

Preclinical safety of AVI-4658, a Phosphorodiamidate Morpholino Oligomer (PMO), being developed to skip Exon 51 in Duchenne muscular dystrophy (DMD).

Shrewsbury SB; Sazani P*

AVI BioPharma Inc. 3450 Monte Villa Parkway, Bothell, Washington, USA *psazani@avibio.com



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Abstract

Background: AVI-4658 is a PMO that skips dystrophin exon 51, restores the reading frame and enables dystrophin expression in selected DMD patients, proven by a single IM dose study in the UK. To enable clinical trials in the US, three 12-week GLP studies in animals were performed. Published data suggests the older phosphorothioate antisense oligonucleotides have dose limiting toxicities.

Objective: The current studies were designed to assess the safety of AVI-4658 (and PMO in general).

Method: (1) *mdx* mice were dosed IV with 0, 12, 120 or 960mg/kg (the maximum feasible dose (MFD)), or subcutaneously at 960mg/kg; wild type C57 mice at 0 and 960 mg/kg (i.e., 7 groups) with AVI-4658. (2) A second identical study with AVI-4225, the PMO to skip exon 23 of in the dystrophic mouse and restore dystrophin (looking for mechanistic toxicity), was also performed. (3) Cynomolgus monkeys were dosed IV with 0, 5, 40 or 320mg/kg (MFD) and 320mg/kg subcutaneously. A 28 day recovery period was included in all studies.

Results: In mice, both AVI-4658 and AVI-4225 were well tolerated at doses including 960 mg/kg/injection, with no adverse effects. Findings were generally limited to the kidney, and were generally reversible, as shown in the 28 day recovery groups. No evidence of kidney function change was detected. In cynomolgus monkeys, AVI-4658 was also well tolerated at all doses including 320 mg/kg/injection, with no adverse effects. Findings were similar to those seen in the mouse studies.

Conclusion: AVI-4658, the first PMO for DMD, was extremely well tolerated at all doses in dystrophic mice, normal mice and non-human primates. In addition, AVI-4225, which restores dystrophin in *mdx* mice, also led to no adverse effects. Based on this preclinical package, and encouraging safety and dystrophin expression results from a concurrent UK clinical study, US clinical studies are anticipated.

PRECLINICAL METHODS:

Species: Cynomolgus monkeys, *mdx* and C57 wild-type mice

Route of Administration: intravenous or subcutaneous injection.

Frequency and Duration of Administration: once weekly for a total of 12 doses.

Terminal and Recovery Phases: Terminal – 1 day following the final dose
Recovery – 28 days following the final dose

Analyses

OBSERVATIONS: Twice daily (mortality/morbidity)

DETAILED CLINICAL OBSERVATION: Weekly **BODY WEIGHTS:** Weekly

FOOD CONSUMPTION: Daily (Monkey); Weekly (Mouse)

PHYSICAL EXAMINATIONS: Conducted by staff veterinarian on all animals prior to initiation of test article administration

OPHTHALMOLOGY: All animals pretest and all survivors at termination and recovery

ELECTROCARDIOGRAMS: Monkey – All surviving animals pretest, at 1 hour post-dose during the last week of dosing, and prior to the recovery necropsy

CLINICAL PATHOLOGY:

Pretest, at Week 4 (Monkey), and prior to the terminal and recovery necropsies

Hematology and Coagulation

erythrocyte count (total and absolute differential), erythrocyte count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular concentration (calculated), absolute reticulocytes, platelet count, blood cell morphology, prothrombin time, activated partial thromboplastin time

Clinical chemistry

alkaline phosphatase, total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, sorbitol dehydrogenase, urea nitrogen, creatinine, creatine kinase (MM and MB isozymes), total protein, albumin, globulin and A/G (albumin/globulin) ratio (calculated), glucose, total cholesterol, triglycerides, electrolytes (sodium, potassium, chloride), calcium, phosphorus

Urinalysis evaluations

Volume, specific gravity, pH, color and appearance, protein, glucose, bilirubin, ketones, blood, urobilinogen, microscopy of centrifuged sediment, creatinine, creatinine/16 hours (calculated), creatinine clearance (calculated)

TOXICOKINETICS:

Monkey **NECROPSY:** All animals

ORGAN WEIGHTS: Adrenals, brain, heart, kidneys, liver, lungs, ovaries with oviducts, pituitary, prostate, salivary glands, spleen, thyroid with parathyroid, thymus, testes, uterus

SLIDE PREPARATION/MICROSCOPIC PATHOLOGY: All main study animals, full set of standard tissues (approximately 70); target organs in recovery animals (to be determined), gross lesions from all animals.

Table 1. Study Design – AVI-4658 in Cynomolgus Monkeys

Group	Animals/Group (M/F)	Study Drug			Necropsy (M/F)		
		Name	Dose (mg/kg)	Route	Frequency	Day 79 (12 doses)	Day 106 (12 doses +28 day recovery)
1	6/6	AVI-4658	0	IV	Weekly (last dose Day 78)	4/4	2/2
2	6/6		5	IV		4/4	2/2
3	6/6		40	IV		4/4	2/2
4	6/6		320	IV		4/4	2/2
5	6/6		320	SC		4/4	2/2

Table 2. Study Design – AVI-4658 and AVI-4225 in mice

Group	Animals/Group (M/F)	Study Drug			Necropsy (M/F)		
		Name	Dose (mg/kg)	Route	Frequency	Day 79 (12 doses)	Day 106 (12 doses +28 day recovery)
1	15/15 (<i>mdx</i>)	AVI-4658 Or AVI-4225	0	IV	Weekly (last dose Day 78)	10/10	5/5
2	15/15 (<i>mdx</i>)		12	IV		10/10	5/5
3	15/15 (<i>mdx</i>)		120	IV		10/10	5/5
4	15/15 (<i>mdx</i>)		960	IV		10/10	5/5
5	15/15 (<i>mdx</i>)		960	SC		10/10	5/5
6	15/15 (WT)		0	IV		10/10	5/5
7	15/15 (WT)		960	IV		10/10	5/5

Results in Primates:

Table 3. Coagulation and Chemistry results with AVI-4658 (Primates)

Endpoint	Time Point	Dose 0 mg/kg (IV)		5 mg/kg (IV)		40 mg/kg (IV)		320 mg/kg (IV)		320 mg/kg (SC)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
APTT (sec)	Pretest	27.7	2.7	28.4	2.6	28.4	3.1	27.6	3.5	28.1	2.0
	Week 4	27.2	2.9	27.7	3.8	27.0	2.4	26.1	2.7	32.8	2.3
	Terminal	25.7	2.0	25.8	2.8	25.1	2.4	24.7	3.3	24.8	2.7
Prothrombin Time (sec)	Pretest	11.0	0.4	10.7	0.3	11.2	0.5	10.6	0.4	10.8	0.6
	Week 4	11.5	0.5	11.3	0.4	11.6	0.3	11.1	0.6	11.1	0.5
	Terminal	11.1	0.3	10.5	0.5	10.8	0.5	10.7	0.6	10.5	0.5
ALT (U/L)	Pretest	41.0	11.8	39.2	10.4	37.8	12.3	33.8	10.2	37.8	15.1
	Week 4	52.0	19.3	42.8	13.3	39.3	14.9	31.3	10.1	39.8	10.9
	Terminal	44.3	10.6	49.8	18.4	50.5	18.5	39.0	9.9	39.8	13.5
Blood Urea Nitrogen (mg/dL)	Pretest	23.5	1.9	25.3	2.9	22.3	2.4	23.7	4.3	25.0	3.0
	Week 4	25.3	3.5	26.0	3.1	20.3	3.3	23.8	2.4	24.8	3.9
	Terminal	25.5	2.4	25.8	5.2	20.5	2.3	22.3	4.5	25.0	4.1
Serum Creatinine (mg/dL)	Pretest	0.68	0.08	0.68	0.08	0.68	0.1	0.73	0.1	0.73	0.10
	Week 4	0.68	0.10	0.66	0.09	0.67	0.1	0.65	0.2	0.62	0.08
	Terminal	0.63	0.08	0.62	0.08	0.65	0.1	0.60	0.1	0.60	0.06
Urine Creatinine (mg/dL)	Pretest	44.5	24.1	43.2	28.1	46.3	16.2	42.9	27.4	51.9	13.7
	Terminal	54.3	30.1	59.0	33.2	74.2	33.2	69.5	40.7	64.0	28.5
	Pretest	22.1	18.7	20.3	12.9	28.3	5.6	25.8	12.5	31.9	7.0
Urine Creatinine (mg/16hr)	Pretest	25.4	4.5	35.7	13.9	34.4	11.0	35.0	10.1	37.2	8.0
	Terminal	3.5	3.0	3.1	2.0	4.3	0.5	3.7	2.0	4.6	1.3
	Terminal	4.3	1.3	6.2	2.8	5.5	1.3	6.3	1.9	6.6	1.6

Figure 1. Mean (SD) serum creatinine in primates over 12 weeks after IV or subcutaneous doses of AVI-4658

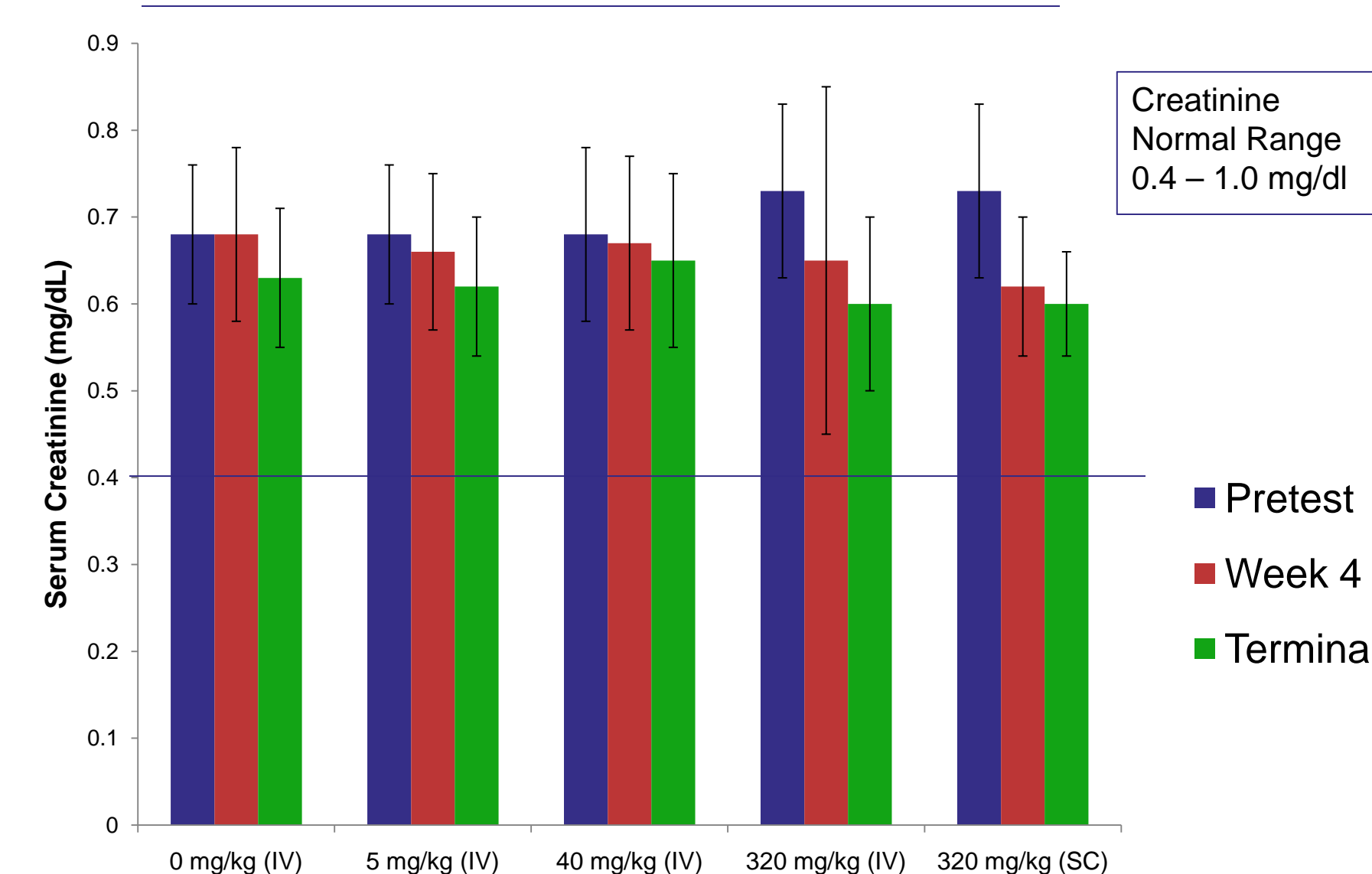


Table 4. Microscopic Evaluation Summary of AVI-4658 (Primates)

Test Article-related Microscopic Observations (Kidney) - Terminal												
Dose level: mg/kg (Route)	0 (IV)		5 (IV)		40 (IV)		320 (IV)		320 (SC)		320 (SC)	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Number Examined	4	4	4	4	4	4	4	4	4	4	4	4
Basophilic granules, tubular cell	-minimal: 0 0 2 0 2 0 0 0 0 0 0 0 0											
Basophilic tubules, cortex	-minimal: 0 0 0 0 2 3 4 4 4 4 4 4 4											
Vacuolation, tubular	-minimal: 0 0 0 0 2 3 0 0 0 0 0 0 0											
	-mild: 0 0 0 0 0 0 0 4 4 4 4 4 1											
	-moderate: 0 0 0 0 0 0 0 0 0 0 0 0 3											
Vacuolation, tubular	-minimal: 0 0 0 0 0 0 0 4 4 4 4 4 4											
	-mild: 0 0 0 0 0 0 0 4 4 4 4 4 3											
	-moderate: 0 0 0 0 0 0 0 0 0 0 0 0 1											

Result: No adverse findings at any dose level

Figure 2. Representative images of kidney tissue from male monkeys treated with IV AVI-4658, showing minimal basophilic granules at all doses and some vacuolization at higher (40 and 320 mg/kg) doses.

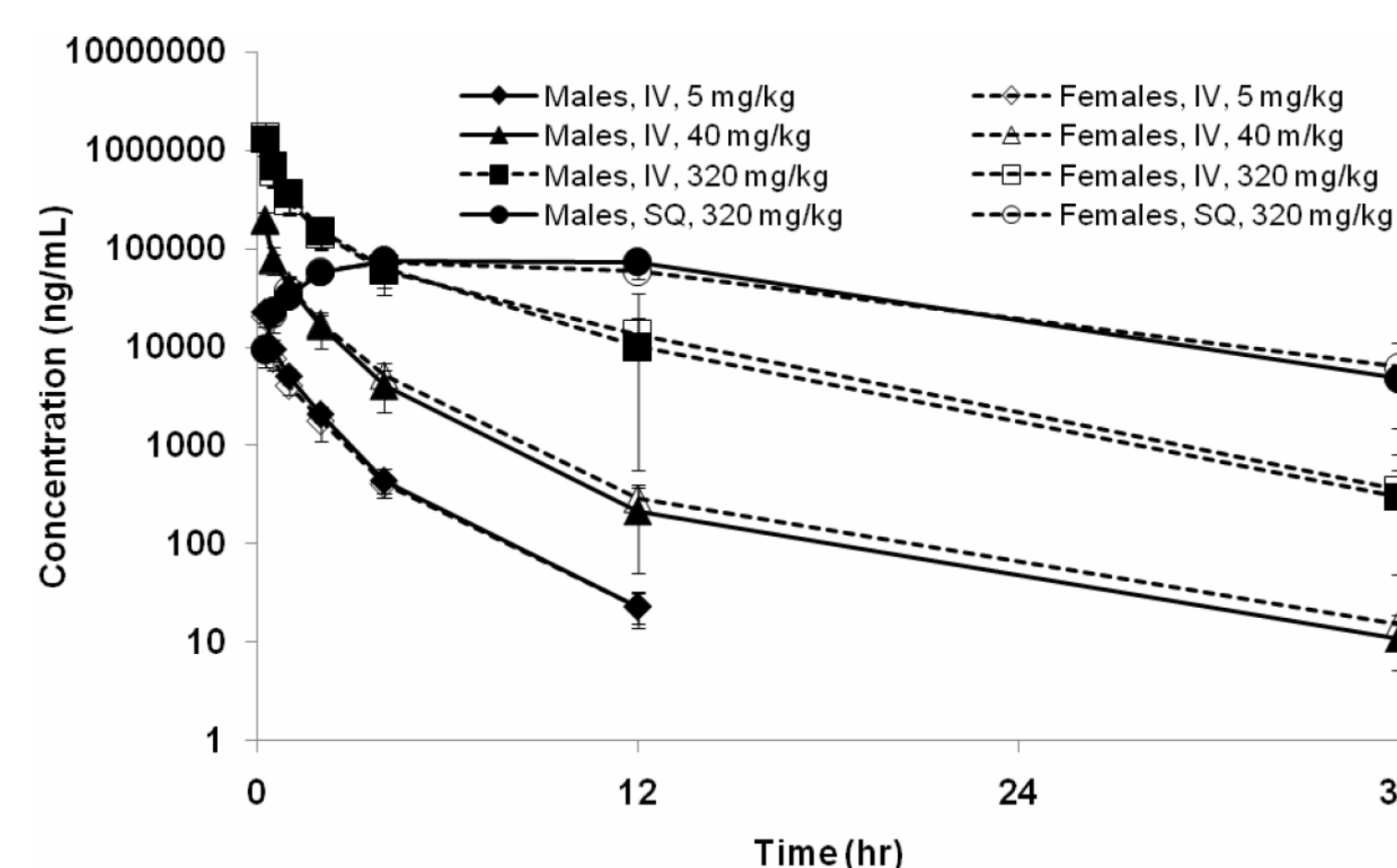
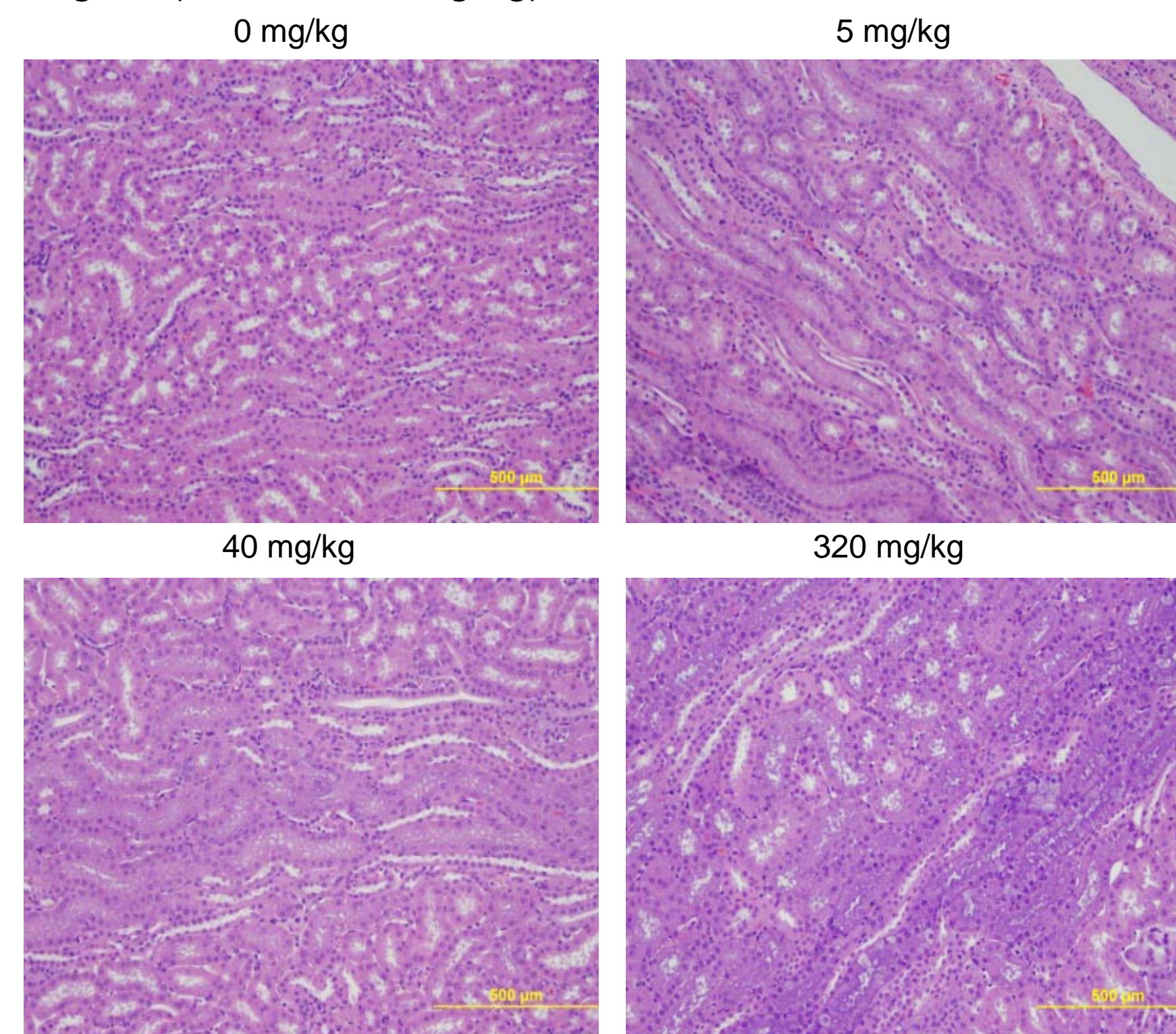


Figure 3. Mean (SD) Plasma concentrations of AVI-4658 in male and female monkeys after a single intravenous (IV) or subcutaneous (SQ) dose

Results in Mice (*mdx* and Wild Type):

Table 5. Chemistry Summary of AVI-4658 and AVI-4225 in *mdx* and healthy male mice

Endpoint	Time Point	Dose 0 mg/kg (IV) <i>mdx</i>		120 mg/kg (IV) <i>mdx</i>		960 mg/kg (IV) <i>mdx</i>		0 mg/kg (IV) C57		960 mg/kg (IV) C57	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AST (U/L)	Terminal	3232	606.3	2633	1275.2	2676†	1102.9	154	65.1	134	54.8
	Recovery	5160	1060.7	4053	1969.2	5797	3424.1	130	19.5	113	22.8
BUN (mg/dL)	Terminal	31	3.8	29	2.7	35	13.4	21	1.8	22	1.5
	Recovery	56	19.8	33	2.1	49	17.6	23	2.5	24	1.2

Table 6. Chemistry Summary of AVI-4658 and AVI-4225 in *mdx* and healthy female mice

Endpoint	Time Point	Dose 0 mg/kg (IV) <i>mdx</i>		120 mg/kg (IV) <i>mdx</i>		960 mg/kg (IV) <i>mdx</i>		0 mg/kg (IV) C57		960 mg/kg (IV) C57	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AST (U/L)	Terminal	2467	1031.3	3410	1204.3	4520	1668.0	386	437.5	150	57.8
	Recovery	5160	1060.7	4053	1969.2	5797	3424.1	130	19.5	113	22.8
BUN (mg/dL)	Terminal	28	2.5	51	11.8	43	16.8	26	14.1	18	3.7
	Recovery	56	19.8	33	2.1	49	17.6	23	2.5	24	1.2

Table 7. Microscopic Evaluation Summary of AVI-4658 and AVI-4225 in *mdx* mice (Terminal and Recovery)

Test Article-related Microscopic Observations (Kidneys) - Terminal						Myofiber Degeneration in <i>mdx</i> mice – Termination																
AVI-4658 Dose level: mg/kg	0 (IV)		12 (IV)		120 (IV)		960 (IV)		960 (SC)		AVI-4225 Dose level: mg/kg		0 (IV)		12 (IV)		120 (IV)		960 (IV)		960 (SC)	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Number Examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Basophilic tubules, cortex	-minimal: 0 0 0 0 1 1 7 5 1 2																					
Vacuolation, tubular	-minimal: 0 0 0 0 1 1 5 5 1 1																					
Basophilic tubules, cortex	-minimal: 0 0 0 0 0 0 3 6 0 0																					
	-mild: 0 0 0 0 0 0 6 2 0 0																					
	-moderate: 0 0 0 0 0 0 0 0 0 0																					
Degeneration/regeneration, tubular	-minimal: 0 0 0 0 0 0 9 4 0 0																					
	-mild: 0 0 0 0 0 0 0 0 0 0																					
	-moderate: 0 0 0 0 0 0 0 0 0 0																					
Vacuolation, tubular	-minimal: 0 0 0 0 0 0 0 4 0 0																					
	-mild: 0 0 0 0 0 0 0 0 0 0																					
	-moderate: 0 0 0 0 0 0 0 0 0 0																					

Result: No adverse findings at any dose level

Conclusions:

- Cynomolgus monkeys were dosed once weekly for 12 weeks with AVI-4658 at levels up to and including the Maximum Feasible Dose (MFD) of 320 mg/kg/injection, either intravenously or subcutaneously
- Wild type and *mdx* mice were dosed once weekly for 12 weeks with either AVI-4658 or AVI-4225 at levels up to and including the MFD of 960 mg/kg/injection, either intravenously or subcutaneously
- AVI-4658 in monkeys and mice and AVI-4225 in mice were well tolerated at MFD, with no adverse effects
- Findings were generally limited to the kidney, and included basophilic granules, basophilic tubules and instances of vacuolation at the highest dose levels. No evidence of kidney function change (clinical chemistry or urinalysis) was detected. Kidney findings were generally reversible in the 28 day recovery groups
- Mouse specific PMO, AVI-4225, led to reduction in muscle pathology in the *mdx* mouse in a dose dependant fashion.
- Formal NOAEL were set at 320mg/kg and 960mg/kg in primates and mice respectively.