Poster 345

HIV DNA Identified in Most Tissues of a Plasma Negative HIV Autopsy Cohort

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Background: While combined antiretroviral therapy (ART) can reduce plasma viral loads to undetectable levels, the degree to which virus is eliminated from other anatomical sites remains unclear. The high frequency of comorbidities in ART^{+}HIV^{+} patients suggests that persistence of virus may contribute to tissue pathologies. The goal of this study was to identify subjects with undetectable plasma and CSF viral load at death, assay a panel of their autopsy tissues for HIV, and assess tissue histopathology to discover the extent of residual anatomical HIV levels during cART and the potential relationship to tissue injury.

Methods: The National Neurological AIDS Bank (NNAB) and AIDS and Cancer Specimen Resource (ACSR) autopsy cohort was screened to identify 20 HIV^{+}/ART-treated participants who had undetectable plasma and CSF viral loads at autopsy. Extensive medical histories were compiled for each participant. Detailed histopathological findings were noted in multisite autopsy specimens (n=212, including up to 6 brain and 6 lymphoid tissues per subject). All tissues were assayed for the presence of HIV DNA using quantitative and digital drop PCR. A subset of tissues was evaluated for HIV RNA using an RNAscope *in situ* hybridization assay.

Table 1. Patient Demographics. 17 male and 3 female participants were included in the study. Their average age was 46.5 years and the average length of infection was 12 years (Figure 1). Sixteen patients had a PMI of 10 hours or less, with an average PMI of 7.77 hours. The cohort reported a mix of HIV risk factors, with ten participants likely contracting HIV by MSM, four through heterosexual transmission, three through IDU, one through an infected blood product, and three participants reported two or more risk factors. Three participants were enrolled post-mortem into the NNAB program.

Patient ID	Gender	# Years Enrolled in NNAB	[1]Pre/ Post Mortem Enrollment	[2]PMI (Hrs)	[4]Years of Known HIV infection	Risk-Factor(s)
1010	Male	5	Pre	3.5	12	Male to male sex
1156	Male	0	Post	7.5	15	Male to male sex
2004	Male	7	Pre	11	17	Male to male sex
4106	Male	2	Pre	10	18	Heterosexual Exposure
4010	Male	1	Pre	3	5	Male to male sex
4013	Male	2	Pre	4.5	6	Intravenous drug user / Male to Male Sex
4124	Male	2	Pre	19.5	16	Intravenous drug user
4129	Female	3	Pre	4	10	Heterosexual Exposure
4130	Male	1	Pre	7	13	Heterosexual Exposure
4143	Male	1	Pre	4	5	Intravenous drug user
4149	Male	0	Post	20.5	20	Male to male sex
4150	Male	0	Pre	8	12	Heterosexual Exposure/ Intravenous drug user/ Male to male sex
4154	Male	0	Post	13	13	Male to male sex
4175	Male	1	Pre	5	1	Male to male sex
4179	Male	1	Pre	5	1	Male to male sex
5024	Male	5	Pre	7	15	Heterosexual Exposure/ Other
5025	Female	0	Pre	4	13	Blood Product recipient
5095	Male	1	Pre	8	4	Male to male sex
6015	Female	1	Pre	6	7	Intravenous drug user
6083	Male	0	Pre	5	6	Male to male sex

1. Designates if the patient was enrolled in the NNAB pre- or post-mortem 2. Post-mortem interval. 3. Estimated year infected.

Table 2. Viral loads.

Subject	CSF-VL[1] at Death	Blood-VL[2] at Death	Last Living VL
1010	<400	<40	<400
1156	<40	<40	<400
2004	<40	<40	<400
4106	<40	failed	<400
4010	<40	<40	<400
4013	<40	<400	<400
4124	<40	<40	<400
4129	<40	<40	<40
4130	<40	<40	<40
4143	<40	<400	<40
4149	<40	<40	<40
4150	<40	<40	<40
4154	<40	<40	<40
4175	<40	<40	<40
4179	<400	<40	<40
5024	<40	<40	<40
5025	942	<40	<400
5095	<400	<40	<400
6015	2061	<40	<400
6083	<40	<40	<400

1. CSF VL load at autopsy. VL are all in cells/mm^3
2. Blood VL obtained from cardiac aspiration after death.

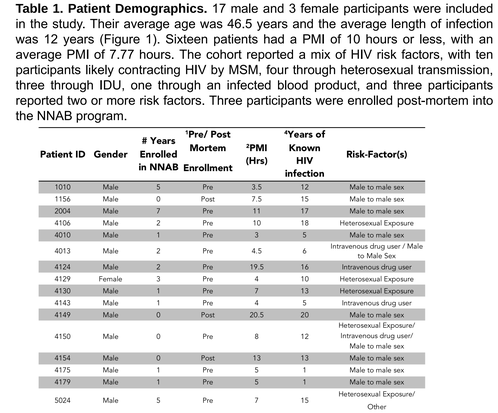


Figure 2. Distribution of available CD4^{+} measurements for study participants. Box and whisker plots show the median, range and outlying CD4^{+} measurements available for each patient in cells/mm^3. Participant IDs are on the x-axis followed by the number of available CD4^{+} measurements in parentheses. CD4^{+} counts should be interpreted with caution since it was common for patients to visit the clinic and be evaluated for CD4^{+} when they were ill.

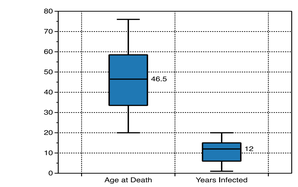


Figure 1. Distribution of cohort age and number of years infected. The box and whisker plot shows the median, upper and lower quartiles and range for the cohort age (left) and number of year infected (right).

Table 3. Antiretroviral Histories. The known ARV history of the participants is shown, with the last known ARV dose obtained either from a medical facility or from questioning the caregiver at the time of death. Fro studying medical records, we noted that 11 of the participants were on cART until death. Of the remaining participants, 4 may have stopped their medications within 4 weeks of death, 3 within 8 weeks of death, and participants 4010 and 1010's last prescribed ARV dose was 4 months prior to death. While we have no records to show ARV adherence immediately prior to death in these patients, it is likely that some form of compliance took place due to their low viral loads at autopsy and the known ability of HIV to rapidly rebound during treatment interruption.

ID	Visit[1]	ARVExp
1010	0	ddC,3TC,ZDV,IDV,d4T
	11	RTV,SQV,CBV,NVP
	38	TFV,RTV,3TC
	44	TFV,3TC,KTA
	50	TFV,3TC,KTA
	62	NONE
	66	TFV,RTV,3TC,ATV
	72	Death
2004	0	3TC,NVP,IDV,ddI,NFV,ddC,ZDV
	7	3TC,NVP,IDV
	38	TFV,3TC,NVP
	53	TFV,RTV,3TC,KTA,NVP
	67	TFV,3TC,NVP
	83	ABC,3TC,NVP
	83	Death
4013	0	NFV,3TC,ZDV
	1	NFV,3TC,ZDV,CBV
	6	NFV,3TC,CBV,d4T
	13	NFV,3TC,FTV,IDV,d4T
	18	3TC,FTV,d4T
	25	3TC,FTV,d4T
	27	3TC,FTV,d4T
	32	3TC,FTV,d4T
	39	3TC,FTV,d4T
	50	3TC,FTV,d4T
	50	Death
4106	0	ZDV
	1	ZDV
	40	ZDV
	61	ZDV
	68	3TC,ZDV
	72	3TC,ZDV
	80	NFV,3TC,ZDV,IDV
	91	3TC,ZDV
	192	ABC,RTV,3TC,ATV
	203	ABC,RTV,3TC,ATV
	205	ABC,RTV,3TC,ATV
	207	ABC,RTV,3TC,ATV
	210	ABC,RTV,3TC,ATV
	212	ABC,RTV,3TC,ATV
	214	ABC,RTV,3TC,ATV
	215	Death
5095	0	KTA,TRU
	3	NONE
	9	KTA,TRU
	10	KTA,TRU
	21	KTA,TRU
	21	Death
4149	0	RTV,DAR,RGV,TMC
	0	Death
4010	0	NFV,3TC,ZDV,EFV,CBV
	3	EFV,CBV
	5	EFV,CBV
	10	EFV,CBV
4124	12	EFV,CBV
	13	EFV,CBV
	16	EFV,CBV
	17	EFV,CBV
	18	Death
4129	0	RTV,CBV,NVP,ATV,TRU
	6	TFV,CBV,RGV
	14	TFV,3TC,CBV,RGV
	23	TFV,CBV,RGV
	29	TFV,CBV,RGV
	36	TRU,RGV
	38	Death
4143	0	ABC,CBV,TRU
	0	KTA,TRU
	4	KTA,TRU
	5	KTA,TRU
	4	RTV,DAR,RGV,TMC
	6	Death
4150	0	3TC,ZDV,IDV
	52	KTA,CBV
	126	ATR
	128	RTV,ATV,TRU
	133	RTV,ATV,EPZ
	136	ATV,EPZ
	145	RTV,DAR,EPZ
	146	RTV,DAR,EPZ
	146	Death
4154	0	RTV,ATV,FPV,EPZ,RGV
	0	Death
4175	0	ATR
	6	ATR
	6	ATR
	7	ATR
	7	ATR
	8	ATV,TRU,ATR
	8	RTV2,ATV,TRU
	8	NFV,ATV,TRU
	8	Death
5024	0	TFV,3TC,EFV
	9	TFV,ddI,KTA
	23	KTA,TRU
	24	NONE
	41	KTA,TRU
	44	KTA,TRU
	67	RTV,DAR,TRU
	69	Death
4179	0	RTV,DAR,TRU
	3	RTV,DAR,TRU
	5	RTV,DAR,TRU
	6	RTV,DAR,TRU
	6	Death
5025	0	TFV,ddI,3TC,KTA
	4	TFV,3TC,KTA
	5	TFV,3TC,KTA
	7	TFV,3TC,KTA
	8	TFV,3TC,KTA
	9	TFV,3TC,KTA
	10	TFV,3TC,KTA
	12	TFV,3TC,KTA
	13	TFV,3TC,KTA
	14	TFV,3TC,KTA
	15	3TC,KTA
	15	TFV,3TC,KTA
	28	Death
6015	0	NVP,TRU
	2	NVP,TRU
	12	3TC,CBV,d4T
	17	NVP,TRU
	21	NVP,TRU
	24	NVP,TRU
	29	Death
6083	0	ZDV
	13	3TC,NVP,d4T
	14	ddI,NVP,d4T
	46	ddI,d4T
	46	ddI,NVP,d4T
	48	ddI,RTV,SQV,NVP,d4T
	50	Death
1156	0	EFV,CBV
	6	CBV,NVP
	9	CBV,NVP
	13	ABC,TFV,KTA,CBV,NVP
	21	KTA,CBV,NVP,TZV
	25	TFV,3TC,NVP
	31	TFV,3TC,NVP
	38	TFV,NVP,ETC
	47	TFV,NVP,TRU,ETC
	50	NVP,TRU
	51	Death
1156	0	3TC,FTV,CBV,d4T
	0	Death
4010	0	3TC,ZDV,FTV,d4T
	2	3TC,ZDV,SQV
	15	3TC,ZDV,FTV
	15	3TC,ZDV,FTV
	19	Death

1. Month since first available ARV record.

Table 4. Patient pathologies.

Subject	Tumors	Infection	Atherosclerosis	Nephrosclerosis
1010	X		X	
1156	X	X	X	X
2004			X	
4106	X		X	X
4010			X	
4013		X		X
4124	X		X	X
4129		X		
4130	X			
4143	X	X	X	
4149	X	X	X	
4150	X		X	
4154	X		X	
4175	X	X	X	
4179	X	X	X	
5024	X			
5025	X	X	X	
5095	X		X	X
6015				
6083	X	X	X	

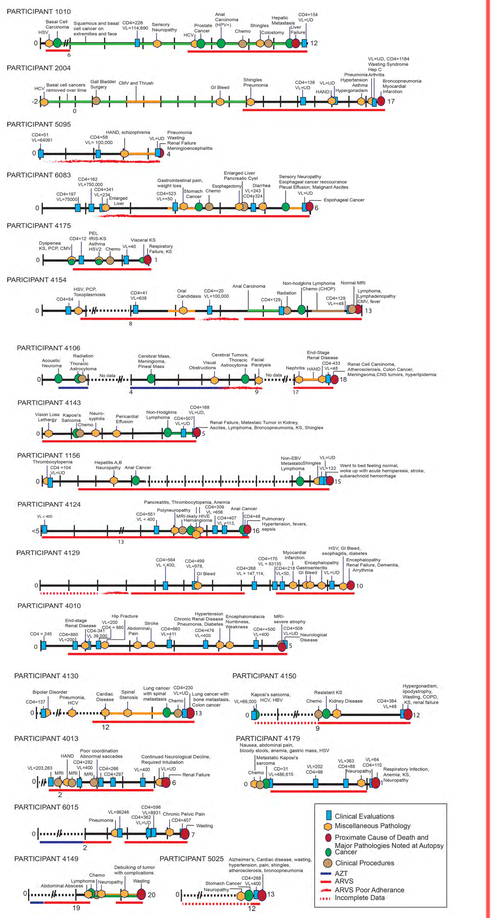


Figure 3. Participant Timelines. The timelines highlight major clinical evaluations, procedures, and pathology diagnosed during the course of each NNAB-participant's HIV infection. A symbol legend for all timelines is shown. The year the participant was infected is indicated as a "0", usually at the beginning of the timeline and ends with the number of years infected. Hash marks indicate gaps in available data. Abbreviations are VL=viral load, CD4=CD4counts, UD=undetectable, HSV=herpes simplex virus, HPV=human papilloma virus, Chemo=chemotherapy, CMV=cytomegalovirus, GI=gastrointestinal, HAND=HIV-associated neurological disorders, which encompasses ANI (asymptomatic neurocognitive disorder), MND (minor neurocognitive disorder, similar to MCMD) and HIV associated dementia (HAD), PCP=pneumocystis pneumonia, PEL= primary effusion lymphoma, IRIS-KS=immune reconstitution inflammatory syndrome leading to Kaposi's sarcoma, EBV=Epstein Barr Virus, HCV=Hepatitis C Virus, COPD=chronic obstructive pulmonary disease, KS = Kaposi's sarcoma.

One clear observation from this data was that all participants presented with numerous serious pathologies during their infection despite cART. Fourteen of participants in the cohort were diagnosed with at least one cancer prior to death (one patient was also deemed cancer+ post-mortem), which included basal cell carcinoma, prostate cancer, anal carcinoma, stomach cancer, malignant ascites, Kaposi's sarcoma (both dermal and visceral), Non-Hodgkin's lymphoma, B-cell and Burkett's lymphoma, brain cancers, EBV-lymphoma, hemangioma and lung cancer. Other pathologies observed in the cohort included varying neurological disorders, miscellaneous infections and organ disease.

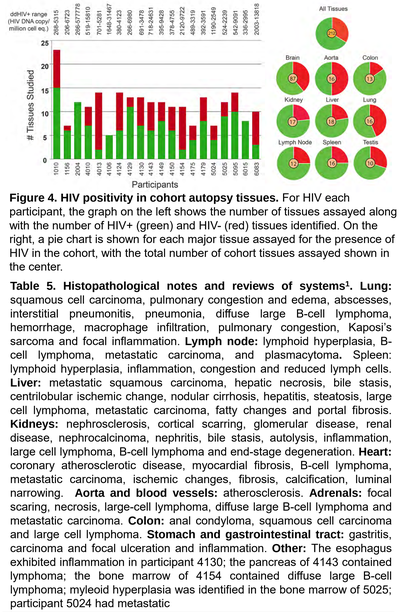


Figure 4. HIV positivity in cohort autopsy tissues. For HIV each participant, the graph on the left shows the number of tissues assayed along with the number of HIV+ (green) and HIV- (red) tissues identified. On the right, a pie chart is shown for each major tissue assayed for the presence of HIV in the cohort, with the total number of cohort tissues assayed shown in the center.

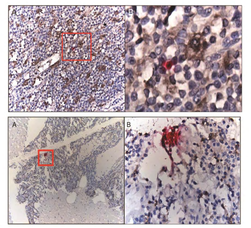
Table 5. Histopathological notes and reviews of systems[1]. Lung: squamous cell carcinoma, pulmonary congestion and edema, abscesses, interstitial pneumonitis, pneumonia, diffuse large B-cell lymphoma, hemorrhage, macrophage infiltration, pulmonary congestion, Kaposi's sarcoma and focal inflammation. **Lymph node:** lymphoid hyperplasia, B-cell lymphoma, metastatic carcinoma, and plasmacytoma. Spleen: lymphoid hyperplasia, inflammation, congestion and reduced lymph cells. **Liver:** metastatic squamous carcinoma, hepatic necrosis, bile stasis, centrilobular ischemic change, nodular cirrhosis, hepatitis, steatosis, large cell lymphoma, metastatic carcinoma, fatty changes and portal fibrosis. **Kidneys:** nephrosclerosis, cortical scarring, glomerular disease, renal disease, nephrocalcinoma, nephritis, bile stasis, autolysis, inflammation, large cell lymphoma, B-cell lymphoma and end-stage degeneration. **Heart:** coronary atherosclerotic disease, myocardial fibrosis, B-cell lymphoma, metastatic carcinoma, ischemic changes, fibrosis, calcification, luminal narrowing. **Aorta and blood vessels:** atherosclerosis. **Adrenals:** focal scaring, necrosis, large-cell lymphoma, diffuse large B-cell lymphoma and metastatic carcinoma. **Colon:** anal condyloma, squamous cell carcinoma and large cell lymphoma. **Stomach and gastrointestinal tract:** gastritis, carcinoma and focal ulceration and inflammation. **Other:** The esophagus exhibited inflammation in participant 4130; the pancreas of 4143 contained lymphoma; the bone marrow of 4154 contained diffuse large B-cell lymphoma; myeloid hyperplasia was identified in the bone marrow of 5025; participant 5024 had metastatic

Subject	Spleen	Blood Vessels	Heart	Muscle	Lungs	LN	Liver	Kidney	Aorta	Testis	Adrenal	Colon	Stomach	Ovary	Esophagus	Pancreas	Marrow	Thyroid
1010	X	X	X	X	X	X	X											
1156	X				X	X		X	X	X								
2004	X		X		X	X	X		X	X								
4106					X		X	X	X		X	X						
4010	X	X			X	X	X		X	X	X							
4013	X		X	X	X	X	X	X	X	X			X					
4124	X	X	X		X	X	X	X	X	X			X					
4129	X		X		X	X	X	X	X	X					X			
4130	X				X		X	X	X	X		X			X			
4143	X				X	X	X	X	X		X	X				X		
4149	X				X	X	X	X	X	X								
4150	X	X			X	X	X		X	X								
4154	X		X		X	X	X	X	X		X						X	
4175	X				X	X			X	X		X						
4179	X				X	X			X	X								
5024	X		X		X	X	X	X	X		X							X
5025	X	X	X		X	X	X	X									X	
5095	X		X		X	X	X	X	X				X					
6015	X				X	X												
6083	X	X	X		X	X				X	X							

1. An "X" indicates mild to severe pathology noted during histological examination.

Table 6. Brain Pathology

	Brain	Spinal Cord
1010	Alzheimer Type 2 gliosis, corpora amylacea.	No spinal cord available.
1156	Anoxic-ischemic changes	No significant pathology
2004	Reactive astrocytes, corpora amylacea, numerous CD68+ cells in white matter.	No significant pathology
4106	Microglial nodule encephalitis, History of NeuroFibromatosis Type 2 with meningioma plaque-like nodule on dura	Neurofibromas
4010	Focal infarct.	No significant pathology
4013	Alzheimer's Type 2 gliosis, subarachnoid hemorrhage due to pre-terminal event	Focal chronic inflammation of spinal cord and spinal ganglia
4124	Several areas show thickened blood vessel walls and perivascular hemosiderin. Some areas suspicious for very early infarct.	No significant pathology
4129	Anoxic-ischemic encephalopathy, Monckeberg medial calcific sclerosis of arteries, possible microinfarcts	No significant pathology
4130	Focal Hemorrhage- in anterior basal ganglia with extension into ventricle and subarachnoid space.	No significant pathology
4143	Alzheimer type 2 gliosis	No significant pathology
4149	Lymphoma	Lymphoma Infiltrate
4150	Mild acute hypoxic ischemic encephalopathy	No significant pathology
4154	Focal involvement of leptomeninges by metastatic diffuse B cell lymphoma.	No significant pathology
4175	No significant pathology, some histologic suggestion of early infarct.	No significant pathology
4179	Aseptic leptomeningitis.	Mild focal meningothelial proliferation
5024	Anoxic ischemic encephalopathy.	No significant pathology
5025	Alzheimer's Disease.	No significant pathology
5095	Neoplastic plasma cell infiltrate.	Neoplastic plasma cell infiltrate.
6015	Alzheimer type 2 gliosis, multiple cerebral infarcts andor hemorrhages. Cerebellar Purkinje cell loss and gliosis.	No significant pathology
6083	Moderate white matter gliosis.	No significant pathology



4149 5095 4143 1010

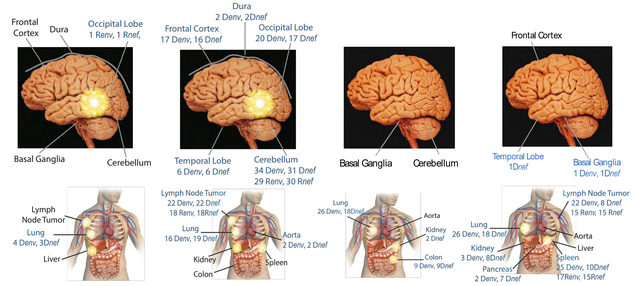


Figure 6. HIV DNA and RNA sequences amplified from anatomical sites. HIV was amplified from tissues from four participants using a single genome sequencing approach targeting *env-nef* sequences. The number of sequences and their tissue of origin are shown in the images. D=DNA and R=RNA. A yellow flare in the images indicates the presence of cancer identified post-mortem.

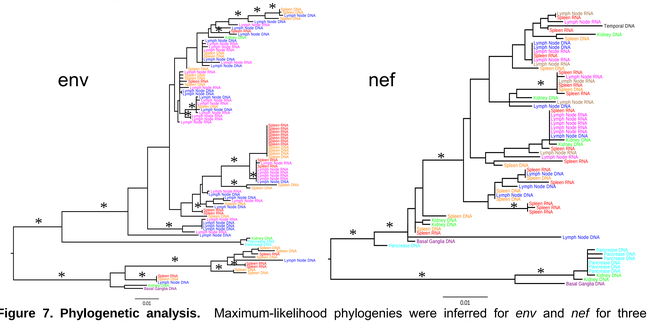


Figure 7. Phylogenetic analysis. Maximum-likelihood phylogenies were inferred for *env* and *nef* for three participants shown in Figure 6 (4149 was omitted due to limited sequence data). Phylogenies are shown only for participant 1010; however, other participant's sequence data generated similar branching patterns. Branches with a bootstrap of >90% are indicated with an asterisk. Only one env sequence was hypermutated (indicated), thus indicating a "dead-end" sequence. RNA and DNA sequences were dispersed within the tree. Two distinct patterns of branching were observed: 1) clades with little or no diversity, 2) clades with long branches indicating a potential source of on-going replication. While sequences in some clades were limited to one distinct tissue, other clades showed closely related sequences from multiple sites, thus suggesting virus migrating between tissues during cART. ***More information concerning patient sequence evolution can be viewed on poster 1167***

Table 7. Drug resistance mutations. In an ongoing analysis, HIV pol sequences covering RT and P24 were generated to identify if single drug resistance mutations (SDRM) were present in anatomical sites. To date, only one sequence with a SDRM have been identified. This work suggests that ART is not penetrating anatomical reservoirs (anatomic isolation), or that cells (i.e. macrophages, dendritic cells) in anatomical reservoirs do not respond to current cART.

Resistance category	5095 4 tissues		4143 2 tissues		1010 3 tissues	
	Sequences Analyzed	Containing SDRM	Sequences Analyzed	Containing SDRM	Sequences Analyzed	Containing SDRM
Sequences with any SDRM	49	0	9	0	26	1
PR Sequences with any PI SDRM	49	0	9	0	25	0
RT Sequences with any NRTI SDRM	47	0	9	0	25	0
RT Sequences with any NNRTI SDRM	47	0	9	0	25	1
RT Sequences with any NRTI + any NNRTI SDRM	47	0	9	0	25	0
PRRT Sequences with any NRTI + any NNRTI + PI SDRM	47	0	9	0	24	0

Conclusions: This study confirms the presence of HIV within diverse anatomical tissues in virally suppressed ART^{+} patients. Persistent virus replication in tissues could promote inflammatory diseases, including cancer, atherosclerosis and other organ-associated diseases. These ACSR/NNAB cohorts, along with others of their kind, are highly valuable resources for future studies of HIV reservoirs and persistence.