrations of this triphosphorylated form of ALS-008112, which has an intracellular half-life of approximately 29 hours. The pharmacokinetic population was defined as all participants who received at least one dose of ALS-008176 (ITT population) and from whom at least one measurement of drug concentration was obtained. Actual sample times were used for all analyses. For each of the three regimens, ALS-008176 was administered orally every 12 hours for 5 days. Sampling for pharmacokinetic data occurred on all 5 days of the study. If the first daily dose was administered in the morning, sampling for pharmacokinetic data occurred before administration and at 0.5, 1, 2, 4, 6, 8, 10, and 12 hours after administration. If the first daily dose was administered in the evening, sampling for pharmacokinetic data occurred before administration and at 0.5, 1, 2, 4, and 6 hours after administration. Steady-state pharmacokinetic sampling occurred after a morning dose (maintenance dose 9 or 10) at the following time points: before administration of the dose and at 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 12 hours after administration. Plus–minus values are means ±SD. $AUC_{0–12\ hr}$ denotes area under the concentration–time curve from time 0 to 12 hours, C_{max} the maximum observed concentration, C_{min} the minimum concentration (trough), NA not assessed, and T_{max} the time to reach the maximum plasma concentration.

quantitation at most time points. After administration of the first dose of 750 mg or 375 mg, the T_{max} of the parent nucleoside, ALS-008112, was approximately 1.0 hour and, at steady state (doses 9 or 10), exposures of ALS-008112 appeared to increase in a dose-proportional manner (Table 4). The terminal half-life of ALS-008112 was 63 hours. The pharmacokinetic assessments of the major metabolite of ALS-008112 are shown in Table S1 in the Supplementary Appendix.

DISCUSSION

As compared with placebo, the administration of ALS-008176 resulted in a reduction of viral load, the AUC for viral load, peak viral load, and duration of viral shedding in the 23 infected participants. These effects were associated with concomitant reductions in the severity of clinical disease.

The reason for the antiviral effect probably reflects the mechanism of action of ALS-008176, which inhibits RSV replication within cells already infected in addition to protecting uninfected host respiratory epithelial cells. Other RSV antiviral drugs (e.g., monoclonal antibodies[19-21] and small-molecule fusion inhibitors[22]) prevent the infection of uninfected cells by blocking cellular entry of the virus, but these drugs do not inhibit viral replication within cells that have already been infected.

Consistent with this mechanism of action, treatment with ALS-008176 resulted in antiviral effects that were different from those observed with a fusion inhibitor in an RSV challenge study with a similar design.[17] First, reduction in viral load was observed to occur more rapidly. The first assessment of viral load, performed 12 hours after ALS-008176 dosing, revealed that the load was already lower than the predose load by 0.5 $\log_{10}$ PFUe per milliliter. In contrast, administration of the fusion inhibitor GS-5806 was associated with an increase in viral load for 24 hours after administration, at which point the load began to decline. Second, the extent of suppression of viral replication was greater with ALS-008176. The reduction in the AUC for viral load in participants treated with ALS-008176 was 73 to 88% as compared with the AUC for viral load for placebo, and the virus was undetectable in all but one participant within 4.5 days of the start of treatment. In contrast, in participants treated with the fusion inhibitor, mean

reduction in the AUC for viral load as compared with placebo was 38 to 67%, and participants continued to have mean viral loads of approximately 0.5 $\log_{10}$ PFUe per milliliter for up to 9.5 days after the initiation of treatment. These differences in antiviral effects may translate into clinical benefit or expansion of the therapeutic window, but therapeutic activity and dosing regimens must be determined in clinical trials in naturally infected populations that include immunocompromised patients.

Nucleoside analogues typically have higher barriers to emerging resistance for viruses with higher mutation rates, including the hepatitis C and hepatitis B viruses.[23,24] A higher barrier to resistance was also observed with ALS-008176 in vitro but not with RSV fusion inhibitors.[25,26] Although we observed no emergence of resistant viruses, the sample size was quite small for the purposes of such detection. The potential for the emergence of resistance is lower in immunocompetent hosts than in immunocompromised patients, who are known to have prolonged and elevated levels of RSV replication. The emergence of resistance during therapy with RSV monoclonal antibodies has been documented.[27] In such situations, nucleoside analogues may offer advantages. More definitive conclusions regarding viral resistance to ALS-008176 should be drawn from patients who become infected in natural settings.

Our proof-of-concept trial had several limitations in addition to the small number of participants. Despite waiting approximately 12 hours after viral detection before starting treatment, we did provide treatment early in the course of infection. In addition, the participants' infections were limited to their upper respiratory tract, and all had preexisting immune memory, which may have aided the eradication of the virus. Patients, particularly infants, who are infected in natural circumstances are likely to present later in the course of disease, with greater disease severity and with more limited preexisting immunity. Therefore, it may be inappropriate to directly extrapolate the results of the study to a clinical setting. The relatively small study size limited our ability to identify potential concerns about the safety of ALS-008176.

In conclusion, this anti-RSV compound shows potential for inhibiting viral replication, reducing clinical disease severity, and clearing virus in infected patients. Clinical trials evaluating ALS-008176 in these patient populations are needed.

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REFERENCES

1. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010;375:1545-55.
2. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med 2009;360:588-98.
3. Leader S, Kohlhase K. Recent trends in severe respiratory syncytial virus (RSV) among US infants, 1997 to 2000. J Pediatr 2003;143:Suppl:S127-S132.
4. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 2003;289:179-86.
5. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med 2005;352:1749-59.
6. Lee N, Lui GC, Wong KT, et al. High morbidity and mortality in adults hospitalized for respiratory syncytial virus infections. Clin Infect Dis 2013;57:1069-77.
7. Widmer K, Zhu Y, Williams JV, Griffin MR, Edwards KM, Talbot HK. Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults. J Infect Dis 2012;206:56-62.
8. Ventre K, Randolph AG. Ribavirin for respiratory syncytial virus infection of the lower respiratory tract in infants and young children. Cochrane Database Syst Rev 2007;1:CD000181.
9. American Academy of Pediatrics Committee on Infectious Diseases, American Academy of Pediatrics Bronchiolitis Guidelines Committee. Updated guidance for palivizumab prophylaxis among infants and young children at increased risk of hospitalization for respiratory syncytial virus infection. Pediatrics 2014;134:415-20.
10. American Academy of Pediatrics Committee on Infectious Diseases, American Academy of Pediatrics Bronchiolitis Guidelines Committee. Updated guidance for palivizumab prophylaxis among infants and young children at increased risk of hospitalization for respiratory syncytial virus infection. Pediatrics 2014;134(2):e620-e638.
11. Buckingham SC, Bush AJ, Devincenzo JP. Nasal quantity of respiratory syncytical virus correlates with disease severity in hospitalized infants. Pediatr Infect Dis J 2000;19:113-7.
12. DeVincenzo JP, El Saleeby CM, Bush AJ. Respiratory syncytial virus load predicts disease severity in previously healthy infants. J Infect Dis 2005;191:1861-8.
13. El Saleeby CM, Bush AJ, Harrison LM, Aitken JA, Devincenzo JP. Respiratory syncytial virus load, viral dynamics, and disease severity in previously healthy naturally infected children. J Infect Dis 2011;204:996-1002.
14. DeVincenzo JP, Wilkinson T, Vaishnaw A, et al. Viral load drives disease in humans experimentally infected with respiratory syncytial virus. Am J Respir Crit Care Med 2010;182:1305-14.
15. DeVincenzo J, Lambkin-Williams R, Wilkinson T, et al. A randomized, double-

blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus. Proc Natl Acad Sci U S A 2010;107:8800-5.
16. Kim YI, DeVincenzo JP, Jones BG, et al. Respiratory syncytial virus human experimental infection model: provenance, production, and sequence of low-passaged memphis-37 challenge virus. PLoS One 2014;9(11):e113100.
17. DeVincenzo JP, Whitley RJ, Mackman RL, et al. Oral GS-5806 activity in a respiratory syncytial virus challenge study. N Engl J Med 2014;371:711-22.
18. Perkins SM, Webb DL, Torrance SA, et al. Comparison of a real-time reverse transcriptase PCR assay and a culture technique for quantitative assessment of viral load in children naturally infected with respiratory syncytial virus. J Clin Microbiol 2005;43:2356-62.
19. Johnson S, Griego SD, Pfarr DS, et al. A direct comparison of the activities of two humanized respiratory syncytial virus monoclonal antibodies: MEDI-493 and RSHZl9. J Infect Dis 1999;180:35-40.
20. Johnson S, Oliver C, Prince GA, et al. Development of a humanized monoclonal antibody (MEDI-493) with potent in vitro and in vivo activity against respiratory syncytial virus. J Infect Dis 1997;176:1215-24.
21. Wu H, Pfarr DS, Johnson S, et al. Development of motavizumab, an ultrapotent antibody for the prevention of respiratory syncytial virus infection in the upper and lower respiratory tract. J Mol Biol 2007;368:652-65.
22. Douglas JL, Panis ML, Ho E, et al. Small molecules VP-14637 and JNJ-2408068 inhibit respiratory syncytial virus fusion by similar mechanisms. Antimicrob Agents Chemother 2005;49:2460-6.
23. McCown MF, Rajyaguru S, Le Pogam S, et al. The hepatitis C virus replicon presents a higher barrier to resistance to nucleoside analogs than to nonnucleoside polymerase or protease inhibitors. Antimicrob Agents Chemother 2008;52:1604-12.
24. Zoulim F. Hepatitis B virus resistance to antiviral drugs: where are we going? Liver Int 2011;31:Suppl 1:111-6.
25. Lundin A, Bergström T, Bendrioua L, Kann N, Adamiak B, Trybala E. Two novel fusion inhibitors of human respiratory syncytial virus. Antiviral Res 2010;88:317-24.
26. Yan D, Lee S, Thakkar VD, Luo M, Moore ML, Plemper RK. Cross-resistance mechanism of respiratory syncytial virus against structurally diverse entry inhibitors. Proc Natl Acad Sci U S A 2014;111(33):E3441-E3449.
27. Grad YH, Newman R, Zody M, et al. Within-host whole-genome deep sequencing and diversity analysis of human respiratory syncytial virus infection reveals dynamics of genomic diversity in the absence and presence of immune pressure. J Virol 2014;88:7286-93.