Efficacy and Complementarity of HIV-1 Suppression by CD4-binding site bNAbs

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BACKGROUND & RATIONALE

Broadly neutralizing antibodies (bNAbs) show potential as complementary immunotherapy to combination anti-retroviral therapies for HIV-1 treatment. However, infusion of a single bNAb selects for resistant variants. We developed an *ex vivo* assay that predicts bNAbs potential to achieve *in vivo* virologic response in people with HIV and allows investigation of viral resistance signatures. Further, *ex vivo* investigation of CD4 binding site (CD4bs) bNAbs revealed complementary virologic suppression by the next-generation C4bs bNAbs 1-18, N6 and VRC01v23, suggesting these bNAbs represent ideal candidates for future combined therapies or multi-specific bNAb designs.

STUDY DESIGN & METHODS

PBMCs were obtained from healthy donors and viremic individuals enrolled in the phase I VRC607/ACGT5378 study investigating antiviral efficacy of CD4bs bNAbs VRC01LS or VRC07-523LS (**Table 1**). The development of the *ex vivo* assay utilized cells from pre-infusion timepoint and is described in **Fig. 1.** bNAbs tested are listed on **Table 2**. Viral replication was measured by p24 ELISA and sequencing was performed by single genome amplification (SGA) (**Fig. 2**).

Table 1. VRC607/ACTG5378 study and subject description. Sixteen subjects were infused once with either VRC01LS or VRC07-523LS at indicated dose. S.D.: single dose. * Participants consisted of viremic adults

VRC07 607/ A5378 Study Enrollment			
PART	Participants	Product	Administration
PART A	7	VRC01LS	40mg/kg IV S.D.
PART B	9	VRC07-523LS	40mg/kg IV S.D.
Total	16*		

Table 2. Ex vivo assay bNAb panel

Antibody Panel (Ab conc. 75 ug/ml)
9114 (Influenza, assay control)
VRC01LS
VRC07-523LS
VRC01v23
1-18LS
N6LS

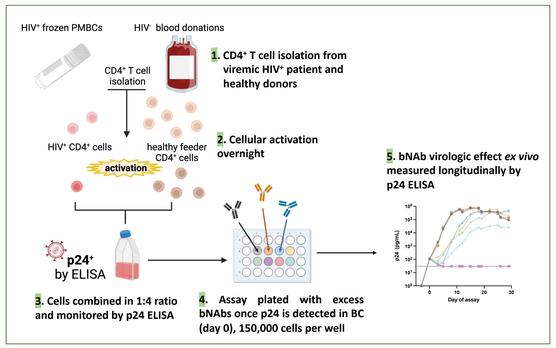

Figure 1. *Ex vivo* assay workflow. Assays were maintained for approximately 21-28 days. CD4+ T cells from healthy donors were added twice a week, supernatants were collected 3 times a week with reposition of media + bNAbs.

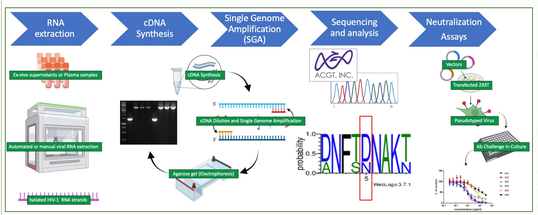

Figure 2. SGA workflow. Viral RNA was extracted using automated platform Biomek and the RNAdvance viral extraction kit (Beckman Coulter). Viral RNA was used for cDNA synthesis and sequenced on Illumina platform. Sequences from outgrowth viruses were inserted in pseudovirus expression vectors and tested against different bNAbs in TZM-bl neutralization assay.

RESULTS

Clinical trial and *ex vivo* assay outcomes: In VRC607/ACGT5378, 2 patients infused with VRC01LS and 8 with VRC07-523LS showed viremia decrease > 1 log10. *Ex vivo* assays using samples from 9 patients whose viremia decreased after VRC01LS (n=2) or VRC07-523LS (n=7) infusion evidenced >10-fold reduction in viral replication by the corresponding bNAb relative to assay control **(Fig. 3)**.

Neutralization data: Assessment of more potent and broader next-generation CD4bs bNAb VRC01v23, 1-18 and N6 evidenced complementary suppression profiles for these 3 bNAbs with some assays showing suppression by 1 or 2 but not all 3 bNAbs. Most VRC01LS or VRC07-523LS escape strains were neutralized by 1-18, N6 or VRC01v23, supporting potential for combined therapy with these bNAbs (**Fig. 4, Table 3**).

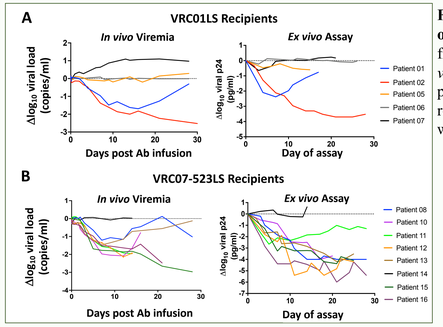

Figure 3. *Ex Vivo* Assay recapitulates virological outcome observed *in vivo*. **(A)** Viremia follow up from patients that received VRC01LS infusion and *ex vivo* assays performed with cells from corresponding patients. **(B)** Viremia follow up from patients that received VRC07-523LS infusion and *ex vivo* assays with cells from corresponding patients.

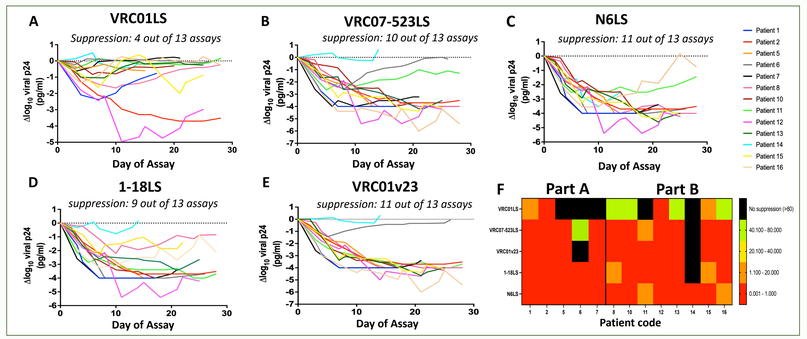

Figure 4. CD4bs bNAbs efficacy in *ex vivo* assay. (A-E) VRC07-523LS shows improved suppression compared to VRC01LS and complementary suppression profile is seen between next generation CD4bs bNAbs 1-18, N6 and VRC01v23. **(F)** Heat map based on p24 ELISA titers by the end of the assay normalized to assay control

Virus	Patient	VRC01LS	VRC07-523LS	VRC01-23LS	VRC01v23	1-18LS	N6LS
pCMVR_RA45_VRC07-523LS_C8_d29	VRC607-06	>50	>50	>50	>50	0.09	0.30
pCMVR_RA45_CAP256-J3LS_C13_d29	VRC607-06	>50	0.18	0.13	1.47	0.26	0.34
pCMVR_RA45_BC_C6_d0	VRC607-06	>50	1.19	0.95	38.75	0.25	0.49
pCMVR_RA45_VRC01LS_C4_d29	VRC607-06	>50	>50	21.35	>50	0.29	0.51
pCMVR_RA45_VRC01-23LS_J1_C4_d29	VRC607-06	>50	>50	>50	>50	0.56	0.72
pCMVR_RA48_VRC01LS_C19_d13	VRC607-07	5.08	0.37	0.98	1.01	0.42	0.93
RA48_1-18LS_C12_d3	VRC607-08	>50	17.03	10.13	10.65	>50	47.60
RA48_1-18LS_C7_d28	VRC607-08	>50	9.96	7.03	9.45	49.62	11.59
RA48_VRC01LS_C2_d28	VRC607-08	>50	10.18	9.88	9.92	>50	18.79
RA48_VRC01LS_C8_d3	VRC607-08	>50	10.25	9.59	10.39	>50	24.94
RA48_VRC01LS_C4_d28	VRC607-08	48.46	9.69	4.52	4.65	16.48	11.14
pCMVR_RA44_VRC01LS_C1_d14	VRC607-11	14.63	0.81	0.42	0.20	0.11	1.58
pCMVR_RA44_N6LS_C15_d14	VRC607-11	>50	2.31	1.62	1.11	0.57	8.24
pCMVR_RA44_VRC01-23LS_J1_C1_d14	VRC607-11	48.18	3.23	1.70	1.36	0.55	8.04
pCMVR_RA44_VRC07-523LS_C4_d14	VRC607-11	43.07	2.57	3.46	2.70	0.39	4.71
pCMVR_RA43_VRC01LS_C7_D4	VRC607-14	>50	>50	>50	>50	>50	4.23
pcDNA3.1_RA43_VRC01-23LS_J1_C2_d8	VRC607-14	>50	>50	>50	>50	>50	3.20
pcDNA3.1_RA43_VRC01-23LS_J1_C10_d8	VRC607-14	>50	>50	>50	>50	>50	2.01
RA58_VRC01LS_C1_d14	VRC607-15	43.36	1.11	1.48	2.00	4.93	1.01
RA58_VRC01LS_C9_d14	VRC607-15	>50	2.41	2.86	3.96	7.71	2.39
RA58_VRC01LS_C9DH983R)_d14 *	VRC607-15	14.83	1.10	0.81	1.09	4.40	4.13
RA56_N6LS_C6_d21	VRC607-16	7.03	1.34	0.51	0.48	1.41	2.02
RA56_1-18LS_C1_d21	VRC607-16	25.07	5.11	2.29	1.33	7.69	5.11
RA56_1-18LS_C1(N315R)_d21 *	VRC607-16	4.53	1.39	0.21	0.23	1.31	1.57

Table 3. Neutralization data of outgrowth viruses from *ex vivo* assays. Outgrowth viral sequences from VRC01LS, VRC07-523LS, N6, 1-18 or VRC01v23 conditions were inserted in either pCMVR or pcDNA3 expression vectors and tested for sensitivity/resistance against the 5 bNAbs above. While neutralization profile was improved for 1-18, VRC0v23 and N6, the later two presented the most improved neutralization of the panel.*represents clones with reverse mutations in 1-18 contact site.

RESULTS (*cont.*)

Sequence Analysis: 1-18, N6 and VRC01v23 escape variants had changes, glycan loss or shifts in the ß23-V5. Resistant strains to 1-18 lacked R308 while N6 escape strains presented N279D mutation (**Fig.5**). Moreover, genetic signatures in *ex vivo* outgrowth viruses were also found in plasma viral sequences, suggesting that the *ex vivo* assay can identify circulating bNAb resistant variants in these patients (**Fig. 6**).

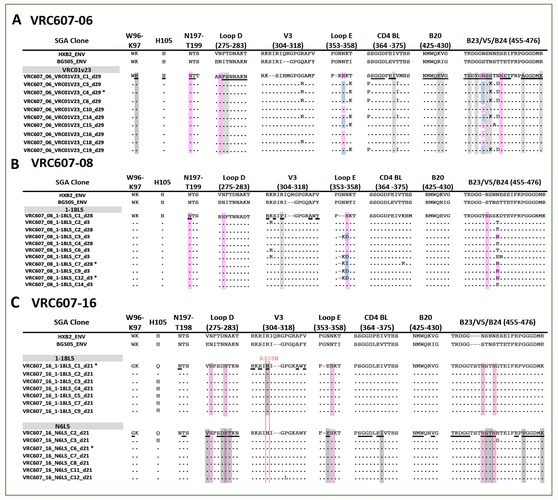

Figure 5. Alignment of representative clones obtained from *ex vivo* assay outgrowth viruses. Viruses here represented showed outgrowth under **(A)** VRC01v23 treatment, patient 06; **(B)** 1-18, patient 08 and **(C)** 1-18 and N6, patient 16. Contact sites for the respective bNAbs are underlined on the first sequence of each alignment. Glycosylation sites are marked in pink, while loss of glycosylation sites is shown in blue. Gray boxes indicate AA variation in the contact site of the respective bNAb. Red box in patient 's 16 clones indicate R308N mutation, which was reverted to R308 for neutralization assay in Fig. 4. *indicates clones tested for neutralization.

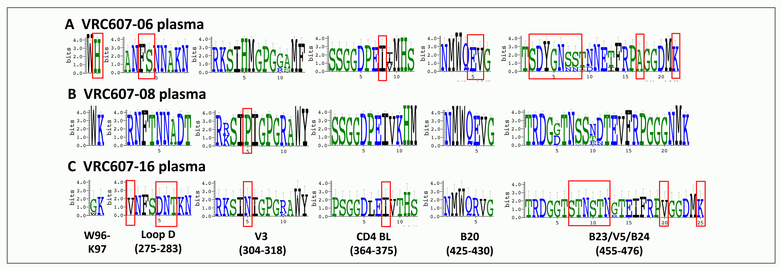

Figure 6. Logo plot analysis of representative plasma sequences depicting contact sites for VRC01v23, 1-18 or N6. (A) patient 06, **(B)** Patient 08 and **(C)** patient 16. Red boxes indicate mutations on respective bNAb contact site also found in *ex vivo* assay outgrowth clones (Fig. 5).

CONCLUSIONS

- ❑ **Complementary suppression profile was seen between 1-18LS, N6LS and VRC01v23**
- ❑ **N6LS and VRC01v23 presented improved virologic control *ex vivo***
- ❑ **Improved neutralization observed for 1-18, N6 and VRC01v23**
- ❑ **Mutations potentially associated with 1-18, N6 and VRC01v23 appear in distinct positions of ENV CD4 binding sites, confirming complementary suppression**
- ❑ **Emergent outgrowth viruses present similarities with patients' sequences from plasma, suggesting *ex vivo* assay can be a useful tool to predict resistance *in vivo***

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