

# Preliminary toxicological evaluation of AVI-7100, a phosphorodiamidate morpholino oligomer with selectively introduced positive charges (PMOplus) targeted to a highly conserved region of Influenza A virus

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## ABSTRACT

Seasonal influenza (H3N2), along with the recently emergent swine origin influenza virus (SOIV), H1N1, are caused by the influenza A virus. Both forms of the disease are major health concerns. To address both emerging multidrug resistant viral strains and the needs of susceptible populations will require new forms of treatments. To meet this need, we have progressed into preclinical development AVI-7100, a phosphorodiamidate morpholino oligomer with selectively introduced positive charges (PMOplus). This novel oligomer is targeted to a highly conserved region of the influenza A virus. In previous studies in the ferret model of H1N1 disease, AVI-7100 reduced the combined average daily viral titer in nasal wash through peak viral load (days 1 - 3) versus saline control (p=0.0012) and oseltamivir control (p=0.0103) by up to 3.9 log. Microscopic and pathology scores also revealed a significant benefit only to animals receiving AVI-7100 versus controls. These data show that AVI-7100 is active against a fully virulent and non-adapted strain of pandemic H1N1 virus in the ferret model. In the current toxicological studies, AVI-7100 was evaluated in rats and cynomolgus monkeys (non-human primate, NHP) following 7 once daily intravenous slow bolus injections, at doses up to 240 and 180 mg/kg/injection, respectively. Clinical observations, mean body weight, organ weights, mean food consumption, macroscopic observations, or ophthalmoscopic clinical chemistry, hematology and coagulation evaluations were performed for all animals. In addition, urinary cystatin C concentrations, an investigational biomarker for nephrotoxicity with increased sensitivity over standard measures, was also evaluated. At all doses tested, including those that are equivalent or greater than the expected clinical dose, AVI-7100 was extremely well tolerated in both species. Overall, the data, which will be discussed in detail, support further development of AVI-7100 as an influenza therapeutic.

## Methods:

### Ferret Studies:

**Procedure:** Ferrets were obtained from an approved source and acclimated to the laboratory for a period of one week. In that time a small telemetry device was implanted in the animals which identified each animal and reported body temperature.

On Day 0, all animals received H1N1 inoculation at  $1-5 \times 10^5$  pfu per ferret (not expected to be a lethal challenge). A nasal wash sample was collected from each animal beginning on Day 1 and then daily through day 6 for determination of viral titer in the upper respiratory system (sample will be taken at the time of dosing to prevent excessive handling of the animals). Lungs were preserved and placed in storage for histologic examination.

Group	Agent	Chemistry	Dose (mg/kg)	Route	Schedule	Day 3	Day 7
1	AVI-7100 <sup>a</sup>	PMOplus	10 <sup>b</sup>	i.p.	-4H, 1D, 3D, 5D	3 <sup>c</sup>	3 <sup>d</sup>
2	AVI-7100	PMOplus	30	i.p.	-4H, 1D, 3D, 5D	3	3
3	Scramble	PMOplus	30	i.p.	-4H, 1D, 3D, 5D	3	3
4	AVI-7100	PPMO	0.5	i.n.	-4H, 1D, 3D, 5D	3	3
5	AVI-7100	PPMO	1.5	i.n.	-4H, 1D, 3D, 5D	3	3
6	Scramble	PPMO	1.5	i.n.	-4H, 1D, 3D, 5D	3	3
7	Tamiflu	-	10	p.o.	-4H, 1D, 3D, 5D	3	3
8	Saline	-	-	i.n.	-4H, 1D, 3D, 5D	3	3
<b>TOTALS</b>						24	24

Representative study design

## Toxicology Studies:

### 7 Day toxicology study in rats

- Based on good tolerability in 4 Day pilot in Rats at 170 mg/kg
- Regimen: Daily
- Dose range: 24 – 240 mg/kg
- Clinical Observations, Hematology, Serum Chemistry, Coagulation, Organ weights
- Special kidney biomarkers – Urine cystatin C and GGT
- Recovery, toxicokinetic and histopathology analysis to be performed in GLP study

Group	Animals (M/F)	Study Drug				Necropsy	
		Name	Dose (mg/kg)	Route	Frequency	Terminal	Recovery
1	5/5	0	0	IV	5/5	0	0
2	5/5	AVI-7100	24	IV	Daily	5/5	0
3	5/5	AVI-7100	120	IV	7 Days	5/5	0
4	5/5	AVI-7100	240	IV	7 Days	5/5	0

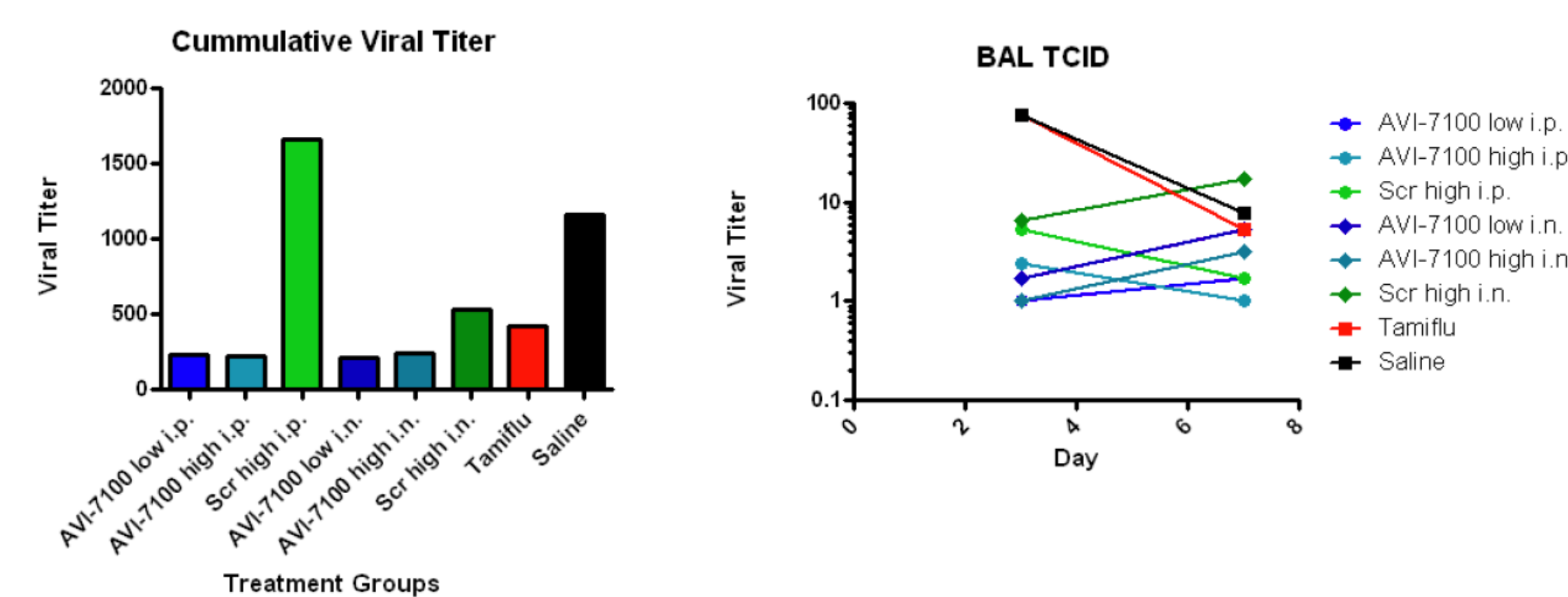
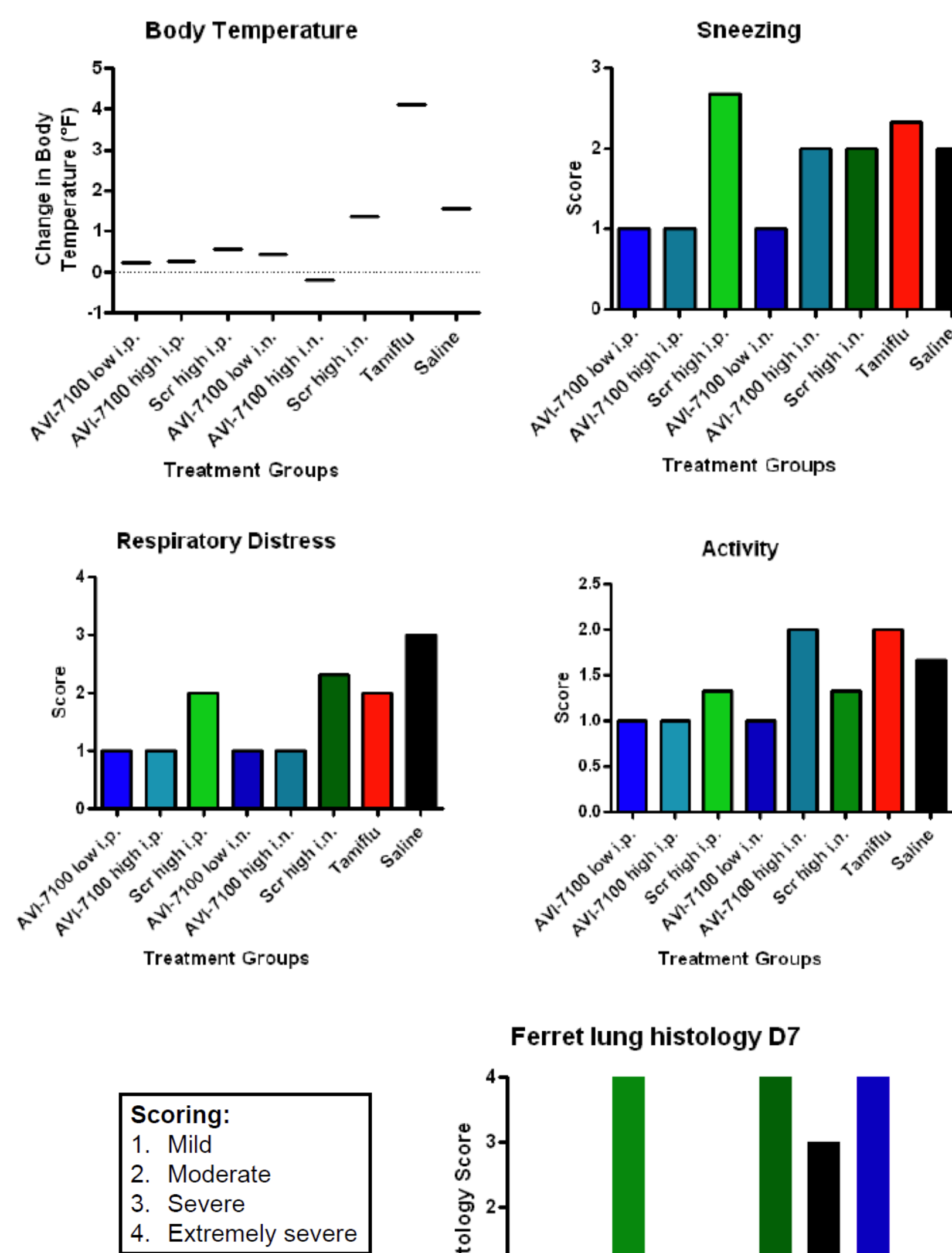
### 7 Day toxicology study in cynomolgus monkeys

- 1st toxicological evaluation of AVI-7100 in non-human primates (NHP)
- Regimen: Daily
- Dose range: 12 – 180 mg/kg
- Clinical observations, hematology, serum chemistry, coagulation, organ weights
- Special kidney biomarkers – Urine cystatin C and GGT
- Recovery, toxicokinetic and histopathology analysis to be performed in GLP study

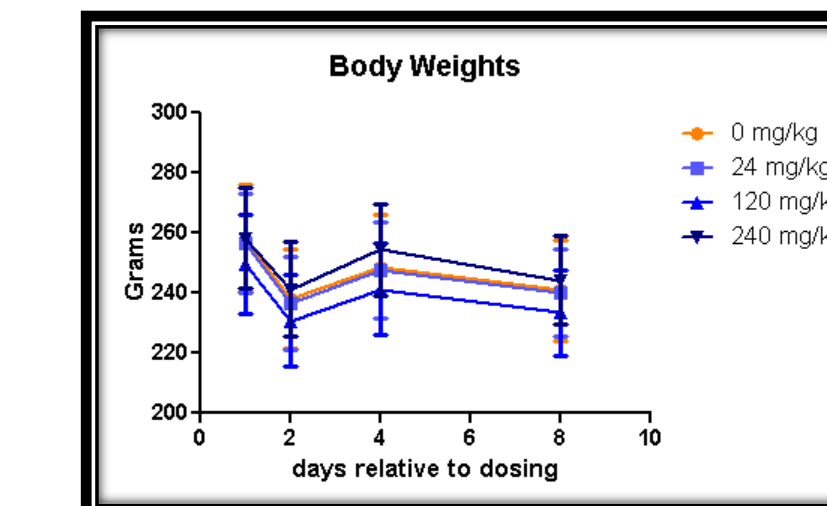
Group	Animals (M/F)	Study Drug				Necropsy	
		Name	Dose (mg/kg)	Route	Frequency	Terminal	Recovery
1	2/2	0	0	IV	2/2	0	
2	2/2	AVI-7100	12	IV	Daily	2/2	0
3	2/2	AVI-7100	60	IV	7 Days	2/2	0
4	2/2	AVI-7100	180	IV	7 Days	2/2	0

## Oligomer Chemistries

### Activity of AVI-7100 in a Ferret Influenza Model Study

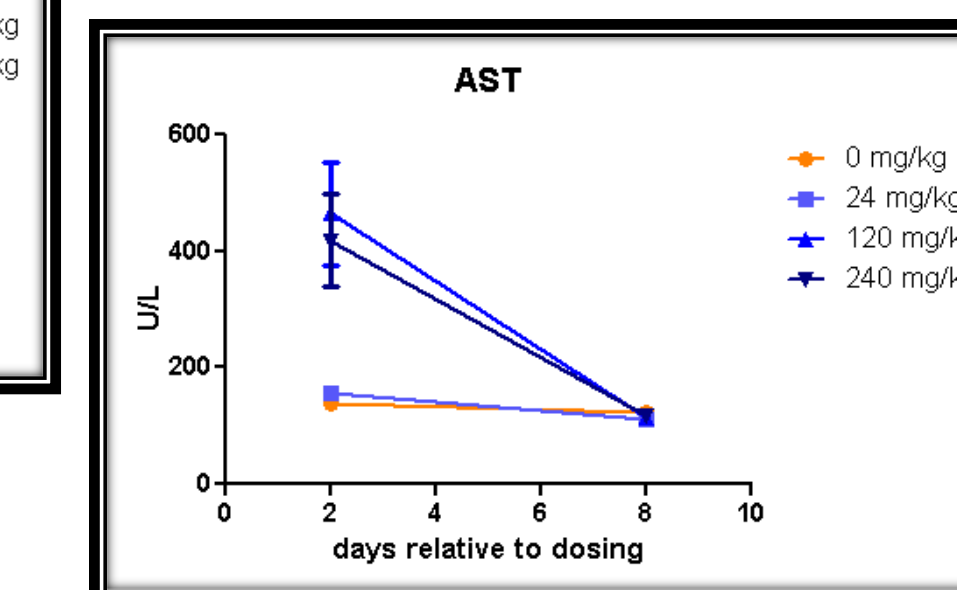
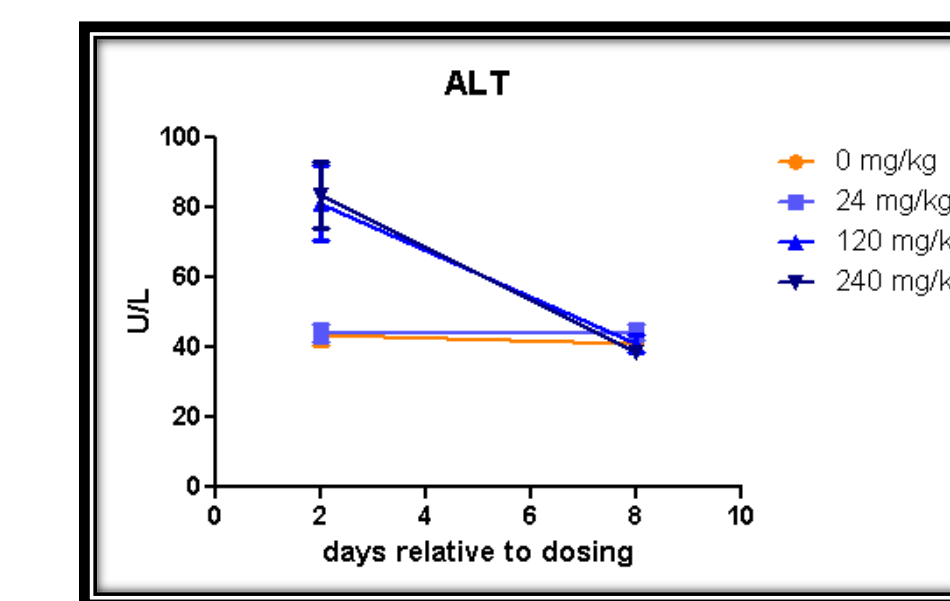


## 7 Day Toxicological Evaluation of AVI-7100 In Rats

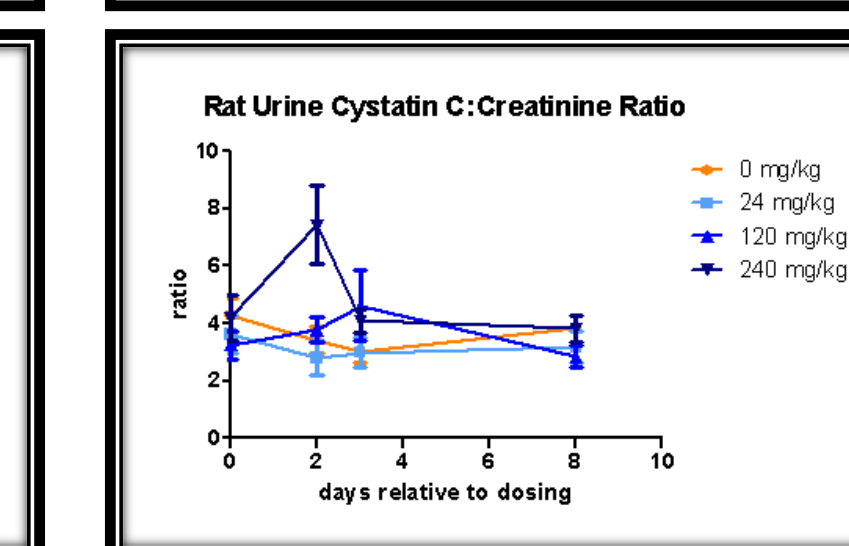
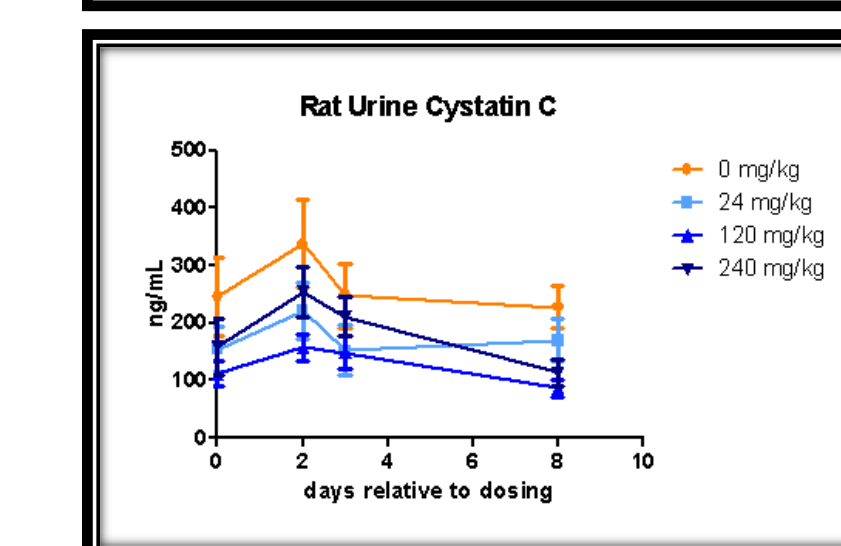
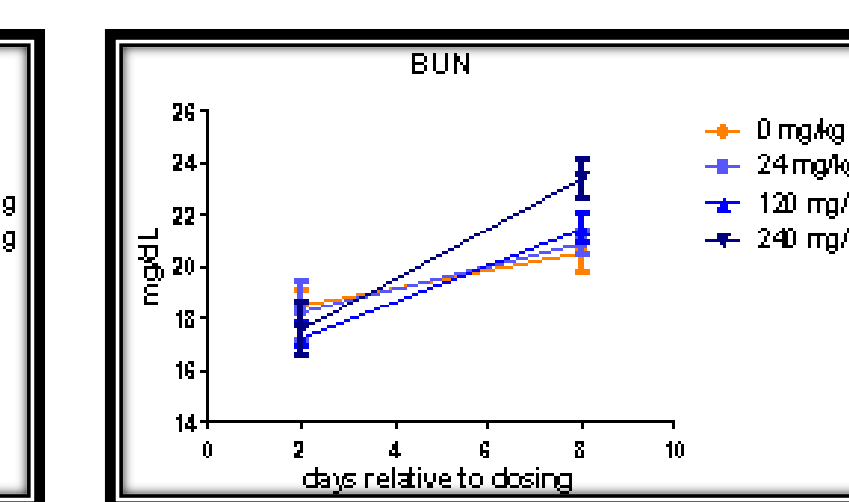
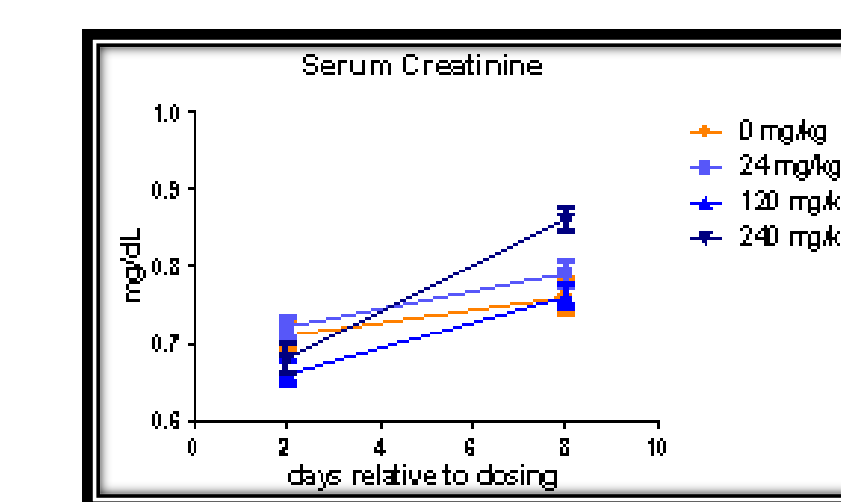


Observation – Day 1 Only	Dose Level
Hypoactivity	2/10 rats 120 mg/kg
Ataxia	4/10 rats 240 mg/kg
Irregular respirations	2/10 rats 240 mg/kg
Recumbent position	2/10 rats 240 mg/kg

At high doses only, clinical signs were transient and did not appear to affect the overall health of the animal, therefore were considered compound related but not adverse



At high doses only, transient moderate changes in ALT and AST, indicative of damage to liver hepatocytes  
o Resolved by the last day of dosing  
o Not necessarily adverse, histopathology in GLP studies to confirm

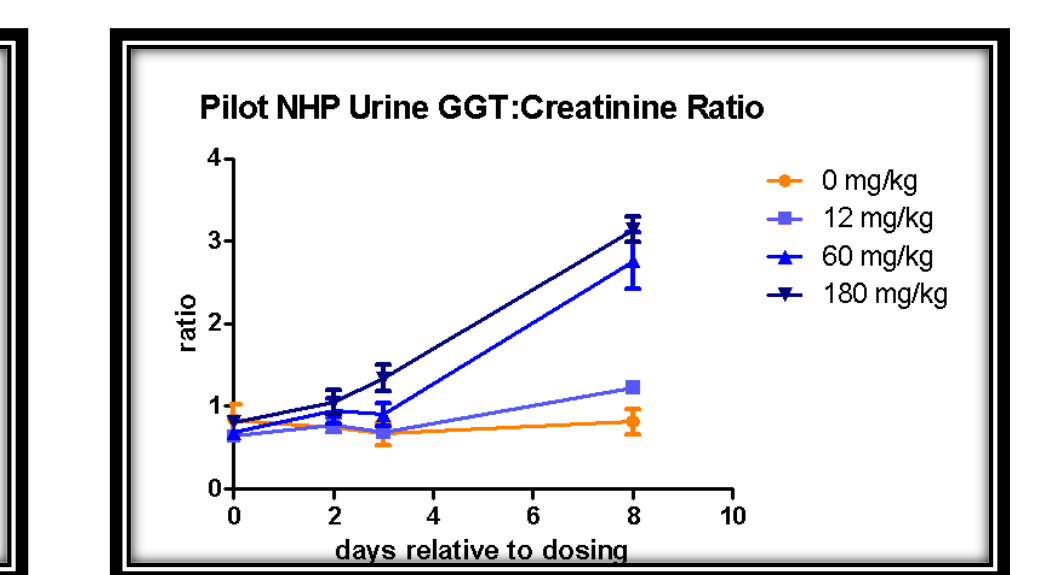
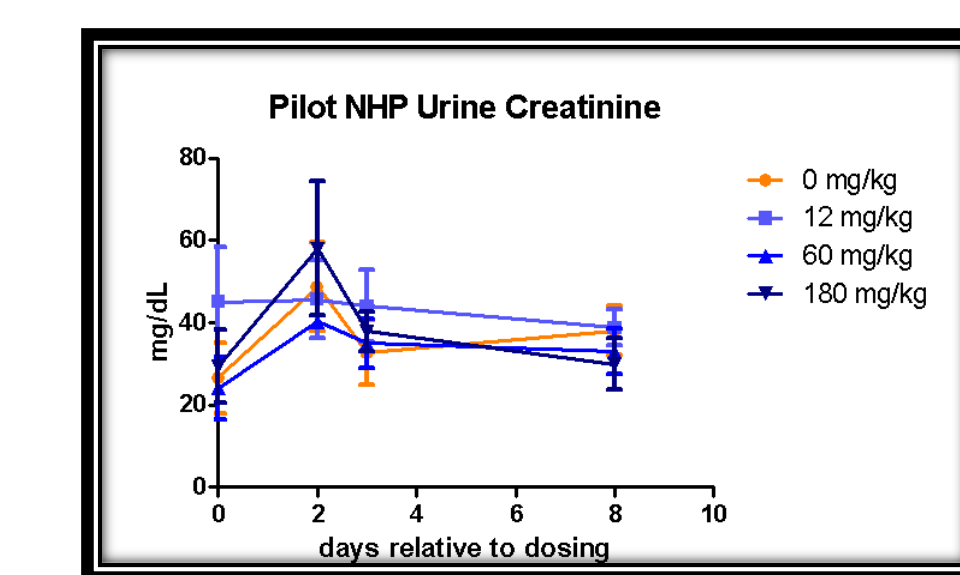


No adverse changes in any kidney biomarkers tested

## 7 Day Toxicological Evaluation of AVI-7100 In Cynomolgus Monkeys

- No related clinical observations at any dose level
- Human equivalent dose of at least 60 mg/kg
- Observed few animals with liquid feces; not considered clinically relevant
- No related change in standard clinical pathology parameters
- Hematology, Clinical Chemistry, Coagulation
- No changes in kidney markers: BUN, serum Creatinine
- No change in liver markers: ALT, AST

AVI-7100 was extremely well tolerated in NHP up to 180 mg/kg



No functional impact of kidneys in cynomolgus monkeys up to 180 mg/kg/day for 7 days.

Hematology	
red blood cell (erythrocyte) count	platelet count
hemoglobin	white blood cell (leukocyte) count
hematocrit	differential blood cell count
mean corpuscular volume	blood smear
mean corpuscular hemoglobin	reticulocyte count
mean corpuscular hemoglobin concentration	
Coagulation	
prothrombin time	activated partial thromboplastin time
Clinical Chemistry	
glucose	alkaline phosphatase
urea nitrogen	gamma glutamyltransferase
creatinine	aspartate aminotransferase
total protein	calcium
albumin	inorganic phosphorus
globulin	sodium
albumin/globulin ratio	potassium
cholesterol	cholesterol
total bilirubin	chloride
alanine aminotransferase	triglycerides
Urino Biomarkers	
Total protein	Creatinine
Gamma glutamyl transferase	Cystatin C
Urinalysis	
appearance (clarity and color)	ketones
volume	bilirubin
specific gravity	urobilinogen
pH	blood
protein	microscopic examination of sediment
glucose	

## Conclusions:

- AVI-7100 is active against fully virulent and non-adapted pandemic H1N1 virus in the ferret model following intraperitoneal or (i.p.) intranasal (i.n.) delivery.
- AVI-7100 was generally well tolerated in rats up to 240 mg/kg
  - Human equivalent dose of at least 40 mg/kg
  - Ataxia/Hyperactivity noted following Day 1 dosing, which resolved thereafter
  - Transient changes noted in some clinical pathology parameters
- AVI-7100 was extremely well tolerated in NHP up to 180 mg/kg
  - Human equivalent dose of at least 60 mg/kg
  - Observed few animals with liquid feces
  - No related clinical observations or standard clinical pathology parameters
  - GTT:Creatinine ratio slightly elevated at mid- and high-dose