The Preclinical Profile of Maturation Inhibitor VH3739937

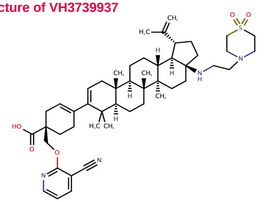
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Introduction

- During virion maturation, HIV-1 Gag is cleaved by the HIV-1 protease in a process that reorganizes the virion core in preparation for the early steps of the HIV-1 life cycle[1]
- HIV-1 maturation inhibitors (MIs) interfere with maturation by blocking the removal of Spacer Peptide 1 from the C-terminal end of Capsid, and consequently make virus particles non-infectious[1,2]
- An earlier investigational MI, GSK3640254, demonstrated robust activity against a range of HIV-1 isolates with diverse Gag sequences, including most of those with polymorphisms that had reduced sensitivity to other MIs; however, the A364V substitution remained less susceptible to inhibition[2]
  - No treatment-emergent resistance to GSK3640254 was observed through Week 24 of the phase 2b DOMINO and DYNAMIC clinical trials[3,4]
- VH3739937 (VH-937 [also known as GSK3739937]; Figure 1) is a relative of GSK3640254 and has a long oral half-life in humans of approximately 3 days supportive of less than once-daily dosing, differentiating it from previous investigational MIs with shorter half-lives[5]
- A phase 1 study in healthy participants found no unexpected safety or tolerability issues with VH-937 and that it is suitable for further development as a weekly administered oral treatment[5]
- Here, the spectrum of antiviral activity and in vitro resistance profile of VH-937 were determined

Figure 1. Chemical Structure of VH3739937



Methods

Drug Susceptibility Assays

- Susceptibility of site-directed mutant (SDM) viruses to VH-937 were performed in MT-2 cells (multi-cycle) or HEK-293T cells (single-cycle); reporter-free laboratory strains and clinical isolates were evaluated in phytohemagglutinin-stimulated peripheral blood mononuclear cells or CEM-NKR-CCR5-Luc cells
- SDM viruses were derived from NLRepRluc-P373S, a variant of NL_{4-3} that includes a *Renilla* luciferase gene in place of a portion of the HIV-1 *nef* region[6] and a P373S substitution in the Spacer Peptide 2 region of Gag to match the dominant polymorphism in HIV-1 subtype B viruses

Dose-Escalating Resistance Selection

- MT-2 cells were infected with full-length HIV-1 NL_{4-3}, treated with VH-937 at 16 hours, post-infection and monitored for cytopathic effects (CPEs) every 1-3 days; in the absence of CPE, cultures were replenished with fresh media and the VH-937 concentration kept constant
- When CPE was apparent, the culture supernatant was used to infect fresh MT-2 cells and the VH-937 concentration in the culture was doubled; after the seventh passage, genotypic analysis of the *gag* gene was performed

Dissociation of a VH-937 Surrogate from Virus-Like Particles

- Non-infectious virus-like particles (VLPs) were collected from the culture supernatant of HEK-293T cells transfected with plasmids expressing the full-length HIV-1 LAI Gag sequence or derivatives containing polymorphisms of interest
- VLPs were bound with an H^3-labeled surrogate of VH-937 and dissociative half-lives were determined using a scintillation proximity assay (SPA) by chasing off radiolabeled compound from pre-formed complexes with a >50-fold molar excess of VH-937

Pre-clinical Pharmacokinetic Analysis

- Pharmacokinetic parameters were obtained by standard non-compartmental analyses of plasma concentration vs time data (Pharsight Phoenix WinNonlin® Version 8.1; Certara Inc, Princeton, NJ)

The long half-life HIV-1 maturation inhibitor VH3739937 demonstrated low nanomolar potency against most viruses containing Gag polymorphisms associated with reduced susceptibility to other maturation inhibitors and was partially active against a replication-competent virus harboring A364V

Results

Antiviral Activity of VH-937 Against Laboratory Strains and Clinical Isolates

- VH-937 was highly potent against a panel of 8 HIV-1 laboratory strains, with half-maximal effective concentration (EC_{50}) values ranging from 1-5 nM against both CXCR4- and CCR5-tropic viruses (Table 1)
- All 42 HIV-1 clinical isolates examined were susceptible to VH-937 at EC_{50} values ≤5 nM with no discernible difference in susceptibility among the 6 subtypes examined
- Activity was also observed against 1 of 2 HIV-2 laboratory strains examined

Table 1. Antiviral Activity of VH-937 Against HIV-1 and HIV-2 Laboratory Strains[a] and Representative Clinical Isolates[b]

Laboratory strain	Tropism	EC_{50} (SD), nM
NL_{4-3}	CXCR4	3.1 (1.0)
HXB2	CXCR4	2.0 (1.6)
IIIB	CXCR4	1.6 (0.3)
LAI	CXCR4	1.3 (0.3)
MN	CXCR4	1.4 (0.4)
RF	CXCR4	4.4 (2.9)
BaL	CCR5	2.0 (0.5)
JRFL	CCR5	2.4 (2.0)
ROD (HIV-2)	CXCR4	1.3 (0.2)
287 (HIV-2)	CCR5/CXCR4	>750
Clinical isolate	**HIV-1 subtype**	**EC_{50}, µM**
UG275	A	0.002
I-2496	A	0.003
92HT593	B	0.001
ASM61	B	0.004
UG268	C	0.003
97ZA009	C	0.005
CM235	CRF01_AE	0.001
CM243	CRF01_AE	0.002
UG270	D	0.002
SE365	D	0.002
BZ126	F	0.002

SD, standard deviation. [a]Average of 2 experiments, each performed in triplicate. [b]Representative examples shown; in total, 42 clinical isolates were examined for their ability to infect peripheral blood mononuclear cells or CEM-NKR-CCR-luc cells.

Antiviral Activity Against Gag Polymorphs

- VH-937 potently inhibited viruses carrying SDM polymorphisms known to reduce the inhibitory activities of previous MIs (Table 2)
- In a multi-cycle assay, VH-937 remained active against a virus harboring the A364V polymorphism (EC_{50}, 5 nM; maximum percent inhibition [MPI], 95%)
- In a single-cycle assay, the A364V substitution caused a 6.4-fold increase in VH-937 EC_{50} (32 nM) and lowered the MPI to 57%

Table 2. Antiviral Activity of VH-937 Against Polymorphic Viruses on an NLRepRluc-P373S Background

	Multi-cycle assay			Single-cycle assay		
Mutation[a]	**EC_{50}, µM**	**FC-EC_{50}**	**MPI, %**	**EC_{50}, µM**	**FC-EC_{50}**	**MPI, %**
Wild type	0.002	Ref	99.1	0.005	Ref	99
V370A[7]	0.004	2	98.5	0.006	1.2	99
ΔV370[7]	0.004	2	97.6	0.012	2.4	100
V370A/ΔV371[7]	0.005	2.5	92.2	ND	ND	ND
V362I/V370A[2]	0.002	1	94	0.011	2.3	98
T332S/V362I/prR41G[8]	0.008	4	98	0.024	4.8	96
A326T/V362I/V370A[2]	0.004	2	95	ND	ND	ND
A364V[2,7,8]	0.005	2.5	95.2	0.032	6.4	57
ΔV370/T371A	ND	ND	ND	0.007	1.4	99
A366V[2,9]	ND	ND	ND	>3	>600	-141[b]

FC-EC_{50}, fold change in EC_{50} relative to the wild-type NLRepRluc-P373S virus; ND, no data. [a]Known Gag polymorphisms identified in studies with bevirimat, GSK3532795, and/or GSK3640254. [b]A negative MPI indicates virus replication is enhanced compared with samples without VH-937.

Selection of Viruses With Reduced Susceptibility to VH-937

- A364V was selected under escalating VH-937 conditions in 1 of the 4 resistance selection assays (Table 3)
- Other mutations selected by VH-937 included T332P, L363W, and the triple mutant H144Y/V362I/R384K
  - Whereas viruses with each of these mutations exhibited highly reduced susceptibility in a single-cycle assay, incorporation of any of these mutations into a replication-competent NL_{4-3}-based clone did not produce a functional virus

Table 3. Substitutions in Gag After Dose-Escalating Resistance Selection[a]

	Gag variants, %[b,c]							
Virus	**D93N[d]**	**H144Y**	**D298D/N**	**T332P**	**V362I**	**L363L/W**	**A364A/V[e]**	**R384K[f]**
IIIB #1	—	—	—	—	—	20/80	—	—
IIIB #2	—	—	—	—	—	70/30	20/80	—
NL_{4-3} #1	100	—	—	100	—	—	—	—
NL_{4-3} #2	—	100	70/30	—	100	—	—	100

[a]Final concentration of VH-937 was 64x the EC_{50}. [b]Substitutions are in the capsid protein unless otherwise noted. [c]Percentages estimated from sequence traces of PCR amplicons. [d]Located in the matrix protein. [e]Located in spacer peptide 1. [f]Located in the nucleocapsid protein.

Dissociation of a VH-937 Surrogate Compound From VLPs

- The H^3-radiolabeled VH-937–like surrogate compound demonstrated a similar antiviral profile to its unlabeled counterpart
- Based on displacement of the radiolabeled compound from wild-type VLPs with a 50-fold molar excess of VH-937, the estimated dissociative half-life of VH-937 was ~3 days (4125 minutes; Table 4), showing the compound dissociates slowly
- However, the dissociative half-life with A364V-containing VLPs was relatively rapid at 29 minutes
- In a cleavage assay with A364V-containing VLPs, the VH-937 surrogate transiently inhibited cleavage, suggesting a loss of inhibition over time

Table 4. Binding Affinities and Dissociative Half-lives of Radiolabeled VH-937 to VLPs

VLP	$t_{1/2}$, min
Wild type[a]	4125
V362I/V370A	2894
A364V	29

$t_{1/2}$, dissociative half-life. [a]NL_{4-3} gag.

Pre-clinical Pharmacokinetic Profile of VH-937

- VH-937 was ~93.3% protein bound in 100% human serum; based on a value of 3 times the protein binding–adjusted EC_{90} of a series of viruses carrying different Gag polymorphisms, a target trough (C_{min}) value of 312 nM was established for clinical use of VH-937
- VH-937 was orally bioavailable in all animal species tested, ranging from 44% in monkey to 59% in dog, and plasma clearance was low (Table 5)
- Aggregate pharmacokinetic and metabolism data suggest VH-937 is suitable for clinical development

Table 5. Single-Dose Pharmacokinetic Parameters From Various Non-clinical Species

			Parameters, mean (SD)		
Species	**Route**	**Dose, mg/kg**	**$t_{1/2}$, h**	**Cl_p, mL/min/kg**	**F, %**
Mouse	IV	1	10.9 (2.4)	0.63 (0.08)	NA
	Oral	5	14.5 (7.8)	NA	46 (3.2)
Rat	IV	1	8.6 (0.3)	1.23 (0.06)	NA
	Oral	5	8.6 (2.5)	NA	45.1 (15.3)
Dog	IV	1	26.9 (4.0)	0.21 (0.04)	NA
	Oral	2	26.9 (2.0)	NA	59 (17)
Monkey	IV	1	12.3 (1.2)	0.32 (0.09)	NA
	Oral	2	11.4 (5.5)	NA	43.5 (7.8)

Cl_p, systemic plasma clearance; F, absolute bioavailability; IV, intravenous; NA, not applicable; $t_{1/2}$, apparent elimination half-life.

Conclusions

- VH-937 exhibited low nanomolar potency against HIV-1 laboratory strains, clinical isolates, and viruses with a broad range of Gag polymorphisms associated with resistance to prior MIs, including A364V
- However, VH-937 had a lower MPI in a single-cycle assay against HIV-1 harboring A364V and dissociated relatively rapidly from A364V-containing VLPs, suggesting viruses with the A364V polymorphism could replicate in the presence of VH-937, similar to other MIs
  - In agreement, A364V developed in 1 of 4 cultures undergoing dose-escalating resistance selection
- Based on protein-binding studies and inhibitory activities against a series of viruses with different polymorphisms in Gag, a C_{min} of 312 nM is expected to provide antiviral coverage against the vast majority of viruses
- Overall, these findings demonstrate the robust antiviral properties of VH-937 against HIV-1 strains with diverse Gag sequences and polymorphisms conferring resistance to prior MIs, with rare exception, and support the continued clinical development of VH-937
  - See poster 634 in poster session G2 for an analysis of GSK3640254 potency in the DOMINO clinical trial

Acknowledgments: This study was funded by ViiV Healthcare. Editorial assistance and graphic design support for this poster were provided under the direction of the authors by MedThink SciCom and funded by ViiV Healthcare.

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