AAC Accepts, published online ahead of print on 25 August 2014
Antimicrob. Agents Chemother. doi:10.1128/AAC.03442-14

Safety and Pharmacokinetic Profiles of Phosphorodiamidate Morpholino Oligomers with Activity against Ebola Virus and Marburg Virus: Results of Two Single Ascending Dose Studies

Alison E. Heald[1#], Patrick L. Iversen[1,4], Jay B. Saoud[1], Peter Sazani[1], Jay S. Charleston[1], Tim Axtelle[1,5], Michael Wong[1], William B. Smith[2], Apinya Vutikullird[3], and Edward Kaye[1]

[1] Sarepta Therapeutics, Inc., 215 First Street, Cambridge, MA 02142

[2] New Orleans Center for Clinical Research-Knoxville, 1928 Alcoa Highway, Suite G-50, Knoxville, TN 37920

[3] West Coast Clinical Trials, 5630 Cerritos Avenue, Cypress, CA 90630

[4] Oregon State University, Corvallis, OR 97331

Address correspondence to:
Alison E. Heald, MD
Sarepta Therapeutics, Inc.
215 First Street
Cambridge, MA 02142
healda@uw.edu

Running title: **Safety and Pharmacokinetics of Morpholino Oligomers**

[5] Current Address: Cocrystal Discovery, Inc., 19805 North Creek Parkway, Bothell, WA 98011

ABSTRACT [234 words; Limit: 250]

Two identical single ascending dose (SAD) studies evaluated the safety and pharmacokinetics (PK) of AVI-6002 and AVI-6003, two experimental combinations of phosphorodiamidate morpholino oligomers with positive charges (PMO*plus*®) that target viral messenger RNA (mRNA) encoding Ebola virus and Marburg virus proteins, respectively. Both AVI-6002 and AVI-6003 were found to suppress disease in virus-infected nonhuman primates (NHPs) in previous studies. AVI-6002 (a combination of AVI-7537 and AVI-7539) or AVI-6003 (a combination of AVI-7287 and AVI-7288) were administered as sequential intravenous (IV) infusions of a 1:1 fixed dose ratio of the two subcomponents. In each 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 IV infusion of active study drug (0.005, 0.05, 0.5, 1.5, 3, and 4.5 mg/kg per component) or placebo in a 4:1 ratio. Both AVI-6002 and AVI-6003 were safe and well tolerated at the doses studied. A maximum tolerated dose (MTD) was not observed in either study. The four chemically similar PMO*plus* components exhibited generally similar PK profiles. The mean peak plasma concentration (C_{max}) and area under the concentration curve (AUC) values of the four components exhibited dose-proportional PK. The estimated plasma half-life of all four components was 2 to 5 hours. The safety of the two combinations and the PK of the four components were similar, regardless of the target RNA sequence.

INTRODUCTION

Ebola virus (EBOV) and Marburg virus (MARV) are filamentous, single stranded, negative sense ribonucleic acid (RNA) viruses of the family Filoviridae and causative agents of viral hemorrhagic fever (1). Clinically, filovirus infections are characterized by the acute onset of illness after a typical incubation period of 4 to 10 days, with symptoms initially consisting of fever, chills, myalgia, and malaise (2). Disease features may evolve to encompass anorexia, nausea, vomiting, abdominal pain, diarrhea, respiratory complaints, conjunctival injection, hypotension, edema, prostration, confusion, and coma. Hemorrhagic manifestations, coagulopathy, maculopapular rash, cytopenias, and increased transaminase levels may also be observed (2-4). Infections caused by Ebola and Marburg viruses are associated with a very high mortality rate (1, 2). Ebola virus is associated with case fatality rates of 47% to 89% (5), and of the 2,387 known cases of Ebola virus reported up to April 2014, 1,590 (66.6%) have been fatal (6). Death rates in Marburg hemorrhagic fever outbreaks have ranged from 24% to 88%, and of the 571 cases of Marburg Hemorrhagic Fever (MHF) reported to date, 82.3% have been fatal (7).

Both viruses may be transmitted to the host by exposure of mucosal surfaces or abraded skin to infected body fluids or through parenteral inoculation; the contribution of aerosol or respiratory droplet transmission in the setting of natural epidemics is unknown (8). Because they are highly lethal and readily transmitted from person to person, both Ebola virus and Marburg virus have been classified by the US CDC as potential “Category A” agents of bioterrorism, and deemed of high priority for focused preparedness efforts, including the development of effective counter measures to infection (9). Other than supportive care, no vaccine or established effective therapy is currently available to treat Ebola and Marburg virus infections.

AVI-6002 and AVI-6003 are two combination drugs under evaluation for post-exposure prophylaxis of Ebola virus and Marburg virus, respectively. Both drugs were developed in 1:1 combinations: AVI-6002 consists of AVI-7537 and AVI-7539 (**Figure 1**); and AVI-6003 consists of AVI-7287 and AVI-7288 (**Figure 2**). Both drugs consist of phosphorodiamidate morpholino oligomers that have positive charges in the form of piperazine residues at defined locations along the backbone (PMO*plus*®). The oligomers target specific messenger RNA (mRNA) sequences of viral proteins and physically block their translation. The addition of the piperazine residues impart positive charges to the anti-sense oligonucleotide and are thought to enhance binding to negatively charged viral RNA, possibly subverting the consequences of individual viral resistance mutations, should they evolve.

Studies of the efficacy of AVI-6002 and AVI-6003 in the mouse, guinea pig and nonhuman primate lethal challenge models were performed at the United States Army Medical Research Institute for Infectious Diseases (10, 11). In these studies, treatment with AVI-6002 resulted in high levels of survival in mice, guinea pigs and rhesus monkeys after exposure to Ebola virus. Similarly, treatment with AVI-6003 resulted in high levels of survival in mice, guinea pigs and cynomolgus macaques after exposure to Marburg virus. None of the untreated control animals and none of the animals that were treated with scrambled control survived in either the Ebola or Marburg virus nonhuman primate lethal challenge models. Dose-dependent survival was observed in both models, with high levels of survival observed on nonhuman primates treated with AVI-6002 and AVI-6003 at doses of 28 mg/kg to 30 mg/kg. The human equivalent dose, based on scaling by body surface area, was approximately 9 mg/kg, or 4.5 mg/kg per component.

Two identical, first-in-man, single ascending dose studies of AVI-6002 and AVI-6003 were completed to assess the safety, tolerability, and pharmacokinetics (PK) of these compounds in healthy volunteers. The doses tested ranged from a very low dose, 0.005 mg/kg per component, a dose 100-fold lower than the human equivalent dose observed to have minimal adverse effects in toxicology studies, to 4.5 mg/kg per component, the human equivalent dose, as noted above.

MATERIALS AND METHODS

Study agents. The chemical properties of the components of AVI-6002 (AVI-7537 and AVI-7539) and AVI-6003 (AVI-7287 and AVI-7288) are shown in **Table 1**. Each component is composed of morpholino analogs of A, C, G, T and I nucleosides arranged on a phosphorodiamidate linkage platform in which five or six dimethylamine linkage sites have been replaced with a piperazine ring to confer a net 5+ positive charge to AVI-7537, AVI-7539 and AVI-7288, and a net 6+ charge to AVI-7287 (**Figures 1** and **2**). The I (inosine) nucleoside was included because it pairs with A, C and T, potentially maintaining target binding if a mutation emerges.

Doses of both AVI-6002 and AVI-6003 of 0.01, 0.1, 1, 3, 6, and 9 mg/kg made up of equal parts of their respective individual components were administered as sequential intravenous (IV) infusions. Each component was diluted in 150 mL normal saline prior to IV infusion over 30 minutes each. Placebo for both studies consisted of two sequential infusions of 150 mL normal saline, administered IV over 30 minutes. Placebo and active drug preparations were indistinguishable.

Study design. The designs of the two randomized, double-blind, placebo-controlled studies were identical. Each study protocol was reviewed and approved by the Institutional Review Board overseeing the research at the participating institution. In each study, a total of 30 subjects were enrolled into six cohorts of five subjects each. Within each cohort, subjects were randomized in a 4:1 ratio to active drug or placebo. The dose of active drug in the first cohort of each study was 0.005 mg/kg per component, and was escalated in each subsequent cohort to 0.05, 0.5, 1.5, 3, and 4.5 mg/kg per component, after cumulative blinded safety data were reviewed by an independent Data Safety Monitoring Board, prior to enrollment of subjects into the subsequent dose cohort.

Study subjects. In both studies, subjects were males and females between the ages of 18 and 50 in good general health who were willing to use barrier methods of contraception or were of non-childbearing potential. Major exclusion criteria included pregnancy; breastfeeding; body mass index (BMI) <18 or >35 kg/m^2; any clinically relevant abnormalities in physical examinations, vital signs, ECG, clinical chemistry, hematology or urinalysis; an elevated serum creatinine level and/or a urine albumin-to-creatinine ratio (UACR) > 30 mg/g; glomerular filtration rate (GFR) of <80 mL/min, based on Cockcroft-Gault calculation using lean (ideal) body weight; or positive test for human immunodeficiency virus, hepatitis B or hepatitis C or known history of HIV infection.

Study procedures. Study procedures were identical for both studies. After providing informed consent, subjects underwent screening procedures up to 21 days prior to study drug

administration. Eligible subjects were admitted to the Phase 1 unit the day prior to study drug administration and underwent Day -1 assessments to confirm eligibility. Subjects received a single IV dose of AVI-6002 or AVI-6003 or placebo. Subjects were observed in the study unit for 96 hours after study drug administration for serial plasma and urine PK sampling. Safety was monitored through adverse event (AE) collection, telemetry, clinical laboratory assessments (hematology, coagulation, chemistry, urinalysis, and complement levels), oximetry, and electrocardiograms (ECGs). Subjects returned for follow-up visits 14, 21, and 28 days after study drug administration. AEs were coded by preferred term and system organ class (SOC) using MedDRA Version 14.1. The intensity of adverse events and laboratory abnormalities was determined using the criteria specified in the FDA's guidance document, "Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials" (September 2007) (12).

Pharmacokinetic evaluations. Blood samples for pharmacokinetic determinations were drawn pre-dose (within 30 minutes before dosing); at 5, 15, and 28 minutes after the start of the infusion of the first component of the study drug; at 5, 15, and 28 minutes after the start of the infusion of the second component of the study drug; at 5, 10, 15, 30, 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72 and 96 hours after the completion of the study drug infusions. Urine was collected in aliquots for pharmacokinetic determinations at each of the following intervals: start of dosing through completion of the infusions, completion of the infusions through 1 hour, >1 hour through 4 hours, >4 hours through 8 hours, >8 hours through 12 hours, >12 hours through 24 hours, >24 hours through 48 hours, >48 hours through 72 hours and >72 hours through 96 hours.

Plasma and urine concentrations were determined using a validated capillary gel electrophoresis and fluorescent probe hybridization assay for each component (Helix Diagnostics, Inc., Madison WI). Briefly, plasma and urine samples were prepared for hybridization using TriZOL LS extractions followed by alcohol precipitation, and then hybridized to a fluorescent-labeled oligonucleotide probe. The probe-analyte species were separated and detected using capillary gel electrophoresis with laser-induced fluorescence.

The pharmacokinetics of the individual components of AVI-6002 and AVI-6003 were determined from blood and urine samples, using a model-independent (non-compartmental) approach appropriate for a constant rate infusion (13). The pharmacokinetic parameters characterized included the maximum plasma concentration (C_{max}), the plasma half-life, the time at which C_{max} occurs (T_{max}), the area under the plasma concentration-time curve from time 0 to 24 hours after the start of infusion of study drug (AUC_{0-24}), the volume of distribution under steady-state conditions (V_{ss}), plasma clearance (CL_P) and renal (i.e., urinary) clearance (CL_r). Pharmacokinetic and exploratory analyses were performed using WinNonlin V 5.2 (Pharsight Corp., Mountain View, CA), SigmaPlot V 11.0 (Systat Software Inc, San Jose, CA), GraphPad Prism V 5.0 (La Jolla, CA) and Microsoft Excel 2003 (Microsoft Corp, Redmond, WA).

RESULTS

Study enrollment and disposition. Demographics and baseline characteristics of the subjects who participated in the two studies are outlined in **Table 2**. Fifteen males and 15 females took part in the AVI-6002 study. The majority of the subjects were white (66.7%), 8 were black or African American (26.7 %), and two were American Indian or Alaska Native (6.7%). The mean

age was 26.4 years (range: 19 to 42) and was comparable across dose groups. Mean weight and BMI were also similar across dose groups. All subjects were dosed and all but two completed the 28 day study. One subject in the 1 mg/kg cohort withdrew consent on Day 15 and one subject in the 6 mg/kg cohort was lost to follow up after Day 5; neither discontinuation was treatment-related.

Sixteen males and 14 females took part in the AVI-6003 study; 18 (60.0%) subjects were white, 9 (30.0%) were black or African American, and 3 (10.0%) were Asian. The overall mean age was 31.1 years (range: 18–49) and was comparable across dose groups. Mean height, weight, and BMI also were similar across does groups. All subjects were dosed and all completed the 28-day study except for 1 subject in the 0.1 mg/kg cohort who withdrew consent after experiencing a non-treatment related serious adverse event (SAE).

Tolerability. Both AVI-6002 and AVI-6003 were safe and well-tolerated at all doses studied. Treatment-emergent adverse events (TEAEs) occurring two or more times in a given System Organ Class in either study are summarized in **Table 3**. In the AVI-6002 study, 12 (50%) of the AVI-6002-treated subjects experienced 20 TEAEs, 15 of which were considered treatment-related. Overall, gastrointestinal disorders were most common, followed by nervous system disorders. No TEAEs related to abnormal laboratory results were reported. Treatment-emergent AEs occurring in more than 1 subject included headache (4 AVI-6002 subjects; 0 placebo subjects), nausea (3 AVI-6002 subjects; 0 placebo subjects) and sinus congestion (2 AVI-6002 subjects; 1 placebo subject). No dose-dependent pattern was observed. All TEAEs were mild in severity, with the exception of four subjects who experienced one moderate episode each of

204 headache, uveitis, contact dermatitis, and laceration. Nine AVI-6002-treated subjects
205 experienced 15 treatment-related TEAEs with headache and nausea the only TEAEs occurring in
206 more than one subject. One subject in the AVI-6002 9 mg/kg group developed moderate uveitis
207 that was considered related to drug. A retinal specialist suggested recurrent toxoplasmosis as the
208 underlying cause of the uveitis based on the subject's country of origin (West Africa) and the
209 presence of retinal scarring. The subject was treated, and the AE resolved on Day 20. No SAEs,
210 discontinuations due to AEs or deaths were reported.
211
212 Similarly, AVI-6003 was safe and well tolerated at all doses studied. Thirteen (54.2%) AVI-
213 6003-treated subjects experienced 27 TEAEs (**Table 3**), 6 of which were considered treatment-
214 related. Overall, nervous system disorders were most common, followed by gastrointestinal
215 disorders. No TEAEs related to abnormal laboratory results were reported. Treatment-emergent
216 AEs occurring in more than 1 subject included headache (2 AVI-6003 subjects; 0 placebo
217 subjects) and dizziness (2 AVI-6003 subjects; 0 placebo subjects). No dose-dependent pattern
218 was observed. All TEAEs were mild in severity, except for anxiety and cataplexy of moderate
219 severity and exacerbation of schizophrenia of severe intensity, all reported in one subject, who
220 had not disclosed his preexisting diagnosis of schizophrenia at the time of screening.
221 Exacerbation of schizophrenia was considered a SAE, was assessed as severe, was unrelated to
222 study drug, but was considered related to study procedure (i.e., confinement to the study site),
223 and led the subject to withdraw consent on Day 21. Five (20.8%) AVI-6003 subjects experienced
224 six treatment-related TEAEs with headache the only TEAE occurring in more than one subject.
225 No deaths or discontinuations due to study treatment were reported.
226

No clinically significant or dose dependent effects of AVI-6002 or AVI-6003 were reported on any of the safety endpoints evaluated, including clinical laboratory assessments, vital signs, ECGs, physical examinations, pulse oximetry, and cardiac telemetry, nor were any gross abnormalities in fluid status, nephrotoxicity, changes in renal function or changes in biomarkers of renal dysfunction observed. Specifically, no subjects in either study developed a confirmed increase in serum creatinine level of ≥0.3 mg/dL from baseline or had a confirmed percentage increase of ≥50% from baseline in serum creatinine level, where baseline was defined as the mean of the screening and Day -1 value, and no subjects in either study developed a consistent or durable > 3-fold increase in UACR from predose on Day 1 and to an absolute value > 30 mg/g, which were the individual stopping rules based on renal function for each study. Mild (grade 1) increases in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) to 1.1 to 2.5 times the upper limit of normal were observed in 2 subjects in the AVI-6002 study and 2 subjects in the AVI-6003 study, but no dose-dependent pattern was observed. Mild (grade 1) increases in amylase to 1.1 to 1.5 times the upper limit of normal were observed in 2 subjects in the AVI-6002 study and 8 subjects in the AVI-6003 study, but no dose-dependent pattern was observed. No grade 2 or greater increases in AST, ALT or amylase were observed in either study.

Pharmacokinetics. The four PMO*plus* components displayed similar plasma PK properties including similar time-concentration curves (**Figure 3**), and absolute values of C_{max} (**Figure 4**) and AUC (**Figure 5**) over the range of dosing from 0.005 to 4.5 mg/kg. These parameters changed in a dose proportional manner. Other PK parameters (e.g., T_{max}, half-life, clearance, and volume of distribution) were nearly identical, particularly at the higher dose levels (**Table 4**).

250

251 For all four components, most of the drug was excreted in the urine within the first 24 hours.
252 AVI-7537 CL_r was unmeasurable for the two lower doses but CL_r/CL_P averaged 1.7%, 21.6%,
253 26.8%, and 44.0% for the 0.5, 1.5, 3, and 4.5 mg/kg dose levels, respectively. Similarly, AVI-
254 7539 CL_r was unmeasurable for the two lower doses but CL_r/CL_P averaged 1.3%, 20.0%, 30.7%,
255 and 27.1% for the 0.5, 1.5, 3, and 4.5 mg/kg dose levels, respectively. AVI-7287 CL_r was
256 unmeasurable for the 3 lower doses, but CL_r/CL_P averaged 11.0%, 38.7%, and 37.7% for the 1.5,
257 3, and 4.5 mg/kg dose levels. AVI-7288 CL_r was unmeasurable for the two lower doses (0.005
258 and 0.05 mg/kg) but CL_r/CL_P averaged 2.2%, 25.4%, 51.5%, and 49.5% for the 0.5, 1.5, 3, and
259 4.5 mg/kg dose levels. A consistent trend of increasing renal clearance with increasing dose was
260 observed in CL_r/CL_P with an average of 1.7% at 0.5 mg/kg, 19.5% at 1.5 mg/kg, 31.9% at 3.0
261 mg/kg and 39.6% at 4.5 mg/kg which is likely to be due to low affinity interactions between the
262 four different PMO*plus* agents and plasma proteins.

263

264 **DISCUSSION**

265 In demographically similar study subjects, the safety of the two combinations (AVI-6002 and
266 AVI-6003) and the PK of the four components (AVI-7537, AVI-7539, AVI-7287, and
267 AVI-7288) in identical single ascending dose studies were similar. In both studies, all subjects
268 received one dose of active study drug or placebo as planned.

269

270 Both AVI-6002 and AVI-6003 were shown to be safe and well tolerated at doses of 0.005 mg/kg,
271 0.05 mg/kg, 0.5 mg/kg, 1.5 mg/kg, 3 mg/kg, and 4.5 mg/kg per component. A maximum
272 tolerated dose was not observed in either study. More subjects in the AVI-6002 study group

273 experienced treatment-emergent adverse events than in the AVI-6003 study group (50% vs
274 21%), but no dose dependence was observed. Of note, more subjects in the placebo group of the
275 AVI-6002 study experienced AEs than in the placebo group of the AVI-6003 study (83% vs
276 17%). In the AVI-6002 study, headache and nausea were reported more frequently with
277 AVI-6002 than with placebo. However, these results were reported across the cohorts who
278 received AVI-6002 with no apparent relationship to dose.
279
280 The chemically similar components of AVI-6002 and AVI-6003 exhibited generally similar PK
281 profiles. The mean C_{max} and AUC values of the four components showed a dose-dependent
282 increase suggestive of dose-proportional PK. The estimated plasma half-life of all four
283 components was 2 to 5 hours. Most of the drug that was excreted in the urine was excreted
284 within the first 24 hours. Renal clearance increased linearly with dose, and urinary excretion of
285 intact drug accounted for no more than 44.0% of AVI-7537 total elimination, 30.7% of AVI-
286 7539 total elimination, 38.7% of AVI-7287 total elimination, and 51.5% of AVI-7288 total
287 elimination. The plasma protein binding of these components is likely to be weak resulting in a
288 greater filtered fraction in the kidney. The observed increase in renal excretion with dose may be
289 reflected in the increase in V_{ss} observed at higher doses.
290
291 As expected, the safety of the two combinations and the PK of the four components were similar,
292 given their similar chemical properties. The molecular weights and nucleobase compositions of
293 the four components differ, but these differences do not appear to affect the pharmacokinetic
294 properties of the compounds.
295

296 Initially, AVI-6002 and AVI-6003 were developed as the two drug combinations because early
297 mouse and guinea pig lethal challenge models suggested they were the best candidates for post
298 exposure prophylaxis for Ebola and Marburg, respectively. However, subsequent studies in the
299 nonhuman primate lethal virus challenge models demonstrated that AVI-7537 for Ebola and
300 AVI-7288 for Marburg were the active components of the parent combination compounds (10).
301 Therefore, the single agents are now considered the lead clinical candidates. Additional studies
302 in NHPs and humans are underway to estimate the protective human dose, based on appropriate
303 PK parameters such as AUC and CL.
304
305 Through these single ascending dose studies, four PMO*plus* compounds directed against Ebola
306 and Marburg viruses demonstrate human safety profiles that are equivalent to placebo. One of
307 the major advantages of the PMO*plus* platform technology is the ability to very rapidly respond
308 to new or emerging infectious disease threats with safety demonstrated in the chemistry while
309 efficacy and specificity are based upon the sequence used for pathogen specific mRNA
310 translation inhibition.
311

312 **ACKNOWLEDGMENTS**

313 This work was conducted under contract with the Joint Product Management Office of
314 BioDefense Therapeutics (BD-Tx). BD-Tx is a component of the Medical Countermeasure
315 Systems Joint Project Management Office (JPM-MCS) within the U.S. Department of Defense's
316 Joint Program Executive Office for Chemical and Biological Defense. The views expressed here
317 are those of the authors and do not necessarily represent the views or official position of the
318 JPM-MCS or BD-Tx. JPM-MCS aims to provide U.S. military forces and the nation with safe,
319 effective, and innovative medical solutions to counter chemical, biological, radiological, and
320 nuclear threats. JPM-MCS facilitates the advanced development and acquisition of medical
321 countermeasures and systems to enhance our nation's biodefense response capability. For more
322 information, visit www.jpeocbd.osd.mil. Editorial assistance was provided by Richard S. Perry,
323 PharmD and was supported by Sarepta Therapeutics, Cambridge, MA.

324

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FIGURE LEGENDS

FIG 1 The components of AVI-6002 are AVI-7537 and AVI-7539, each of which is a phosphorodiamidate morpholino oligomer in which dimethylamine linkage sites have been replaced with a piperazine rings at defined locations along the backbone to confer a net positive charge.

FIG 2 The components of AVI-6003 are AVI-7287 and AVI-7288, each of which is a phosphorodiamidate morpholino oligomer in which dimethylamine linkage sites have been replaced with a piperazine rings at defined locations along the backbone to confer a net positive charge.

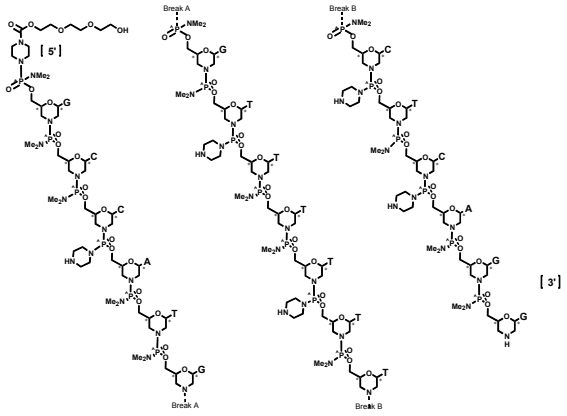
FIG 3 Time-concentration curves of the components of AVI-6002: (A) AVI-7537 and (B) AVI-7539; and the components of AVI-6003: (C) AVI-7287 and (D) AVI-7288.

FIG 4 Mean C_{max} by dose of components of AVI-6002 (AVI-7537, open circles, and AVI-7539, open squares) and AVI-6003 (AVI-7287, plus sign, and AVI-7288, open diamond). Linear regression R^2 0.96 for AVI-7537, 0.94 for AVI-7539, 0.96 for AVI-7287, and 1.00 for AVI-7288. The slopes in ng/mL Cmax per mg/kg dose are 5701±568 for AVI-7537, 6069±780 for AVI-7539, 5545±534 for AVI-7287, and 6122±182 for AVI-7288. Individual linear regression line for each agent plotted.

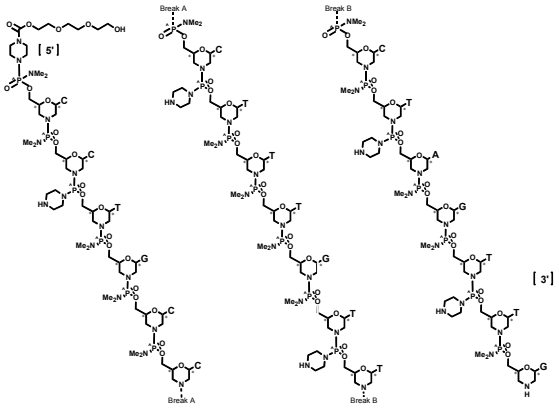
FIG 5 Mean AUC_{0-24} by dose of components of AVI-6002 (AVI-7537, open circles, and AVI-7539, open squares) and AVI-6003 (AVI-7287, plus sign, and AVI-7288, open diamonds).

379 Linear regression R^2 0.99 for AVI-7537, 0.98 for AVI-7539, 0.99 for AVI-7287, and 1.00 for
380 AVI-7288. The slopes in hr*ng/mL AUC_{0-24} per mg/kg dose are 8095±442 for AVI-7537,
381 8612±635 for AVI-7539, 7961±325 for AVI-7287, and 7244±154 for AVI-7288. Individual
382 linear regression line for each agent plotted.

Figure 1

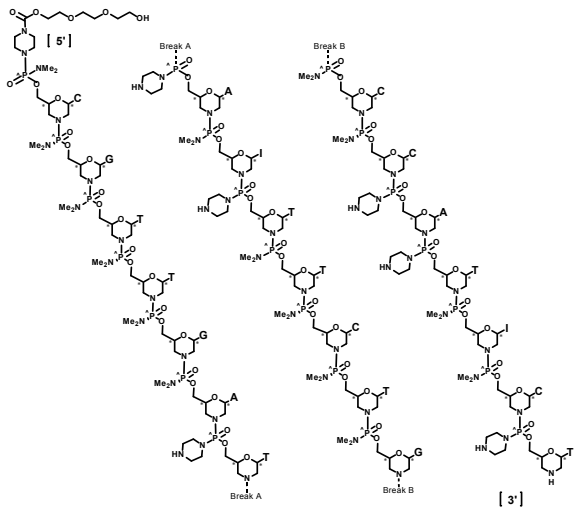


AVI-7357: 5′-GCC $^{+}$ATG GT^{+}T TT^{+}T TC^{+}T C^{+}AG G-3′

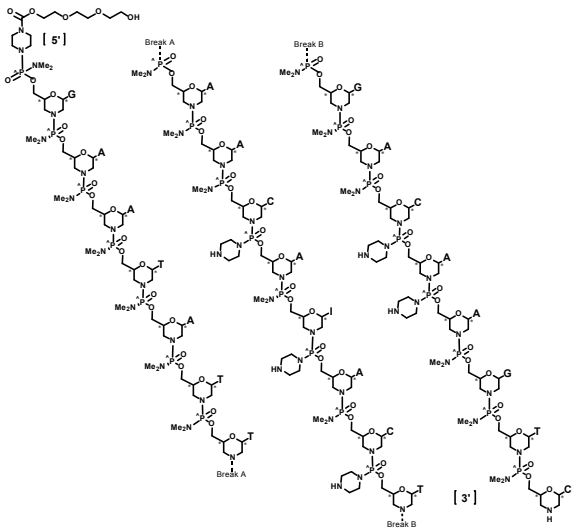


AVI-7539: 5′-CC^{+}T GCC C^{+}TT TGT $^{+}$TCT $^{+}$AGT $^{+}$TG-3′

Figure 2



AVI-7287: 5'-CGT TGA $^{+}$T^{+}AI $^{+}$TTC TGC C^{+}A^{+}T ICT-3'



AVI-7288: 5'-GAA TAT TAA C^{+}AI $^{+}$AC^{+}T GAC $^{+}$A^{+}AG TC-3'

Figure 3

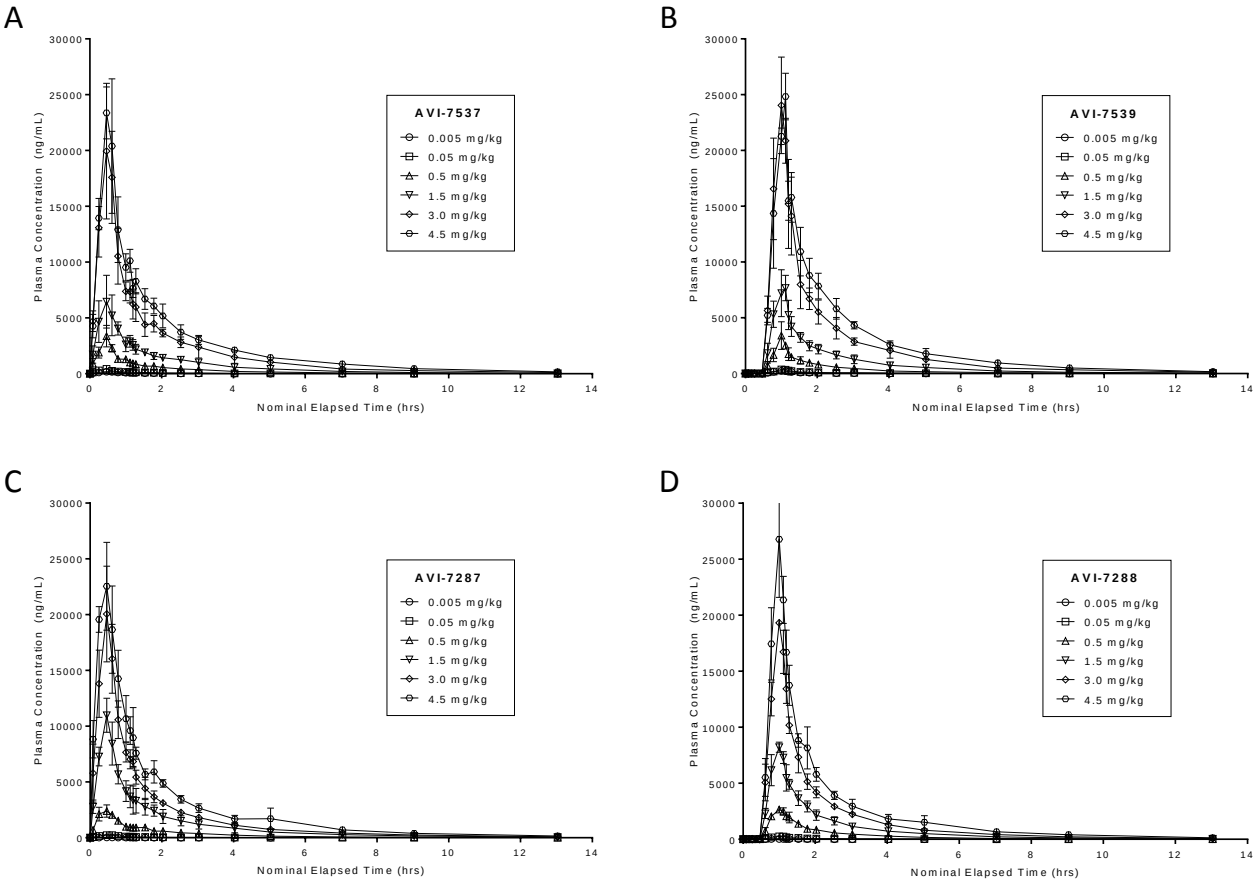


Figure 4

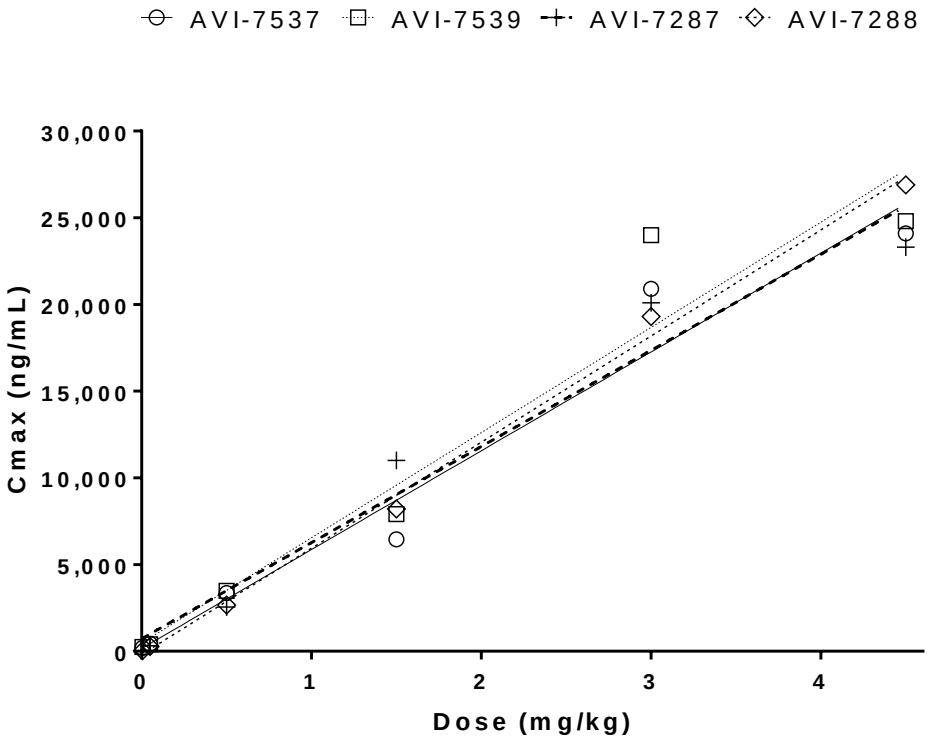


Figure 5

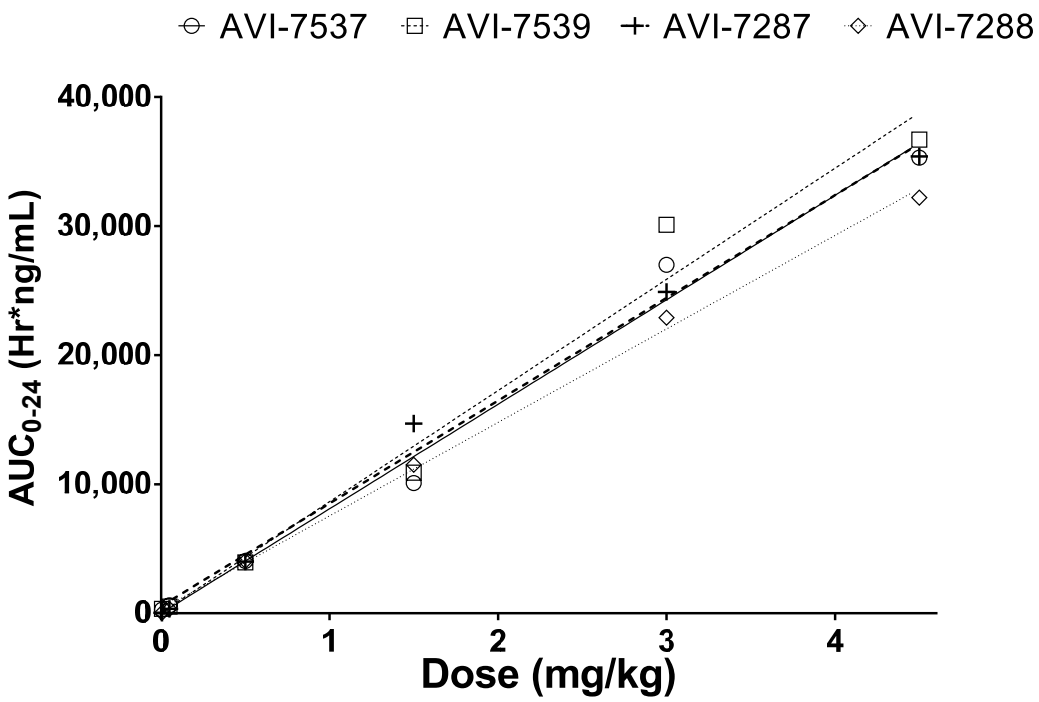


TABLE 1 Study agents

Parent compound	PMO*plus* component	Molecular weight (Daltons)	Number of nucleobases	Nucleobase composition (%)					Number of piperazine rings	Target
				A	C	G	I	T		
AVI-6002	AVI-7357	6825.9	19	11	21	26	0	42	5	Ebola viral protein 24, viral matrix protein that inhibits interferon signaling
	AVI-7359	7092.1	20	5	30	20	0	45	5	Ebola viral protein 35, component of the RNA-dependent RNA polymerase that antagonizes the interferon pathway
AVI-6003	AVI-7287	7491.4	21	14	24	14	10	38	6	Marburg viral protein 24, viral matrix protein that inhibits interferon signaling
	AVI-7288	8179.0	23	43	17	13	4	22	5	Marburg nucleoprotein, the major nucleoprotein involved in RNA encapsulation

TABLE 2 Demographic and baseline characteristics of study subjects

	AVI-6002 Study								AVI-6003 Study							
		Dose (mg/kg)								Dose (mg/kg)						
	Placebo (n=6)	0.01 (n=4)	0.1 (n=4)	1 (n=4)	3 (n=4)	6 (n=4)	9 (n=4)	Total (n=30)	Placebo (n=6)	0.01 (n=4)	0.1 (n=4)	1 (n=4)	3 (n=4)	6 (n=4)	9 (n=4)	Total (n=30)
Gender [n (%)]																
Male	2	2	2	2	2	2	3	15	5	1	2	2	2	2	2	16
	(33)	(50)	(50)	(50)	(50)	(50)	(75)	(50)	(83)	(25)	(50)	(50)	(50)	(50)	(50)	(53)
Female	4	2	2	2	2	2	1	15	1	3	2	2	2	2	2	14
	(67)	(50)	(50)	(50)	(50)	(50)	(25)	(50)	(17)	(75)	(50)	(50)	(50)	(50)	(50)	(47)
Race [n (%)]																
White	4	2	2	3	4	3	2	20	6	2	1	1	3	3	2	18
	(67)	(50)	(50)	(75)	(100)	(75)	(50)	(67)	(100)	(50)	(25)	(25)	(75)	(75)	(50)	(60)
Black or African	1	2	1	1	0	1	2	8	0	2	2	2	0	1	2	9
American	(17)	(50)	(25)	(25)		(25)	(50)	(27)		(50)	(50)	(50)		(25)	(50)	(30)

	AVI-6002 Study								AVI-6003 Study							
	Dose (mg/kg)								Dose (mg/kg)							
	Placebo (n=6)	0.01 (n=4)	0.1 (n=4)	1 (n=4)	3 (n=4)	6 (n=4)	9 (n=4)	Total (n=30)	Placebo (n=6)	0.01 (n=4)	0.1 (n=4)	1 (n=4)	3 (n=4)	6 (n=4)	9 (n=4)	Total (n=30)
Asian	0	0	0	0	0	0	0	0	0	0	1 (25)	1 (25)	1 (25)	0	0	3 (10)
American Indian or Alaska Native	1 (17)	0	1 (25)	0	0	0	0	2 (7)	0	0	0	0	0	0	0	0
Mean age (yrs) [a]	28.0 (5.8)	28.3 (5.1)	30.3 (9.0)	26.5 (6.0)	23.5 (6.5)	24.0 (5.7)	23.3 (3.3)	26.4 (6.0)	33.2 (10.9)	29.0 (8.9)	35.0 (13.5)	26.5 (5.3)	32.8 (5.7)	26.3 (7.5)	33.8 (6.9)	31.1 (8.7)
Mean weight (kg) [a]	81.6 (10.9)	77.8 (3.3)	75.9 (14.8)	65.1 (7.1)	71.1 (10.5)	73.0 (16.2)	66.6 (7.7)	73.6 (11.3)	77.3 (14.0)	75.7 (18.4)	76.1 (12.9)	73.7 (21.7)	69.0 (4.5)	69.8 (4.6)	70.1 (5.0)	73.4 (12.3)
Mean body mass index (kg/m^2) [a]	27.0 (3.0)	27.1 (2.9)	26.4 (3.0)	22.6 (2.7)	24.2 (3.1)	26.3 (5.5)	23.7 (1.7)	25.5 (3.4)	24.5 (4.4)	26.3 (4.5)	25.3 (3.3)	24.0 (4.5)	25.2 (3.3)	23.8 (2.1)	25.2 (2.2)	24.9 (3.4)

	AVI-6002 Study								AVI-6003 Study							
	Dose (mg/kg)								Dose (mg/kg)							
	Placebo (n=6)	0.01 (n=4)	0.1 (n=4)	1 (n=4)	3 (n=4)	6 (n=4)	9 (n=4)	Total (n=30)	Placebo (n=6)	0.01 (n=4)	0.1 (n=4)	1 (n=4)	3 (n=4)	6 (n=4)	9 (n=4)	Total (n=30)
Estimated creatinine clearance (mL/min) [a, b]	114.1 (28.4)	107.8 (6.9)	104.3 (21.0)	108.2 (20.4)	112.8 (16.2)	103.2 (12.9)	111.9 (16.8)	109.2 (18.0)	106.7 (14.5)	110.7 (14.4)	109.9 (29.4)	118.0 (4.6)	105.1 (8.3)	118.4 (17.2)	98.9 (14.5)	109.5 (17.1)

[a] Mean (standard deviation)

[b] Estimated creatinine clearance based on Cockcroft-Gault calculation using lean (ideal) body weight: (140 - Age) x Ideal body weight (in kg) x [0.85 if Female] / 72 x Serum Creatinine (mg/dL).

TABLE 3 Treatment-emergent adverse events occurring more than once in a given System Organ Class in either study[a]

	AVI-6002 Study								AVI-6003 Study							
		Dose (mg/kg)								Dose (mg/kg)						
	Placebo (n=6)	0.01 (n=4)	0.1 (n=4)	1 (n=4)	3 (n=4)	6 (n=4)	9 (n=4)	AVI-6002 (n=24)	Placebo (n=6)	0.01 (n=4)	0.1 (n=4)	1 (n=4)	3 (n=4)	6 (n=4)	9 (n=4)	AVI-6003 (n=24)
Any TEAE	5 (83)	3 (75)	0	2 (50)	3 (75)	3 (75)	1 (25)	12 (50)	1 (17)	2 (50)	4 (100)	1 (25)	2 (50)	2 (50)	2 (50)	13 (54)
Gastrointestinal disorders[b]	1 (17)	2 (50)	0	0	2 (50)	1 (25)	0	5 (21)	0	0	2 (50)	0	1 (25)	1 (25)	1 (25)	5 (21)
Nervous system disorders[c]	0	3 (75)	0	0	0	1 (25)	0	4 (17)	0	1 (25)	2 (50)	0	1 (25)	1 (25)	1 (25)	6 (25)
Respiratory, thoracic and mediastinal disorders[d]	1 (17)	1 (25)	0	0	0	1 (25)	0	2 (8)	0	0	1 (25)	0	0	0	1 (25)	2 (8)
General disorders and administrative site	3 (50)	2 (50)	0	0	0	0	0	2 (8)	0	0	0	0	1 (25)	0	0	1 (4)

	AVI-6002 Study								AVI-6003 Study							
		Dose (mg/kg)								Dose (mg/kg)						
	Placebo (n=6)	0.01 (n=4)	0.1 (n=4)	1 (n=4)	3 (n=4)	6 (n=4)	9 (n=4)	AVI-6002 (n=24)	Placebo (n=6)	0.01 (n=4)	0.1 (n=4)	1 (n=4)	3 (n=4)	6 (n=4)	9 (n=4)	AVI-6003 (n=24)
conditions[e]																
Musculoskeletal and connective tissue disorders[f]	0	0	0	0	0	0	0	0	1 (17)	0	0	0	0	0	1 (25)	1 (4)

[a] No. (%) of subjects

[b] Gastrointestinal disorders included nausea in 3 subjects and abdominal discomfort, upper abdominal pain, diarrhea, and vomiting in 1 subject each in the AVI-6002 study; and abdominal distension, abdominal pain, gastroesophageal reflux disease, anorectal discomfort, aphthous stomatitis, nausea and vomiting in 1 subject each in the AVI-6003 study.

[c] Nervous system disorders included headache in 4 subjects and somnolence in 1 subject in the AVI-6002 study; and headache in 3 subjects, dizziness in 2 subjects and cataplexy, dysgeusia and tremor in 1 subject each in the AVI-6003 study.

[d] Respiratory, thoracic and mediastinal disorders included sinus congestion in 3 subjects in the AVI-6002 study; and nasal congestion and rhinorrhea in 1 subject each in the AVI-6003 study.

[e] General disorders and administrative site conditions included chest discomfort, fatigue, feeling cold, and infusion site erythema in 1 one subject each in the AVI-6002 study; and fatigue in 1 subject in the AVI-6003 study.

[f] Musculoskeletal and connective tissue disorders included arthralgia and muscle tightness in 1 subject each in the AVI-6003 study.

TABLE 4 PK parameters of components of AVI-6002 (AVI-7537 and AVI-7539) and AVI-6003 (AVI-7287 and AVI-7288)

	Mean (standard deviation)					
	C_{max} (ng/mL)	AUC_{0-24} (hr*ng/mL)	T_{max} (hr)	Plasma half-life (hr)	Cl_P (mL/hr/kg)	V_{ss} (mL/kg)
AVI-7537						
0.005 mg/kg	185 (108)	285 (173)	0.5 (0.08)	2.0 (0.30)	31 (32.6)	65 (68.2)
0.05 mg/kg	425 (99)	617 (237)	0.5 (0.00)	2.0 (0.57)	89 (29.1)	183 (23.8)
0.5 mg/kg	3360 (974)	4060 (462)	0.5 (0.00)	3.6 (0.25)	122 (11.5)	382 (139.0)
1.5 mg/kg	6460 (2370)	10100 (2140)	0.5 (0.00)	2.8 (0.81)	152 (32.3)	406 (124.0)
3.0 mg/kg	20900 (5220)	27000 (6520)	0.5 (0.07)	4.6 (1.17)	114 (22.9)	334 (42.6)
4.5 mg/kg	24100 (3440)	35300 (3700)	0.5 (0.08)	4.0 (0.71)	126 (12.6)	453 (70.8)
AVI-7539						
0.005 mg/kg	242 (67)	338 (72)	0.5 (0.07)	2.1 (0.09)	15 (2.7)	48 (40.3)
0.05 mg/kg	399 (107)	519 (150)	0.5 (0.09)	1.9 (0.63)	103 (28.5)	201 (13.8)
0.5 mg/kg	3480 (1130)	3950 (658)	0.5 (0.06)	2.9 (0.80)	127 (18.9)	387 (125.0)
1.5 mg/kg	7910 (1440)	10900 (1950)	0.5 (0.07)	2.5 (0.92)	140 (24.4)	329 (91.2)
3.0 mg/kg	24000 (4330)	30100 (6250)	0.5 (0.00)	4.6 (1.21)	101 (19.4)	273 (38.6)
4.5 mg/kg	24800 (2090)	36700 (2420)	0.6 (0.00)	3.9 (1.01)	121 (7.9)	401 (35.1)

	Mean (standard deviation)					
	C_{max} (ng/mL)	AUC_{0-24} (hr*ng/mL)	T_{max} (hr)	Plasma half-life (hr)	Cl_P (mL/hr/kg)	V_{ss} (mL/kg)
AVI-7287						
0.005 mg/kg	34 (8)	48 (11)	0.6 (0.14)	1.6 (0.92)	94 (15.8)	189 (99.8)
0.05 mg/kg	313 (89)	338 (65)	0.5 (0.08)	1.8 (0.26)	151 (31.0)	299 (65.3)
0.5 mg/kg	2550 (486)	4000 (729)	0.5 (0.14)	1.9 (0.46)	129 (27.0)	278 (33.9)
1.5 mg/kg	11000 (1530)	14700 (3470)	0.5 (0.0)	2.2 (0.33)	105 (24.7)	267 (50.4)
3.0 mg/kg	20100 (4290)	24900 (3200)	0.5 (0.0)	5.0 (2.04)	120 (17.0)	354 (88.9)
4.5 mg/kg	23300 (2770)	35400 (3720)	0.4 (0.11)	3.7 (1.46)	123 (12.3)	537 (169.0)
AVI-7288						
0.005 mg/kg	36 (7)	45 (7)	0.4 (0.12)	1.2 (0.48)	104 (17.6)	158 (21.0)
0.05 mg/kg	272 (97)	322 (105)	0.5 (0.06)	1.6 (0.19)	171 (67.9)	303 (142.0)
0.5 mg/kg	2660 (271)	4060 (638)	0.5 (0.01)	1.7 (0.46)	126 (23.4)	241 (30.5)
1.5 mg/kg	8210 (460)	11500 (1680)	0.5 (0.01)	2.4 (0.53)	133 (19.2)	277 (38.4)
3.0 mg/kg	19300 (290)	22900 (1170)	0.5 (0.01)	4.1 (1.95)	130 (7.2)	315 (131.0)
4.5 mg/kg	26900 (5040)	32200 (3480)	0.5 (0.06)	5.5 (2.51)	136 (11.6)	569 (298.0)