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WIDE ANATOMIC DISTRIBUTION OF HIV-INFECTED CELLS IN INDIVIDUALS WITH COMORBID CANCER

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BACKGROUND

HIV persistence during combination antiretroviral therapy (ART) is the principal challenge preventing viral eradication. We and others reported that HIV infected cells undergo clonal expansion during ART, and we reported that clones of infected cells are present in tissues and in a neoplasm. The CD4 cell subset and tissue distribution of infected cells and their roles in HIV persistence are not well understood. To determine the distribution of clones of infected cells during ART, we analyzed CD4 T cell subsets and tissues obtained from individuals who underwent autopsy after expiring with comorbid neoplasms.

Hypotheses:

Clones of HIV infected cells are present in CD4 T cell subsets

HIV populations are not uniformly distributed in tissues. Tumor tissues have distinct HIV populations

METHODS

Study Participants

HIV infected undergoing cART for 5-15 years

Participants (N=3) underwent cytopheresis and cells sorted into naïve, central transitional memory, and effector memory cell subsets.

Naïve: CD27+CD45RO-CCR7+CD57- (includes naïve and stem cell memory)
Central and transitional memory (CTM): CD27+CD45RO+
Effector Memory (EM): CD27- cells

Autopsies (N=2)

Two patients with comorbid neoplasms (Primary effusion lymphoma, undifferentiated carcinoma) underwent autopsy after expiration, and samples for analysis were stored at -70° C

Integration site analysis

HIV was extracted from cell associated DNA and integration sites identified as previously described Integration sites were identified and quantified (*Maldarelli et al., 2014*)

HIV DNA sequence analysis

HIV was extracted from cell associated DNA and single genome sequences of pro-pol (1100 nt) obtained

Sequences were aligned, and subjected to phylogenetic analysis using neighbor joining (NJ) algorithm. Topology support was investigated by bootstrap analysis (1000 bootstraps)

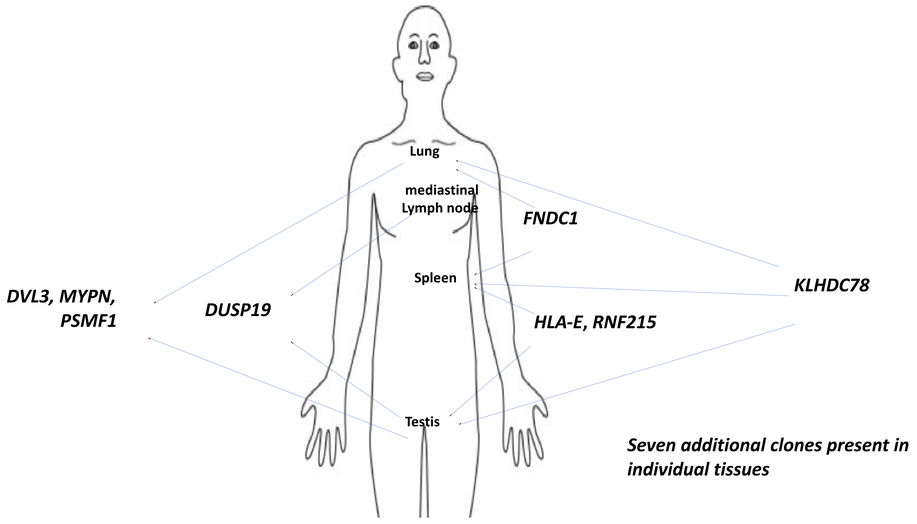
Clones of HIV infected cells are present in multiple CD4 T subsets and in diverse anatomic compartments in infected individuals undergoing combination antiretroviral therapy

Which Cell Subsets Contain HIV?

Participant	Subset	Number of unique Insertion Sites	Number of Clones	Percentage of clones in unique IS(%)	Notes
AVBIO2-21					
	Naïve	33	0	0	*BACH2*
	EM	307	25	8.1	*HORMAD2, BACH2*
	CTM	463	20	4.3	*HORMAD2, BACH2*
AVBIO2-18					
	Naïve	6	2	33.3	
	EM	50	11	22.0	*BACH2, STAT5B*
	CTM	140	11	7.9	*BACH2*
AVBIO2-16					
	Naïve	27	4	14.9	
	EM	48	12	25.0	*STAT5B*
	CTM	131	17	13.0	*STAT5B*
Total		1205	102	8.5	

Sorted cells were subjected to integration site analysis and the numbers of clones present in each subset were determined
HIV infected cells were identified in naïve, CTM, and EM subsets More cells were in clones in EM subsets than in CTM (p =0.03, Fisher exact test)

Clones of HIV Infected Cells Have a Wide Anatomic Distribution



DNA from cells from tissue samples obtained at autopsies (expiration from primary effusion lymphoma) were extracted and subjected to integration site analysis. Clones were identified in lymphoid and non lymphoid tissues; proviruses with identical integration sites were identified in various tissues as indicated by the host gene into which the provirus integrated.

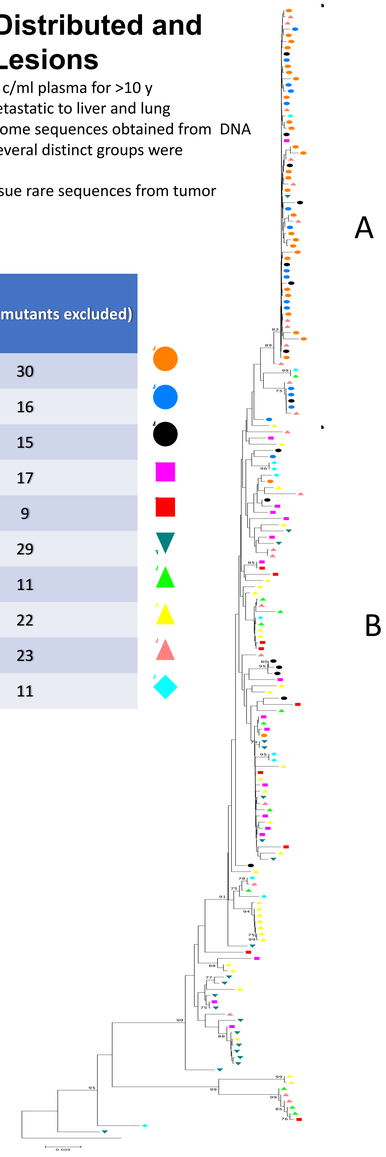
HIV Infected Cells are Widely Distributed and Present in Metastatic Lesions

63 year old man with longstanding HIV infection with HIV <50 c/ml plasma for >10 y
With undifferentiated adenocarcinoma unknown primary, metastatic to liver and lung
Samples were obtained from tissues at autopsy and single genome sequences obtained from DNA extracted from tissues. NJ tree shown with bootstrap values Several distinct groups were identified

A. Most sequences derived from mediastinal lymphoid tissue rare sequences from tumor tissue

B. Most sequences from tumor tissue

Tissue	HIV DNA (copies/10^6 cells)	SGS (Hypermutants excluded)
Mediastinal lymph node 1-2	330	30
Mediastinal lymph node 2-1	140	16
Mediastinal lymph node 5	110	15
Liver metastasis 1	11	17
Liver metastasis 2-1	25	9
Lung cancer lesion	21	29
Liver 1	68	11
Lung 2	35	22
Spleen 3	23	23
Bone marrow 2	15	11



Summary

- **Deleted proviruses are enriched in most individuals undergoing cART**
- **Clones are distributed within and across cell subsets**
- **Sources of clonal expansion**
  - **Homeostatic proliferation**
  - **Antigen induced proliferation**
- **In general effector memory population has more cells in clones**
- **Clones are present in numerous tissues**
  - **Clinical events (e.g., neoplasms) may drive clonal expansion**

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