Assessment of Resistance Development to Therapeutic mAb VIS410 in an International Phase 2a Study (VIS410-202) in Adults with Uncomplicated Influenza A Infection

AWA0039

Kristin Narayan*[1], Ph.D., Bharathi Sundaresh[1], Emily Helger[1], Saskia Smits[2], Ph.D., Rienk Jeeninga[2], Ph.D., David Oldach[1], M.D., and Susan Sloan[1], Ph.D.

*Presenting author: knarayan@visterrainc.com
[1]Visterra, Inc., Waltham, MA, USA
[2]Viroclinics B.V., Rotterdam, The Netherlands

Visterra (an Otsuka company)

ABSTRACT

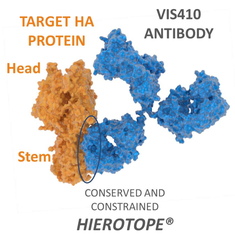
Background: VIS410 is a human monoclonal antibody (mAb) that targets influenza A hemagglutinin (HA) with broad antiviral activity. In a human challenge study utilizing H1N1 virus, VIS410 proved safe and efficacious without resistance emergence. Study VIS410-202 evaluated VIS410 versus placebo for treatment of uncomplicated influenza A infection in patients not requiring hospitalization. Treatment with VIS410 was demonstrated to be safe and was associated with significant reduction in time to symptom resolution and culturable virus. Genotypic and phenotypic analyses were performed to assess the emergence of VIS410 resistance in nasopharyngeal virus populations.

Methods: Sanger sequencing of full-length hemagglutinin (HA) sequences was performed. Sequences were derived from all study subjects for the pre-therapy baseline sample and all post-baseline samples that contained sufficient viral load for this method. Samples were selected for phenotypic testing based on HA genotype or presence of culturable virus post-treatment. Phenotypic methods included (1) IC50 analysis of virus after initial expansion in culture and (2) a direct phenotypic assay in which virus-containing samples are cultured with VIS410 and virus outgrowth is detected (ViroSpot™).

Results: HA sequence was determined for 131 out of 138 influenza A-confirmed subjects [123 H3N2; 8 H1N1] including 107 paired baseline and post-baseline sequences. Only 15 HA amino acid changes were observed post-baseline. The 25 HA residues comprising the VIS410 epitope were inspected for alterations. Only two subjects harbored viruses (both were H3N2 isolates) with changes at VIS410 epitope contact positions, HA2 N53D and HA2 G57R, respectively. Both changes were present in pre-treatment baseline specimens. Both subjects resolved infection without evidence of delayed response or viral rebound. IC50 analysis of the variant isolates revealed reduced VIS410 susceptibility in the HA2 N53D virus [IC50 39.4 µg/mL] while HA2 G57R virus was fully VIS410-sensitive [IC50 2.4 µg/mL]. All other pre- and post-treatment viral isolates were VIS410 sensitive [IC50 range: 0.1-3.1 µg/mL]. Virospot™ analyses are ongoing.

Conclusion: Reduced susceptibility to VIS410, a mAb targeting a highly conserved HA stem epitope, was rarely observed in Study VIS410-202 and no treatment-emergent resistant viruses were observed based on genotypic assessment. Most HA sequences (129 out of 131) were identical to the vaccine strain at VIS410 epitope contact positions, with two variants identified. A single variant, HA2 N53D, which has been identified in 0.06% of HA sequences in the GISAID database, demonstrated reduced susceptibility to VIS410 in vitro. VIS410 shows promise as a potential therapy for influenza A infection.

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.**
- **In a Phase 2a Challenge Study (VIS410-201, NCT02468115) with A/California/04/2009 (H1N1) virus:**

  VIS410 was safe and efficacious; No resistance to VIS410 was observed using genotypic and phenotypic resistance monitoring.

The goal of this study was to assess the development of resistance in patients receiving VIS410 vs placebo in the VIS410-202 study (NCT02989194).

METHODS

VIS410-202 Virology Sample Collection: Nasopharyngeal (NP) swab samples were collected at Baseline (Day 1) prior to receiving study drug, and days 3, 5, and 7. The first 50 subjects had an additional NP sample collected on day 14.

Genotypic Resistance Testing: The full-length HA gene sequence from NP samples was determined using ultrasensitive Sanger sequencing.

Phenotypic IC_{50} Assay: Virus isolates are propagated for one passage to generate sufficient titer, then tested against a series of VIS410 concentrations to determine 50% inhibitory concentration.

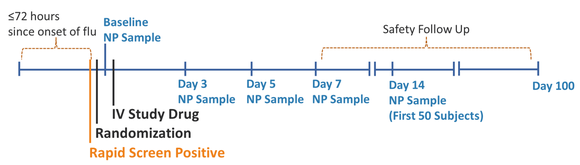
Direct Phenotypic Assay (ViroSpot™): Clinical specimens are cultured in the presence of high concentrations of VIS410 and assessed for virus outgrowth. ViroSpot™ analyses are ongoing and not presented here.

HA Binding: Full length HA genes from VIS410-202 virus isolates were cloned into a mammalian expression vector, expressed in Expi293 cells, and tested for binding to VIS410 using flow cytometry.

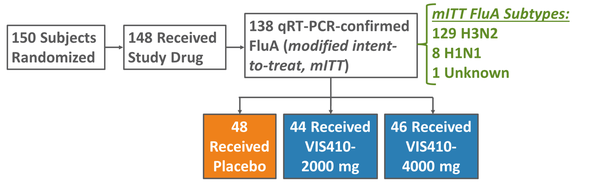
VIS410-202 STUDY OVERVIEW

- **The VIS410-202 Study evaluated 2 doses of VIS410 (2000 mg and 4000 mg) vs Placebo in the treatment of influenza A infection in otherwise healthy adults.**
- **Enrollment occurred in the 2017 Northern and Southern Hemisphere Influenza seasons.**

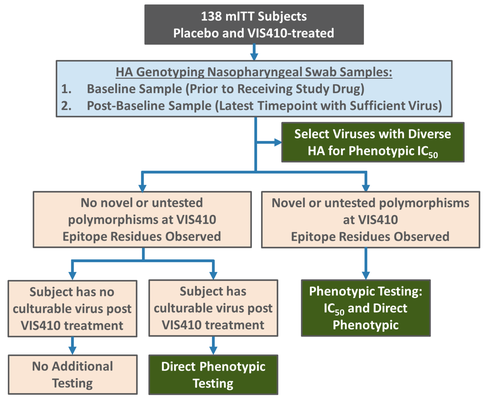
VIS410-202 Key Events and Virology Sampling Schedule



VIS410-202 Summary of Subjects Enrolled



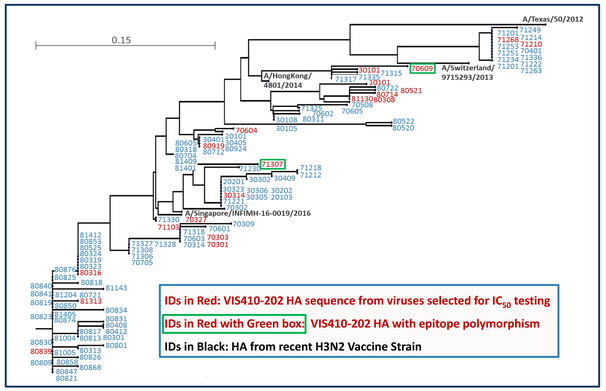
RESISTANCE MONITORING STRATEGY



GENOTYPIC RESISTANCE TESTING

HA GENOTYPING SUMMARY	TOTAL
Influenza A-confirmed Subjects with HA sequence information H3N2 Subtype H1N1 Subtype	131 123 8
Influenza A-confirmed Subjects with no HA sequence	7
Subjects with Paired Baseline and post-baseline sequences	107
Subjects with Post-Baseline HA changes from Baseline sequence	15
Subjects with Post-Baseline HA changes from Baseline sequence at VIS410 epitope positions	0
Subjects with VIS410 epitope residues on HA identical to Vaccine Strain (Baseline and Post-Baseline samples)	129
Subjects with Baseline HA changes from Vaccine Strain HA sequence at VIS410 epitope positions.	2

CLINICAL H3N2 ISOLATES FROM VIS410-202 HAVE DIVERSE HA SEQUENCES



- **HA sequences of 123 H3N2 clinical isolates from VIS410-202 were phylogenetically compared. Scale bars represent the frequency of amino acid variations within the specified distance. Phylogenetic tree was constructed using the Neighbor-Joining Method with Jukes-Cantor protein distance measurement.**

PHENOTYPIC IC_{50} ANALYSIS

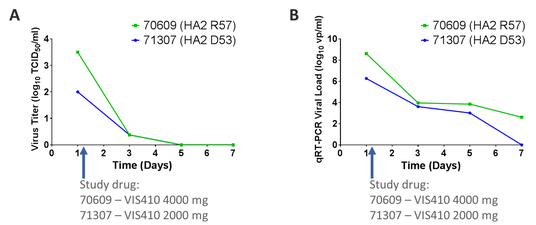
Phenotypic IC_{50} Testing of VIS410-202 Clinical Isolates

Clinical Isolate or Vaccine strain?	Virus	Timepoint	Subtype	IC_{50} (µg/mL)	VIS410 Epitope Polymorphisms
Vaccine strain	**A/Hong Kong/4801/2014**	**n/a**	**H3N2**	**2.5**	**none**
VIS410-202 Isolate	A/Bulgaria/10101/2017	Baseline	H3N2	0.22	none
VIS410-202 Isolate	A/Latvia/30101/2017	Baseline	H3N2	0.28	none
VIS410-202 Isolate	A/Latvia/30314/2017	Baseline	H3N2	1.00	none
VIS410-202 Isolate	A/Florida/70303/2017	Baseline	H3N2	0.34	none
VIS410-202 Isolate	A/Florida/70327/2017	Baseline	H3N2	4.52	none
VIS410-202 Isolate	A/Florida/70604/2017	Baseline	H3N2	1.23	none
VIS410-202 Isolate	A/Florida/70609/2017	Baseline	H3N2	2.41	**HA2 R57**
VIS410-202 Isolate	A/Florida/71210/2017	Baseline	H3N2	0.97	none
VIS410-202 Isolate	A/Florida/71268/2017	Baseline	H3N2	3.14	none
VIS410-202 Isolate	A/North Carolina/71307/2017	Baseline	H3N2	39.4	**HA2 D53**
VIS410-202 Isolate	A/North Carolina/71330/2017	Baseline	H3N2	0.92	none
VIS410-202 Isolate	A/South Africa/80308/2017	Baseline	H3N2	0.91	none
VIS410-202 Isolate	A/South Africa/80316/2017	Baseline	H3N2	0.57	none
VIS410-202 Isolate	A/South Africa/80521/2017	Baseline	H3N2	1.09	none
VIS410-202 Isolate	A/South Africa/80714/2017	Baseline	H3N2	0.13	none
VIS410-202 Isolate	A/South Africa/80839/2017	Day 3	H3N2	0.41	none
VIS410-202 Isolate	A/South Africa/80919/2017	Baseline	H3N2	2.55	none
VIS410-202 Isolate	A/South Africa/81130/2017	Baseline	H3N2	1.00	none
VIS410-202 Isolate	A/South Africa/81313/2017	Baseline	H3N2	1.38	none
Vaccine strains	**A/California/7/2009**	**n/a**	**H1N1**	**0.52**	**none**
VIS410-202 Isolate	A/Florida/70702/2017	Baseline	H1N1	0.41	none
VIS410-202 Isolate	A/South Africa/80811/2017	Baseline	H1N1	0.38	none
VIS410-202 Isolate	A/South Africa/80836/2017	Baseline	H1N1	0.73	none
VIS410-202 Isolate	A/South Africa/80837/2017	Baseline	H1N1	0.25	none
VIS410-202 Isolate	A/South Africa/80860/2017	Baseline	H1N1	0.26	none

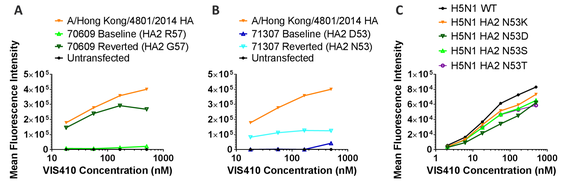
IC_{50} = 50% inhibitory concentration

- **All viruses from VIS410-202 subjects were within the range of previously observed VIS410 IC_{50} against a large panel Influenza A viruses from 1934 to 2012 (Previous IC_{50} range = 0.03 – 64.2 µg/ml).**
- **23 VIS410-202 viruses with no VIS410 HA epitope polymorphisms (*i.e.* epitope residues were identical to the current vaccine strain) demonstrated potent IC_{50} similar to current H1N1 and H3N2 vaccines strains (IC_{50} range = 0.1 – 3.1 µg/ml).**
- **Of 2 Viruses containing untested HA polymorphisms at VIS410 epitope residues, the HA2 R57 virus was fully VIS410-sensitive [IC50 2.4 µg/mL], and the HA2 D53 virus [IC50 39.4 µg/mL] demonstrated reduced VIS410 susceptibility. Genotyping of virus stocks is pending.**

NO CLINICAL IMPACT OF BASELINE VARIATIONS AT VIS410 EPITOPE RESIDUES



- **Viral load curves from 2 subjects harboring baseline HA polymorphisms at VIS410 epitope residues HA2 R57 and HA2 D53 showed no evidence of virus rebound by $TCID_{50}$ (A) or qRT-PCR (B) and demonstrated typical resolution of viral infection and influenza symptoms.**



- **Binding to HA variants with polymorphisms at VIS410 epitope residues was disrupted in the context of H3 HA (A, B) but not group 1 HA (C).**
- **Reversion of H3 HA2 polymorphisms to consensus amino acid restores VIS410 binding in H3N2 indicating a residue preference for binding at HA2 53 and HA2 57 for H3 HA (A and B).**
- **Bioinformatics analysis indicates non-conserved HA polymorphisms are very rare in GISAID HA sequence database: HA2 D53 present in 0.06% of all Group 1 and Group 2 HA sequences, and HA2 R57 is present in 0.13% of all Group 2 HA sequences.**

CONCLUSIONS AND FUTURE DIRECTIONS

- **HA Genotyping demonstrated most HA sequences (129 out of 131) were identical to the vaccine strain at VIS410 epitope contact positions, and no treatment-emergent resistant variants were observed based on genotypic assessment.**
- **Reduced Influenza A susceptibility to VIS410 was rarely observed in VIS410-202.**
- **A baseline virus isolate with HA2 D53 polymorphism showed reduced VIS410 sensitivity by IC_{50} analysis but was still within a previously determined efficacy range for a large panel of Influenza A virus strains.**
- **The 2 Subjects harboring non-conserved polymorphisms at VIS410 epitope residues (H3 HA2 D53 and H3 HA2 R57) resolved influenza with no evidence of virus rebound, worsening disease, or negative clinical impact.**
- **Additional direct phenotypic analysis (ViroSpot™) of virus isolates from VIS410-202- including H3 HA2 D53 and H3 HA2 R57 variants - is ongoing.**
- **VIS410 shows promise as a potential therapy for influenza A infection.**

ACKNOWLEDGEMENTS

We thank the subjects who participated in the study.

This project has been funded in whole with Federal funds from the Department of Health and Human Services; Office of the Assistant Secretary for Preparedness and Response; Biomedical Advanced Research and Development Authority, under Contract No. HHSO100201500018C