Increasing resistance of inducible, infectious HIV-1 in the latent reservoir to autologous neutralizing antibodies after long-term ART

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BACKGROUND

- Antiretroviral therapy (ART) prevents new cycles of HIV-1 replication and disease progression but is not curative. If ART is stopped, viral rebound will occur due to the persistence of a latent reservoir in resting memory CD4$^+$ T cells.
- We previously showed that the reservoir does not decay over 20 years of ART due to infected cell proliferation (McMyn et al., JCI, 2023) and that autologous neutralizing antibodies (aNAbs) can prevent outgrowth of some reservoir viruses (Bertagnolli et al., PNAS 2020).

Goal

It is unclear whether viruses persisting after long-term ART (~20 years) are sensitive to neutralization by aNAbs and if sensitivity changes over time on ART. In this study, we compared the neutralization sensitivity of reservoir viruses in people with HIV (PWH) on ART intermittently, on ART for <14 years, and on long-term ART.

METHODS

We performed **quantitative viral outgrowth assays** using resting CD4$^+$ T cells of PWH on ART intermittently (1-2 periods of nonadherence over 15 years of ART), for <14 years (mean 8.3 years), and on long-term ART (>14 years, mean 21.8 years). Next, as shown in Fig. 1, **single genome sequencing** of *env* was done on outgrowth positive supernatants to **generate pseudoviruses** with the *env* sequences of inducible, replication-competent proviruses. Pseudoviruses were tested in **neutralization assays**. In each neutralization assay, virus was incubated with autologous IgG purified from plasma and then used to infect TZM.bl cells. After a single round of infection, neutralization was measured as the reduction in expression of the HIV-1 Tat-regulated luciferase reporter gene.

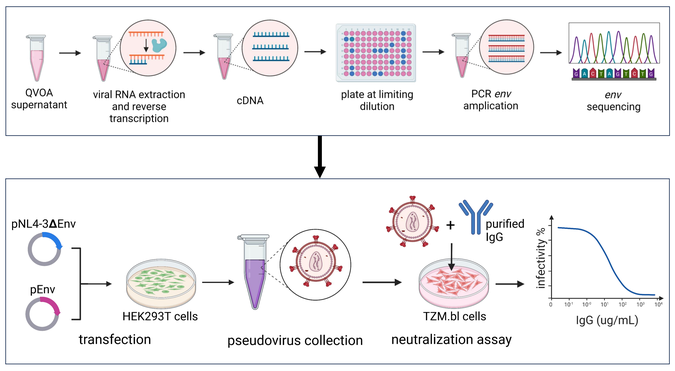


Figure 1. Experimental workflow for evaluating aNAb neutralization of outgrowth viruses.

After long-term ART, outgrowth viruses are resistant to a functional autologous neutralizing antibody response

RESULTS

Most outgrowth isolates from PWH on long-term ART (76%) were resistant to neutralization by aNAbs with IC50 values >100 ug/ml, while a higher fraction of isolates from PWH on ART <14 years (71%) and intermittently (93%) were sensitive to neutralization.

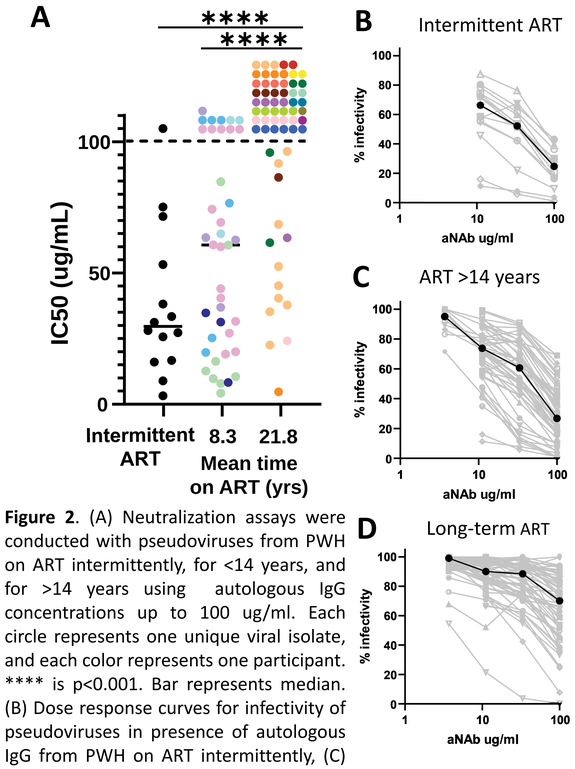


Figure 2. (A) Neutralization assays were conducted with pseudoviruses from PWH on ART intermittently, for <14 years, and for >14 years using autologous IgG concentrations up to 100 ug/ml. Each circle represents one unique viral isolate, and each color represents one participant. **** is p<0.001. Bar represents median. (B) Dose response curves for infectivity of pseudoviruses in presence of autologous IgG from PWH on ART intermittently, (C) for <14 years, and (D) for >14 years with the median curve overlayed in black.

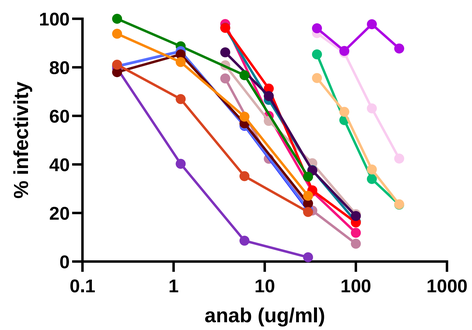
Long-term ART isolates showed variable sensitivity to neutralization by bNAbs that did not correlate with aNAb sensitivity.

aNAb IC50 (ug/ml)		bNAb IC50 (ug/ml)		
	Viral Isolate	VRC01	10-1074	PGDM1400
4.73	DW108-A03	1.9	1.26	0.1
24.6	CIRC0323 B46	4	4	0.531
61.51	DY275-A02	0.74	0.102	4
63.4	OM5418 A14	1.27	0.038	4
86.41	SCOPE2114-B21	1.29	4	4
95.87	DY275-B15	0.87	0.376	0.032
>100	SCOPE2256-A15	0.34	4	4
>100	SCOPE2256-A24	1.48	4	4
>100	RC47-A07	1.74	4	0.49
>100	SCOPE2046-A24	0.75	0.53	0.16
>100	LC246-A34	1.82	0.12	4
>100	LC246-A22	4.32	0.093	0.09
>100	LC246-A03	1.45	0.61	4
>100	LC246-A01	0.85	0.14	0.26
>100	LL218-A09	0.93	0.258	4
>100	LL218-A03	4	1.43	0.382
>100	SCOPE2114-B42	0.66	4	2.19
>100	SCOPE2114-B06	1.97	4	0.097
>100	SCOPE2114-B01	2.78	4	0.89
>100	SCOPE2114-B09	3.42	4	0.39
>100	DW108-A04	4	2.2	2.1
>100	DW108-A02	3.66	0.62	0.25
>100	DW108-A14	4	3.18	0.086
>100	DW108-A01	3.45	3.31	1.57
>100	CIRC0113-A03	4	4	4
>100	CIRC0113-A09	4	4	4
>100	DY275-A05	0.5	0.28	0.032
>100	DY275-B45	0.97	0.284	0.24
>100	OM5418 A03	2.14	0.19	4
>100	OM5418 A10	0.91	0.035	4
>100	OM5418 B39	1.05	0.021	4
>100	OM5418 A07	0.42	0.056	4
>100	CIRC0323 A06	4.06	0.131	1.09
>100	CIRC0323 A05	2.48	0.586	1.58
>100	CIRC0323 B03	4	4	1.61
>100	CIRC0323 B18	0.61	4	4
>100	CIRC0323 A08	2	0.062	0.44
>100	VM27 A04	0.78	4	0.5
>100	VM27 A09	0.82	4	0.261
>100	VM27 A11	0.27	1.08	0.266
>100	VM27 A21	0.81	4	0.22
>100	VM27 B22	1.22	2.29	0.531

0.032 >4 ug/ml

Table 1. Neutralization was tested with long-term ART pseudoviruses and aNAbs or bNAbs (broadly neutralizing antibodies).

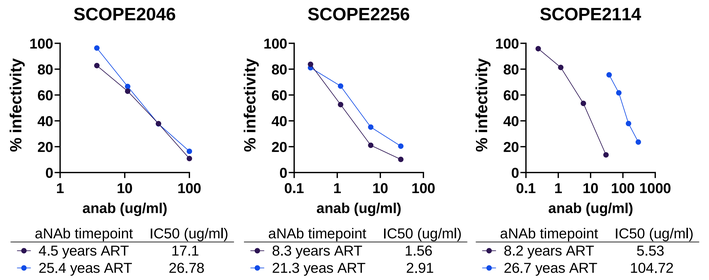
To determine whether the low fraction of reservoir viruses neutralized by autologous IgG in PWH on long term ART was due to a waning of the neutralizing antibody response, we examined neutralization of SF162, a highly neutralization-sensitive tier 1 virus. SF162 was neutralized by aNAbs isolated from 16 of 17 long-term ART participants.



	aNAb IC50 (ug/ml)
SCOPE2006	8.91
MT167	22.41
RH24	25.25
SCOPE2256	2.91
RC47	7.24
SCOPE2046	26.78
SCOPE2114	104.72
LC246	9.6
LL218	0.806
DW108	6.75
CIRC0113	6.62
DY275	19.04
OM5418	>300
CIRC0232	234.75
CIRC0323	23.38
VM27	110.99
CIRC0036	17.21

Figure 3. Neutralization assays were conducted with SF162 virus in the presence of IgG isolated from long-term ART participants at concentrations up to 300 ug/ml. Each colored line represents data from one participant. IC50 levels are shown on the right.

Longitudinal analysis showed waning of antibody titers over time in some but not other participants.



aNAb timepoint	IC50 (ug/ml)
4.5 years ART	17.1
25.4 yeas ART	26.78

aNAb timepoint	IC50 (ug/ml)
8.3 years ART	1.56
21.3 yeas ART	2.91

aNAb timepoint	IC50 (ug/ml)
8.2 years ART	5.53
26.7 yeas ART	104.72

Figure 4. Neutralization assays were conducted with SF162 virus in the presence of aNAbs isolated at different timepoints from representative long-term ART participants. aNAb collection timepoints during suppressive ART and IC50s are indicated.

CONCLUSIONS

- Most outgrowth viruses that persist after long-term ART are resistant to neutralization by aNAbs. This may reflect gradual Ab-mediated immune selection and/or waning of Ab titers.
- Long-term ART outgrowth viruses remain sensitive to some bNAbs.
- aNAb titers against SF162 may wane over time in some participants but persist after >20 years and remain functional.

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