Pre-Clinical Profiles of HIV-1 Capsid Inhibitors VH4004280 (VH-280) and VH4011499 (VH-499)

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Key Takeaways

- The HIV-1 capsid inhibitors VH4004280 (VH-280) and VH4011499 (VH-499) demonstrated multistage mechanisms of action and achieved picomolar half-maximal effective concentrations (EC_{50}s) against a broad range of HIV-1 sequences
- None of the individual substitutions associated with reduced susceptibility to VH-280 or VH-499 are common, suggesting that pre-existing resistance to these inhibitors should be rare
- The pre-clinical profiles of VH-280 and VH-499 support their continued clinical development as potential long-acting antiretroviral agents

Introduction

- The HIV-1 capsid protein fulfills multiple roles in the HIV-1 life cycle, contributing to key functions during reverse transcription, nuclear entry, chromosome integration and virus particle assembly[1-4]
- HIV-1 capsid inhibitors bind to a pocket formed between adjacent capsid proteins within capsid hexamers and demonstrate potent antiviral activity in vitro[5,6]
- Here, we report on the pre-clinical profile of two capsid inhibitors (VH-280 and VH-499), both of which are currently in clinical development

Methods

- Drug susceptibility assays were conducted in MT-2 cells infected with replication-competent reporter viruses or Rev-A3R5-GFP HIV reporter cells infected with HIV-1 laboratory strains; cytotoxicity was assayed in MT-2 cells
- Binding affinities of VH-280 and VH-499 to capsid hexamers, as defined by equilibrium dissociation, were determined using surface plasmon resonance (SPR)
- Early and late antiviral activity was determined with VSV-G pseudotyped viruses generated in 293T cells in the absence (early assay) or presence (late assay) of a capsid inhibitor, which were then used to infect a reporter cell line in the presence (early assay) or absence (late assay) of a capsid inhibitor
- For time-of-addition assays, MT-2 cells infected with a reporter virus pseudotyped with HIV-1 LAI gp160 were treated with inhibitors at specific time points post-infection; percent inhibition was determined relative to DMSO-treated controls
- Quantitative polymerase chain reaction (qPCR) assays were used to determine levels of total reverse transcripts, 2-long terminal repeat (2-LTR) circles, and integrated proviruses
- Dose-escalating resistance selection studies were conducted in MT-2 cells infected with a cell culture–adapted NL_{4-3} strain

Results

Antiviral Activity and Cytotoxicity of VH-280 and VH-499

- Both VH-280 and VH-499 potently inhibited the reporter virus with mean EC_{50} values in the picomolar range (Table 1)
- Half-maximal cytotoxicity concentrations (CC_{50}s) were >20 µM for both inhibitors, yielding high therapeutic indices

Table 1. Antiviral Activity of VH-280 and VH-499 Against HIV-1 NLRepRluc-WT[a] and Cytotoxicity in MT-2 Cells

	VH-280	N	VH-499	N
Antiviral activity				
EC_{50}, mean ± SD, nM	0.093 ± 0.021	74	0.023 ± 0.008	113
EC_{90}, mean ± SD, nM	0.23 ± 0.08	74	0.061 ± 0.022	111
EC_{90}/EC_{50} ratio	2.5		2.7	
Cytotoxicity				
CC_{50}, mean, µM	>20	8	>20	4
Therapeutic index[b]	>220,000		>870,000	

EC_{90}, 90% effective concentration; N, number of assays, each run in duplicate. [a]Reporter virus derived from NL_{4-3} in which *nef* has been replaced with a gene encoding *Renilla* luciferase. [b]Calculated as CC_{50}/EC_{50}.

- All EC_{50}s determined for VH-280 and VH-499 against a panel of seven HIV-1 laboratory strains were in the picomolar range (Table 2)

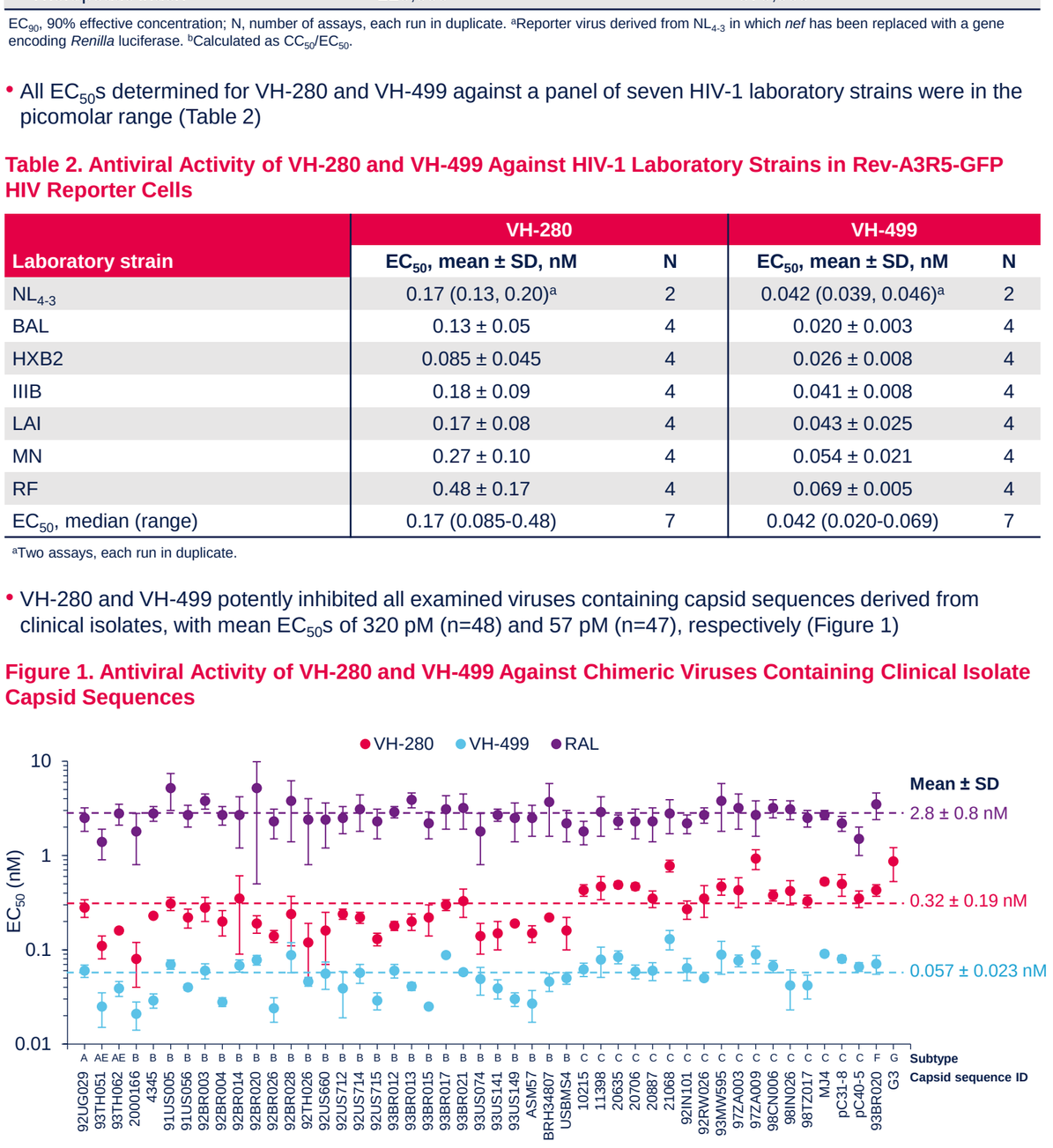
Table 2. Antiviral Activity of VH-280 and VH-499 Against HIV-1 Laboratory Strains in Rev-A3R5-GFP HIV Reporter Cells

	VH-280		VH-499	
Laboratory strain	EC_{50}, mean ± SD, nM	N	EC_{50}, mean ± SD, nM	N
NL_{4-3}	0.17 (0.13, 0.20)[a]	2	0.042 (0.039, 0.046)[a]	2
BAL	0.13 ± 0.05	4	0.020 ± 0.003	4
HXB2	0.085 ± 0.045	4	0.026 ± 0.008	4
IIIB	0.18 ± 0.09	4	0.041 ± 0.008	4
LAI	0.17 ± 0.08	4	0.043 ± 0.025	4
MN	0.27 ± 0.10	4	0.054 ± 0.021	4
RF	0.48 ± 0.17	4	0.069 ± 0.005	4
EC_{50}, median (range)	0.17 (0.085-0.48)	7	0.042 (0.020-0.069)	7

[a]Two assays, each run in duplicate.

- VH-280 and VH-499 potently inhibited all examined viruses containing capsid sequences derived from clinical isolates, with mean EC_{50}s of 320 pM (n=48) and 57 pM (n=47), respectively (Figure 1)

Figure 1. Antiviral Activity of VH-280 and VH-499 Against Chimeric Viruses Containing Clinical Isolate Capsid Sequences



Error bars represent SD. Dashed lines represent mean. RAL, raltegravir.

VH-280 and VH-499 Mechanism of Action

- VH-280 and VH-499 bind to the mature capsid hexamer with mean dissociation constants (K_d) of 60 and 190 pM, respectively
- Both early and late steps of the HIV-1 life cycle were potently inhibited, with inhibition of the early stages ~9- to 14-fold greater than that for the late stages (Table 3)

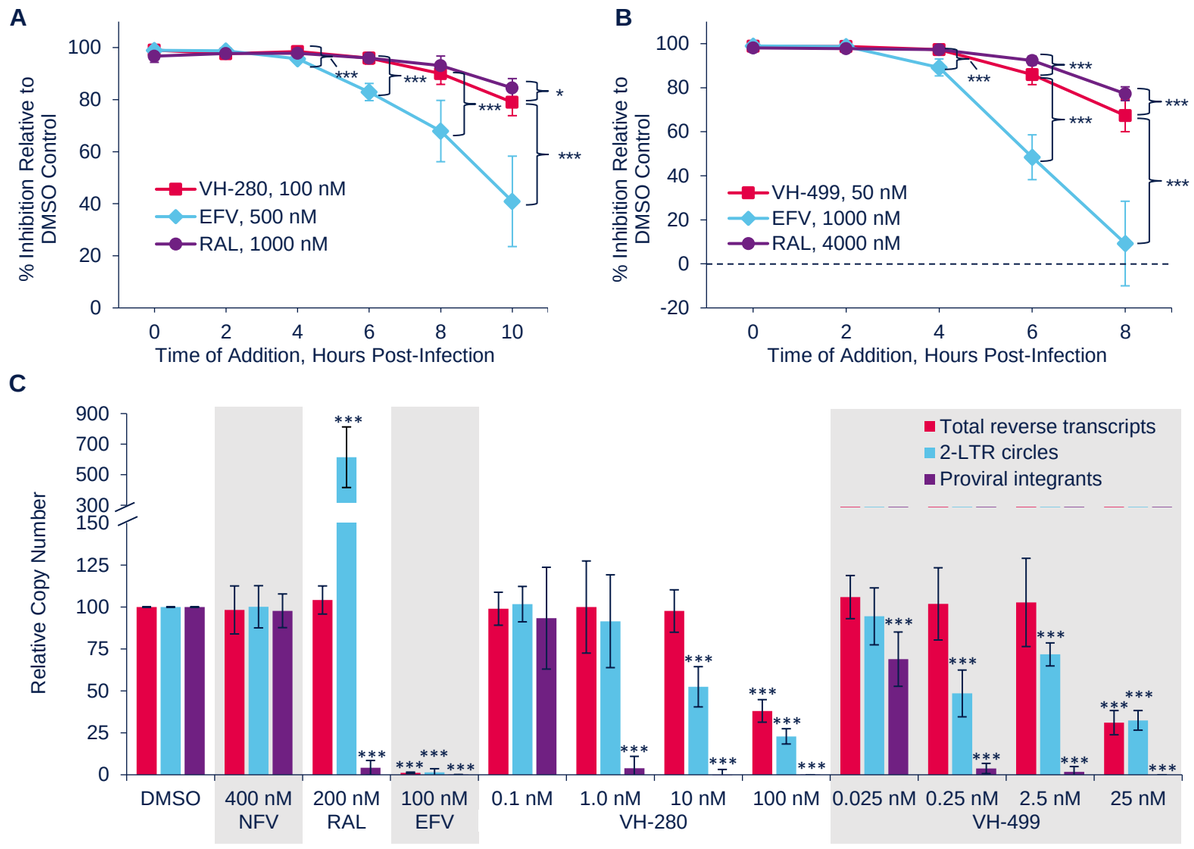
Table 3. Antiviral Activity of VH-280 and VH-499 in an Early–Late Assay[a]

	VH-280 EC_{50}, mean ± SD, nM	N	VH-499 EC_{50}, mean, nM	N
Early activity	0.25 ± 0.04	3	0.036 (0.033, 0.039)	2
Late activity	2.3 ± 1.3	3	0.50 (0.31, 0.68)	2

[a]EC_{50} values are from two or three independent experiments performed in triplicate.

- In time-of-addition experiments, the inhibition profile of VH-280 and VH-499 resembled that of raltegravir, indicating that they primarily block HIV-1 replication after reverse transcription and slightly before integration (Figure 2A-B)
- Analyses of replication intermediates confirmed that VH-280 and VH-499 primarily block a post-nuclear import step of the virus life cycle but also demonstrated that they disrupt multiple other early HIV-1 replication events (Figure 2C)
  - The most pronounced effect of VH-280 and VH-499 was the reduction in number of integrated proviruses; both capsid inhibitors also caused a marked reduction in the number of 2-LTR circles, implying that they also block nuclear import

Figure 2. Time-of-Addition Experiments for (A) VH-280 and (B) VH-499 and (C) Effect of VH-280 and VH-499 on HIV-1 Replication Intermediates

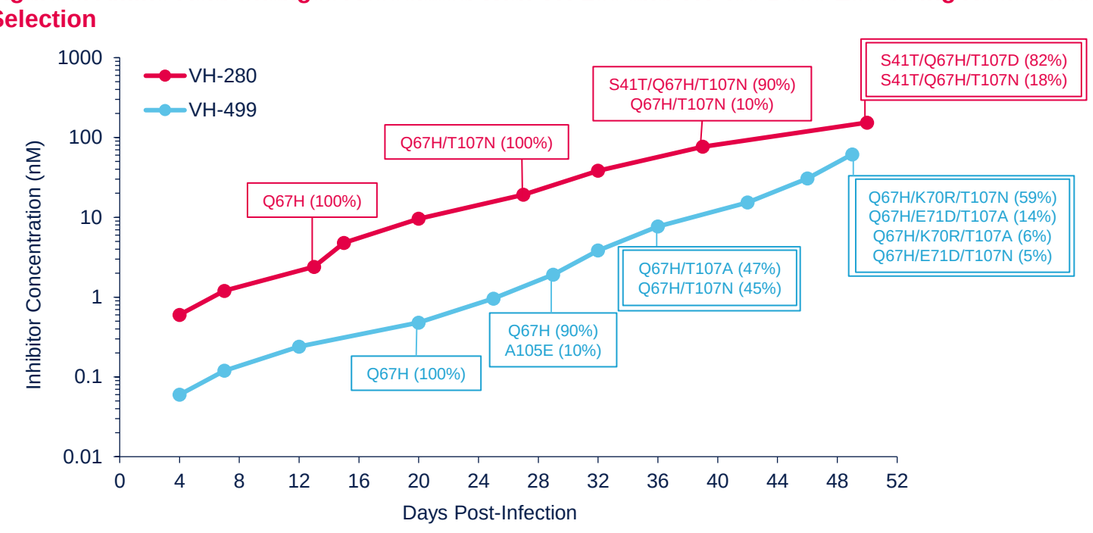


In panels A and B, time post-infection correlates with progression through post-entry steps of the virus life cycle; decreases in mean percent inhibition are observed if the inhibitor is added after the viruses complete inhibitor-relevant stage(s) of the HIV-1 life cycle. Error bars represent SD. *P* values for comparison between indicated capsid inhibitor and EFV or RAL at each time point. *$P<0.05$. ***$P<0.001$. In panel C, plotted data represent number of copies of replication intermediates relative to DMSO-treated control cells. Error bars represent SD from ≥3 experiments. Significance of each value determined relative to DMSO control. ***$P<0.001$. DMSO, dimethyl sulfoxide; EFV, efavirenz; NFV, nelfinavir; RAL, raltegravir.

In Vitro Resistance of VH-280 and VH-499

- For both VH-280 and VH-499, Q67H was the first substitution to emerge and was maintained throughout the remaining passages (Figure 3)
- Additional amino acid changes were observed at capsid positions 41 and 107 for VH-280 or positions 70, 71, 105, and 107 for VH-499; all these positions are located in or close to the capsid inhibitor binding pocket

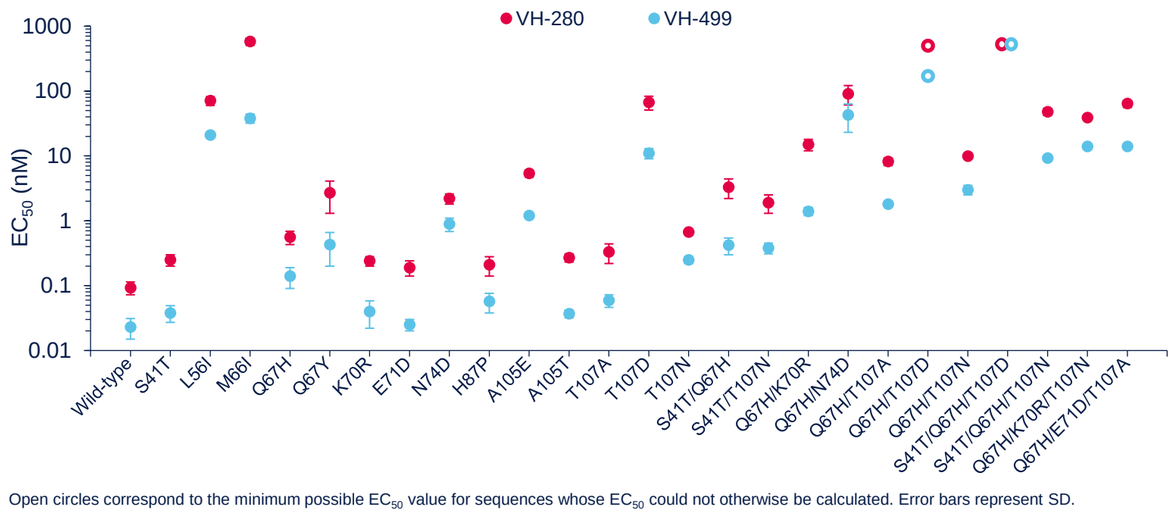
Figure 3. Amino Acid Changes Associated With VH-280 and VH-499 Dose-Escalating Resistance Selection



Amino acid changes in single boxes represent results of population sequence analyses and frequencies estimated from sequence traces. Changes in double boxes represent results generated from next-generation sequencing analyses. Genotypes of reads present at <5% are not shown.

- Single amino acid substitutions selected in this analysis or by other capsid inhibitors[7-10] that resulted in a >3-fold reduction in susceptibility to both VH-280 and VH-499 included L56I, M66I, Q67H, Q67Y, N74D, A105E, T107D, and T107N (Figure 4)
  - No substitutions conferring >4-fold reduced susceptibility were present in >0.06% of Los Alamos National Laboratory database sequences[11]
- Q67H/K70R double substitution, seen in the CALIBRATE and CAPELLA clinical trials,[12,13] reduced virus susceptibility to VH-280 and VH-499 by 160- and 61-fold, respectively

Figure 4. Effect of Capsid Inhibitor Resistance-Associated Mutations on VH-280 and VH-499 Potency



Open circles correspond to the minimum possible EC_{50} value for sequences whose EC_{50} could not otherwise be calculated. Error bars represent SD.

Conclusions

- Both VH-280 and VH-499 demonstrated multistage mechanisms of action and potently inhibited HIV-1 replication with EC_{50} values against laboratory strains and viruses containing capsid sequences from clinical isolates in the picomolar range
- In vitro resistance selections identified S41T, Q67H, K70R, E71D, A105E, and T107N/D/A as emergent substitutions of interest, though only amino acid changes rarely observed in clinical sequences caused >4-fold changes in EC_{50} values as individual substitutions
- Overall, the pre-clinical virology profiles and other favorable drug-like properties of VH-280 and VH-499 supported their progression into clinical development
  - Data on the pharmacokinetics and safety of orally administered VH-280 and VH-499 in participants without HIV are presented in Poster THPEB093 (VH-280)[14] and Poster WEPEB105 (VH-499)[15]
- VH-280 and VH-499 are being investigated in a phase 2a proof-of-concept study (NCT06039579)

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