Design of a Broadly Neutralizing Antibody Targeting Influenza A

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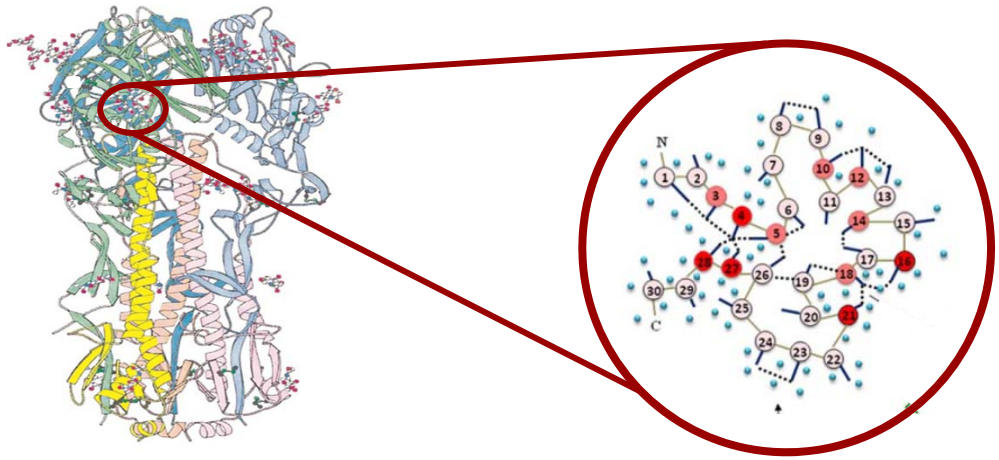


Financial Disclosures

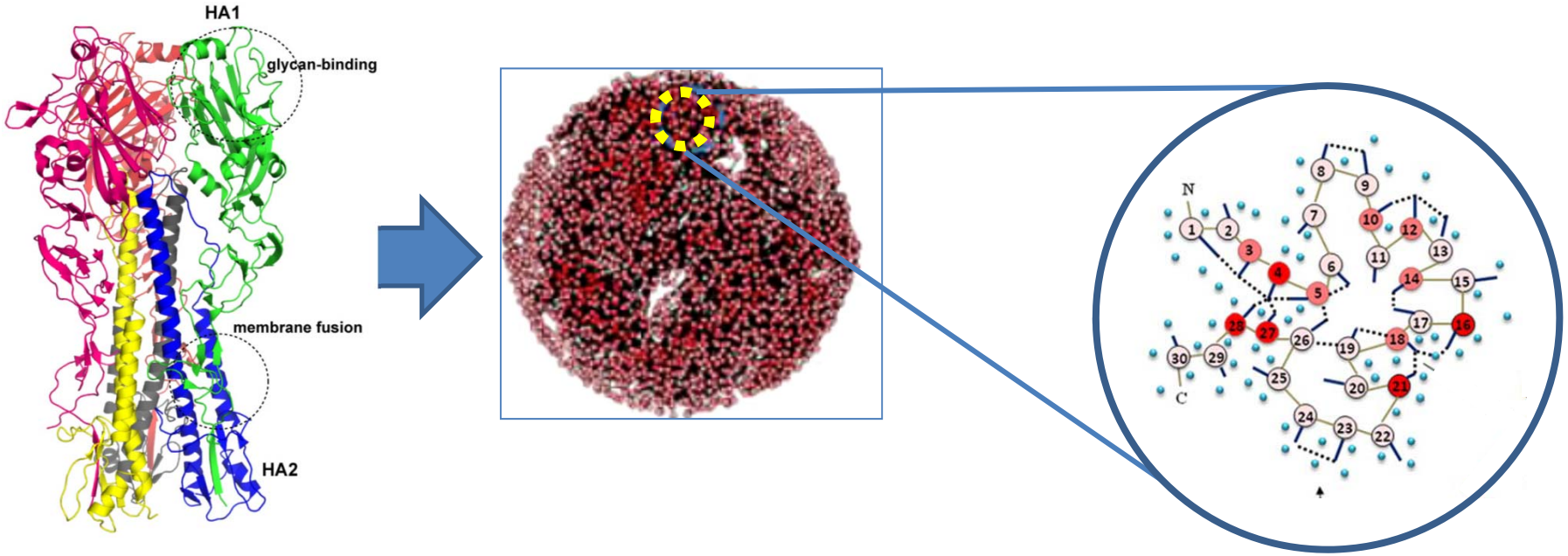
- Zach Shriver and Karthik Viswanathan are named as co-inventors on relevant patents with rights assigned to MIT or to Visterra, Inc.

- Zach Shriver and Karthik Viswanathan are employees of Visterra, Inc.

Atomic Interaction Network Analysis

Evaluate protein sequence in the context of its atomic interaction network

*Describe target protein in terms of **nodes** (amino acids) and **edges** (set of weighted interactions for each node)*



Source: Soundararajan et al., Sci Rep. 1, 200; 2011

Conventional View ***Atomic Interaction Network View***

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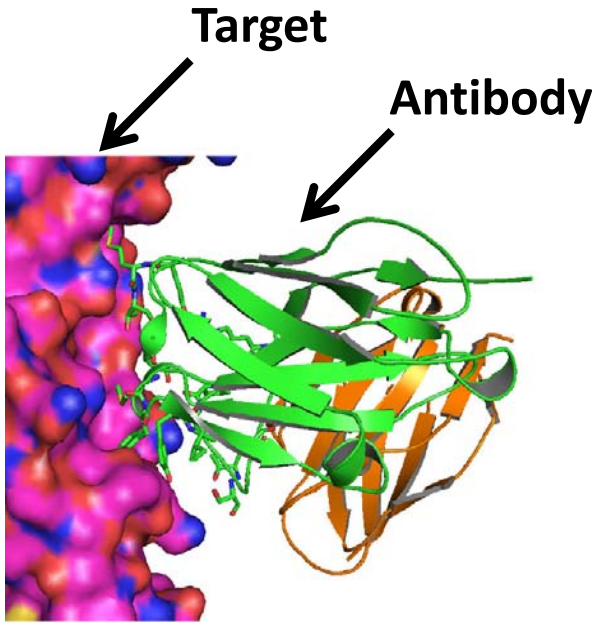
Background

Atomic Interaction Network Analysis

- **Quantitatively score each amino acid of a protein in terms of its network of interactions with other amino acids**
  - Includes both covalent and non-covalent interactions
- **Analysis of various influenza A subtypes enables three dimensional structural determination and target definition**
  - Identification of epitopes that are structurally conserved
- **Guide the engineering of human antibodies**

Approach to Engineering Human Antibodies



Step 1

- Identify target by network analysis

Step 2

- Identify an antigen from data base which best matches the target network characteristics
- Use the antibody against that antigen as Starting Antibody Scaffold

Step 3

- Selectively change V_H and V_L residues using network analyses

Approach does not rely on screening, panning or affinity maturation.

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Design Objectives

Design a fully human monoclonal antibody to influenza A using network analysis:

- ✓ **Human IgG1 Antibody**
- ✓ **Targeting a unique, highly conserved epitope on hemagglutinin, differentiated from other mAbs**
- ✓ **High affinity binding and neutralization**
- ✓ **Broad coverage across both group 1 and 2 influenza sub-types**
- ✓ **Potent, fast acting, single-dose efficacy**
- ✓ **Refractory to resistance development**
- ✓ **Effective for both prophylactic and therapeutic treatment**

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Design Cycles Build in Specificity and Affinity

From Target Identification to Development Candidate

	Starting Antibody		Design Cycle 1		Design Cycle 2		Design Cycle 3		**VIS410**	
	HA	Binding Affinity (K_d)	HA	Binding Affinity (K_d)	HA	Binding Affinity (K_d)	HA	Binding Affinity (K_d)	HA	Binding Affinity (K_d)
Group 1	H1	No binding	H1	630 pM	H1	74 pM	H1	73 pM	H1	76 pM
	H5	No binding	H5	1,000 pM	H5	101 pM	H5	79 pM	H5	98 pM
	H9	No binding	H9	Low binding	H9	84 pM	H9	85 pM	H9	52 pM
Group 2	H3	No binding	H3	No binding	H3	37,000 pM	H3	450 pM	H3	101 pM
	H7	No binding	H7	No binding	H7	33,000 pM	H7	513 pM	H7	400 pM

In silico design and experimentation guides each design cycle

- **Activity** – Binding (ELISA); Kd (Surface Plasmon Resonance) KinExA; in vitro Neutralization (CPE)
- **Integrity** – Conformational Stability (Circular Dichroism); Biophysical Characterization (SEC, RP-HPLC); Expression
- **Immunogenicity** – Proximity from human germ line; IFNg; CD4+; Computational Identification of Epitopes

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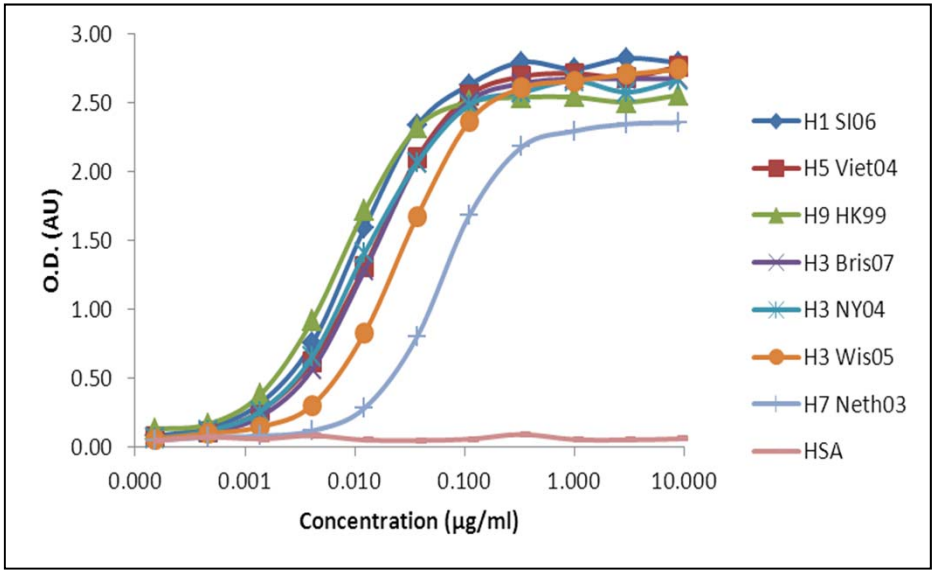
Measurement of Affinity and Avidity for Group 1 and 2 Influenza Sub-Types



	HA	Binding Affinity (K_d)
Group 1	H1N1 (A/Solomon Island/3/2006)	76 pM
	H5N1 (A/Vietnam/1203/2004)	98 pM
	H9N2 (A/Hong Kong/1073/99)	52 pM
Group 2	H3N2 (A/Brisbane/10/2007)	101 pM
	H3N2 (A/New York/55/2004)	104 pM
	H3N2 (A/Wisconsin/67/2005)	239 pM
	H7N7 (A/Netherlands/219/2003)	400 pM

- Picomolar binding to hemagglutinin across Group I and Group 2
- Results confirmed by a variety of binding assays- including ELISA, Surface Plasmon Resonance, and KinExA

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Neutralization of Group 1 and 2 Virus Strains

Virus		Inoc.[a]	VIS410 (µg/mL)			Ribavirin (µg/mL)		
			EC_{50}[b]	CC_{50}[c]	SI[d]	EC_{50}[b]	CC_{50}[c]	SI[d]
H1N1	California 04/2009	300	5.1	>500	>98	5.4	>100	>195
H1N1	Solomon Islands	160	1.2	>500	>420	11	>100	>9.1
H3N2	Brisbane/10/2007	40	0.3	>500	>1700	18	>100	>5.6
H3N2	Fujian/411/2003	65	0.3	>500	>1800	8.7	>100	>12
H3N2	Panama/2007/99[e]	13	11	>500	>45	8.7	>100	>12
H3N2	Shangdong/09/ 93	100	0.6	>500	>890	3.2	>100	>31
H3N2	Victoria/3/75[e]	12	4.9	>500	>100	9.9	>100	>10
H3N2	Wyoming/03/2003	10	6.8	>500	>74	22	>100	>4.5
H5N1	Duck/MN/1525/81	160	1.5	>500	>330	5.3	>100	>19

- Assays performed using standard protocols
- No cellular cytotoxicity observed with VIS410
- High selectivity index against a wide panel of influenza strains

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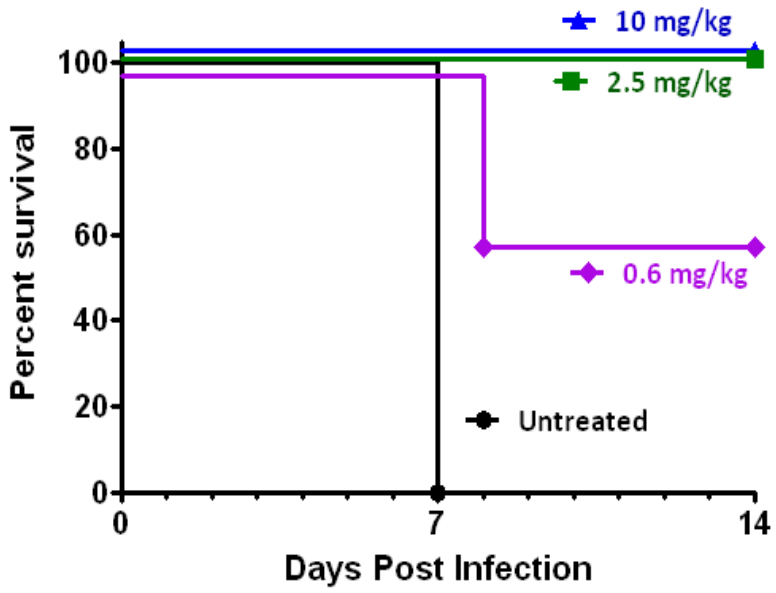
Mouse Models of Prevention and Treatment

- BALB/c mice (female) 10-11 weeks old
- Virus Challenge
  - H1N1 (PR8 at 100 PFU/dose)
  - H3N2 (VIC75 at 10,000 PFU/dose)
- Monoclonal Administration
  - Prevention Model: One Dose of VIS410 at 24 hrs before challenge
  - Treatment Model: One Dose of VIS410 at 48 or 72 hrs after challenge
- Endpoints
  - Survival (euthanasia at 20% of weight loss)
  - Viral Titer (viral load in lungs by plaque assay)
  - Weight Loss
  - Histology (lung histology 8 days post infection)

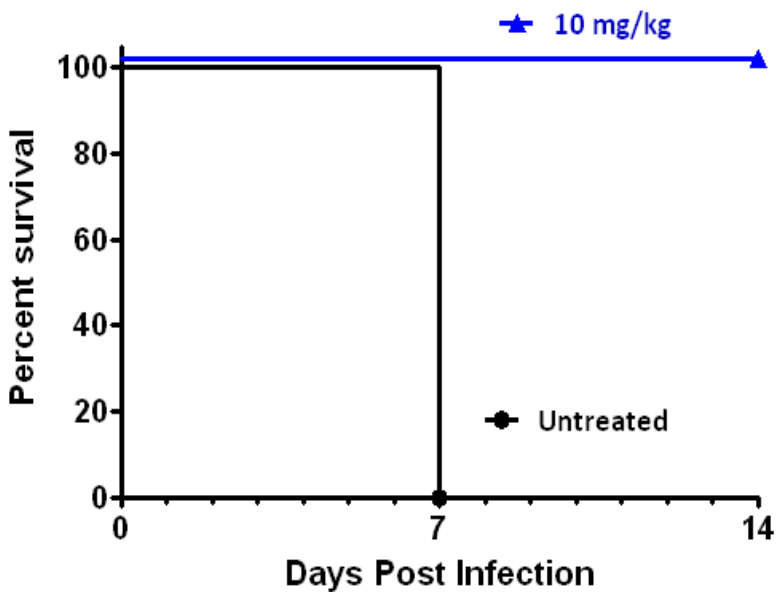
Increased Survival with VIS410 Prophylaxis

H1N1



n=5 per group

H3N2



n=5 per group

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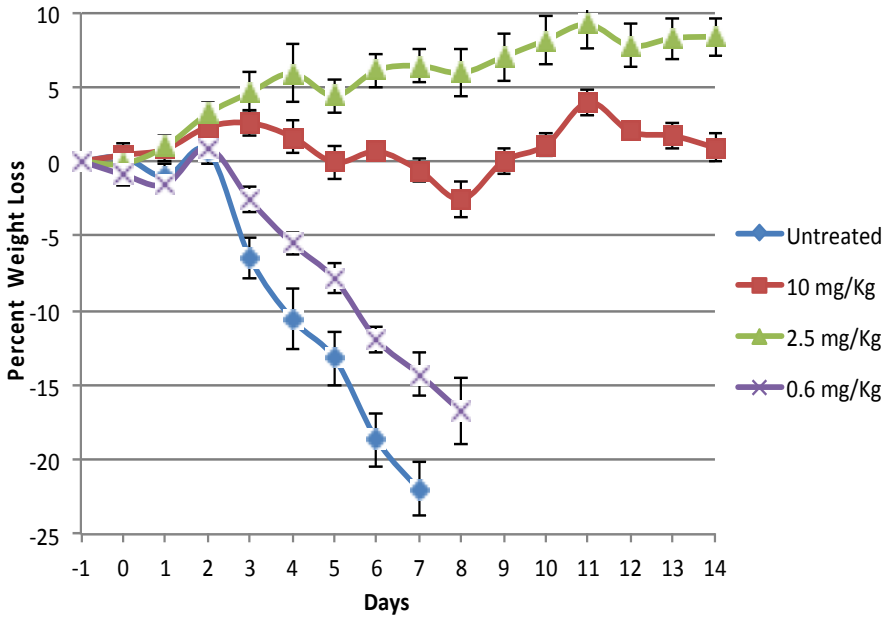
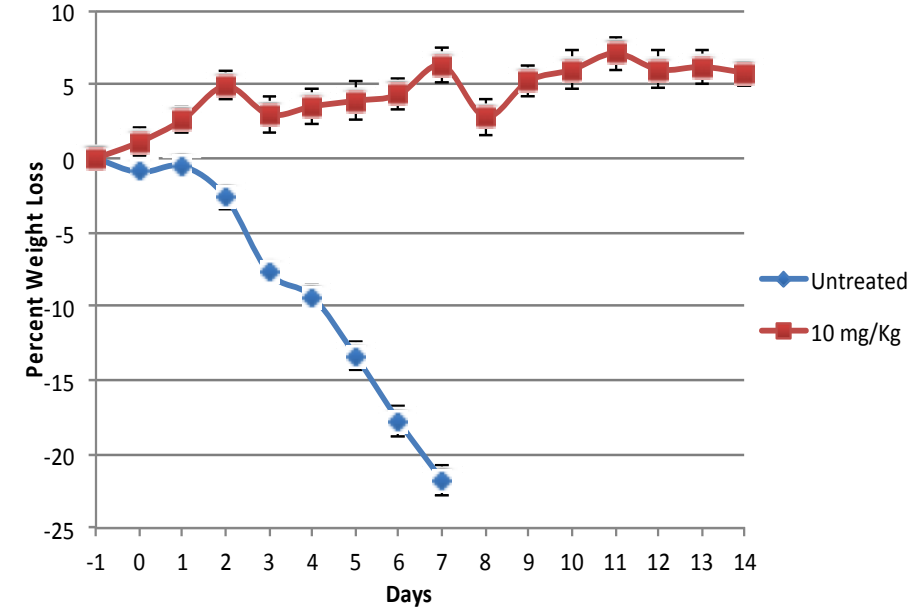
Reduction in Weight Loss with VIS410 Prophylaxis

VIS410 against H1N1



VIS410 against H3N2



- Average percentage weight loss for groups was calculated as long as all mice in group alive. The standard error of the mean is shown for the 5 mice per group.

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Reduction of Lung Viral Load with VIS410 Prophylaxis

VIS410 against H1N1

Treatment Group	Lung Viral Load H1N1 (PFU/ml)
Untreated	6.03
10 mg/Kg	4.45*
2.5 mg/Kg	4.08*
0.6 mg/Kg	5.38*

VIS410 against H3N2

Treatment Group	Lung Viral Load H3N2 (PFU/ml)
Untreated	6.48
10 mg/Kg	5.42*

* Significance ($p<0.05$) as compared to control group determined Mann Whitney U test

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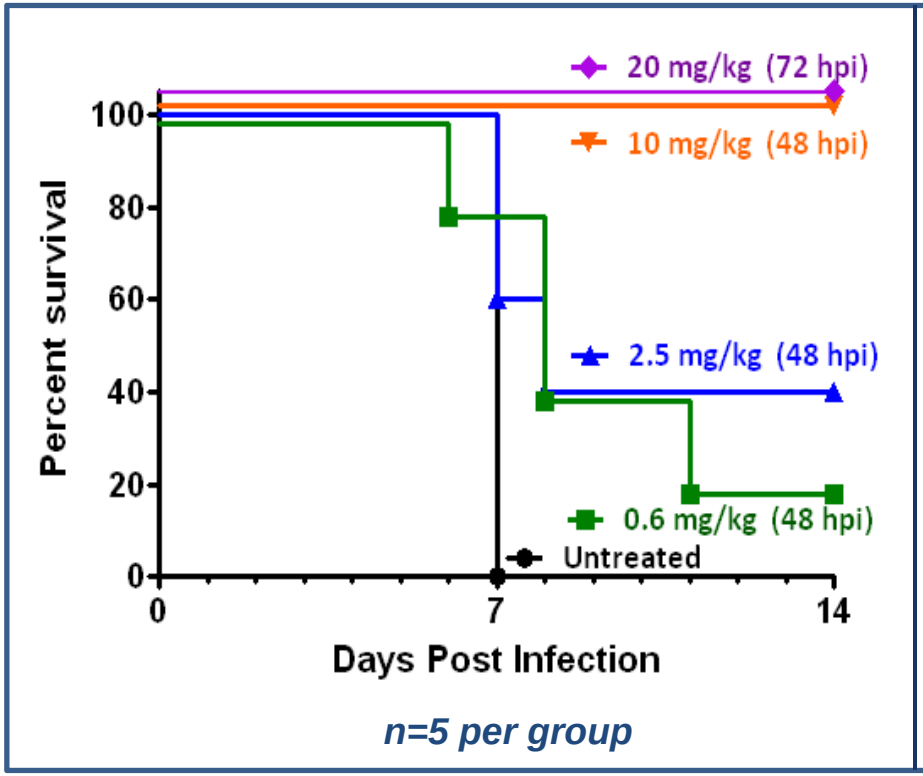
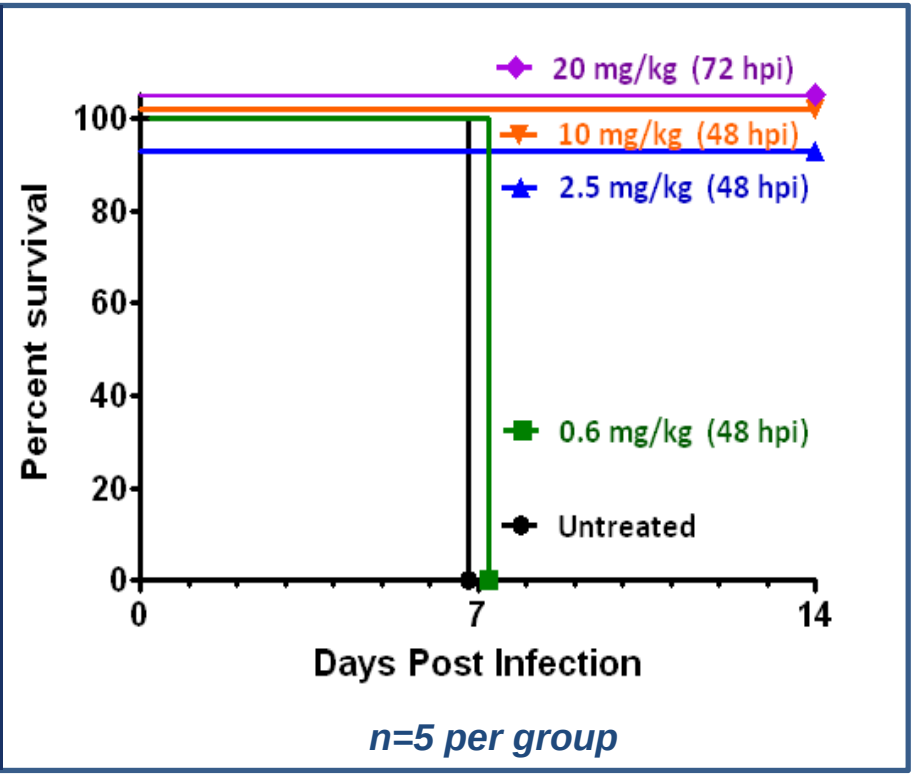
Increased Survival with VIS410 Treatment

VIS410 against H1N1



n=5 per group

VIS410 against H3N2



n=5 per group

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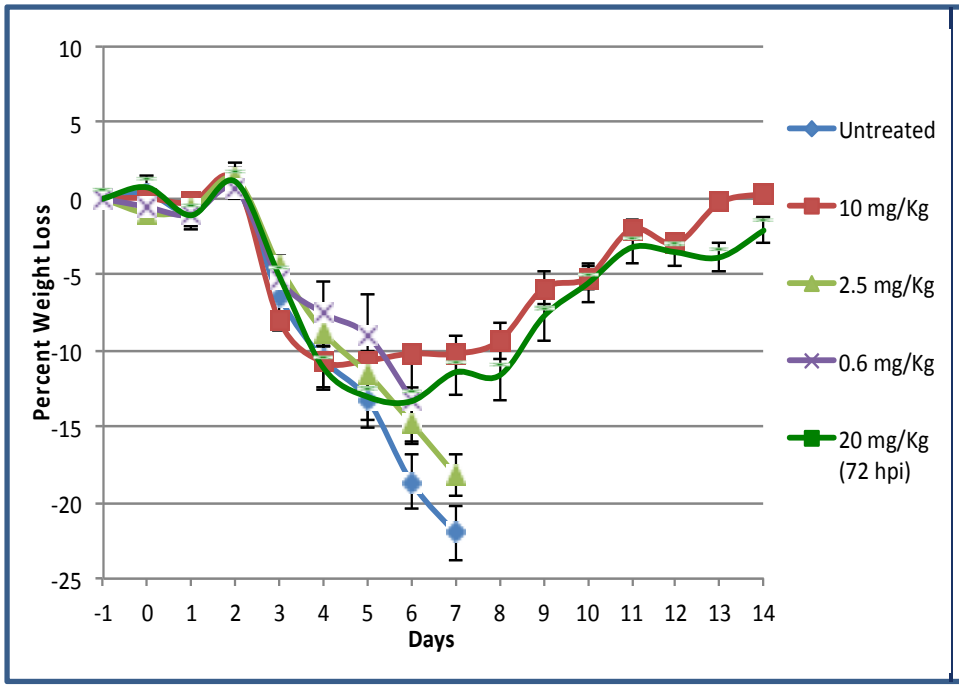
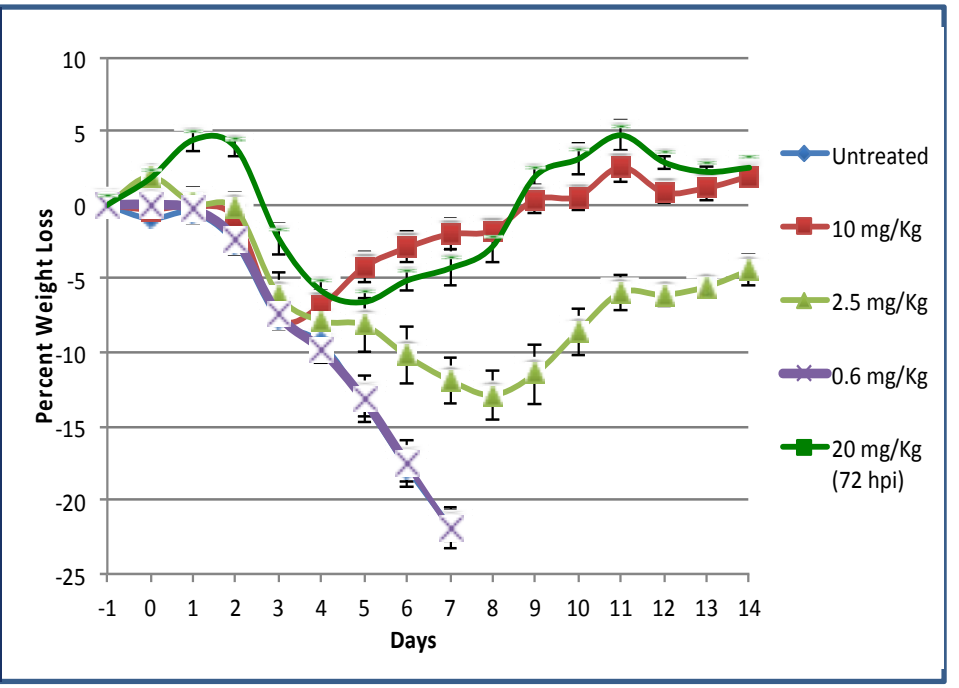
Reduction of Weight Loss with VIS410 Treatment

VIS410 against H1N1



VIS410 against H3N2



- Average percentage weight loss for groups was calculated as long as all mice in group alive. The standard error of the mean is shown for the 5 mice per group

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Reduction in Lung Viral Load with VIS410 Treatment

VIS410 against H1N1

Treatment Group	Lung Viral Load H1N1 (PFU/ml)
Untreated	6.03
10 mg/Kg (48hpi)	5.34*
2.5 mg/Kg (48 hpi)	5.49*
0.6 mg/Kg (48hpi)	5.74
20 mg/Kg (72hpi)	5.29*

VIS410 against H3N2

Treatment Group	Lung Viral Load H3N2 (PFU/ml)
Untreated	6.48
10 mg/Kg (48hpi)	5.46*
2.5 mg/Kg (48 hpi)	5.99*
0.6 mg/Kg (48hpi)	6.44
20 mg/Kg (72hpi)	5.64

* Significance ($p<0.05$) as compared to control group determined Mann Whitney U test

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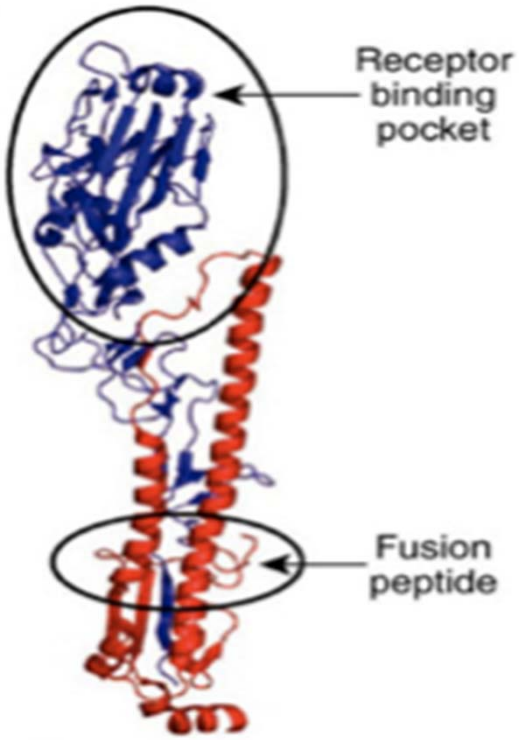
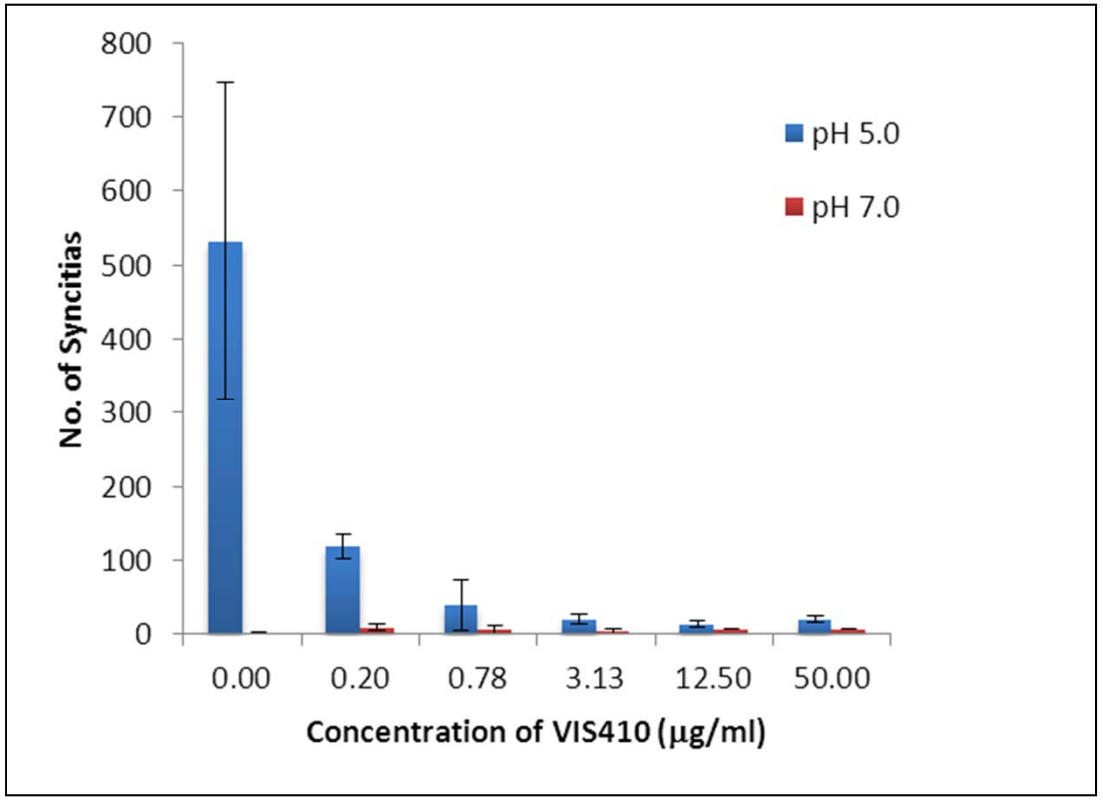
VIS410 Inhibits HA-Mediated Cell Membrane Fusion





- Syncytia formation between HA-expressing cells inhibited with pre-treatment of VIS410 in a dose responsive manner

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Conclusions

- VIS410 is a broadly neutralizing monoclonal antibody designed using a novel approach
- VIS410 is highly effective in mouse models of H1N1 and H3N2
- VIS410 has potential for treatment and prevention of influenza A
- VIS410 targets an unique epitope that has potential for the development of a universal vaccine

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