Optimized LNP-Induced Viral Outgrowth Assay to Quantify bNAb Sensitivity in the HIV Latent Reservoir

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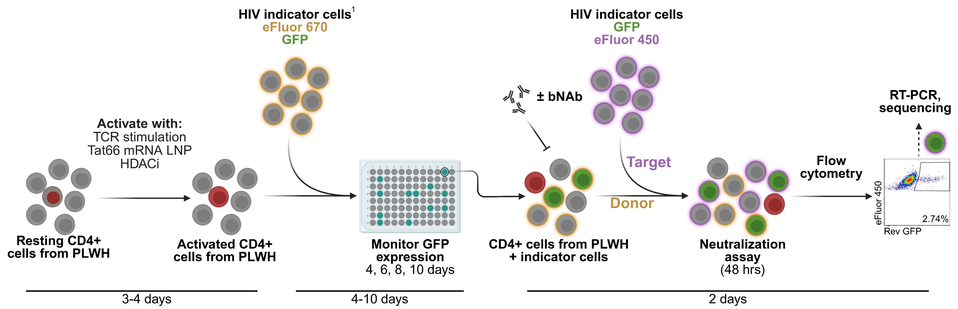
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BACKGROUND

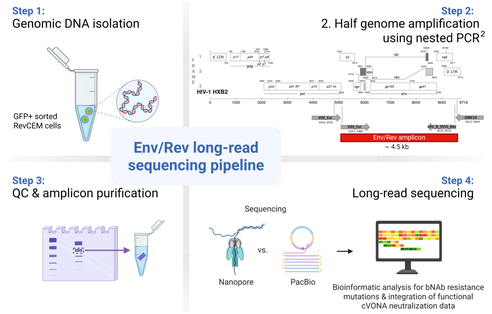
Broadly neutralizing antibodies (bNAbs) are a promising tool for use in HIV treatment and cure strategies. Existing methods are insufficient to accurately predict bNAb sensitivity of HIV-1 *in vivo*, especially for ART-treated individuals. Effective pre-screening for bNAb clinical trials will require a rapid, sensitive, and scalable neutralization assay pipeline. We have developed a co-culture-based infection assay with single-cell resolution that combines viral transmission outgrowth with a tandem neutralization assay to rapidly quantify bNAb sensitivity of HIV-1 reactivated from PBMCs.

METHODS

Single Cell Viral Outgrowth and Neutralization Assay (scVONA)



Long-read sequencing pipeline



CONCLUSIONS

By integrating efficient activation of the functional HIV-1 reservoir with an optimized reporter cell line to detect new infection events at single-cell resolution, we have developed a streamlined single cell viral outgrowth and neutralization assay (scVONA). This platform directly measures cell-to-cell spread, overcoming key limitations of outgrowth approaches that rely on bulk virus release. This assay yields a functional bNAb sensitivity readout 16-days after PBMC isolation for timely, scalable assessment of reservoir susceptibility. These innovations provide a powerful tool to accelerate pre-screening and advance development of bNAb-based therapies.

RESULTS

1. Combination Tat66 mRNA LNP and HDACi promotes reactivation of latent HIV-1 provirus

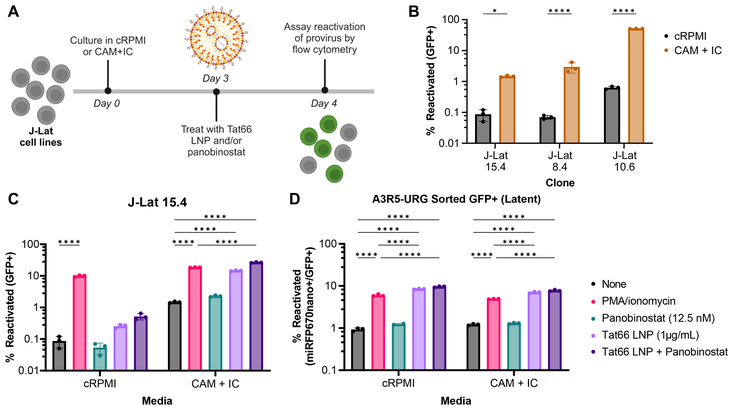


Figure 1. Combination Tat66 LNP and HDACi[3] treatment triggers reactivation of HIV-1 provirus. (A) Reactivation protocol schematic. Cells were cultured for 72 hours in cRPMI or CAM (cRPMI + IL-2, T-cell growth factor) with Immunocult T Cell Activator (IC). Cells were cultured for an additional 24 hours in **(B)** cRPMI or CAM alone or **(C-D)** with LRAs as shown. Experiments were performed using **(C)** J-Lat clone 15.4 and **(D)** A3R5-URG cells infected with NL-miRFP670nano-CreI-JRFL. Data are expressed as mean ± SD. Statistical analyses were performed using 2-way ANOVA (*p<0.05, ****p<0.0001).

2. bsAb$^{hCD5\text{-}HA}$ increases target cell infection efficiency, favors donor-target interactions

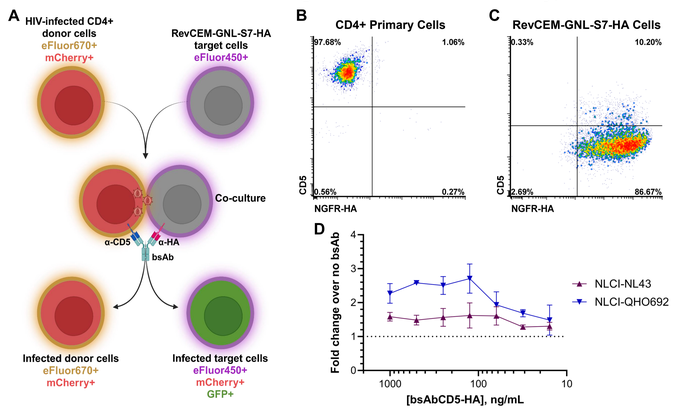


Figure 2. Co-culture in the presence of bsAb$^{hCD5\text{-}HA.4}$ increases efficiency of target cell infection. (A) Experimental setup for bsAb-facilitated cell-cell infection. **(B-C)** CD5 and NGFR-HA expression were measured by flow cytometry for **(B)** primary CD4+ T-cells and **(C)** RevCEM-GNL-S7-HA cells. **(D)** Primary CD4+ cells were infected with NLCI-NL43 or –QH0692 for 24 hours prior to co-culture with RevCEM-GNL-S7-HA cells and serially diluted bsAb$^{hCD5\text{-}HA}$ starting at 1 µg/mL. After 48 hours of co-culture, infection rates of target RevCEM-GNL-S7-HA cells were determined based on mCherry expression via flow cytometry. Data points represent mean ± SD of duplicate wells.

5. Env mutations identified by scVONA correlate with known 10-1074 resistance genotypes

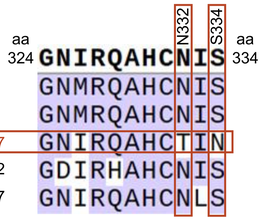


Figure 5. Alignment of participant Env consensus sequence at 10-1074 binding site. gDNA was isolated from 120-4000 sorted GFP+ target' cells and amplicons containing Env were generated by nested PCR. Purified amplicons were sequences using an Oxford Nanopore platform. Amino acid sequences based on a consensus Env DNA sequence for each sample were aligned using Clustal Omega (residues 324-334 shown here). Two sites associated with 10-1074 resistance mutations (N332 and S334)[5] are present in the consensus Env sequence of a sample that exhibited resistance by scVONA (highlighted in red).

3. Reactivation pipeline results in viral outgrowth from PBMCS of viremic, suppressed PLWH in 2 weeks

A

	Viremic	Suppressed
CD4+ cells assayed/ participant	0.2-1.2 million	1-9 million
Total participants	4	13
Outgrowth observed	100% (4/4)	85% (11/13)
Assayed for 10-1074 sensitivity	100% (4/4)	64% (7/11)
Sequenced	25% (1/4)	14% (1/7)

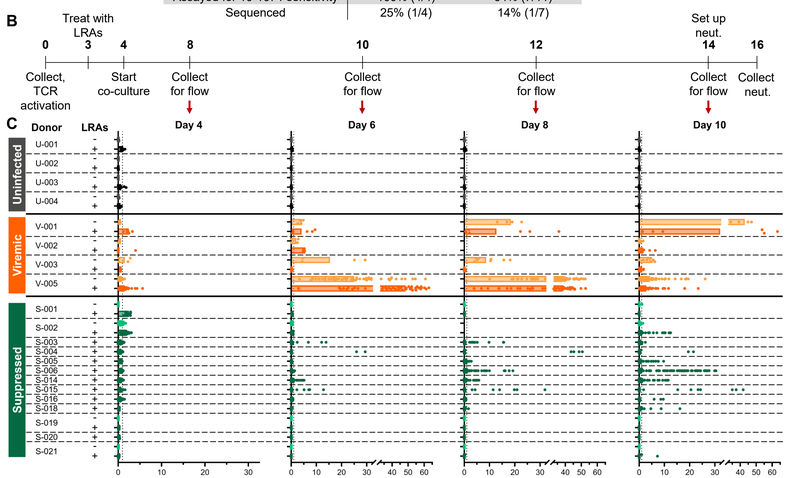


Figure 3. Outgrowth of HIV-1 from CD4+ T cells of PLWH. (A) Summary of scVONA outgrowth and neutralization assay efficiency. **(B)** Activation and co-culture protocol timeline. CD4+ cells from viremic ("V") and virologically suppressed ("S") PWLH or uninfected donor controls ("U") were activated using IC and IL-2 and cultured in the presence or absence of LRAs Tat66 LNP and panobinostat. After 24 hours, cells were co-cultured with eFluor670-labeled RevCEM-GNL-S7-HA indicator cells in the presence of bsAb$^{hCD5\text{-}HA}$ (100,000 target cells/well). **(C)** Cultures were monitored for GFP expression in target cells by flow cytometry. Bars represent median values, with each data point representing a single well in a 96-well plate.

4. Assaying neutralization by 10-1074 identifies resistant virus from outgrown samples

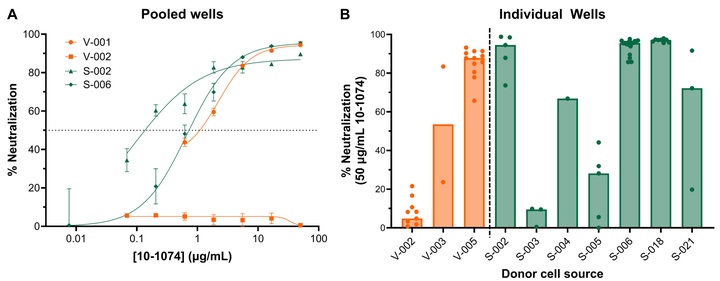


Figure 4. Neutralization of HIV-1 from CD4+ T cells of PLWH by 10-1074. (A) Wells with >1% GFP+ target cells were pooled and co-cultured with fresh, eFluor450-labeled RevCEM-GNL-S7 (target') cells. Pooled samples were assayed for neutralization by serially diluted bNAb 10-1074 (starting at 50 µg/mL). **(B)** Cells from each well with >1% GFP+ target cells was assessed individually at a high concentration of 10-1074 (50 µg/mL) to determine maximum percent inhibition (MPI). Each data point represents the MPI from neutralization of a single outgrown well. **(C)** Summary table of participant reservoir sensitivity to 10-1074. Resistance to 10-1074 based on a given parameter is highlighted in red.

C

Donor	Pooled IC50 (µg/mL)	Pooled MPI (%)	Median single-well MPI (%)	Maximum single-well MPI (%)	Minimum single-well MPI (%)	% single-well MPIs >90%
V-001	2.24	94.36	N/A	N/A	N/A	N/A
V-002	34.01	5.78	4.83	21.59	0.79	0 (0/11)
V-003	N/A	N/A	53.52	83.4	23.65	0 (0/2)
V-005	N/A	N/A	87.77	93.18	65.71	33 (4/12)
S-002	0.09	89.66	94.52	98.82	73.68	60 (3/5)
S-003	N/A	N/A	9.51	9.88	0.48	0 (0/3)
S-004	N/A	N/A	66.82	66.82	66.82	0 (0/1)
S-005	N/A	N/A	28.17	44.19	0.00	0 (0/5)
S-006	0.67	95.42	95.58	97.67	85.71	78 (14/18)
S-018	N/A	N/A	97.18	98.02	95.62	100 (8/8)
S-021	N/A	N/A	72.22	91.65	19.77	33 (1/3)

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PLAIN LANGUAGE SUMMARY

We created a new way to test people living with HIV for resistance to antibody treatments, with the potential to increase efficiency of screening for clinical trials.