AVI-7100: Novel Therapeutic in Response to Pandemic Influenza (H1N1-SOIV)

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ABSTRACT

Background. A new triple reassortant swine origin influenza virus (SOIV), H1N1, emerged and reached pandemic alert stage 6 in the summer of 2009. The H1N1 virus, like the H5N1 avian flu, presents a new challenge to the human population. A broadly applicable flu therapeutic is needed given the emergence of multidrug resistance influenza strains. The urgency for such a therapeutic is linked to the capacity for influenza reassortants to acquire viral segments that will confer drug resistance.

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. Lead candidates were then evaluated in a non-adapted H1N1 (SOIV) ferret infection model.

Results. The H3N2 infected mouse model led to identification of a PMO*plus* 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 mk/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 may have a broad therapeutic index.

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.

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.

PMO*plus*

METHODS

Number of Ferrets in this Study: A total of 48 male ferrets will be utilized in each study. Ferrets, male with matched body weight ~ 700g at study initiation. All of the ferrets used for this study will be evaluated pre-test (at the animal supplier prior to shipping and again at Tulane animal facility) and only animals that seronegative for influenza and are healthy will be used on the study. Animals will be randomized to one of 8 treatment groups. The ferrets are housed in Hepa filtered cages (four per cage) to minimize cage to cage transmission of virus and the cages are maintained within the Tulane University Medical Center BSL-2 laboratory.

Challenge: All ferrets will be challenged with intranasal inoculation of a freshly thawed and diluted aliquot of the influenza H1N1 (SOIV) strain obtained from the CDC, 5 X 10^5 pfu/ferret in a volume of 0.2 mL.

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 be implanted in the animals which will identified each animal and reported body temperature.

On Day 0, all animals received H1N1 inoculation at 1-5 x 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.

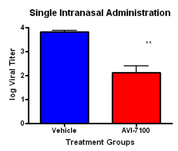
MOUSE STUDIES (H3N2 Port Chalmers)

Screening Targets in the Mouse Model

Treatment	Chemistry	Route	Daose	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

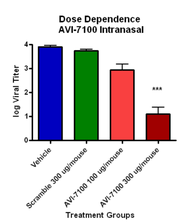
Investigate Intranasal Route of Administration
In the Mouse Model

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



Confirm Intransal 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 300 ug/mouse	i.n. i.n.	1 1	6 6

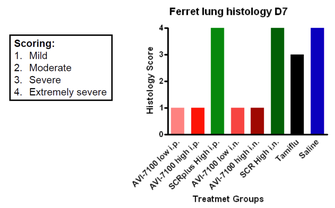


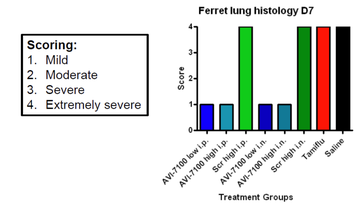
Ferret Study 1

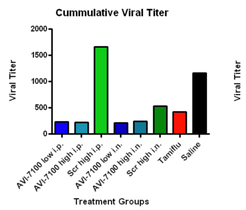
Group	Agent	Chemistry	Dose (mg/kg)	Route	Schedule	Day 3	Day 7
1	AVI-7100[a]	PMOplus	10^b	i.p.	-4H, 1D, 3D, 5D	3^c	3^d
2	AVI-7100	PMOplus	30	i.p.	-4H, 1D, 3D, 5D	3	3
3	Scramble	PMOplus	30	i.p.	-4H, 1D, 3D, 5D	3	3
4	AVI-7100	PPMO	0.5	i.n.	-4H, 1D, 3D, 5D	3	3
5	AVI-7100	PPMO	1.5	i.n.	-4H, 1D, 3D, 5D	3	3
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

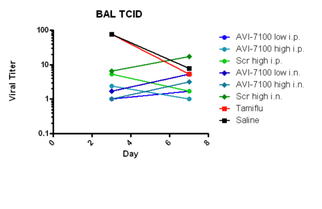
Ferret Study 2

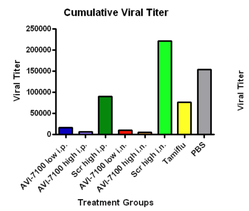
Group	Agent	Chemistry	Dose (mg/kg)	Route	Schedule	Day 3	Day 7
1	AVI-7100	PMOplus[a]	10	i.p.	-4H, 1D, 3D, 5D	3^c	3^d
2	AVI-7100	PMOplus	10^b	i.p.	-4H, 1D, 2D, 3D, 4D, 5D	3	3
3	Scramble	PMOplus	10	i.p.	-4H, 1D, 2D, 3D, 4D, 5D	3	3
4	AVI-7100	PMOplus	2.0	i.n.	-4H, 1D, 3D, 5D	3	3
5	AVI-7100	PMOplus	2.0	i.n.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

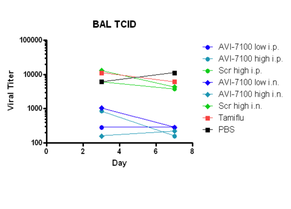












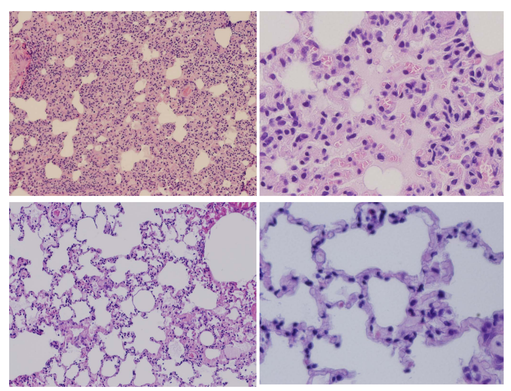
Oseltamivir Resistant H1N1pdm (OP[r]) Study in Ferrets

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]

[a]The 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

Lung - Group 1: Tamiflu
20X L, 40X R

Lung - Group 2: AVI-7100
20X L, 40X R



Conclusions:

1. 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.
2. AVI-7100 is a 20-mer containing three PMO*plus* cationic linkages. AVI-7100 prevents viral titer expansion in cell culture and reduction in targeted viral protein synthesis.
3. AVI-7100 is active against fully virulent and non-adapted pandemic H1N1 virus in the ferret model.
4. AVI-7100 is effective against non-adapted H1N1 following intraperitoneal or (i.p.) intranasal (i.n.) delivery in the ferret.
5. AVI-7100 protects against viral damage in the lung caused by Oseltamivir resistant H1N1-SOIV.