VIS410, A BROAD-SPECTRUM, ANTI-INFLUENZA A MONOCLONAL ANTIBODY NEUTRALIZES BALOXAVIR RESISTANT INFLUENZA A VIRUS *IN VITRO*

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ABSTRACT

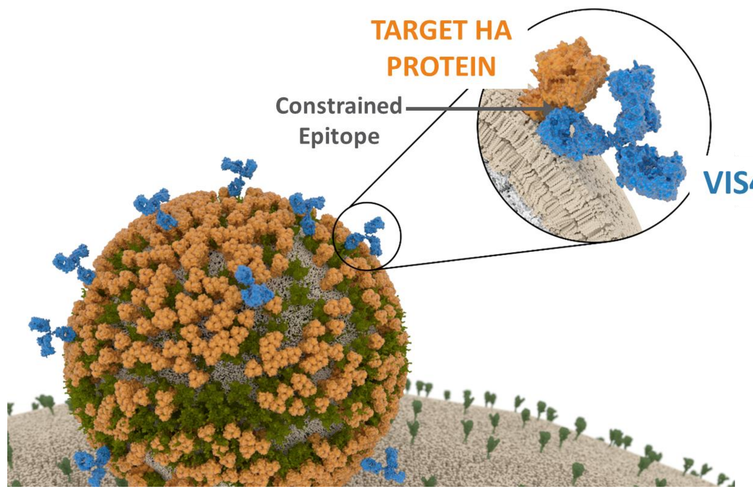
Introduction: Current antiviral therapies for influenza include the neuraminidase inhibitors (NAIs) and, since 2018, baloxavir marboxil, which targets the PA subunit of the viral RNA polymerase. NAI resistant viruses have circulated in the last decade, and viruses resistant to baloxavir have been isolated from baloxavir-treated patients. Thus, there is a need for antivirals that target diverse influenza virus proteins and possess different mechanisms of action. VIS410 is a hemagglutinin (HA) stem binding, broadly active anti-influenza A monoclonal antibody (mAb) currently in clinical development for treatment of patients hospitalized with influenza A. VIS410 was previously shown to be synergistic with baloxavir *in vitro*, and effective against oseltamivir-resistant influenza A strains both *in vitro* and *in vivo*. Here, we assessed the antiviral activity of VIS410 against baloxavir-resistant influenza virus *in vitro*.

Methods: Reverse-genetically engineered wild-type (WT) influenza A/PR/8/1934 (H1N1) virus and a baloxavir-resistant counterpart carrying the PA I38T substitution were generated. Virus stocks were sequence-confirmed and tested for susceptibility to baloxavir and VIS410 using microneutralization assay in MDCK cells. Data were normalized to infected virus controls.

Results: The baloxavir-resistant PA I38T mutant exhibited ~100-fold lower susceptibility to baloxavir as compared to the WT virus. The observed EC_{50} for baloxavir was consistent with other studies (WT EC_{50} ~1 nM and PA I38T virus ~130 nM). In contrast, both WT and PA I38T viruses showed comparable susceptibility to VIS410 (with EC_{50} ~1.1-2.2 µg/mL). Notably, in previous studies VIS410 was found to both neutralize A/PR/8/1934 (H1N1) virus and protect against lethal challenge, indicating that *in vitro* results may predict *in vivo* efficacy.

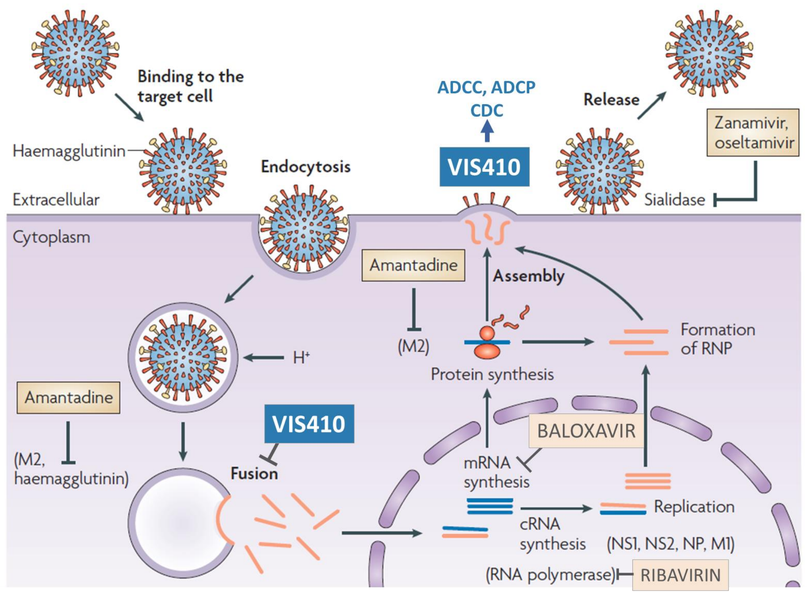
Conclusions: VIS410 demonstrated antiviral activity against baloxavir-resistant influenza A virus *in vitro*. These data support the clinical development of VIS410 that is active against influenza viruses resistant to currently licensed antiviral therapies.

INTRODUCTION



- VIS410 is a human IgG1 monoclonal antibody that targets a highly constrained epitope on HA.
- VIS410 demonstrates broad coverage across Groups 1 & 2 Influenza A, including pandemic H7N9 and H5N1 viruses.
- VIS410 Mechanisms of Anti-Influenza Action include:
  - Inhibits viral/endosomal membrane fusion
  - Antibody-Dependent Cellular Cytotoxicity activity (ADCC)
  - Antibody-Dependent Cellular Phagocytosis activity (ADCP)
  - Complement-Dependent Cytotoxicity activity (CDC)
- VIS410 demonstrated significant antiviral activity against influenza A in three Phase 2 studies with no treatment-emergent resistance observed. [1-3]

VIS410 and Small Molecule Antivirals Function via Different Mechanisms of Action (MOA)



From von Itzstein, Nature Reviews Drug Discovery volume 6, p. 967–974 (2007) with Baloxavir and VIS410 MOA added.

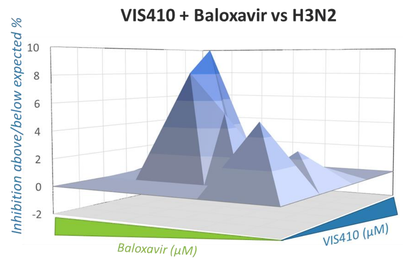
- Most common polymorphisms associated with oseltamivir resistance are NA-H275Y (H1N1) and NA-R292K (H3N2). Oseltamivir-resistant viruses have been detected in circulating strains [4].
- PA I38T is the most common polymorphism associated with baloxavir resistance [5-7].
- Baloxavir-resistance has been identified in treated patients [5,7,9] and, in children prior to treatment (*i.e.*, in circulating strains) [10].

METHODS

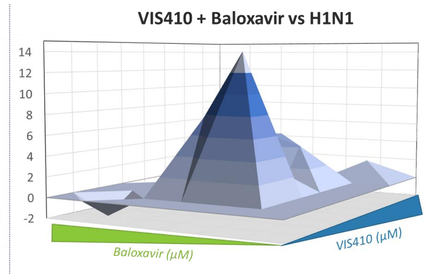
- Reverse-genetics A/Puerto Rico/8/1934_H1N1 (PR8) wild-type and PA I38T mutant viruses were obtained from St. Jude and virus stocks were generated. Full-length HA and PA sequencing was performed on stocks to confirm genotypes.
- In vitro antiviral activity was assessed using an NP-ELISA method derived from the WHO protocol [8]. A second NP-ELISA method using removal of virus following absorption was used to compare EC_{50} values. Drug EC_{50} and curves were determined using Graph Prism 7.

RESULTS

VIS410 and Baloxavir in Combination Synergistically Inhibit Influenza A

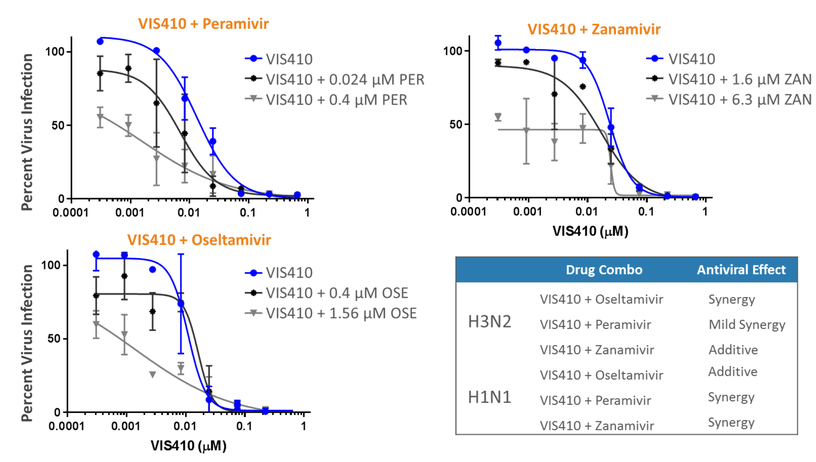


Synergy Plot (95%)	
SYNERGY	63.14
log volume	-9.07
ANTAGONISM	-0.4
log volume	0.06



Synergy Plot (95%)	
SYNERGY	78.55
log volume	-11.28
ANTAGONISM	-28.88
log volume	4.15

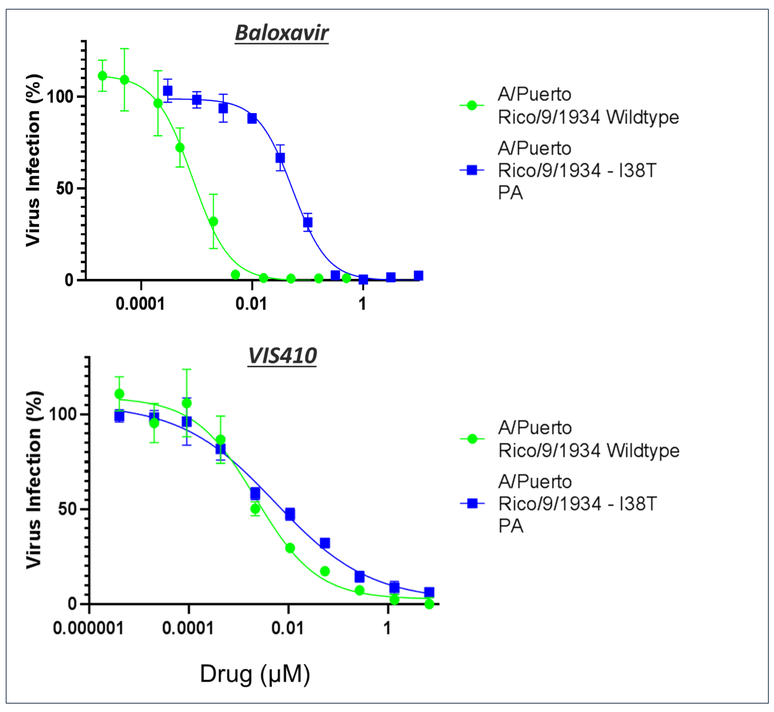
VIS410 and NAIs in Combination Demonstrate Improved Antiviral Activity over Individual Compounds



	Drug Combo	Antiviral Effect
H3N2	VIS410 + Oseltamivir	Synergy
	VIS410 + Peramivir	Mild Synergy
	VIS410 + Zanamivir	Additive
H1N1	VIS410 + Oseltamivir	Additive
	VIS410 + Peramivir	Synergy
	VIS410 + Zanamivir	Synergy

- VIS410 in combination with baloxavir demonstrated *in vitro* synergistic antiviral activity that may be clinically significant for both H1N1 and H3N2 viruses [11].
- Antiviral effects of VIS410 in combination with NAIs (oseltamivir, peramivir, and zanamivir) ranged from additive to moderately synergistic across conditions and viruses tested.
- No significant antagonistic antiviral effects were observed for any of the VIS410-small molecule antiviral combinations.

VIS410 Neutralizes Baloxavir Resistant Virus In Vitro



Comparison of VIS410 and Baloxavir Antiviral Activity against WT and PA I38T Viruses

	NP-ELISA Method 1		NP-ELISA Method 2	
Virus	VIS410 EC_{50}	Baloxavir EC_{50}	VIS410 EC_{50}	Baloxavir EC_{50}
A/Puerto Rico/8/1934_ H1N1 WT	1.1 µg/ml	0.001 µM	0.3 µg/ml	0.0008 µM
A/Puerto Rico/8/1934_ H1N1 PA I38T	2.2 µg/ml	0.13 µM	0.7 µg/ml	0.054 µM
Fold change in EC_{50} (PA I38T: WT virus)	2-fold	130-fold	2-fold	68-fold

- The baloxavir resistant PA I38T mutant virus exhibited ~100-fold lower susceptibility to treatment with baloxavir alone compared to the WT virus.
- WT and PA I38T mutant viruses showed comparable sensitivity to VIS410.
- NP-ELISA method 1 and 2 yielded similar results.
- In previous studies, VIS410 was found to both neutralize A/PR/8/1934 (H1N1) virus and protect against lethal challenge, indicating that *in vitro* results may predict *in vivo* efficacy.

VIS410 Neutralizes Oseltamivir Resistant Virus In Vitro

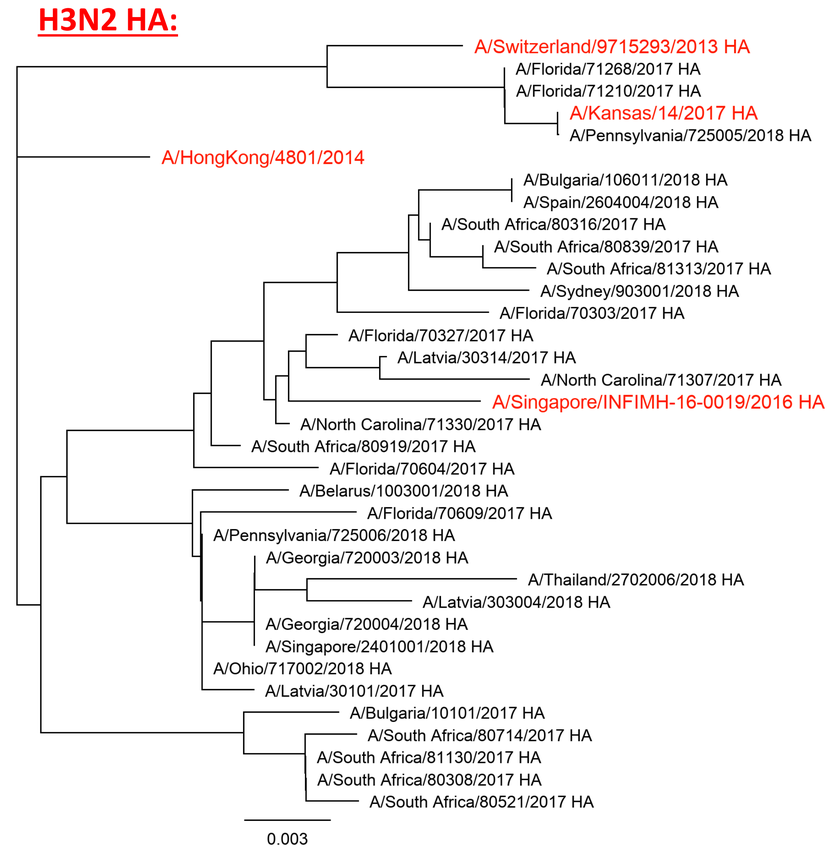
*VIS410 Antiviral Activity Against Oseltamivir-Resistant Viruses**

Virus	VIS410 EC_{50}	Oseltamivir Resistance Mutation**
A/WSN/1933 (H1N1)	0.07 µg/ml	NA H275Y
A/Mississippi/3/2001 (H1N1)	0.69 µg/ml	NA H275Y
A/Hawaii/31/2007 (H1N1)	0.75 µg/ml	NA H275Y
A/Pennsylvania/30/2009 (H1N1)	1.6 µg/ml	NA H275Y
A/Hong Kong/2369/2009 (H1N1)	1.2 µg/ml	NA H275Y

*Data previously reported [2]
**N1 Numbering

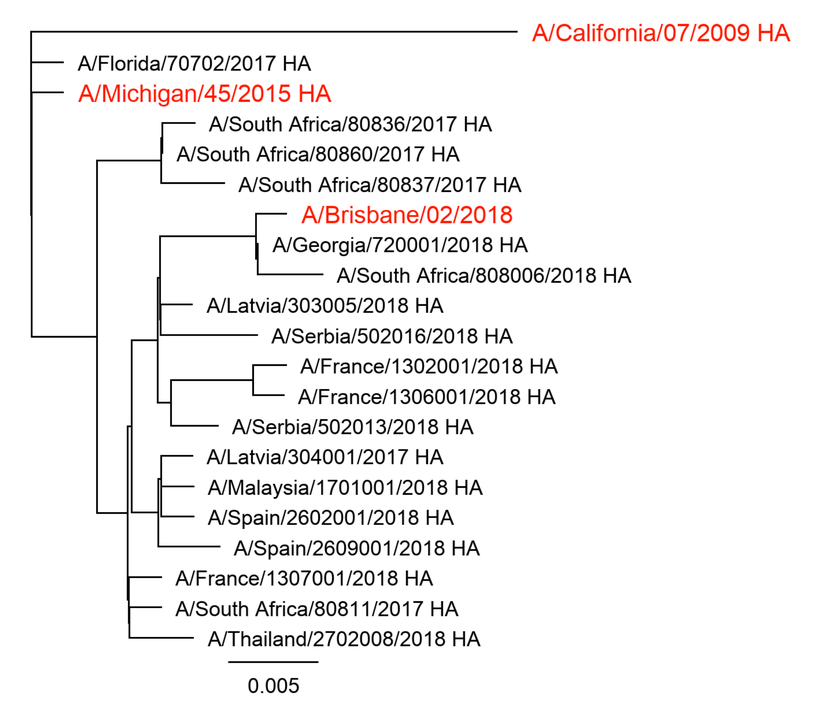
VIS410 Broadly Neutralizes Influenza A Viruses

H3N2 HA:



- H3N2 viruses with diverse HA genotypes (n=35 total; 4 vaccine strains (red) and 31 clinical circulating strains) were tested for VIS410 IC_{50} by NP-ELISA.
- An IC_{50} range of 0.1 – 4.5 µg/ml was observed across 34 viruses with a mean IC_{50} = 1.3 µg/ml.
- This range excludes A/North Carolina/71307/2017* (IC_{50} = 39.4 µg/ml) which possessed a rare polymorphism at VIS410 epitope residue HA2 D53.

H1N1 HA:



- H1N1 viruses with diverse HA genotypes (n=21 total; 3 vaccine strains (red) and 18 clinical circulating strains) were tested for VIS410 IC_{50} by NP-ELISA.
- An IC_{50} range of 0.3 – 0.9 µg/ml was observed across 21 viruses, with a mean IC_{50} = 0.6 µg/ml

CONCLUSIONS AND FUTURE DIRECTIONS

- Baloxavir resistant influenza A virus is susceptible to VIS410 *in vitro*.
- Oseltamivir resistant influenza A viruses are susceptible to VIS410 *in vitro*.
- VIS410 demonstrated synergistic antiviral activity in combination with Baloxavir and enhanced antiviral activity in combination with NAIs.
- VIS410 broadly neutralizes recent vaccine and circulating influenza A strains.
- The emergence of baloxavir resistant viruses highlights the need for orthogonal therapies; VIS410 synergizes with approved therapies and provides antiviral coverage upon emergence of resistance. Dual therapy including VIS410 should be investigated to determine if it can reduce antiviral (baloxavir, oseltamivir) resistance emergence.
- VIS410 in combination with small molecule antivirals is a promising therapy for treatment of severe influenza.

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