A Phase 1, Single Ascending-Dose Study of AVI-6002, a Combination of Two PMO*plus*™ Compounds with Activity against Ebolavirus

A. Heald[1]*, T. Axtelle[1], J. Thimmarayappa[1], W. Smith[2]
[1]AVI BioPharma, Inc., Bothell, WA, USA; [2]New Orleans Center for Clinical Research, Knoxville, TN, USA

Abstract

Objective: Ebola hemorrhagic fever (EHF) is a rare human disease caused by ebolavirus, a filamentous single-stranded, negative-sense RNA virus of the family *Filoviridae.* No vaccine or established effective therapy is currently available for this catastrophic disease. AVI-6002 is an experimental combination of two phosphorodiamidate morpholino oligomers with positive charges (PMO*plus*™) that specifically target two viral messenger RNAs (mRNAs) encoding ebolavirus proteins. AVI-6002 has demonstrated evidence of protection against lethal infection in experimental mouse, guinea pig and non-human primate models of ebolavirus infection. The objective of this clinical study is to determine the safety, tolerability and pharmacokinetics of intravenous administration of AVI-6002 in healthy human subjects over a dose range predicted to cover a therapeutic dose.

Methods: In this first-in-man study, 30 healthy male and female subjects between 18 and 50 years of age were enrolled in 6 dose escalation cohorts of 5 subjects each and received a single intravenous (IV) infusion of AVI-6002 (0.01, 0.1, 1.0, 3.0, 6.0 and 9.0 mg/kg) or matched placebo in a 4:1 ratio. Safety was monitored through adverse event collection, telemetry, oximetry and serial blood tests, urine tests and electrocardiograms. The study was overseen by an independent Data Safety Monitoring Board (DSMB).

Results: No significant safety concerns arose upon review of blinded study data from the first 5 cohorts by the independent DSMB. While 14 of the first 25 subjects dosed experienced a variety adverse events such as headache (n=4), nausea (n=3) or fatigue (n=2), almost all were mild or moderate in severity. The only exception was one episode of severe hypertension, which was not considered related to study drug. No changes in kidney function related to study drug were observed.

Conclusion: The study has been completed. Final results of this first-in-man phase 1 study suggest that single IV administrations of AVI-6002 are well-tolerated up to a dose level of 9 mg/kg. No changes in kidney function related to study drug were observed. The pharmacokinetics of the components of AVI-6002 (AVI-7537 and AVI-7539) are similar.

ClinicalTrials.gov ID: NCT01353027

Purpose

To determine the safety, tolerability and pharmacokinetics of a single intravenous (IV) administration of AVI-6002 (sequential infusions of AVI-7537 and AVI-7539) in healthy human subjects over a dose range predicted to cover a therapeutic dose.

Methods

Study Design

In this randomized, double-blind, placebo-controlled study, 30 qualifying subjects were randomized into 6 cohorts of 5 subjects each such that 4 subjects received AVI-6002 and 1 received placebo in each cohort. The dose levels were 0.01, 0.1, 1.0, 3.0, 6.0 and 9.0 mg/kg. The study was overseen by a DSMB, who reviewed blinded data prior to enrollment of the subsequent dose cohort.

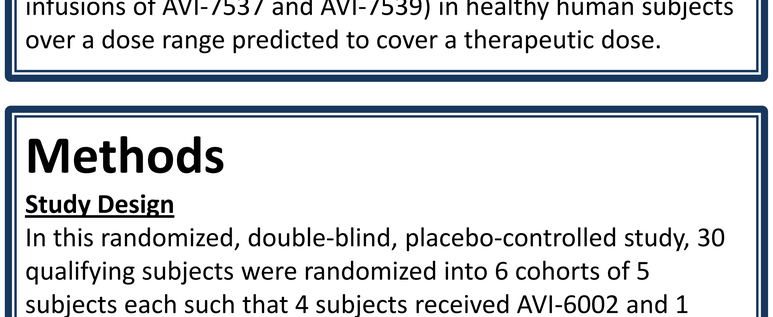
Major Inclusion Criteria

- Male or female and between the ages of 18 and 50 years in good general health
- Willing to use barrier methods of contraception or be of non-childbearing potential
- Willing to undergo a urine screen for drugs of abuse

Major Exclusion Criteria

- Pregnancy or breastfeeding.
- Any clinically relevant abnormalities in physical examinations, vital signs, ECG, clinical chemistry, hematology or urinalysis
- Positive test for human immunodeficiency virus, hepatitis B or hepatitis C or known history of HIV infection

Dosing and Evaluation Schedule



[1]Sequential, 30 minute IV infusions of AVI-7537 followed by AVI-7539.
[2]After reviewing safety, clinical laboratory and renal biomarker results through Study Day 5, the DSMB issued a recommendation to either proceed as planned, proceed with modification of the dosing schedule or discontinue the study.

Support

This study was conducted under contract with the Department of Defense Joint Project Manager Transformational Medical Technologies.

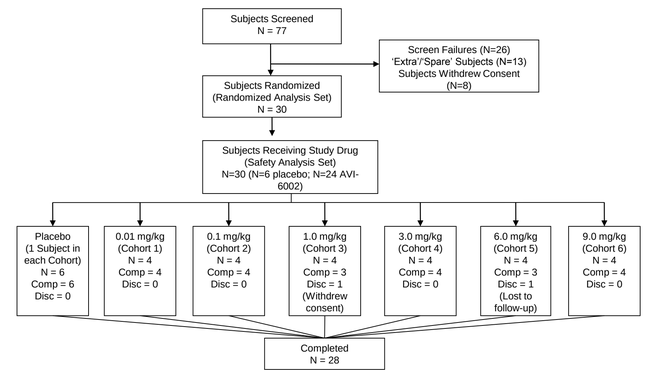
Conclusions

- No significant safety concerns were identified after single IV administration of AVI-6002 at doses up to 9.0 mg/kg.
- No changes in kidney function related to study drug were observed.
- The pharmacokinetics of the components of AVI-6002 (AVI-7537 and AVI-7539) are similar.
- A multiple ascending dose study is planned.

Results

Enrollment

- 15 males and 15 females
- 20 Caucasians, 8 African-Americans, 2 American Indian or Alaska Native
- Mean age 26.4 years (range 19 to 42 years)
- Mean weight at screening 73.6 kg (range 53.1 to 95.7 kg)



Adverse Events

Table 1: Number of Subjects with Adverse Events by System Organ Class and Treatment Group

	Placebo (N = 6)	AVI-6002						
		0.01 mg/kg (N = 4)	0.1 mg/kg (N = 4)	1.0 mg/kg (N = 4)	3.0 mg/kg (N = 4)	6.0 mg/kg (N = 4)	9.0 mg/kg (N = 4)	Total (N = 24)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Any TEAE	5 (83.3)	3 (75.0)	0	2 (50.0)	3 (75.0)	3 (75.0)	1 (25.0)	12 (50.0)
Eye disorders	0	0	0	0	0	0	1 (25.0)	1 (4.2)
Gastrointestinal Disorders	1 (16.7)	2 (50.0)	0	0	2 (50.0)	1 (25.0)	0	5 (20.8)
General Disorders &Administration Site Conditions	3 (50.0)	2 (50.0)	0	0	0	0	0	2 (8.3)
Infections & Infestations	0	0	0	1 (25.0)	0	0	0	1 (4.2)
Injury, Poisoning & Procedural Complications	0	0	0	1 (25.0)	0	0	0	1 (4.2)
Investigations	1 (16.7)	0	0	0	0	0	0	0
Nervous System Disorders	0	3 (75.0)	0	0	0	1 (25.0)	0	4 (16.7)
Renal & Urinary Disorders	1 (16.7)	0	0	0	0	0	0	0
Respiratory, Thoracic & Mediastinal Disorders	1 (16.7)	1 (25.0)	0	0	0	1 (25.0)	0	2 (8.3)
Skin & Subcutaneous Tissue Disorders	0	0	0	0	1 (25.0)	1 (25.0)	0	2 (8.3)

Safety Assessments

- No clinically significant or dose-dependent changes were observed post-treatment in hematology, chemistry, urinalysis, coagulation parameters, reticulocyte counts, complement levels, vital signs, ECG findings, physical examination findings, pulse oximetry, and cardiac telemetry.
- No nephrotoxicity, changes in renal function, or changes in biomarkers of renal dysfunction were observed.
- No deaths or serious adverse events (SAEs) were observed.
- No relationship was observed between AVI-6002 dose level and the incidence of AEs or treatment-related AEs (**Table 1**).
- The most frequently observed AEs in AVI-6002 subjects were headache (n=4), nausea (n=3) and sinus congestion (n=2).
- One subject (9.0 mg/kg) developed moderate uveitis on Day 23, likely related to recurrence of ocular toxoplasmosis, given the retinal scarring and his country of origin (West Africa).

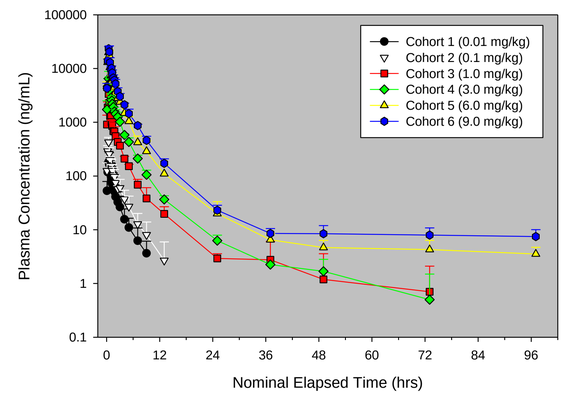


Figure 1: Semi-log Plot of mean (+SD) Plasma Concentrations of AVI-7537 versus Nominal Elapsed Time

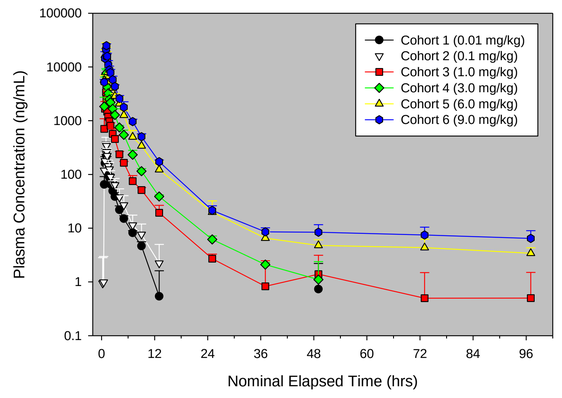


Figure 2: Semi-log Plot of mean (+SD) Plasma Concentrations of AVI-7539 versus Nominal Elapsed Time

Pharmacokinetics

- Pharmacokinetic profile of each of the two individual components demonstrated that both components declined in a multi-phasic manner over time (**Figures 1 & 2**).
- Plasma pharmacokinetic parameters were similar for AVI-7537 and AVI-7539 (**Table 2**).

Table 2: Summary of Mean AVI-7537 and AVI-7539 Plasma Pharmacokinetic Parameters

AVI-6002 Dose	AUC_{0-t} (hr*ng/mL)		C_{max} (ng/mL)		CL_P (mL/hr/kg)		Vss (mL/kg)		Half-life (hr)	
(mg/kg)	AVI-7537	AVI-7539	AVI-7537	AVI-7539	AVI-7537	AVI-7539	AVI-7537	AVI-7539	AVI-7537	AVI-7539
0.01	277	337	185	242	30.6	14.8	65.1	47.9	2.00	2.06
0.1	602	507	425	399	89.4	103	183	201	1.98	1.87
1.0	4120	4010	3360	3480	122	127	382	387	3.60	2.87
3.0	10200	11000	6460	7910	152	140	406	329	2.83	2.45
6.0	27400	30500	20900	24000	114	101	334	273	4.60	4.61
9.0	36000	37400	24100	24800	126	121	453	401	4.00	3.92

- Renal clearance tended to increase with dose and urinary excretion of intact drug accounted for no more than 44.0% of AVI-7537 total elimination and 30.7% of AVI-7539 elimination.

Background

PMO*plus*™ Chemistry

- Phosphorodiamidate morpholino oligomers (PMOs) are compounds in which the nucleobases are linked to a morpholino group, not ribose, and the nucleotide subunits are linked through charge-neutral dimethylamino phosphorodiamidate moieties (**Figure 3**).
- A derivative of the PMO chemistry developed by AVI BioPharma, Inc. replaces one or more of the dimethylamino groups in the phosphorodiamidate backbone with piperazine. The piperazine moiety is capable of bearing a positive charge, and these molecules as a class are named PMO*plus*™ (**Figure 4**).

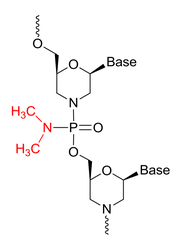


Figure 3: PMO

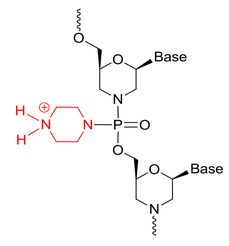


Figure 4: PMO*plus*

AVI-6002 Targets 2 Essential Viral Proteins

Name of Drug Substance	Protein Encoded by Target mRNA		PMO*plus* Sequence[a]	Base Length
	Name	Description		
AVI-7537	VP24	Matrix Viral Protein 24	GCC+ATG GT+T TT+T TC+T C+AG G	19
AVI-7539	VP35	Viral RNA Polymerase Complex Cofactor	CC+T GCC C+TT TGT+TCT+AGT+TG	20

[a]The + indicates the position of the positively charged linkages.

AVI-6002 Improves Survival in Ebolavirus Lethal Challenge Models

Studies of AVI-6002 in the mouse, guinea pig, and nonhuman primate lethal challenge models demonstrated significantly increased survival after exposure to Ebolavirus (**Figure 5**).

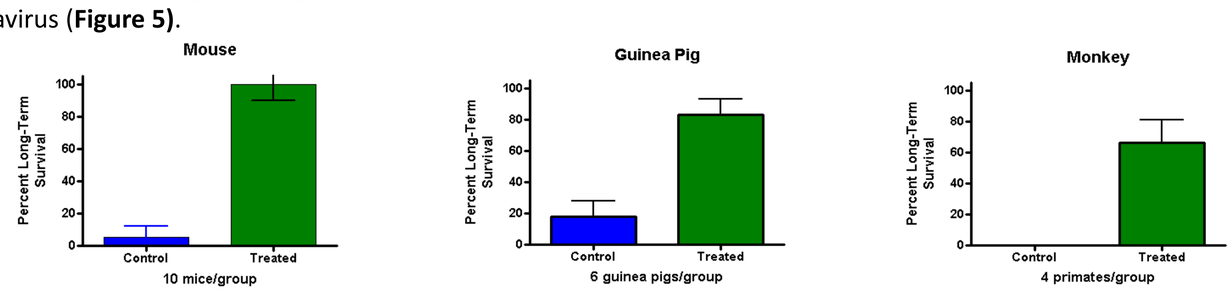


Figure 5: Mice were challenged with mouse-adapted ebolavirus isolates and treated with AVI-6002 (5 mg/kg IP) or vehicle control. Guinea pigs were challenged with ebolavirus and treated with AVI-6002 (40 mg/kg IP). Cynomolgus monkeys were challenged with ebolavirus and treated with AVI-6002 (40 mg/kg IV or SC). Percent survival at 28 days post challenge is shown.

Toxicology Findings

In GLP toxicology studies, AVI-6002 was generally well tolerated at doses up to 400 mg/kg/day IV for 28 days in rats and 200 mg/kg/day IV in cynomolgus monkeys. In both species, the primary adverse histopathology finding was renal tubular degeneration in high dose groups. The severity and duration of renal tubular degeneration correlated with transient increases in urinary cystatin C (a biomarker of early renal dysfunction) and urinary total protein.