

Rapid Response Therapeutic for Pandemic (H1N1-SOIV) and Seasonal Influenza

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ABSTRACT

Objective: Emergence of novel influenza viruses including the recent pandemic H1N1 strain presents a new challenge to the human population. An exercise was initiated to identify an effective therapeutic from design to synthesis in one week.

Methods: Conserved influenza A genome sequences were identified by extensive alignment using the NCBI influenza database. Potential homologies with human genome sequences were excluded using BLAST for expressed sequences. Twelve active candidates and three scramble sequence controls were prepared and evaluated in a mouse influenza infection model with A/Port Chalmers H3N2 infection with endpoints of lung viral titer and preservation of body weight. Lead candidates were then evaluated in a non-adapted H1N1 (SOIV) ferret infection model with endpoints that include clinical signs, nasal wash viral titer and lung viral titer. Pilot toxicology studies were conducted in the rat and studies to evaluate sequence-specificity, IC50, and CC50 were conducted in cell culture.

Preliminary Results: The H3N2 infected mouse model led to identification of a PMOplus oligomer (AVI-7100) targeting a viral segment translation start site as a lead candidate. The reduction of viral titer was dose dependent over a range of 2 to 6 mg/kg by the intraperitoneal route and greater than 10 mg/kg oseltamivir positive control. This lead compound also demonstrated significant reduction in viral titer, improvement in clinical signs and reduction in inflammatory cells in the H1N1 ferret model at a dose of 10 mg/kg by the intraperitoneal route. Pilot toxicology studies indicate AVI-7100 is well tolerated at doses over 150 mg/kg in the rat. Inhibition of viral titer is sequence specific, the CC50 is greater than 150uM, and the inhibition of virus is proportional to the oligomer concentration in cells.

Preliminary Conclusions: A rapid response to the pandemic H1N1 influenza was accomplished in less than two weeks. The studies identified AVI-7100 which appears to have a broad safety margin and is effective in both mouse and ferret influenza models challenged by H3N2 and H1N1, respectively.

Potential Impact: Rapid therapeutic antiviral design for influenza represents a template for therapeutic design for emerging infectious disease or engineered bioterror agents.

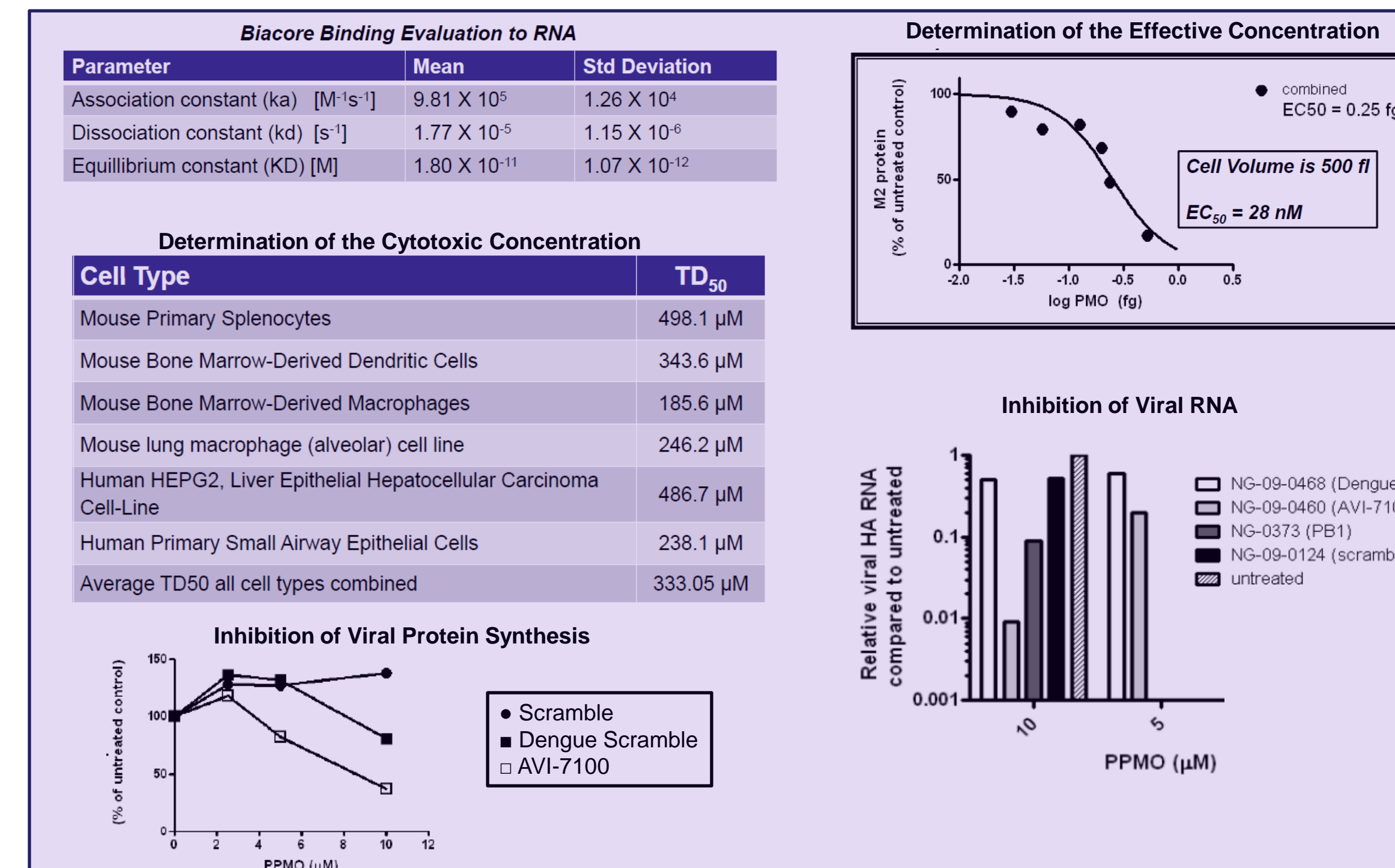
INTRODUCTION

Influenza A, a member of the *Orthomyxoviridae* family, is composed of a negative-sense, single-stranded and segmented RNA genome. The virus is enveloped in a lipid membrane derived from the host cell which is embedded with viral hemagglutinin (HA), neuraminidase (NA), and M2 proteins. A matrix (M1) protein is found just below the lipid envelope and the core is made of the eight RNA segments, the polymerase proteins (PB1, PB2 and PA) and the nucleoprotein (NP). Two nonstructural proteins are also present internally. The virus particle is composed of approximately 1 % RNA, 5 % carbohydrate, 20 % lipid, and 70 % protein.

A triple-reassortant influenza A (H1) virus has been circulating since 1998 with segments from pigs (HA, NP, NA, M and NS), humans (PB1), and birds (PB2 and PA). A newly described and novel swine-origin influenza A (H1N1) virus (SOIV) is a triple reassortant virus that includes genetic elements of this preexisting virus that have reassorted with the neuraminidase (NA) and matrix (M) segments of a Eurasian swine virus. The previous influenza A (H1) triple-reassortant virus was occasionally transmitted to humans but not spread efficiently from human-to-human but the new SOIV is very efficient in human-to-human transmission.

While the SOIV is currently sensitive to the neuraminidase inhibitors oseltamivir and zanamivir, seasonal influenza has previously been documented to evolve mutations that confer neuraminidase inhibitor resistance. Will SOIV replace the human H1 as the seasonal influenza virus or will SOIV reassort with yet another strain of influenza to create another new variant? Will it evolve to become more lethal? These uncertainties are compounded by the time interval from the identification of a new virus to the manufacture and distribution of a new vaccine. Further, a sufficiently novel viral hemagglutinin antigen may necessitate the use of large doses of immunogen and a prime boost schedule, posing practical difficulties for mass vaccination campaigns that must promptly elicit protective immunity. In view of these considerations, there exists an urgent need to create novel forms of prophylaxis and therapy for S-OIV in particular, ideally with broad activity against various influenza viral strains, subtypes and types.

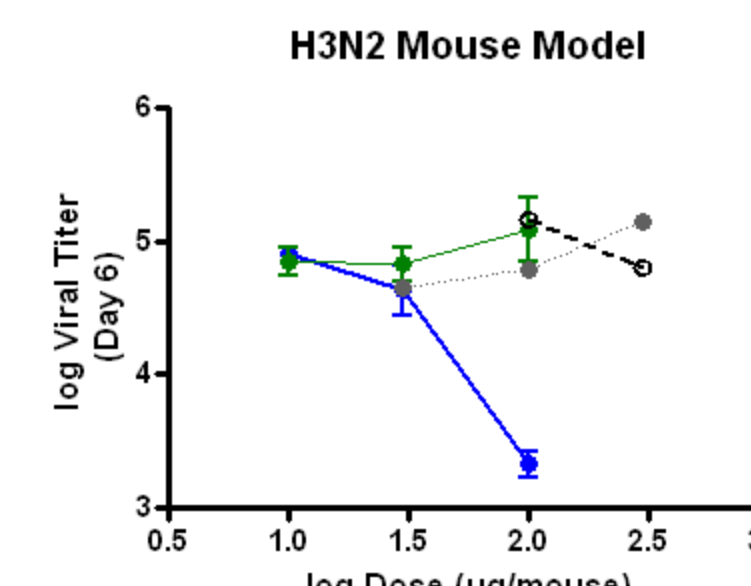
I. In Vitro Studies



II. MOUSE STUDIES (H3N2 Port Chalmers)

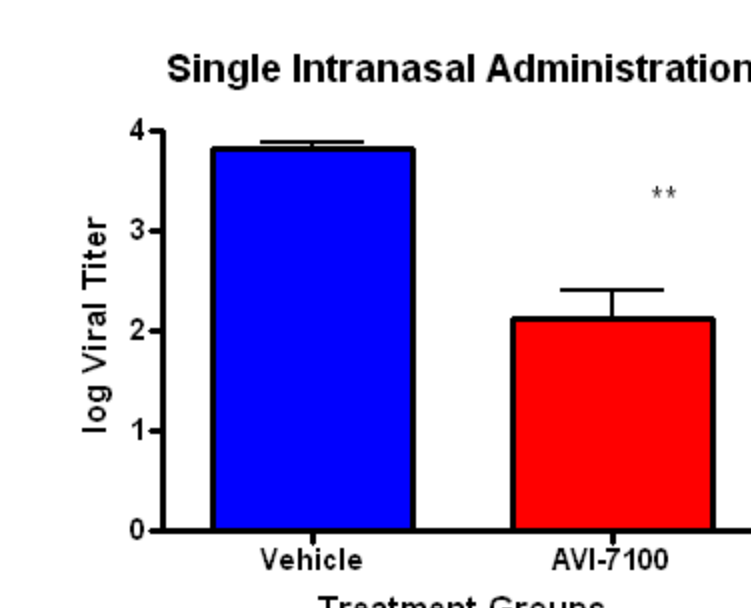
Screening Targets in the Mouse Model

Treatment	Chemistry	Route	Dose	Day 6 No. Mice
AVI-7100	PMOplus	intranasal	10, 30, 100	6
Flu Target 2	PMOplus	intranasal	10, 30, 100	6
Flu Target 3	PMOplus	intranasal	10, 30, 100	6
Dengue Scr	PMOplus	intranasal	100, 300	6



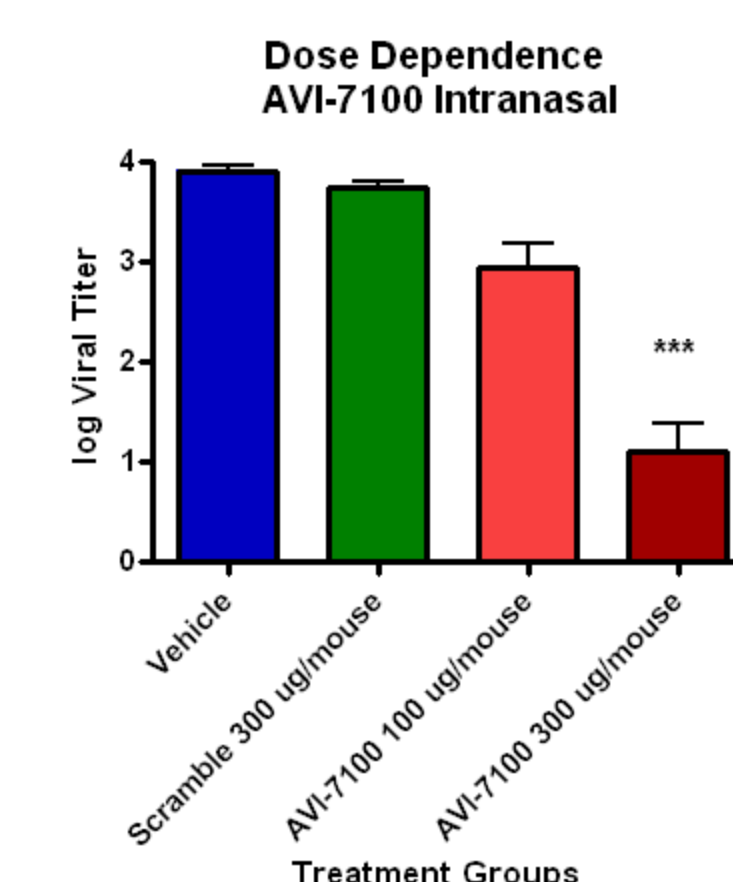
Investigate Intranasal Route of Administration In the Mouse Model

Treatment	Dose	Route	Number Doses	Mice/Group Day 6
PBS	-	i.n.	1	6
AVI-7100	100 ug/mouse	i.n.	1	6



Confirm Intranasal Route of Administration In the Mouse Model and Dose Dependency

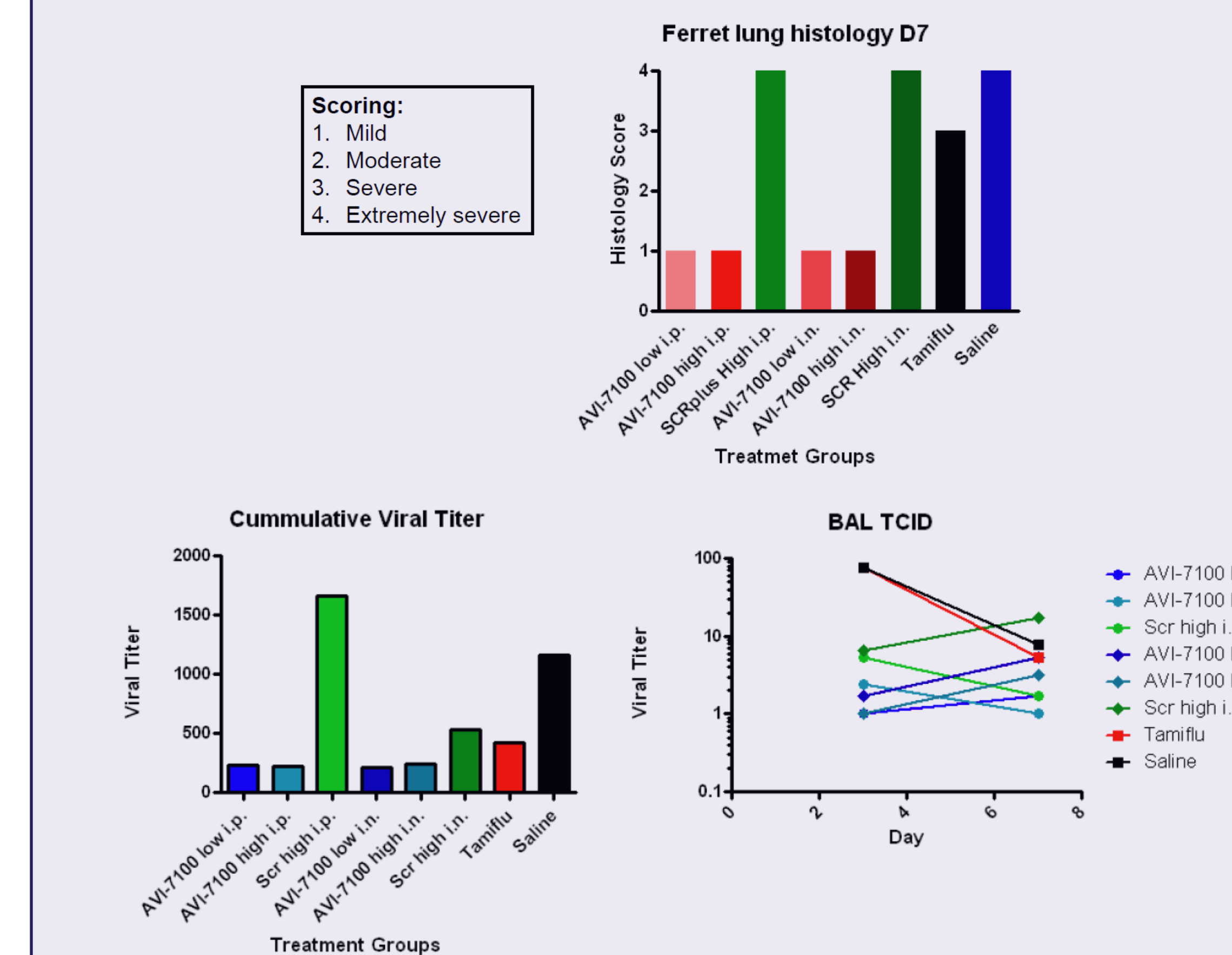
Treatment	Dose	Route	Number Doses	Mice/Group Day 6
PBS	-	i.n.	1	6
Scramble Control	300 ug/mouse	i.n.	1	6
AVI-7100	100 ug/mouse	i.n.	1	6
	300 ug/mouse	i.n.	1	6



III. Ferret Studies

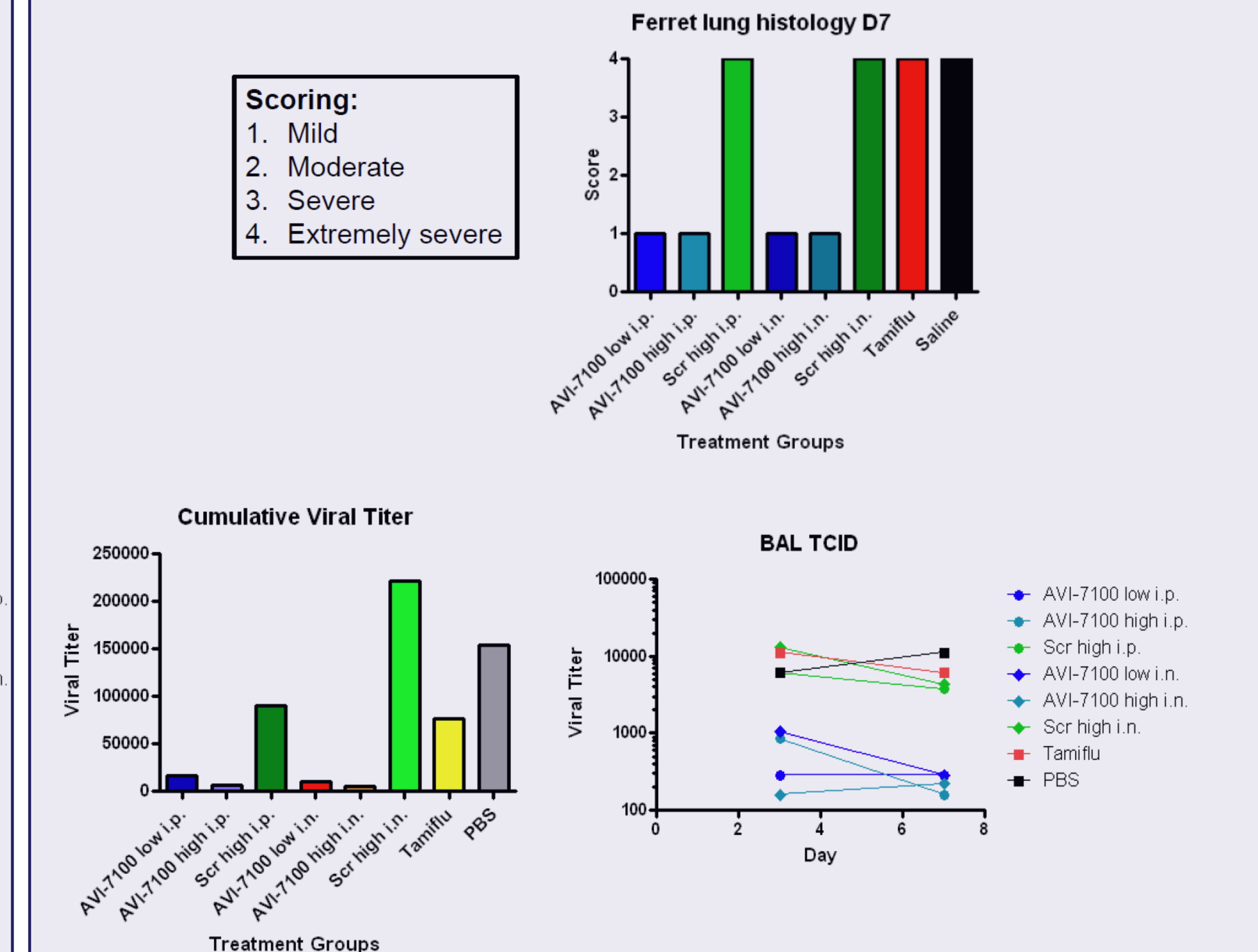
Ferret Study 1 with H1N1 SOIV

Group	Agent	Chemistry	Dose (mg/kg)	Route	Schedule	Day 3	Day 7
1	AVI-7100 ^a	PMOplus	10 ^b	i.p.	-4H, 1D, 3D, 5D	3 ^c	3 ^d
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
TOTALS						24	24



Ferret Study 2 with H1N1 SOIV

Group	Agent	Chemistry	Dose (mg/kg)	Route	Schedule	Day 3	Day 7
1	AVI-7100	PMOplus ^a	10 ^b	i.p.	-4H, 1D, 3D, 5D	3 ^c	3 ^d
2	AVI-7100	PMOplus	10 ^b	i.p.	-4H, 1D, 2D, 3D, 4D, 5D	3	3
3	Scramble	PMOplus	10	i.p.	-4H, 1D, 2D, 3D, 4D, 5D	3	3
4	AVI-7100	PMOplus	2.0	i.n.	-4H, 1D, 3D, 5D	3	3
5	AVI-7100	PMOplus	2.0	i.n.	-4H, 1D, 2D, 3D, 4D, 5D	3	3
6	Scramble	PMOplus	2.0	i.n.	-4H, 1D, 2D, 3D, 4D, 5D	3	3
7	Tamiflu	-	10	p.o.	-4H, 1D, 2D, 3D, 4D, 5D	3	3
8	Saline	-	-	i.n.	-4H, 1D, 2D, 3D, 4D, 5D	3	3
TOTALS						24	24

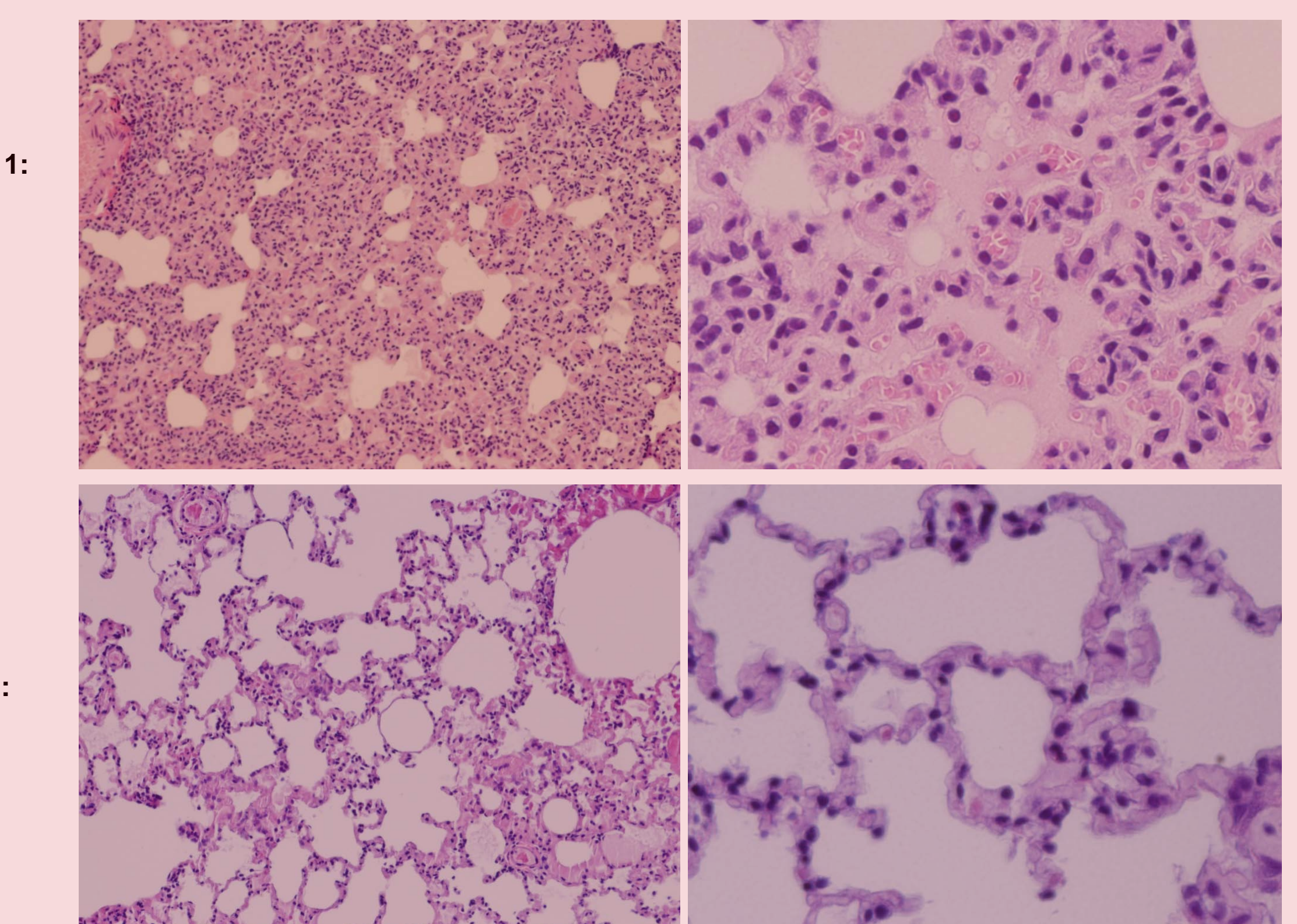


Ferret Study with Oseltamivir resistant H1N1 (SOIV)

Oseltamivir Resistant H1N1pdm (OP) Study in Ferrets

Group	Treatment	Dose (mg/kg)	Route	Regimen	Number
1	Oseltamivir	5	p.o.	-4H, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120H	8
2	AVI-7100	30	i.p.	-4H, 1, 2, 3, 4, 5D	8
3	AVI-7100	10	i.p.	-4H, 1, 2, 3, 4, 5D	8
4	Saline	-	i.n.	-4H, 1, 2, 3, 4, 5D	6 ^a
5	Oseltamivir + AVI-7100	5 + 10	p.o. + i.p.	-4H, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120H -4H, 1, 2, 3, 4, 5D	6 ^a

^aThe study called for 40 ferrets but only, a total of 36 ferrets were utilized in this study. Four of the ferrets shipped were seropositive for influenza and were excluded from the study



Conclusions:

- Multiple oligomers were evaluated in a mouse model to identify AVI-7100 as an effective target and a single dose by the intranasal route is effective.
- AVI-7100 is a 20-mer containing three PMOplus cationic linkages. AVI-7100 prevents viral titer expansion in cell culture and reduction in targeted viral protein synthesis.
- AVI-7100 is active against fully virulent and non-adapted pandemic H1N1 virus in the ferret model.
- AVI-7100 is effective against non-adapted H1N1 following intraperitoneal or (i.p.) intranasal (i.n.) delivery in the ferret.
- AVI-7100 protects against viral damage in the lung caused by Oseltamivir resistant H1N1-SOIV.