Persistent Viremia with vpr/tat-Deleted HIV and No T-cell Responses to HLA-Predicted Peptides

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BACKGROUND

- Observed two children with non-suppressible HIV viremia despite effective antiretroviral therapy (ART) over 14 & 5 years
- Persistent viremia usually due to:
  - Continued HIV replication due to inadequate drug pressure selecting drug-resistant virus; or
  - Production of virus without HIV replication due to clones of HIV-infected cells producing virions paired with immune activation
- We aimed to determine the mechanisms underlying persistent HIV viremia in these children
- **Hypothesis:** Persistent viremia in these children is due to clones of HIV-infected cells harboring HIV epitopes that have escaped immune surveillance

METHODS

Longitudinal blood specimens were analyzed for evidence of:

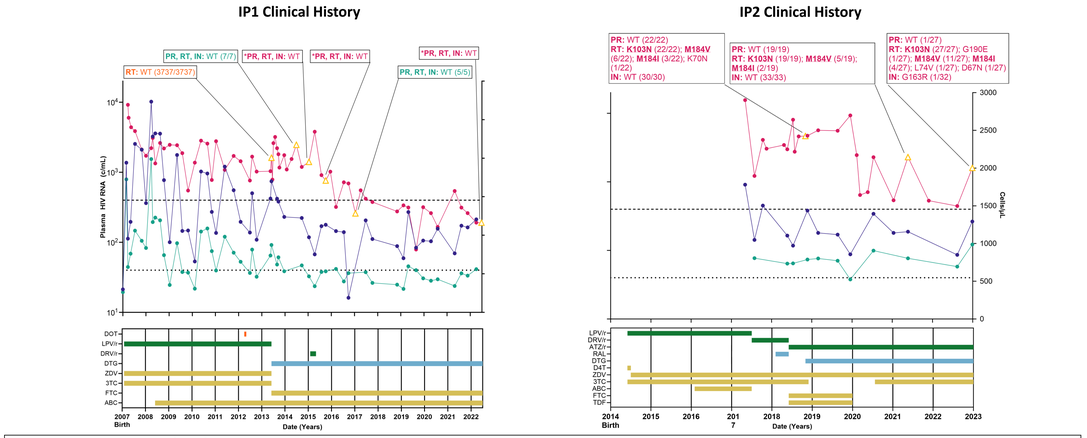
- Virus replication. Generated Sanger and PacBio single-genome-sequencing (SGS) from env, the central region of the viral genome, and from half genomes (HGs) and evaluated for:
  - drug resistance
  - virus evolution
  - viral "landscape"
- HIV-infected cell clones producing virions. Evaluated plasma HIV by SGS and PBMC integration site looping assay (ISLA) or Illuminia sequencing, evaluated for:
  - plasma virus clonality
  - HIV integration sites clonality was determined using ISLA and sequencing on cryopreserved PBMCs
- Immune responses to HIV and control antigens. Performed IFN-γ ELISpot using HIV predicted binding epitopes, based on participants HLA type and NFL plasma sequences
- Biomarkers of inflammation. Determined by flow cytometry
- Replication-competent HIV reservoir. Determined by dQVOA
- HIV-specific CD8+ T-cell peripheral tolerance. Evaluated by IFN-γ ELISpot using autologous dendritic cell presentation of HIV peptides, Treg depletion or checkpoint molecule inhibitors or blocking antibodies

Conclusions

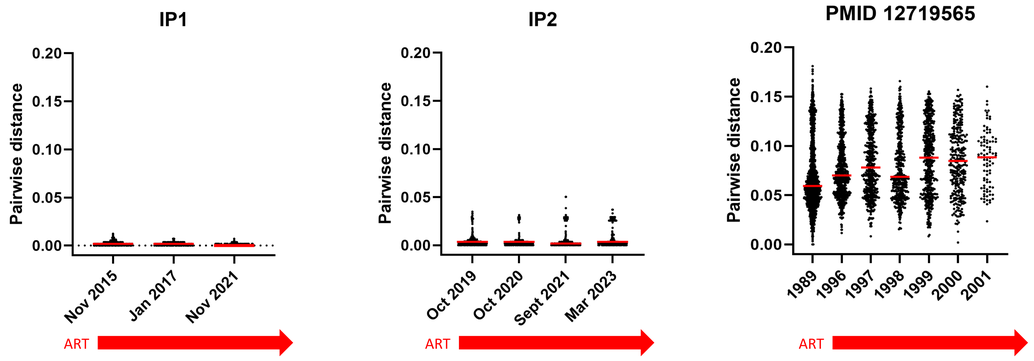
- The persistent viremia without drug resistance or evidence of HIV evolution in two children over years suggests that HIV is not replicating
- Dominance of vpr/tat-1 deleted viruses in both participants suggests that cells infected with these viruses have a survival advantage potentially because of resistance to intrinsic apoptosis
- Their lack of T-cell responses to non-escaped HIV peptides combined with persistently high viral loads suggests ineffective T-cell immunity, potentially due to central tolerance (due to in utero HIV acquisition)

RESULTS

- Clinical viremia documented without loss of CD4+ T-cells



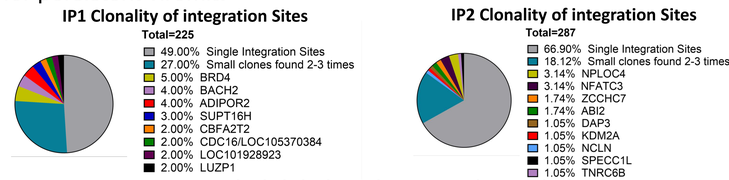
- Virus evolution not detected



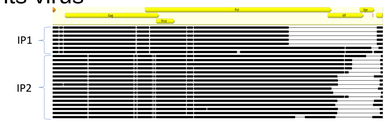
- No evidence of drug resistance to current treatment

Participant ID	HIV-1 Subtype	Number of Sequences	Protease		Reverse Transcriptase		Integrase	
			Major	Minor	NRTI	NNRTI	Major	Accessory
IP1	B	174	Not Done	Not Done	None	None	Not Done	Not Done
	B	7	None	None	None	None	None	None
	B	1	None	None	None	V90I	None	None
	B	1	None	None	None	V90I	None	None
	B	5	None	None	None	None	None	None
	B	1	None	None	None	None	None	None
	B	1	None	None	S68N	L100LI	None	None
IP2	C	16	None	None	M184V/I	K103N	None	None
	C	14	None	None	M184V/I	K103N	None	None
	C	24	None	None	M184V/I	K103N	None	None
	C	19	None	None	M184V/I	K103N	None	None
	C	27	None	None	M184V/I	K103N	None	None

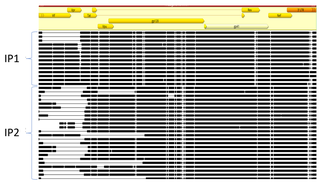
- Integration site analysis showed no large clones that could account for persistent viremia



- NFL sequences revealed deletions in vpr and tat-1 in both participants virus



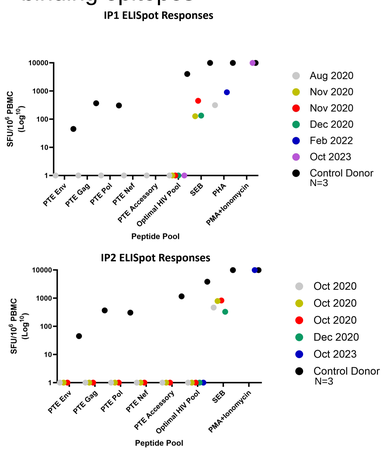
3Kb Amplicon	IP1	IP2
pol-vpu sequences	449	815
Likely defective	448 (99.8%)	808 (99.1%)
Deletion/Insertion/Stop/ATG start codon mutation	444/0/1/3	800/2/3/3
Total with indels	444 (98.9%)	802 (98.4%)
Unique indels	68	175
Unique 5' indel ends	65 (95%)	146 (83.0%)
in Frame indels	16 (23.5%)	66 (37.5%)



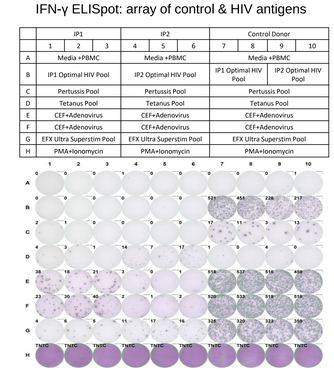
- Biomarkers of immune activation -- all normal
  - Myeloid derived suppressor cells (MDSC) – elevated in IP1; potentially immunosuppressive

Biomarker	Normal Levels	IP1	IP2
MDSC	≤2.52% or ≤1.3%	8.50%	0.02% and 0.1%
sCD163	≤996 ng/mL	618	525
sCD14	≤2600 ng/mL	1976	2408
suPAR	≤3829 pg/mL	2184	3380
CRP	≤10 mg/L	2.5 mg/L	0.3 mg/L

- Neither participant had T-cell responses to HLA-predicted HIV binding epitopes



IFN-γ ELISpot: array of control & HIV antigens



PMID: 28081174 (Sette et al. 2017)
PMID: 27212461 (Sette et al. 2016)