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HIV Drug Resistance Across Anatomical Tissues

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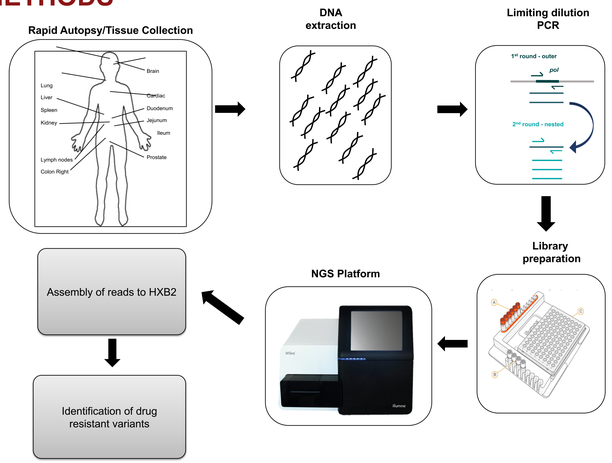
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BACKGROUND

How drug resistant variants emerge and spread within individuals with HIV is not well defined. Clinical testing of drug resistance mutations focuses on sequencing of specific regions within the HIV *pol* from circulating blood plasma or PBMCs. ***We wanted to ask, in people with HIV receiving ART, where do drug resistant variants reside outside of the blood?***

To this end we leveraged anatomical tissues available from the Last Gift (LG) study to determine the presence of drug resistant variants among several anatomical tissues in two LG participants. The LG cohort is made up of altruistic terminally-ill participants with HIV who donate their entire bodies upon death. Participant LG03 had no previous clinical record of drug resistant mutations (DRMs). DRMs were identified in LG05 previously in two clinical tests spanning 14 years. Here, we present single genome amplification (SGA) variants spanning the full-length *pol* region sequenced by Illumina MiSeq to identify DRMs in multiple anatomical tissues within two individual participants.

METHODS



- Two participants were selected from the Last Gift study cohort based on the presence or absence of drug resistance in clinical drug resistance testing.
- Tissues samples were isolated from LG participants utilizing a rapid autopsy protocol
- HIV-1 DNA was extracted from tissues, and coding regions within *pol* (HXB2 1736-5074) were PCR-amplified under single genome conditions.
- HIV *pol* amplicons from tissues for each participant were library prepped and amplicons were sequenced on a Illumina MiSeq instrument to produce reads encompassing the HIV *pol* region (~3 kilobase pairs).
- Reads were mapped to a HIV reference (HXB2) sequence to produce consensus sequences for each individual sample
- Consensus sequences were used to assemble corresponding reads
- Sequences were analyzed for DRMs using the Stanford HIV Drug Resistance Database: (https://hivdb.stanford.edu/).

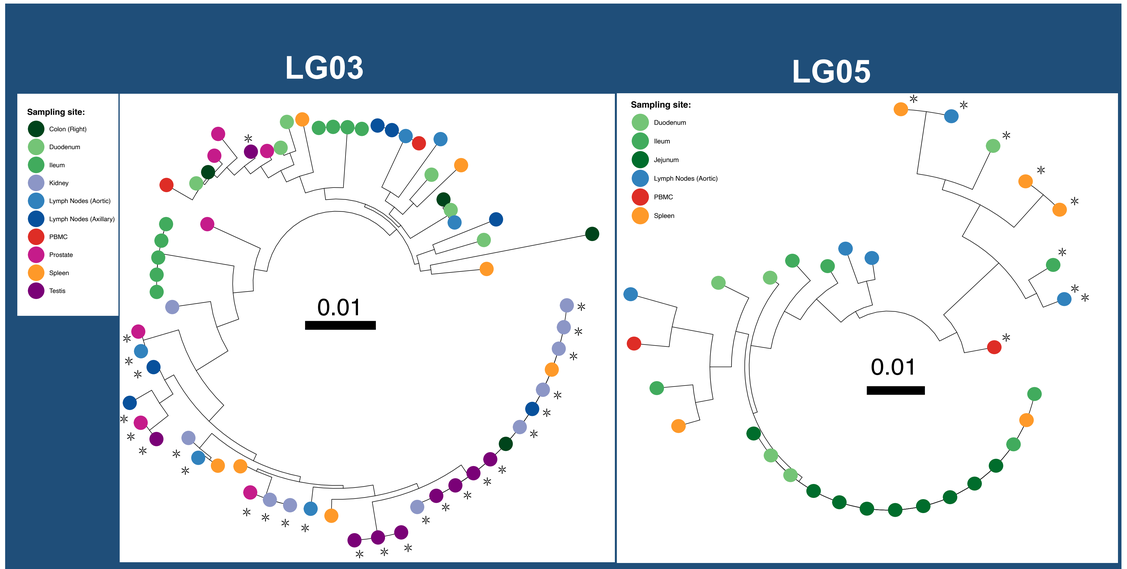


Figure 1. Phylogeny of FL HIV-1 *pol* sequences estimated by maximum likelihood from single genome amplification of HIV DNA from multiple tissue compartments in two participants of the Last Gift study. Phylogeny consists of intact FL *pol* sequences with SNPs at a frequency of less than 1% suggesting single genome amplification. ⁕ = *pol* variants with DRMs.

RESULTS

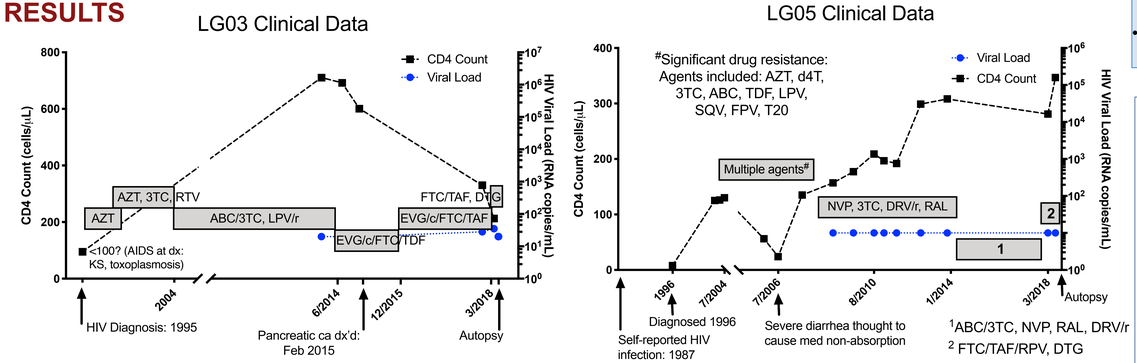


Figure 2. Clinical history for LG participants 3 and 5 who remained virally suppressed on ART until death. CD4⁺ T-cell counts, HIV viral load, and ART history for both participants were collected. Both LG03 and LG05 remained on ART until death.

Table 1. Participant characteristics

ID	Age	Diagnosis	Stopped ART	Last ART Regimen	Last CD4 count (cells/mm^3)	Last HIV RNA level	Deceased
LG03	72	Pancreatic Tumor	No	FTC/TAF+DTG	330 (time of death)	undetectable	May 2018
LG05	57	ALS	No	TAF/FTC/RPV+DTG	347 (time of death)	undetectable	March 2019

Table 2. Summary of HIV-1 *pol* sequences with DRM

Participants	Tissue	# of SGA variants	PI	NRTI	NNRTI
			% of variants with DRM	% of variants with DRM	% of variants with DRM
LG03	PBMC	2	0	0	0
LG03	Prostate	7	0	42.85	0
LG03	Colon right	4	0	25	50
LG03	Spleen	7	0	71.43	28.57
LG03	Kidney	10	0	90	60
LG03	Axillary LN	6	0	50	16.67
LG03	Aortic LN	6	0	50	16.67
LG03	Duodenum	6	0	0	0
LG03	Ileum	8	0	0	0
LG03	Testes	9	0	11.11	77.78
LG05	PBMC	2	50	50	0
LG05	Axillary LN	5	40	40	40
LG05	Spleen	5	60	60	60
LG05	Duodenum	5	20	20	20
LG05	Jejunum	9	0	0	0
LG05	Ileum	6	16.7	16.67	0

Table 4. Comparison of DRM M184V among tissues in LG03

Tissue	PBMC	Kidney	Duodenum	LN
PBMC		0.0455	>0.9999	0.4633
Kidney	**0.0455**		**0.0009**	0.1181
Duodenum	>0.9999	**0.0009**		**0.01818**
LN	0.4633	0.1181	**0.01818**	

Table 3. List of DRMs present in LG participants

Inhibitor and DRM	LG03		LG05	
	#	%	#	%
NRTI				
M41L	32	50	7	21
M184V	31	48	8	24
D67N	31	48	8	24
K70R	31	48	8	24
T215F	30	47	7	21
K219E	23	36	0	0
K219Q	6	9	7	21
L74I	0	0	5	15
NNRTI				
Y181C	18	28	0	0
V108I	13	20	0	0
G190A	2	3	0	0
M230I	1	2	0	0
A98G	0	0	2	6
PI				
M46I	0	0	7	21
I54V	0	0	7	21
V82T	0	0	7	21
I84V	0	0	7	21
L90M	0	0	7	21
L33F	0	0	5	15
G73T	0	0	5	15
G73S	0	0	2	6

- Two participants were selected based on prior clinical history of drug resistance (Table 1)
- We produced full-length HIV-1 *pol* sequences from PCR products amplified under single genome amplification conditions from multiple anatomical sites (Figure 1)
- Sequences analyzed for DRMs was based on the Stanford HIV drug resistance database (Table 2 and 3)
- The presence of DRM M184 varied significantly between PBMC and the gut (duodenum) and the kidneys (Table 4) in LG03 .

CONCLUSIONS

- Last Gift Study provides rare opportunity to study HIV reservoirs in bodily tissues
- Gut tissues of both LG03 and LG05 had significantly reduced number of sequences harboring DRMs relative to other anatomical sites
- We observed drug resistance mutations in tissues of LG03 not found in PBMC
- This study suggests surveillance of additional tissue compartments outside of blood may be necessary to capture DRMs present in PWH
- This study provides a more accurate snapshot of DRMs present within viral reservoirs distributed throughout the body

Acknowledgments

This work was supported by San Diego CFAR, including the Translational Virology Core; supported by NIH P30 A1036214.