

A Phase II Study of DAS181, a Novel Host Directed Antiviral for the Treatment of Influenza Infection

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(See the editorial commentary by Ison, on pages 1806–8.)

Background. DAS181, a novel host-directed antiviral in development for influenza treatment, was assessed in this phase II clinical trial.

Methods. This study was a double-blind, placebo-controlled phase II clinical trial assessing influenza viral load and patient safety in otherwise healthy influenza-infected participants. Participants were randomized to a single-dose, multiple-dose, or placebo group and were followed for safety and virologic outcomes.

Results. A total of 177 laboratory-confirmed influenza-infected participants were enrolled in the trial, which encompassed 3 influenza seasons from 2009–2011 in both the Northern and Southern Hemispheres. Thirty-seven percent of participants had confirmed infection with influenza B, 33% with seasonal H3N2, 29% with pandemic 2009 H1N1, and 1 participant was positive for both influenza B and pandemic 2009 H1N1. Significant effects were observed in regard to decreased change from baseline viral load and viral shedding in the multiple-dose group compared with placebo as measured by quantitative polymerase chain reaction ($P < .05$). No instances of H274Y were observed among viral isolates from this trial. Overall, the drug was generally well tolerated.

Conclusions. DAS181 significantly reduced viral load in participants infected with influenza, thus warranting future clinical development of this novel host-directed therapy.

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Influenza remains an infection with potential for significant morbidity and mortality. The 1918 influenza pandemic resulted in 40 million to 100 million deaths [1]. Persistent genetic changes in the virus result in highly susceptible populations and a need to develop epitope-specific vaccines yearly [2]. Additionally, immunization can be less effective in older individuals and those with compromised immune status [3, 4]. Antiviral drugs are available to treat influenza but can have reduced efficacy depending on the prevalence of viral resistance and the population being treated. Furthermore, the appearance of avian influenza (H5N1),

with mortality possibly reaching 50% of those infected, supports the need for more effective treatment modalities [5].

Because all influenza viruses bind to sialic acids on respiratory epithelial cells, blockage of this interaction has the potential to decrease the magnitude of infection of all influenza variants [6]. DAS181 (Fludas) is a sialidase catalytic domain/amphiregulin glycosaminoglycan binding sequence fusion protein that cleaves both the Neu5Ac $\alpha(2,3)$ - and Neu5Ac $\alpha(2,6)$ -Gal linkages of sialic acid on host cells. DAS181 is administered as an inhalable dry powder to deliver sialidase to the pulmonary epithelium for cleavage of sialic acids, which renders the cells inaccessible to infection by virus [7]. Given the conserved nature of influenza binding to respiratory epithelium, a host-directed approach to the treatment of influenza may be applicable to the treatment of all influenza subtypes. Preclinical in vitro and in vivo studies demonstrated that DAS181 has activity against a number of seasonal influenza strains including those containing the H274Y

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mutation (conferring resistance to oseltamivir), highly pathogenic avian influenza strains (H5N1), and pandemic 2009 influenza A (H1N1) [8–10]. Three phase 1 studies have examined different formulations of DAS181 administered as single and multiple doses in healthy participants. In these phase 1 clinical trials, DAS181 was found to be well tolerated in all treatment groups, and no serious adverse events were observed.

METHODS

Study Design

This was a randomized, double-blind, placebo-controlled phase II study to examine the effects of single-day vs multiple-day dosing of inhaled DAS181 compared with placebo in otherwise healthy adult participants with laboratory-confirmed influenza. The primary objective of the study was to determine the safety and tolerability of DAS181 in participants diagnosed with laboratory-confirmed influenza and to assess the effect of DAS181 on influenza viral load. The primary endpoints were change in viral load as measured by polymerase chain reaction (PCR) using area-under-the-curve (AUC) metric as well as safety. Secondary endpoints included change in viral load, time to decreased viral shedding, and time to clinical symptom resolution. The safety analysis population was defined as all randomized participants who received at least 1 dose of the study drug or placebo. The modified-intent-to-treat (mITT) population was defined as all participants randomized who received at least 1 dose of study drug or placebo with influenza confirmed by quantitative PCR (q-PCR) testing. Participants with a q-PCR result of ≥ 500 viral RNA copies/mL at day 1 from either the pharyngeal wash (PW) or nasal wash (NW) were considered to have confirmed influenza.

Male and female participants who gave informed consent, were in generally good health, aged 18 to 70 years, were febrile with oral temperature $>37.8^{\circ}\text{C}$ or reported temperature $>37.8^{\circ}\text{C}$ or were feeling “feverish” in the past 24 hours and who had either 1 or more respiratory symptoms (cough, sore throat, nasal symptoms) or constitutional symptoms (headache, myalgia, sweat/chills, prostration) were considered eligible for the study. Participants were required to have a positive rapid antigen test for influenza using any US Food and Drug Administration–approved and Clinical Laboratory Improvement Amendments (CLIA)–waived commercially available kit.

The study was conducted over a period of 3 influenza seasons: 2 Northern Hemisphere seasons (2009–2010, 2010–2011) and 1 Southern Hemisphere season (summer 2011). Eligible participants were randomized equally into 1 of 3 groups: DAS181 10 mg daily for 3 days (multiple dose), DAS181 10 mg for 1 day (single dose), or placebo. PW samples were collected from all participants on days 1, 2, 3 (each prior to that day’s dose) and days 5 and 8. NW samples were obtained at baseline and on day 5 for all participants. Respiratory PW and

NW samples were analyzed by q-PCR assays performed by ViraCor-IBT (Lees Summit, MO). The limit of detection (LOD) for each q-PCR assay specific to influenza subtypes ranged from 150 to 500 viral RNA copies/mL. A conservative approach was taken in that the LOD utilized in the statistical analysis was 500 viral RNA copies/mL for all assays. Median tissue culture infective dose (TCID₅₀) was measured by Viro-Clinics (The Netherlands); the LOD for this assay was 0.8 log₁₀ TCID₅₀. Decreased virologic shedding was defined as at least a 1-log decrease in virus levels from baseline that had to be sustained so that subsequent timepoints reached the same or lower threshold. Time-to-event analysis was analyzed using day 1 as baseline and was also adjusted for time-of-symptom onset. Diaries with flu-like (IFV) symptoms of nasal congestion, sore throat, cough, aches and pains, fatigue (tiredness), headache, and chills/sweats (feeling feverish) were collected on days 1, 2, 3, 5, 8, and 14. The flu-like symptom score assessed by the participants in this trial ranged from 0 to 3, where 0 represented the lowest value (no impact) and 3 represented the highest value (severe). Forced expiratory volume in 1 minute percent of predicted (Fev1% predicted) was obtained on day 1 pre- and post-dose as well as on days 5 and 28. Acetaminophen was provided to the participants, counted by the study nurses, and documented on the case report form (CRF). The sample size calculations were based on Treanor et al [11]. A conservative estimate of the mean difference of AUC_{d1–d5} between control and treatment group was given as 1 log₁₀TCID₅₀ or 1 log₁₀ viral RNA copies/mL. A minimum of 60–80 participants per group were to give a greater than 80% power to detect the difference.

Safety and Activity Assessments

Safety and tolerability data on all randomized participants were analyzed and summarized by treatment group. Safety and tolerability assessments conducted during the study included assessment of serious adverse events (SAEs), treatment-emergent AEs (TEAEs), clinical laboratory test results, vital sign measurements, and physical examination findings. A TEAE was one that was observed or reported after the administration of study drug that was not present prior to the administration of study drug or an exacerbation of an event that was present prior to the administration of study drug. The intensity of the event was recorded. If a participant had events with multiple severities, only the maximum severity was recorded. A treatment-related TEAE was defined as a TEAE for which the relationship to study drug was indicated on the CRF as “possibly related,” “probably related,” or “definitely related.” An SAE was an event that was indicated on the CRF as a serious (life-threatening, disabling or incapacitating, hospitalization, or important medical event). Fev1% predicted was recorded on days 1 and 5. Throat swabs were collected on days 1, 5, and 14 and cultured for bacterial flora.

Study Oversight

This study was approved by the institutional review board at each participating site and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice Guidelines, and local regulatory requirements. The study was designed and conducted by the sponsor in collaboration with the Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), clinical research organizations, and the principal investigator. An independent data safety monitoring board (DSMB) reviewed the data 3 times during the study period and made determinations based on stopping criteria at each meeting. The clinical research organizations collected the data, monitored the study conduct, and performed statistical analysis. The first draft of the manuscript was prepared by the principal investigator and lead sponsor author. All authors approved major changes to the manuscript, approved the final changes, and assume responsibility for the veracity and completeness of the reported data and for the fidelity of the study to the protocol.

Statistical Analysis

Data were summarized using descriptive statistics. For continuous variables, descriptive statistics included the number of nonmissing values and mean, SD, median, minimum, and maximum values. For categorical variables, descriptive statistics included counts and percentages per category.

Time-to-event analysis was performed using a log-rank test comparing the treatment groups to placebo. All statistical comparisons were performed using 2-sided tests at an alpha

level of .05 unless specifically stated otherwise. All statistical null hypotheses assumed no differences between treatments. A decrease in sustained viral shedding was defined a 1-log drop in viral load that persisted at later timepoints. Sustained decreases required subsequent timepoints to reach the same threshold. Analysis that adjusted to time-of-symptom onset was also completed. Pearson correlation was utilized for comparison between NWs and PWs.

RESULTS

Participants

A total of 298 participants were randomized to either DAS181 or placebo, although 4 participants were excluded from the safety analysis, as noted in Figure 1. Ninety-nine participants were randomized to multiple-dose DAS181, 100 participants to the single-dose DAS181, and 95 participants to placebo. A total of 177 participants who had PCR-confirmed influenza at baseline were included in the mITT population: 56 in the multiple-dose, 69 in the single-dose, and 52 in the placebo group. Baseline demographics were similar among the treatment groups (Table 1).

Virologic and Clinical Outcomes

In the mITT population, analysis of participants with laboratory (q-PCR)-confirmed influenza indicated that 3 subtypes of virus were encountered during this phase II study, including seasonal H3N2, pandemic 2009 H1N1, and influenza B. During the first season in the Northern Hemisphere, only 2009 H1N1 was observed, while all 3 types were observed in

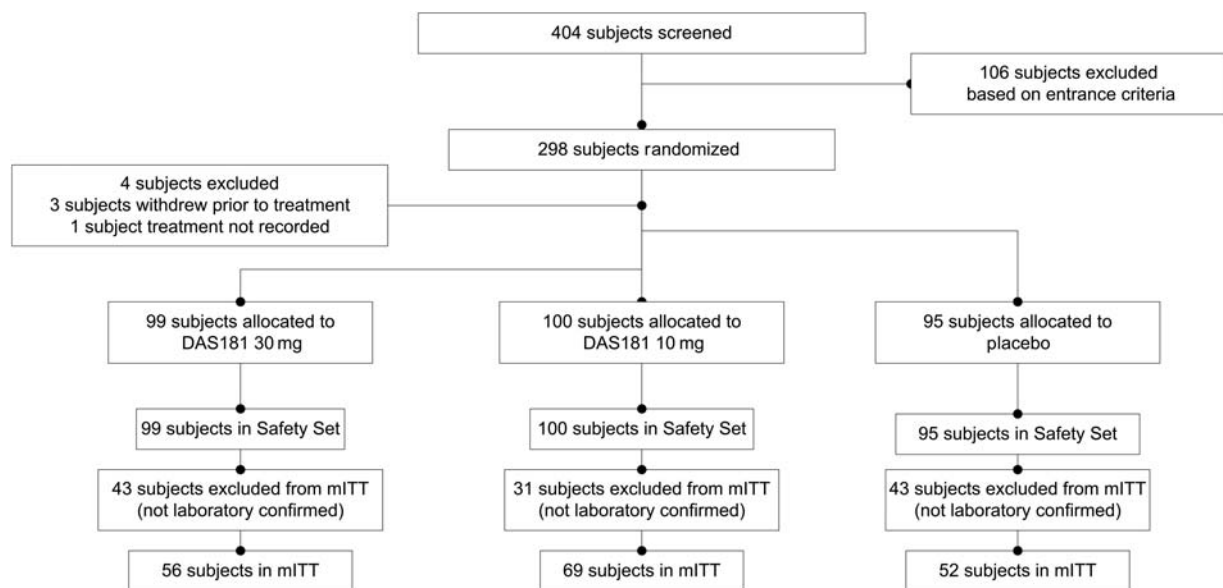


Figure 1. Randomization by treatment group. Abbreviation: mITT, modified intent to treat.

Table 1. Summary of Demographic and Baseline Characteristics (Modified Intent to Treat)

Characteristic	Multiple Dose DAS181 (N = 56)	Single Dose DAS181 (N = 69)	Placebo (N = 52)
Age (y)			
Mean (SD)	31.7 (11.29)	32.2 (10.46)	33.0 (11.50)
Median	28.0	31.0	30.5
Minimum, maximum	18, 68	19, 62	18, 60
Sex			
Female	32 (57.1%)	44 (63.8%)	22 (42.3%)
Male	24 (42.9%)	25 (36.2%)	30 (57.7%)
Race			
White	36 (64.3%)	45 (65.2%)	35 (67.3%)
Black or African American	17 (30.4%)	20 (29.0%)	15 (28.8%)
Asian	3 (5.4%)	3 (4.3%)	2 (3.8%)
Native Hawaiian or other Pacific Islander	0 (0.0%)	1 (1.4%)	0 (0.0%)
Height (cm)			
Mean (SD)	169.09 (10.304)	167.08 (11.406)	171.55 (10.715)
Median	167.32	166.00	171.23
Minimum, maximum	152.0, 190.5	127.0, 190.5	145.0, 198.1
Weight (kg)			
Mean (SD)	76.46 (17.925)	76.98 (18.547)	80.11 (18.438)
Median	73.75	70.49	78.96
Minimum, maximum	52.6, 115.7	54.9, 132.9	54.9, 130.6
Body mass index (kg/m²)			
Mean (SD)	26.576 (4.6580)	27.374 (4.6363)	27.148 (5.0020)
Median	25.555	26.300	26.660
Minimum, maximum	18.80, 34.90	19.80, 39.20	18.52, 39.50

the subsequent seasons in both the Southern and Northern Hemispheres. Thirty-seven percent of participants had confirmed infection with influenza B, 33% with seasonal H3N2, 29% with pandemic 2009 H1N1, and 1 participant was positive for both influenza B and pandemic 2009 H1N1. No cases of H274Y were found among these isolates. Importantly, the combined phase IIA study included analysis of all 3 subtypes encountered from wide geographic areas, allowing assessment of DAS181 activity on a diversity of isolates.

As shown in Table 2, baseline (day 1) viral load from PW was higher in the multiple-dose group compared with placebo ($P = .03$) by q-PCR. From day 1 to day 2, there were statistically significant decreases in the log-transformed viral load for DAS181 treatment groups compared with placebo ($P = .002$ and $P = .006$ for the multiple-dose and single-dose groups, respectively). No significant differences between treatment groups were observed for viral load data obtained from NW samples, although significant correlations ($P < .05$) with PW viral loads at day 1 and at day 5, as measured by q-PCR, were observed. Overall, PW and NW day 1 samples had 88.1% agreement in regard to detection of virus from these samples (using the statistical LOD cutoff of ≥ 500 viral RNA copies/mL; data not shown).

From day 1 to day 3, there was a statistically significant decrease in log-transformed viral load in the multiple-dose

group vs placebo ($P = .009$) but not the single-dose group ($P = .054$). From day 1 to day 5, there was also a statistically significant decrease in log-transformed viral load in the multiple-dose group vs placebo ($P = .008$) but not the single-dose group ($P = .645$), as measured by q-PCR. Analysis using the TCID₅₀ assay also demonstrated a statistically significant decrease in viral load for the multiple-dose group compared with the placebo group ($P = .031$) from day 1 to day 2 but not at other timepoints (see Table 3). As shown in Table 4, a significantly shorter time to sustained decreased viral shedding from day 1 was seen ($P = .007$) for the multiple-dose group compared with the placebo group, as measured by q-PCR in PW samples. A significantly shorter time to sustained decreased viral shedding from the day of onset of symptoms was seen ($P = .003$) for the multiple-dose group compared with placebo group, as measured by q-PCR. No differences in viral load were observed between the treatment groups when examining the AUC metric.

No significant differences were noted in the time to mild or complete resolution of clinical symptoms between the treatment groups. One hundred percent of participants in the multiple-dose group, 96% of participants in the single-dose group, and 94% of participants in the placebo group took acetaminophen at some time during the study. The average daily dose of

Table 2. Summary of Log-Transformed Influenza Viral Load by Polymerase Chain Reaction from Pharyngeal Wash: Baseline (Day 1) to Day 2, Day 1 to Day 3, and Day 1 to Day 5 (Modified Intent to Treat Population)

Study Visit/ Value	Multiple Dose DAS181 (N = 56)	Single Dose DAS181 (N = 69)	Placebo (N = 52)
Baseline (Day 1)			
N	56	67	52
Mean (SD)	5.35 (1.417)	4.85 (1.196)	4.69 (1.468)
Median	5.35	4.87	4.96
Minimum, maximum	2.4, 8.9	2.4, 7.3	2.4, 8.1
Change from day 1 to day 2			
N	56	65	51
Mean (SD)	-1.06 (1.458)	-0.90 (1.285)	-0.25 (1.144)
Median	-1.01	-0.79	-0.34
Minimum, Maximum	-6.2, 2.3	-4.3, 1.6	-2.5, 2.3
t test^a			
P value	.002	.006	
Change from day 1 to day 3			
N	52	64	50
Mean (SD)	-1.46 (1.582)	-1.18 (1.272)	-0.73 (1.183)
Median	-1.46	-1.19	-0.69
Minimum, Maximum	-5.9, 1.5	-4.3, 1.4	-3.7, 1.8
t test^a			
P value	.009	.054	
Change from day 1 to day 5			
N	54	62	49
Mean (SD)	-2.38 (1.359)	-1.78 (1.533)	-1.64 (1.417)
Median	-2.45	-1.96	-1.54
Minimum, maximum	-5.0, 1.3	-4.9, 4.4	-5.7, 1.7
t test^a			
P value	.008	.645	

Log₁₀-transformed viral load data. Undetectable values or those reported as being <500 copies/mL were assumed to be 250 copies/mL.

^a Treatment vs placebo.

acetaminophen was highest in the placebo group (1727 mg) compared with the multiple- (1612 mg) and single-dose groups (1647 mg), but this was not statistically significant.

Safety

As shown in Table 5, during the 28 days of observation, the frequency of participants reporting at least 1 TEAE or treatment-related TEAE was higher in the multiple-dose DAS181 group [(63 participants (63.6%), 29 participants (29.3%), respectively] and in the single-dose group [(54 participants (54.0%), 17 participants (17.0%), respectively] than in the placebo group [(48 participants (50.5%), 11 participants (11.6%), respectively]. Most TEAEs were mild to moderate in

Table 3. Summary of Log-Transformed Influenza Viral Load by TCID₅₀ from Pharyngeal Wash: Baseline (Day 1) to Day 2 (Modified Intent to Treat Population)

Study Visit	Value	Single Dose		
		Multiple Dose DAS181 (N = 53)	DAS181 (N = 64)	Placebo (N = 48)
Day 1	N	52	64	48
	Mean	1.12	1.24	0.99
	SD	1.119	1.246	0.999
	Median	0.40	0.40	0.40
	Minimum Maximum	0.4 3.8	0.4 4.8	0.4 4.0
Day 2	N	53	64	48
	Mean	0.61	0.83	0.86
	SD	0.635	0.937	0.925
	Median	0.40	0.40	0.40
	Minimum Maximum	0.4 4.3	0.4 3.8	0.4 4.0
Change from day 1 to day 2				
N		52	64	48
Mean		-0.50	-0.40	-0.12
SD		0.906	1.003	1.059
Median		0.00	0.00	0.00
Minimum Maximum		-3.4 0.9	-3.6 2.1	-3.6 2.9
t test^a				
	p-value	0.056	0.157	
Wilcoxon rank sum^a				
	p-value	0.060	0.110	

Log₁₀ transformed viral load data. Undetectable values or those reported as being <0.8 TCID₅₀ were assumed to be 0.4 TCID₅₀.

Abbreviation: TCID, median tissue culture infective dose.

^a Treatment vs Placebo.

intensity. SAEs were observed in 2 participants in the single-dose group and in 2 participants in the placebo group. Overall, the most commonly reported treatment-related TEAEs were alkaline phosphatase (ALP) increases. In the multiple-dose group, the most common treatment-related TEAEs (≥5% of participants) were ALP increases (19 participants; 19.2%). In the single-dose group, 6 participants (6.0%) had an ALP increase as a TEAE. Sixteen of 99 (16.2%) participants in the multiple-dose group had a transient grade 1 elevation (DAIDS toxicity scale) of ALP and 3 of 99 (3%) had a transient grade 2 elevation. There were no grade 3 or grade 4 elevations. All elevations normalized to grade 1 or lower by the end of the observation period. Of note, a number of participants had high ALP laboratory measurements (<grade 1) at baseline and 1 participant had a grade 2 elevation of ALP at baseline prior to treatment (data not shown). Most other changes from baseline in hematology, chemistry, coagulation parameters, and urinalysis data were minimal and similar among the multiple-dose, single-dose, and placebo groups.

Table 4. Log Rank Test of Time to Sustained Decreasing Shedding of Influenza Virus (Pharyngeal Wash) as Defined by Time to 1 Log or Greater Decrease from Day 1 (Modified Intent to Treat)

	Multiple Dose DAS181 (N = 56)	Single Dose DAS181 (N = 69)	Placebo (N = 52)
Time to ≥ 1 log drop sustained (days)			
Event ^b /censored ^c	49/7	56/12	39/13
Median time	2	4	4
95% confidence interval	(1, 4)	(2, 4)	(4, 5)
Log-rank test ^a			
P value	.007	.164	

Undetectable values or those reported as being <500 copies/mL were assumed to be 250 copies/mL.

^a Treatment vs placebo.

^b Number of participants who reached sustained 1 log drop at certain date during the study.

^c Number of participants who did not reach sustained 1 log drop during the study and were censored at the date of last follow-up visit.

One SAE of respiratory distress and another SAE of perineal infection were reported in the single-dose group. SAEs of bronchitis and meniscus lesion were reported in the placebo group. There was a single death reported in a 38-year-old African female who received a single dose of DAS181. This patient was documented to have pandemic 2009 H1N1 (94 629 viral RNA copies/mL by PCR on day 1). The viral load observed in this participant on day 1 was comparable with the average viral load obtained from other participants enrolled in the study (Table 2). The participant's Fev1% predicted was 31% prior to receiving the study drug (day 1). On day 2, after 1 dose of DAS181, the viral load, as assessed by q-PCR, revealed a >1 log drop in viral load (4954 copies/mL). On day 2 the participant was hospitalized with a diagnosis of right lower lobe pneumonia. The patient was placed on empiric antibiotics and 40% oxygen face mask because of increasing respiratory distress. Sputum was negative for tuberculosis but positive for *Haemophilus parainfluenza* (β -lactamase negative) and *Staphylococcus aureus*. The participant's respiratory status worsened and she expired 9 days later in the hospital. Serologic testing prior to receiving the study drug documented previously unknown HIV infection. The investigator assessed the pneumonia leading to death as severe and not related to the study drug.

Overall, there were no significant differences in changes in Fev1% predicted or bacterial flora between the treatment groups.

DISCUSSION

This is the first phase II study of DAS181 in otherwise healthy influenza-infected participants encompassing 3 influenza

Table 5. Overview of Treatment Emergent Adverse Events (All Randomized Participants)

	Multiple Dose DAS181 (N = 99)	Single Dose DAS181 (N = 100)	Placebo (N = 95)
At least 1 TEAE	63 (63.6%)	54 (54.0%)	48 (50.5%)
At least 1 treatment-related TEAE	29 (29.3%)	17 (17.0%)	11 (11.6%)
At least 1 TEAE by maximum intensity			
Mild (grade 1)	38 (38.4%)	29 (29.0%)	24 (25.3%)
Moderate (grade 2)	19 (19.2%)	19 (19.0%)	20 (21.1%)
Severe (grade 3)	6 (6.1%)	5 (5.0%)	4 (4.2%)
Potentially life threatening (grade 4)	0 (0.0%)	1 (1.0%)	0 (0.0%)
At least 1 treatment-related TEAE by maximum intensity			
Mild (grade 1)	21 (21.2%)	12 (12.0%)	7 (7.4%)
Moderate (grade 2)	7 (7.1%)	5 (5.0%)	3 (3.2%)
Severe (grade 3)	1 (1.0%)	0 (0.0%)	1 (1.1%)
Potentially life threatening (grade 4)	0 (0.0%)	0 (0.0%)	0 (0.0%)
At least 1 TEAE resulting in temporary interruption of study drug	0 (0.0%)	1 (1.0%)	0 (0.0%)
At least 1 TEAE resulting in permanent discontinuation of the study drug	1 (1.0%)	1 (1.0%)	0 (0.0%)
At least 1 SAE	0 (0.0%)	2 (2.0%)	2 (2.1%)
At least 1 treatment-related serious TEAE	0 (0.0%)	0 (0.0%)	0 (0.0%)

Abbreviation: TEAE, treatment-emergent adverse event.

seasons that provided assessment of 3 strains of influenza (pandemic 2009 H1N1, seasonal influenza A H3N2, and influenza B). The design and sample size calculations for this study were based on assumptive changes in influenza viral load, with the main objective to determine safety and impact on influenza viral load of DAS181. Statistically significant antiviral activity of DAS181 was observed by multiple virologic analyses. A significant decrease in viral load from baseline was observed in the multiple-dose group compared with the placebo group over 5 days. A significant decrease in viral load was observed in the single-dose group compared with the placebo group but only over the first 24 hours. A significantly shorter time to sustained decreased viral shedding was observed in the multiple-dose group compared with the placebo group. No significant differences between the treatment groups were observed using the AUC metric. These results may be explained by the significantly higher viral load in the multiple-dose group at baseline prior to treatment.

PWs were the primary method utilized to measure changes in viral load. For comparative purposes, NWs were also obtained but only on 2 days (day 1 and day 5). The sensitivity of the PW was comparable with the NW, as others have also shown [12]. Furthermore a correlation was observed between quantitation of viral loads in PW samples and the NW samples, as measured by q-PCR. However, for an inhaled antiviral, NW may be inadequate to determine true therapeutic effect, as was observed in some studies of zanamivir that failed to show differences in viral load by NW in spite of clinical effects [13].

The presence of flu-like symptoms, and the alleviation of those symptoms, was similar among groups. In contrast, some previous clinical studies have shown correlations between decreases in viral load and improvements in influenza symptom scores in participants treated with neuraminidase inhibitors [14, 15]. There are a number of possible reasons for the lack of observed clinical effects in this study. This study was not designed, nor sufficiently powered, to assess clinical efficacy of DAS181 on influenza symptoms. Symptom scores were collected only once daily on days 1, 2, 3, 5, 8, and 14. This is in contrast with pivotal studies for oseltamivir and zanamivir, in which clinical assessments were collected twice daily [16]. Hence, more subtle differences in clinical outcomes (especially if less than 24–36 hours) may not have been detected. High antipyretic use in this study may also have obscured a clinical effect as it has in other studies of influenza antiviral drugs. In some studies, analysis of time to symptomatic alleviation was done excluding participants who had taken antipyretics when their use was minimal [12]. Such an analysis was not possible in the current study because antipyretic use was so common.

This study required a positive influenza rapid antigen testing for enrollment, and a lower percentage of participants was determined to have laboratory-confirmed influenza (as documented by q-PCR) than expected. This could be due to the fact that this study was initiated after the peak of the first influenza season. Overall, the PCR positive rate during this first segment of the study was extremely low at a time when there was a low prevalence of influenza. In contrast, the PCR positive rate exceeded 85% by the last segment in the Northern Hemisphere, a period with moderate influenza activity. The majority of participants in this trial were randomized based on results from the Quidel influenza A + B rapid antigen test (Quidel, San Diego, CA).

It is well known that during periods of low prevalence, the positive predictive value is low [17]. Others have found a similar high false-positive rate utilizing the rapid antigen test during the first season of this trial [18,19].

The incidence of SAEs during this study was low. Although 2 participants experienced an SAE in both the single-dose and placebo groups, no participants in the multiple-dose group experienced SAEs. The most common laboratory abnormality observed in this trial was a transient elevation of ALP. In

previous animal studies and phase 1 clinical trials, the most commonly observed pharmacological effect associated with DAS181 systemic exposure was transient elevation of ALP without concomitant transaminitis. In animals, an increase in ALP is typically observed in the absence of any increase in ALT, as well as in the absence of any histological evidence of liver necrosis or other pathology. Interestingly, in animals, ALP elevation can be induced by administration of chemically desialylated serum proteins [20]. Desialylation of the serum proteins may lead to saturation of the asialoglycoprotein receptors in the liver and spleen and to reduced clearance of certain glycoproteins, including ALP [21, 22].

Antiviral activity of DAS181 was observed in influenza-infected participants who received 3 days of treatment in this phase II clinical trial. Overall, the drug was generally well tolerated. Future studies will examine higher dosing levels and will be designed to determine impact on both influenza viral load and clinical outcomes. Because this antiviral is host directed, there is the potential to combine this approach with virus-specific approaches to treat novel and resistant strains of influenza.

There is a continuing public health threat of future influenza pandemics. Recent studies have demonstrated the potential for avian strains to be capable of being transmitted between mammals with high pathogenicity [23]. DAS181 represents a novel host-directed approach to potentially treat and prevent all strains of influenza.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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