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In Vivo Escape Pathways to VRC07-523LS and VRC01LS and Their Effects on CD4bs bNAb Susceptibility

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T. DeStefanis[1], F. Belinky[2], S.H. Ko[2], E. Coates[2], L. Strom[2], M. Happe[2], K. Bar[3], P. Tebas[3], E. Boritz[2], R. Lynch [1]

1. Department of Microbiology, Immunology, and Tropical Medicine, George Washington University 2. Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institute of Health

3. Department of Medicine, Division of Infectious Disease, University of Pennsylvania

Background

The ability of HIV to rapidly mutate remains a major challenge in developing a successful immune-based cure. Despite the potential of broadly neutralizing antibodies (bNAbs) to neutralize a wide breadth of viral strains, viral resistance still occurs in many individuals. To achieve a successful bNAb therapy, it is essential to gain a deeper understanding of the *in-vivo* evolution of HIV under selection pressure imposed by each bNAb. This project aims to comprehensively map *in-vivo* viral escape pathways from VRC01-class bNAbs in order to identify improved bNAb combinations that prevents viral escape.

Methods

Part	Participants	Product	Administration Schedule
			Day 0
Part A	7	VRC01LS	40 mg/kg IV
Part B	9	VRC07-523LS	40mg/kg IV
Total	**16**		

Table 1. NCT02840474 Clinical Trial Schema.

- Plasma viral RNA was isolated before and after bNAb treatment for each participant.
- Full-length *env* genes were sequenced by single-genome sequencing (SGS) (Part A) or High-Throughput Single Genome Sequencing (HT-SGS) (Part B) and representative Envs were cloned to generate pseudoviruses for neutralization assays.
- Single nucleotide variation was quantitated to identify changes in AA frequency post-infusion

$$F_{change} = F_{post} - F_{pre} \qquad F_{ratio} = \frac{F_{post}}{F_adj_{pre}}$$

- A codon-based positive selection analysis was performed on the phylogeny before and after infusion using FUBAR

$$\mathrm{dN/dS}_{\mathrm{ratio}} = \frac{\frac{dN}{dS}post}{\frac{dN}{dS}pre}$$

- Population genetic methods were used to identify selective sweeps

$$\mathrm{H1} = p_1^2$$

p_1 – frequency of the most common haplotype in a sliding window

$$\Delta H1 = H1_{post} - H1_{pre}$$

- To identify residues under positive immune selection, each position was given a combined score (CS) :

$$CS = F_{change} \times F_{ratio} \times \mathrm{dN/dS}_{\mathrm{ratio}}$$

- Combined scores were used to identify putative resistance mutations which were inserted by site-directed mutagenesis in a pre-infusion bNAb-sensitive Env.
- Mutated pseudoviruses were tested for bNAb sensitivity in the standard TZM-bl neutralization assay.

Results

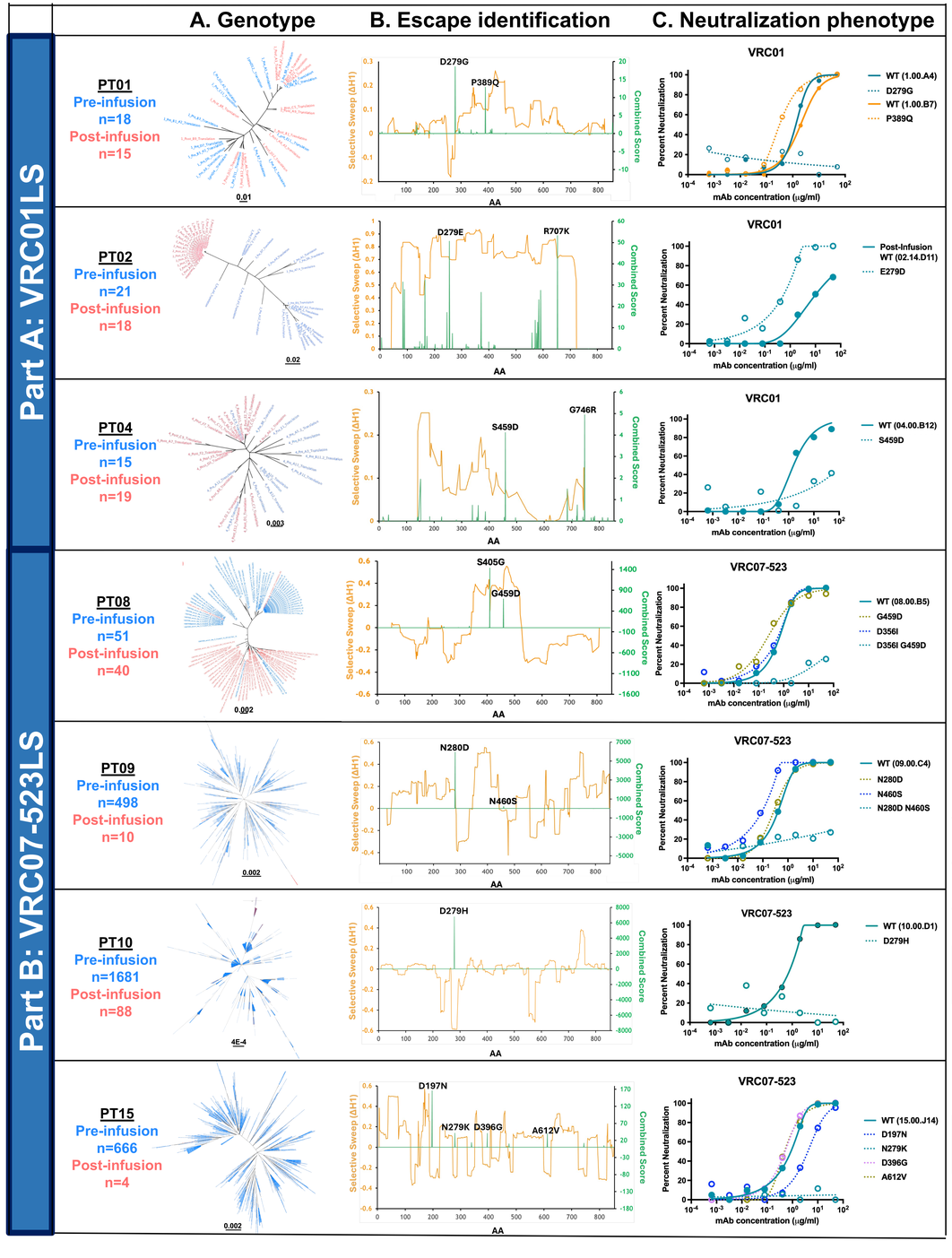


Figure 1. Escape analysis for VRC607 clinical trial participants. A. Phylogenetic trees of intra-host *envs* sequenced pre and post bNAb infusion. Post viruses were obtained from plasma samples taken at least 14 days after bNAb infusion. **B.** Selective sweeps identified via ΔH1 and combined scores for each *env* residue. A positive ΔH1 indicates that a position had less variation post-infusion. Residues were chosen for *in-vitro* testing based on high combined score. **C.** Neutralization curves of wildtype sensitive Envs along with mutated Envs containing the putative resistance residues were generated against the administered bNAb for each participant. As a combination of several putative mutations failed to confer resistance for PT02, a mutation predicted to be associated with sensitivity was inserted into a post-infusion resistant *env*.

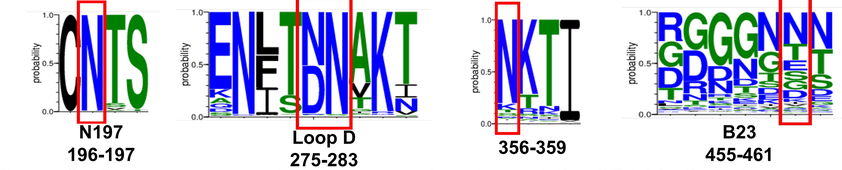


Figure 2. Escape occurs within conserved regions of the CD4 binding site. Logo plot of Env conservation in regions where escape mutations were identified. Conservation was determined among 8735 multi-clade Env sequences using LANL AnalyzeAlign.

		CD4bs								V3	MPER
Participant	Virus	VRC07-523	VRC01	3BNC117	VRC13	1-18	VRC01.23	N49-P9.6	N6	10-1074	10E8
1	WT	0.235	1.32	0.246	0.682	0.290	0.456	0.878	0.405	0.142	32.1
	N279D	0.536	>50	3.82	0.438	0.140	2.06	0.839	0.311	0.127	16.6
2	E279D	0.180	0.544	0.148	0.459	0.042	0.014	0.015	0.130	0.117	0.733
	WT Post	7.24	9.82	>50	NA	0.100	0.020	0.021	0.264	NA	NA
4	WT	0.281	1.57	>50	NA	NA	0.036	0.075	NA	0.152	0.567
	S459D	>50	>50	>50	0.206	0.02	0.087	0.161	0.017	0.054	NA
8	WT	0.712	4.45	3.18	35.9	2.87	0.446	0.910	1.69	0.095	0.766
	D355I G459D	>50	>50	>50	30.1	2.28	0.942	0.910	0.464	0.178	1.61
9	WT	0.418	1.76	0.248	0.377	0.114	0.084	0.068	0.470	0.002	0.019
	N280D N460S	>50	>50	>50	6.37	0.018	>50	0.022	0.053	0.001	0.021
10	WT	0.696	10.9	>50	>50	1.59	1.46	3.03	0.499	0.148	0.605
	N279D	>50	>50	>50	>50	0.319	>50	0.459	5.96	0.087	0.381
15	WT	0.871	21.2	>50	3.24	3.34	0.497	0.463	0.854	>50	1.11
	N279D	>50	>50	>50	10.7	>50	>50	2.48	>50	>50	0.583

Table 2. Impact of VRC01LS and VRC707-523LS escape on sensitivity to other bNAbs. Pseudoviruses containing identified escape mutants were tested for neutralization sensitivity to a panel of bNAbs. The table displays the antibody concentration needed to neutralize 50% of the virus (IC50). Several CD4bs antibodies maintained the ability to neutralize VRC01LS and VRC07-523LS escape variants even though they target the same epitope.

Conclusions

- Combining pre- and post-infusion residue frequency changes, frequency ratios, and dN/dS ratios is a strong predictor of bNAb escape residues.
- Escape emerges through a single residue 279 mutation for PT01, PT10, and PT15 even though they were administered two different but clonally related bNAbs.
- Despite escape emerging in conserved regions of the CD4bs, several CD4bs bNAbs potently neutralize VRC01LS and VRC07-523LS escape variants.

Plain Language Summary

Broadly neutralizing antibodies (bNAbs) are a promising immunotherapy for the treatment and cure of HIV, but pre-existing and new resistance mutations in viruses remains the limiting factor to their success. We have identified several mutations responsible for bNAb resistance, which we can then leverage to inform new bNAb combinations that raise the bar for viral resistance.

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