Ex vivo Assay predicts HIV-1 suppression by bNAbs infused in a Phase I clinical trial

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BACKGROUD

Broadly neutralizing antibodies (bNAbs) target conserved regions of the HIV-1 envelope and show potential as complementary immunotherapy to combination anti-retroviral therapies (cART) for HIV-1 treatment. However, infusion of a single bNAb in viremic individuals results in selection for escape variants that are likely part of the individuals viral quasispecies. As clinical trials can be time-consuming, we developed an *ex vivo* assay to better predict the potential for a bNAb to induce an *in vivo* virologic response which can be a useful predictive model for selection of efficacious bNAbs moving forward to clinical trials. Here we tested the CD4bs bNAbs VRC01LS and VRC07-523LS, assessed by the VRC607/ACTG5378 clinical trial and N6LS and 1-18LS, LS variants of 1-18 and N6, that achieved increased potency and remarkable breadth.

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. The development of the *ex vivo* assay in described in **Figure 1** and the bNAbs herein tested are listed on **Table 1**. HIV p24 ELISA was performed using culture supernatants.

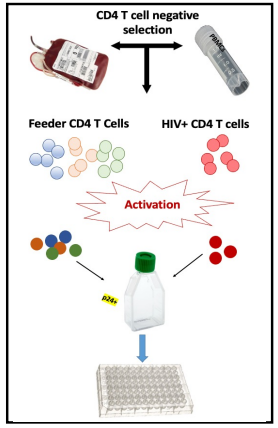

Figure 1. *Ex-vivo* assay workflow. CD4$^+$ T cells were isolated from HIV$^+$ and healthy individuals (feeders) and co-cultured following activation. CD4$^+$ T cells of viremic patients were isolated from PBMCs obtained at day 0, prior to bNAb infusion. Once the Co-culture was p24$^+$ by ELISA, the cells were split and treated with bNAbs. Co-culture supernatants were collected 3 times a week for p24 ELISA assessment while feeders were added once a week to promote viral replication.

bNAb Panel
9114 – Influenza Ab control
VRC01LS
VRC07523LS
1-18LS
N6LS

Table 1. bNAbs tested in ex-vivo assay. The bNAb panel included CR9114, an antibody against influenza, as a control, VRC01LS and VRC07-523LS, and the CD4bs bNAbs 1-18LS and N6LS.

Although bNAbs against HIV-1 show promise in combination therapy with cART, bNAbs may select for viral resistant strains. Here we developed an ex vivo assay investigating bNAb viral suppression efficacy using CD4$^+$ T cells from HIV$^+$ donors. The ex vivo assay was effective in reproducing the virological outcome observed in-vivo, suggesting it could be a predictive model for selection of bNAbs to be tested in future clinical trials.

RESULTS

VRC607/ACTG5378 trial information is described in **table 2.** Results of *ex vivo* assays performed so far are in accordance with the virological outcome observed in these patients following bNAb treatment. Assays using samples from 5 subjects exhibiting decline of viremia to VRC01LS or VRC07-523LS *in vivo* evidenced greater than 10-fold reduction in viral replication by the corresponding bNAb relative to the assay control *ex vivo* (**Figure 3**). In addition, assays using samples from 4 subjects that did not show viral suppression *in vivo* after bNAb infusion also failed to control replication in the *ex vivo* assay (**Figure 3**).

VRC 607/A5378 Study Enrollment			
Part	Participants	Product	Administration Schedule (single dose) Day 0
Part A	7	VRC01LS	40 mg/kg IV
Part B	9	VRC07-523LS	40mg/kg IV
Total	16*		

* Subjects were HIV-infected viremic adults ranging from 18-70 years of age

Table 2. VRC607/ACTG5378 study and subject description. Sixteen subjects were infused once with either VRC01LS or VRC07-523LS at indicated dose.

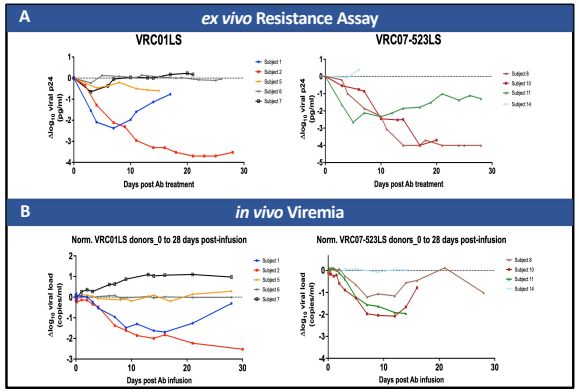

Figure 3. Comparative analysis of viral replication *ex-vivo* and *in-vivo* by the bNAbs tested in clinical trial. A) Values of *ex vivo* p24 ELISA titers were normalized to values of the CR9114 control. **B)** Viremia clinical trial of patients that received either VRC01LS or VRC07-523LS over the course of 28 days of the trial were normalized to viremia values at day 0.

Notably, among subjects that responded to VRC07-523LS infusion with a drop in viremia, 1-18LS promoted complete suppression of viral replication in assays utilizing samples from subjects 10 and 11, which was not observed for N6LS treatment. Conversely, the *ex-vivo* assay using sample from subject 8 evidenced full suppression by N6LS and VRC07-523LS but not by 1-18LS, indicating that CD4bs bNAbs can display complementarity in their resistance profiles. The *ex vivo* assay results for 1-18LS and N6LS treatments are shown in **Figure 4**.

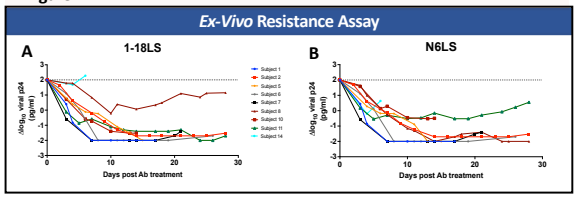

Figure 4. Ex-vivo viral suppression efficacy of A) 1-18LS and **B)** N6LS. 1-18LS and N6LS presented differential viral suppression within donors enrolled in Part B of the VRC607/ACTG trial.

CONCLUSIONS

- Assays from patients that responded to treatment have seen suppression of infection by the corresponding bNAb administered to the patient.
- bNAbs that did not promote drop in viremia *in vivo* also failed to suppress viral replication in the *ex vivo* assay.
- 1-18LS, N6LS and VRC07-523LS show strong promise of broad neutralizing properties with distinct complementary of neutralization activity. Hence, these results indicate that CD4bs bNAbs would be favorable candidates for implementation of a combinatorial immunotherapy approach against HIV-1 infection.

The ex-vivo assay mimicked results observed in clinical trial and allowed efficacy assessment of a diverse range of bNAbs

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