DIFFERENTIAL GENE-SPECIFIC SELECTION PRESSURES ON NEAR-FULL LENGTH DEFECTIVE PROVIRAL POPULATIONS DURING ANTIRETROVIRAL THERAPY

0462

T. Nguyen [1], M.-E. Zipparo [1*], L.Adams [1*], A. Glassey[1], U. Santamaria [2], C. A Rehm [3], J. Earhart [1], Wei Shao [2], C.-Y. Lau [1], F. Maldarelli [1]

[1]National Cancer Institute, National Institutes of Health, USA., HIV Dynamics and Replication Program, Frederick, United States, [2]Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, United States, [3]Clinical Research Section, National Institute of Allergy and Infectious Diseases, Bethesda, United States

*: these authors equally contributed

BACKGROUND

- The error-prone nature of HIV-1 replication and host factors generate defective genomes. Defective proviruses persist and can be transcribed and translated which contribute to the pathogenesis of HIV infection [1-3].
- During antiretroviral therapy (ART), defective proviruses with large deletions and hypermutations are enriched, precluding the investigation of gene-specific selection pressures [4,5].
- Here, we studied the patterns of defects at whole HIV-1 genomic and subgenomic levels of >7kb near-full length (NFL) defective proviruses prior to following ART to understand the selection pressures on these proviral species during ART.

METHODS

- Peripheral mononuclear blood cells were collected from 11 HIV-suppressed participants at pretherapy and at short- (2-8 years), intermediate- (8-15y), and long-term ART (15-21y).
- Single genomes of >7kb proviruses were obtained by limiting dilution and submitted to sequencing on the Illumina MiSeq platform. Sequencing reads were assembled to generate NFL consensus sequences using packages in Geneious Prime (version 2013.03) (https://www.geneious.com) as previously described [6].
- Proviral sequences were annotated as intact or defective based on the algorithm from Proviral Sequencing Database (https://psd.cancer.gov/tools/pvs_annot.php) [7]. Defective proviruses with insertions/deletions, premature stop codons, or G to A hypermutations were identified and identical sequences were collapsed for further analysis.
- The patterns of defects at whole genomic and subgenomic levels of NFL defective proviruses were compared from pretherapy to combined ART using the Wilcoxon sign-rank paired-t test.
- The Major Histocompatibility (MHC) class I binding, immunogenicity, and sensitivity to Broadly Neutralizing Antibodies (bNAbs) from *env* sequences were predicted on the IEDB website (https://www.iedb.org/).

RESULTS

1) Patient characteristics

Characteristic (n=11)	Median (IQR)
Age	61 (59.0-68.5)
Sex, male	9
Log10 (Pretherapy viral load, copies/mL)	4.7 (4.4-5.0)
Nadir CD4 (cells/mm³)	222 (160-241)
CD4 count (cells/mm³)	
➢ **Pretherapy (n=11)**	273 (165-490)
➢ **Short-term ART (2-8 years, n=6)**	637.5 (546.8-1095.0)
➢ **Intermediate-term ART (8-15 years, n=8)**	705.5 (463.0-933.8)
➢ **Long-term ART (15-21 years, n=6)**	421 (380-581)
Suppression time on ART	18.0 (14.0-19.0)

NFL defective proviruses are selected for significant defects on major splice donor/packaging signal and *gag* but against *env* suggesting differential selection pressures on the HIV genome during ART.

RESULTS

1) Significant defects observed in multiple HIV genes during ART

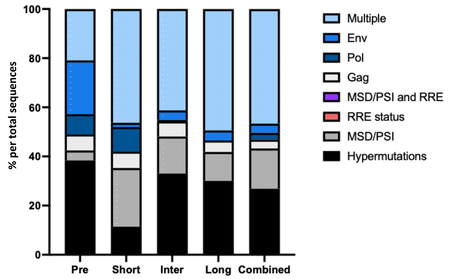


Fig.1: Locations of defects in NFL defective sequences from pretherapy (Pre) to short-, intermediate-(Inter), long-term and all time points on ART (Combined)

2) No significant difference in frequency of hypermutant proviruses at pretherapy and on ART

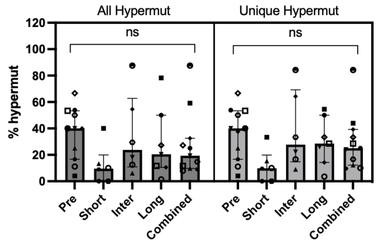


Fig.2: Proportion of all or unique NFL hypermutated proviruses from pretherapy to ART

3) Significant selection FOR proviruses with defects on major splice donor/packaging signal (MSD/PSI) and *gag* but AGAINST *env*

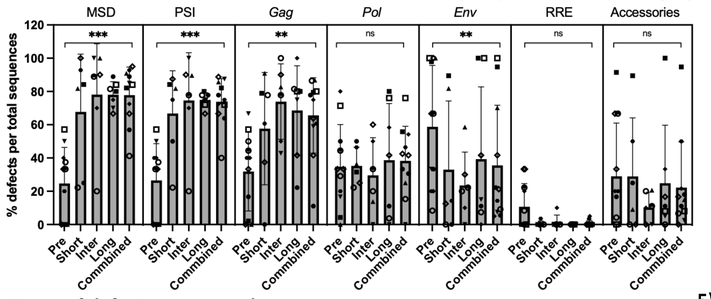


Fig.3: Significant defects such as small deletions/stop codon/frameshift in MSD/PSI, *gag*, and *env* regions from pretherapy to ART

4) Patterns of defects on *gag* and *env*

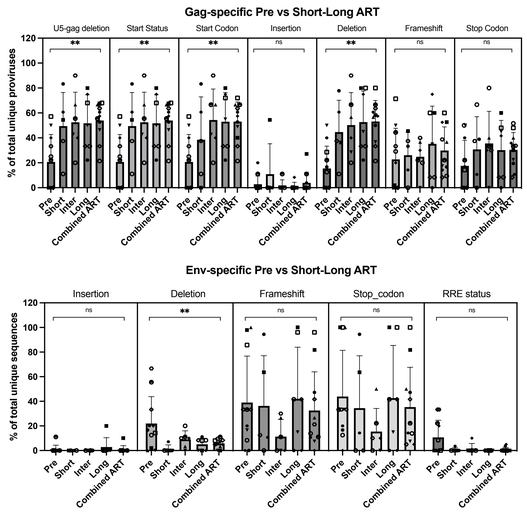


Fig.4: Patterns of defects on *gag* (upper) and *env* (lower)

*: P ≤ 0.05, **: P ≤ 0.01, ***: P ≤ 0.001, ****: P ≤ 0.0001

- *Gag* was mostly defective in LTR-U5 binding and start codon status. Significant deletions on *gag* were also observed but no frameshift or presence of stop codons was detected on *gag*.
- The defect patterns of *gag* are likely linked to defects in MDS/PSI regions
- Significant selection against deletion on proviral *env* during therapy revealed certain immune mechanisms associated with this pattern.
- The NFL defective proviruses harbored little to almost no changes in alternative splicing sites suggesting possible alternative splicing usage to regulate viral protein expression.

5) Broadly Neutralizing Antibody (bNAb) Sensitivity

Significant changes in predicted bNAb sensitivity were observed for the *env* of 2/11 participants.

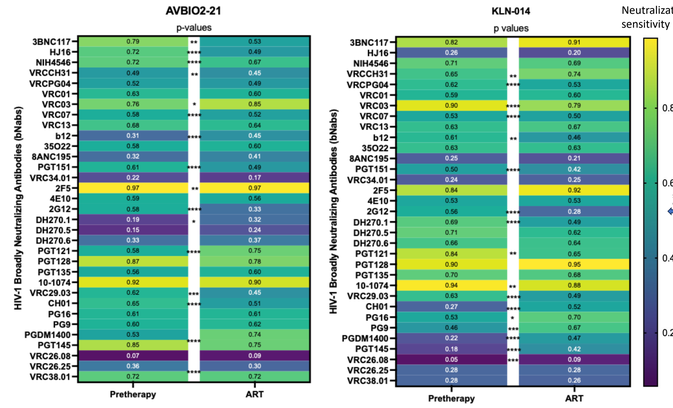


Fig.5: HIV *env* sequences with significant changes in bNAbs sensitivity in two participants

5) Significant changes in HLA-associated mutations during ART

Table 2: Proportion of HLA-associated mutations in *env* during ART

PID		Wildtype epitope	Variant epitope	Pretherapy variant epitope frequency (%)	ART variant epitope frequency (%)	Fisher's exact test P-value
P1	B*07	RPNNNTRKSI	RPNNNTRKgI	76.60	8.70	****
			RPyiNTRKdI	8.51	8.70	
			RPhiNTRKdI	2.13	4.35	
			RPhiNTRKgI	0.00	4.35	
			RPNNNTsKSI	4.26	60.87	
			RPsNNTRKSI	0.00	4.35	
			RPNNNTRKSI	4.26	8.70	
			RPhvNTRKgI	2.13	0.00	
			RPyiNTRKgI	2.13	0.00	
		IPRRIRQGL	vPRRIRQGf	10.64	34.78	*
			IPRRIRQGf	78.72	60.8	
			IPtRvRQGL	4.26	4.35	
P2	A*30	HIGPGRAFY	--GPGRAIY	0	75	****
			--GPGRAFY	100	25	
		KYCWNLLQY	KYwWNLLQY	54.55	82.14	****
			KYwWsLLQY	0	14.29	
			----------	9.09	3.57	
			KYwWNLLrY	36.36	0	
	C*03	RAIEAQQHL	Wildtype	90.91	3.57	****
			RAIEAQQHm	0	96.43	
			----------	9.09	0	
P3	B*38	MHEDIISLW	MHkDIISLW	100	44.44	*
			MqEDIISLW	0	22.22	
			MHEDIvSLW	0	11.11	
			Wildtype	0	22.22	
P4	B*40	QELKNSAVSL	QELKNSAiSL	14.29	4.17	*
			Wildtype	14.29	13.5	
			QELKkSAVSL	57.14	8.33	
			QELqkSAVSL	14.29	70.83	
			----------	0	4.17	
P5	B*27	GRRGWEALKY	GRRGWEILKY	0	83.33	****
			GRRGWEvLKY	20	5.56	
			GRKGWEILKY	20	0	
			GRRGWEILKY	10	0	
			Wildtype	10	11.11	
			Others (deletion)	40	0	
	A*02	(S)LLNATDIAV	(S/)LLNvTavAV	0	5.56	***
			(S/)LLNATaIAV	50	83.83	
			----------	40	0	
			Others	10	5.56	
	A*02	RIRQGLERA	Wildtype	60	100	*
			----------	40	0	

➢ **However, we did not observe consistent changes in the predicted MHC binding affinity or immunogenicity (data not shown).**

CONCLUSIONS

- NFL defective proviruses are subjected to significant differential selection pressures on MSD/PSI, *gag,* and *env* under ART.
- *Gag* defects are linked to deletions on MSD/PSI, indicating the selection for proviruses with impaired replication and infectivity.
- Selection against deletions on *env* is possibly linked to the HLA mechanism but did not lead to significant changes in MHC binding nor immunogenicity, suggesting the possible involvement of other factors.

ADDITIONAL KEY INFORMATION

References

1. Imamichi, H. *et al.* Defective HIV-1 proviruses produce novel protein-coding RNA species in HIV-infected patients on combination antiretroviral therapy. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 8783–8788 (2016).
2. Imamichi, H. *et al.* Defective HIV-1 proviruses produce viral proteins. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 3704–3710 (2020).
3. Singh, K. *et al.* Long-term persistence of transcriptionally-active 'defective' HIV-1 proviruses: Implications for persistent immune activation during antiretroviral therapy. *AIDS Lond. Engl.* (2023) doi:10.1097/QAD.0000000000003667.
4. Ho, Y.-C. *et al.* Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* **155**, 540–551 (2013).
5. Bruner, K. M. *et al.* Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat. Med.* **22**, 1043–1049 (2016).
6. Hiener, B. *et al.* Identification of Genetically Intact HIV-1 Proviruses in Specific CD4+ T Cells from Effectively Treated Participants. *Cell Rep.* **21**, 813–822 (2017)
7. Patro, S. C. *et al.* Combined HIV-1 sequence and integration site analysis informs viral dynamics and allows reconstruction of replicating viral ancestors. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 25891–25899 (2019).

Author Contact Information:

thuy.nguyen2@nih.gov

HIV Dynamics and Replication Program

National Cancer Institute, National Institutes of Health

Acknowledgments:

We thank the study participants.

HIV Dynamics and Replication Program

NCI at Frederick