Identification and Characterization of Broadly Neutralizing Antibodies Targeting Influenza

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Abstract

Background: A longstanding challenge in treatment and prevention of infectious diseases, including influenza and HIV, is the development of efficacious therapeutics that can target constantly evolving pathogens, especially viruses. One such strategy to develop potential next generation therapeutics and vaccines is through identification of broadly neutralizing antibodies (bNAbs), from a B-cell library, that target multiple clades of a given virus. However, many of these antibodies suffer limitations, including possessing highly differentiated, non-germline sequences, which raise the chance of immunogenicity, and demonstrating polyreactivity.

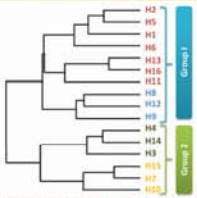
Methods: To develop a structure-function understanding of bNAbs in influenza, we have identified multiple human antibodies that bind to influenza hemagglutinin and neutralize Group 1 and 2 influenza A viruses.

Results: Identified antibodies have high affinity (typically < 100 pM, depending on strain) and demonstrate good physiochemical attributes, including solubility, stability, specificity, and proximity to germline. Additionally, antibodies demonstrate tight binding to both the HA_0 and the mature HA_1-HA_2 form of HA, suggesting the possibility for both direct and indirect, cell-mediated, antiviral effects. Identified antibodies protect mice (n=5 per arm) challenged with H1N1, H5N1 and H3N2 viruses, with prophylactic or therapeutic (48-72 hours post-infection) administration resulting in 100% survival with several of the antibodies. Histological analysis indicates that protection is correlated with substantial reduction in both virally-induced cell damage and presence of inflammatory infiltrate. Antiviral activity and observed efficacy in the mouse correlates with levels of antibody in the lung and bronchioalveolar lavage.

Conclusion: Taken together, these results provide insights into the identification of antibody-based therapeutics for influenza.

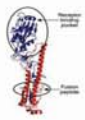
Visterra FluMAbs Display High-Affinity Binding to Group 1 and 2 Influenza

Mabs	ELISA Kd (pM)				
	H1	H5	H9	H3	H7
Ab01	<80	<80	<80	<80	<80
Ab02	143	91	<80	86	<80
Ab03	357	<80	<80	<80	<80
Ab04	<80	<80	<80	<80	<80
Ab05	118	<80	<80	91	<80
Ab06	203	136	121	149	<80
Ab07	83	91	94	139	94
Ab08	86	94	87	125	87
Ab09	79	70	66	111	70
Ab10	76	72	63	145	83
Ab11	<25	51	46	198	51
Ab12	63	89	67	95	85
Ab13	67	70	64	83	76
Ab14	<80	89	<80	87	<80
Ab15	198	95	44	87	81
Ab16	<25	48	<25	95	<25
Ab17	<25	<25	<25	109	79
Ab18	75	82	58	158	91
Ab19	64	<25	87	138	<25
Ab20	81	98	91	103	97
Ab21	110	73	103	164	81
Ab22	44	39	44	177	83



The structure guided design approach has enabled identification of panel of antibodies with high affinity binding to representative hemagglutinins from structural group 1 and group 2.

FluMAbs Neutralize Representative Group 1 and 2 Virus Strains In Vitro

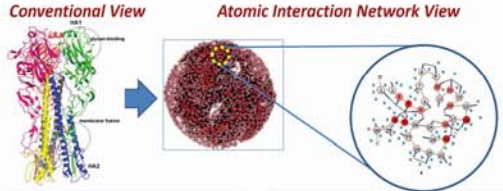


Antibody	Subtype	Strain	EC_{50}	CC_{50}	SI
Ab1	H1N1	A/Solomon Island/3/2006	1.2	>200	>170
	H3N2	A/Brisbane/10/2007	12	>200	>17
Ab2	H1N1	A/Solomon Island/3/2006	<0.059	>180	>3100
	H3N2	A/Brisbane/10/2007	<0.059	>180	>3100
Ab3	H1N1	A/Solomon Island/3/2006	1.2	>500	>420
	H3N2	A/Brisbane/10/2007	0.3	>500	>1700

CC_{50}=50% toxic concentration without virus (μg/mL);
EC_{50}=50% effective concentration (μg/mL); SI= CC_{50}/EC_{50}

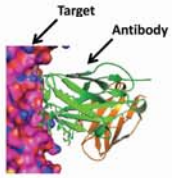
Visterra FluMAbs potently neutralize representative group 1 and group 2 virus in an in vitro neutralization assay. These antibodies bind to a conserved region on the stem and inhibit virus-cell membrane fusion. Data from three such antibodies included above.

Visterra Technology: Characterizing Proteins using Amino Acid Networks



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)*

Structure Guided Design of Antibody



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

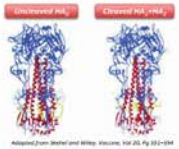
Step 3
- Apply network analyses to selectively engineer V_H and V_L residues for optimal contact

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

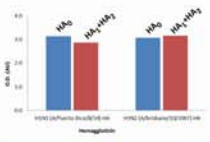
Design Cycles Build in Specificity and Affinity

	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	75 pM
	H5	No binding	H5	1,000 pM	H5	101 pM	H5	79 pM	H5	87 pM
	H9	No binding	H9	Low binding	H9	84 pM	H9	85 pM	H9	59 pM
Group 2	H3	No binding	H3	No binding	H3	37,000 pM	H3	450 pM	H3	114 pM
	H7	No binding	H7	No binding	H7	33,000 pM	H7	513 pM	H7	75 pM

Visterra FluMAbs Bind Uncleaved (HA_0) and Cleaved (HA_1+HA_2)

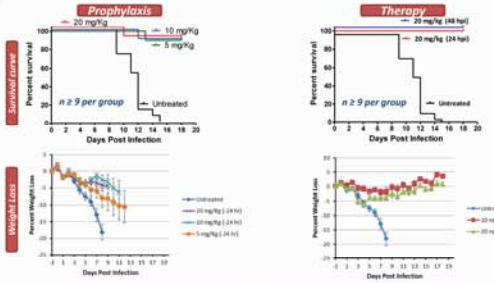


HA is expressed as HA_0, which is then processed on the cell surface or mature virion to form the HA_1-HA_2 heterocomplex.



Biochemical analysis indicates Visterra FluMAbs recognizes both uncleaved (HA_0) and cleaved (HA_1-HA_2) forms of hemagglutinin, indicating the potential for these antibodies in facilitating the clearance of virus and killing of infected cells via Fc-mediated effector functions that include ADCC and CDC.

VIS410 Is a Potent Prophylactic and Therapeutic In H5N1 Mouse Model



- *Average percentage weight loss for groups was calculated as long as all mice in group were alive. The standard error of the mean is shown for at least 9 mice per group.*

VIS410 has previously shown to be a potent prophylactic and therapeutic agent against H1N1 and H3N2 infection (data presented at ICAAC 2012). The above data shows its activity as a prophylactic and as a therapeutic against highly pathogenic 'bird flu' H5N1 virus (A/Vietnam/1203/2004).

Summary

- Visterra's approach has enabled the identification of panel of antibodies targeting conserved epitope on influenza hemagglutinin.
- These FluMAbs showed high affinity binding to HA_0 and HA_1+HA_2 and potently neutralize representative group 1 and group 2 viruses.
- VIS410 represents an optimized influenza antibody and has potential to be a potent therapeutic against seasonal and pandemic influenza.
- The approach used here can be applied to identify novel drug target sites and guide the design of drugs to effectively combat disease.