

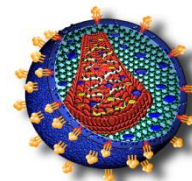


Distribution and Fine Structure Genetic Analysis of HIV in Gut Associated Lymphoid Tissue and Blood after Prolonged Antiretroviral Therapy

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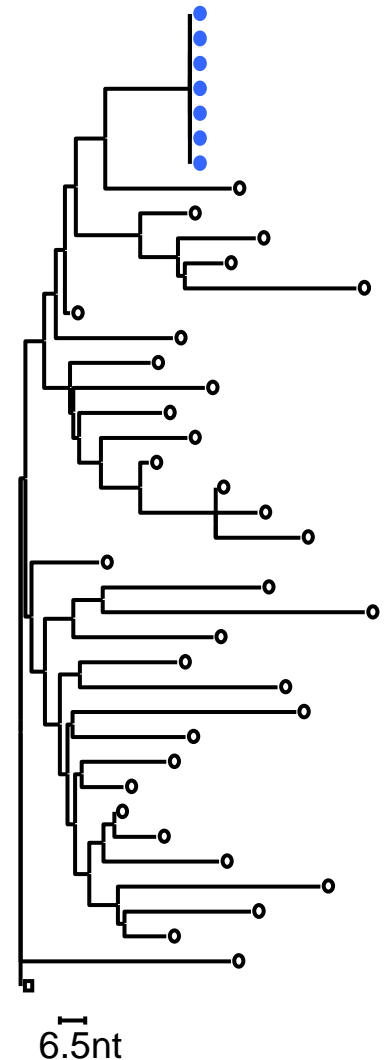
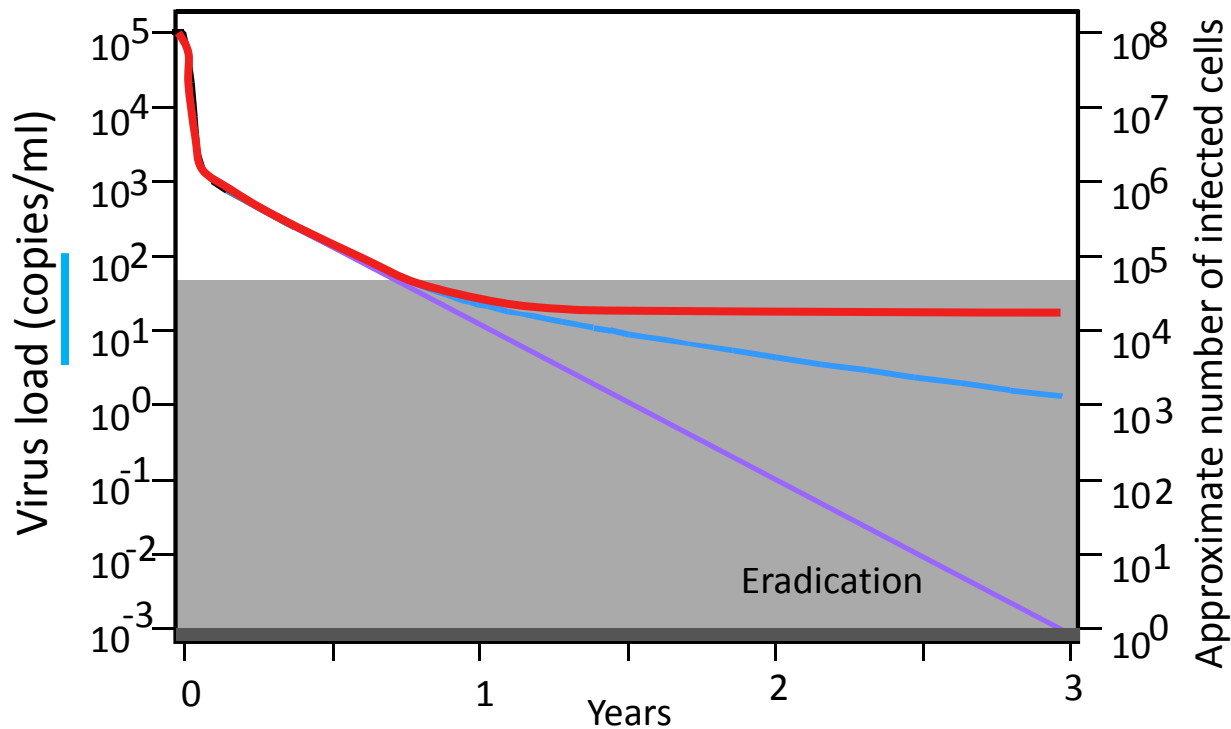
University of Milan, Italy



HIV Drug Resistance Program

National Cancer Institute at Frederick

Persistent HIV can be found in Plasma despite Long Term cART



During long term suppressive cART:

- Viremia reaches a **Plateau** during a 4th phase decay
- Residual viremia includes **Identical Sequences**
- No evidence of molecular evolution from pre-therapy virus

The source of persistent viremia remains uncertain.

*Kearney et al.,
submitted*

The Role of the GALT in HIV Persistence

- Despite long-term cART, GALT immune reconstitution is lower than in periphery
- HIV-DNA levels are 5-10 times higher in GALT than in PBMCs
- HIV in GALT persists in distinct subsets (T_{EM} and T_{naive} vs $T_{CM/TM}$) compared to blood AND shows few genetic changes over time
- Relatively high level of Ileum HIV-RNA and higher RNA/DNA in ileum compared to other gut sites
- cART Intensification with Raltegravir:
 - DECREASE of HIV-RNA in ileum
 - no reduction in plasma RNA

Is GALT a sanctuary of replication/source of persistent viremia?

S Mehandru, PLOSmed 2006

TW Chun, JID 2008

L Josefsson, PNAS 2013

SA Yukl, AIDS 2010

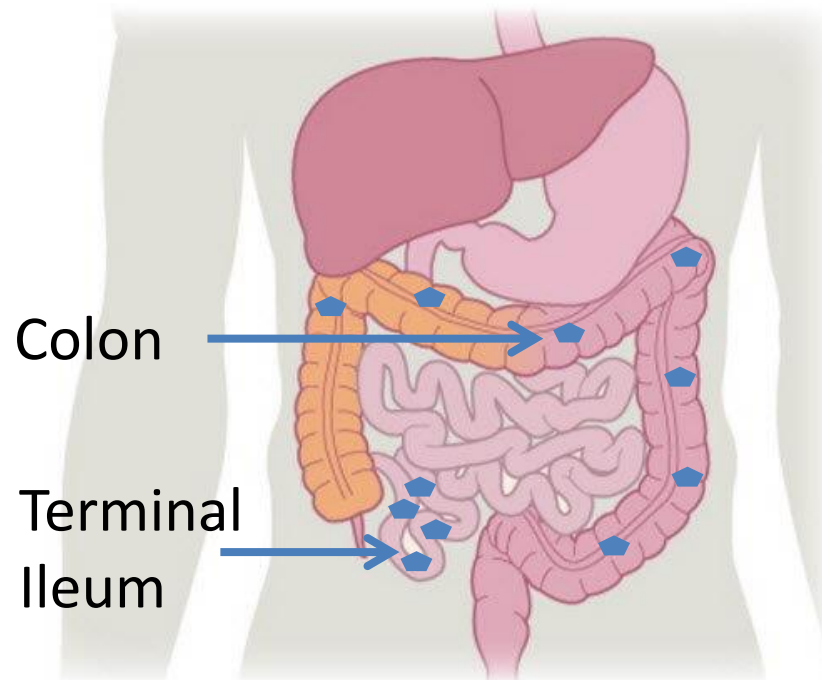
Primary Objectives

- **Determine the fine structure of HIV populations in blood and GALT**
 - **Anatomic distribution in individual or small groups of biopsies**
 - **Single genome sequencing**
 - **Phylogenetic analyses**
 - **Population genetics analyses**
- **Quantify HIV-DNA and RNA in GALT**
 - **Evaluate single pinch biopsies**
 - **Colon**
 - **Ileum**

Study Participants

PT#	Age	Sex	Years Dx	CD4/ μ l nadir(%)	Baseline HIV RNA	Years on ARV	Duration <50 c/ml	ARV regimen	CD4 cells/ μ l (%)	Plasma HIV RNA	Prior evidence of drug resistance
1	46	M	13	364 (19)	27000	12.5	12.	TDF/FTC/EFV	616 (51)	<40	no
2	60	M	14	245 (17)	654577	13	12.5	ABC/3TC/NV	404 (29)	<50	no
3	60	M	16	242 (25)	213349	16	15.1	TDF/FTC/RPV	560 (42)	<40	no
4	56	M	24	8 (1)	284700	18	7.3	TDF/FTC/ATV/rtv	502 (32)	<40	yes
5	64	M	24	180	19000	23	10.4	TDF/FTC/ATV/rtv/RAL	543 (35)	<40	yes
6	60	M	25	164	1400000	23	5.2	TDF/FTC/RAL	1009 (46)	<40	no
7	58	M	26	250 (16)	106300	25	2.5	TDF/FTC/EFV	510 (25)	<40	yes
8	62	M	22	56	76000	22	5.4	TDF/FTC/EFV	427 (35)	<40	no

Experimental Approach



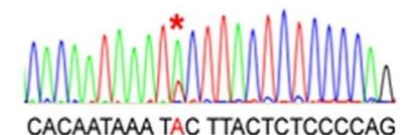
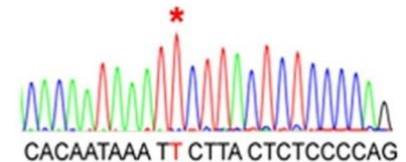
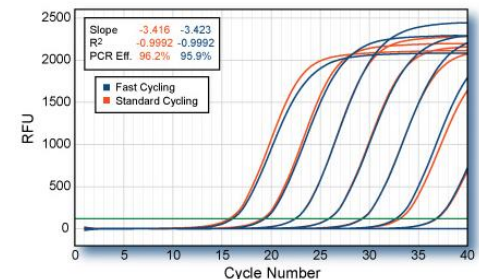
Single pinch
biopsies



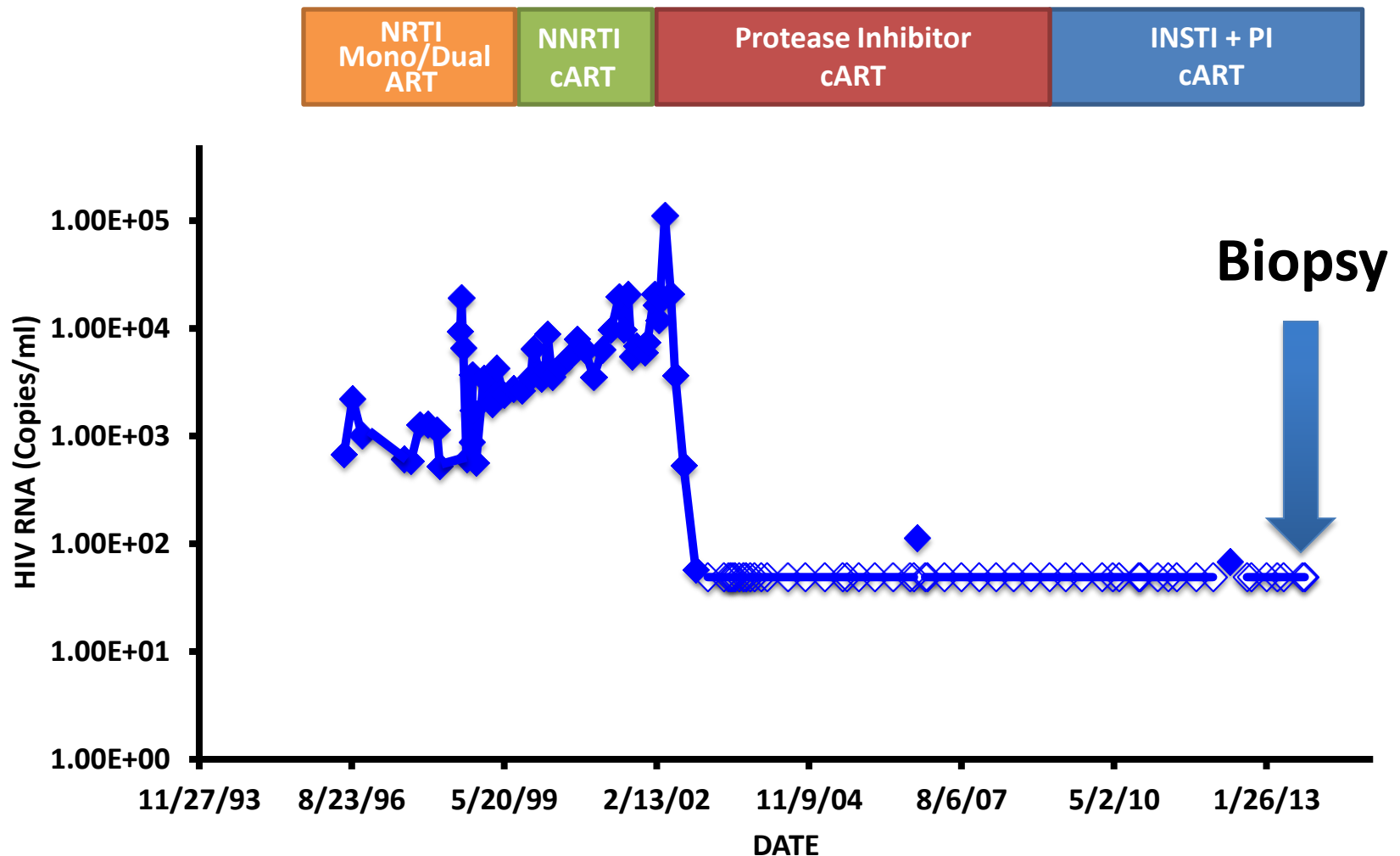
Lower yield in HIV
recovery but can
evaluate sampling
variation

Quantitative and Phylogenetic Analyses of HIV

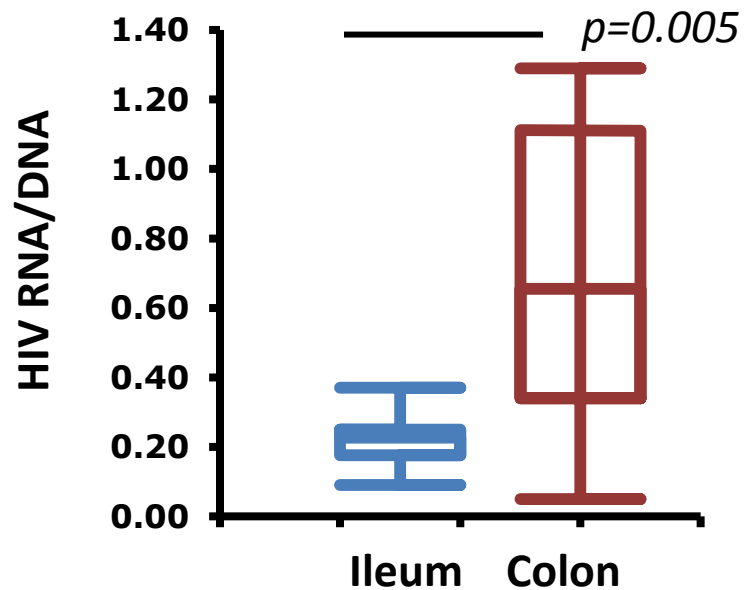
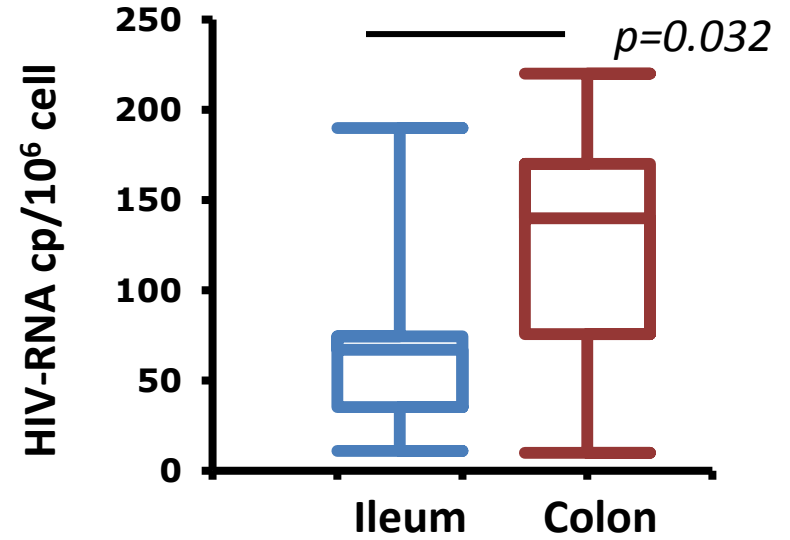
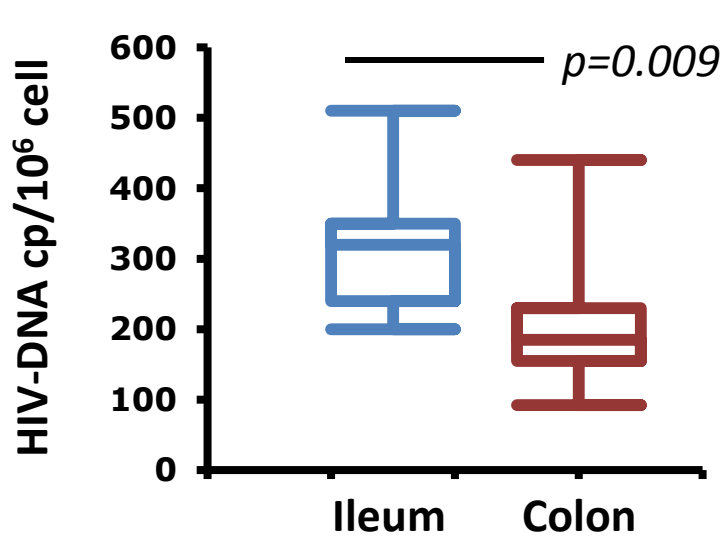
- **Single Biopsy Analyses**
 - Snap frozen single biopsies
 - homogenized in collection tubes containing zirconium beads
 - isolate DNA and RNA
 - qPCR copy number determination
 - CCR5
 - cell associated HIV-DNA and HIV-RNA (*gag*)
 - RNA, DNA Single Genome Sequencing
 - P6-RT region
- **Grouped biopsy and blood analyses**
 - Collagenase digestion and mucosal cell collection from grouped biopsies
- **PBMC**
- **Plasma viremia**



Patient 5: HIV Suppression after Prolonged Viremia on Suboptimal Therapy

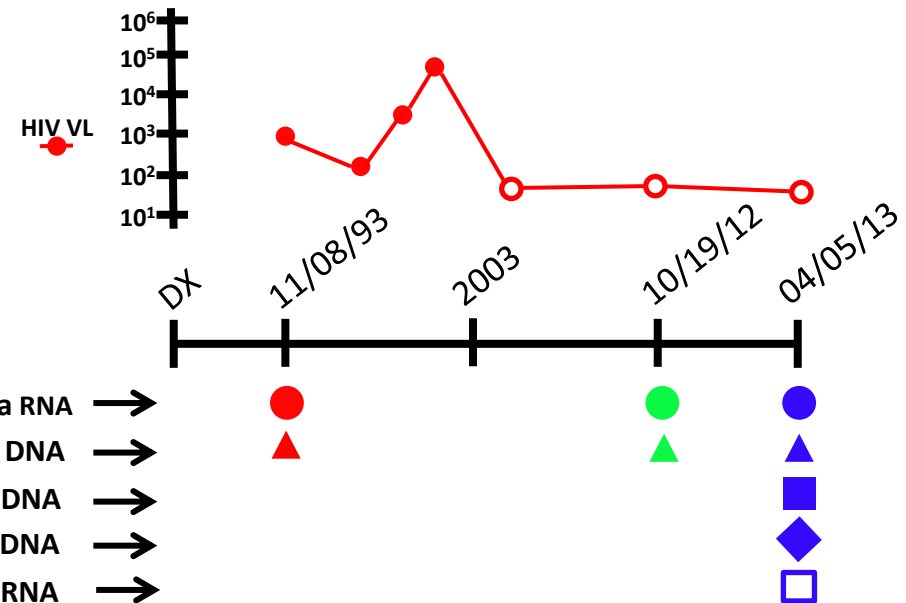
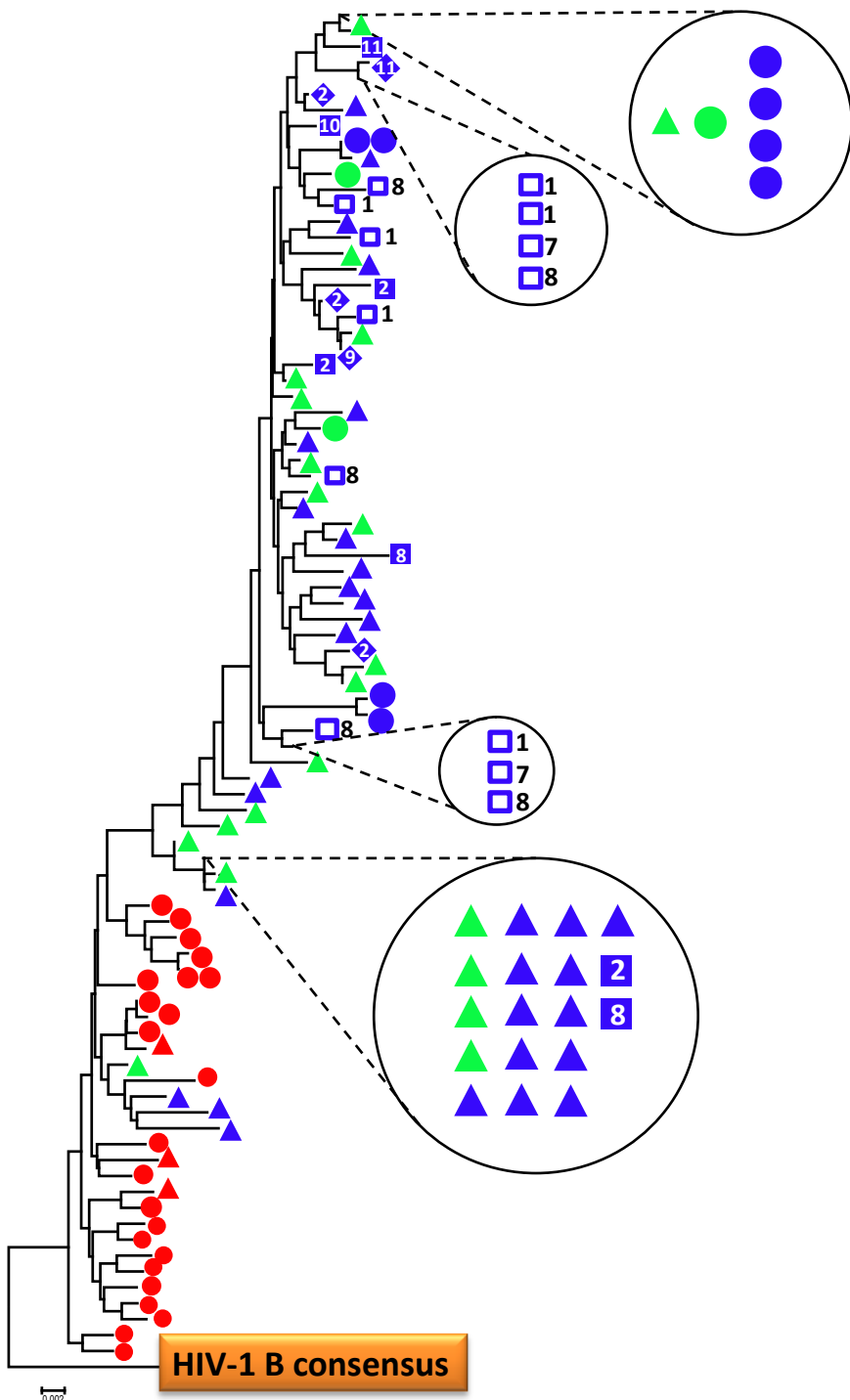


HIV Infected Cells are Present Throughout GALT After Prolonged cART

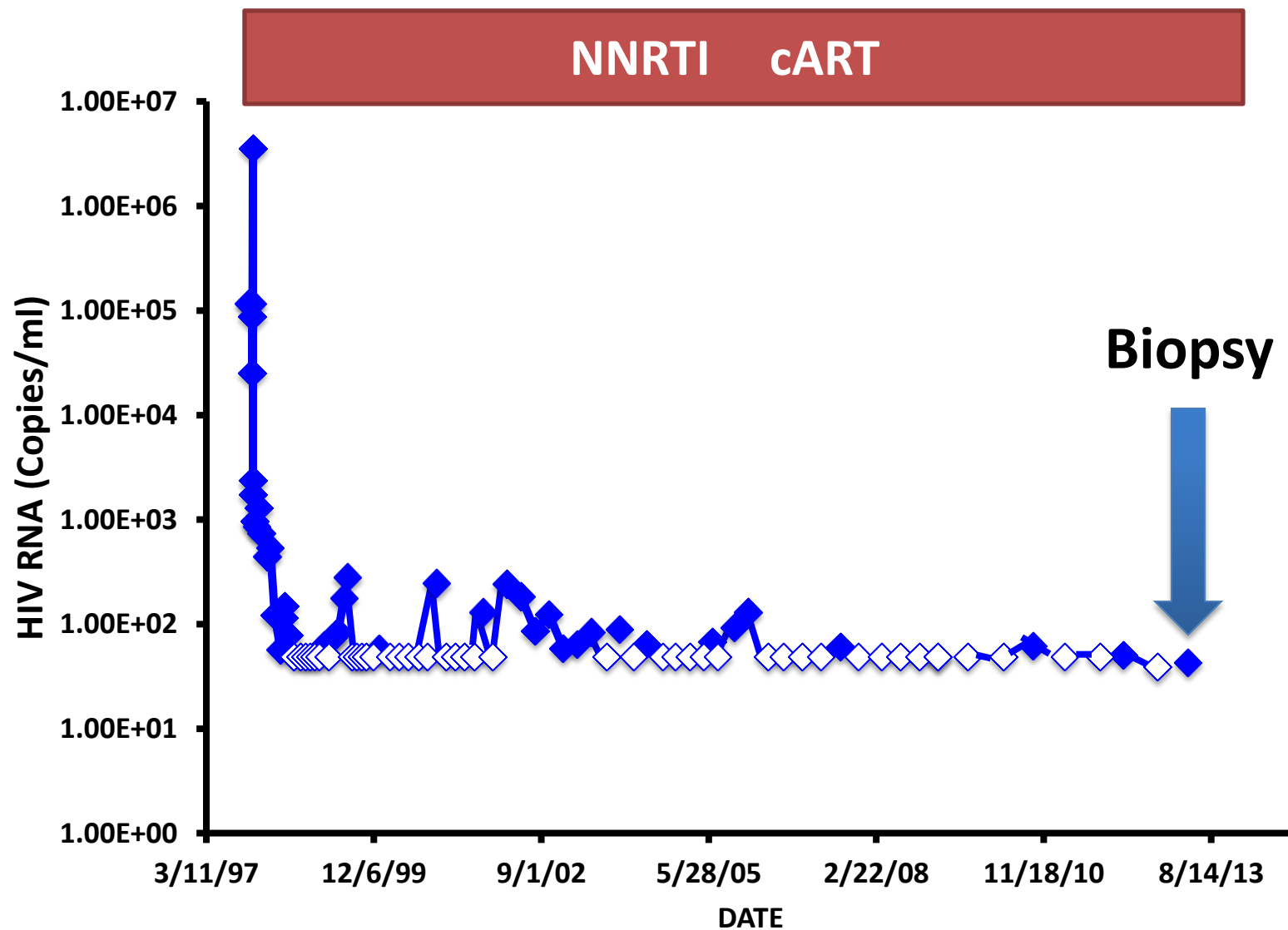


Pt 5: Phylogenetic Analysis

**Clonal Sequences
ARE NOT
Anatomically Restricted**

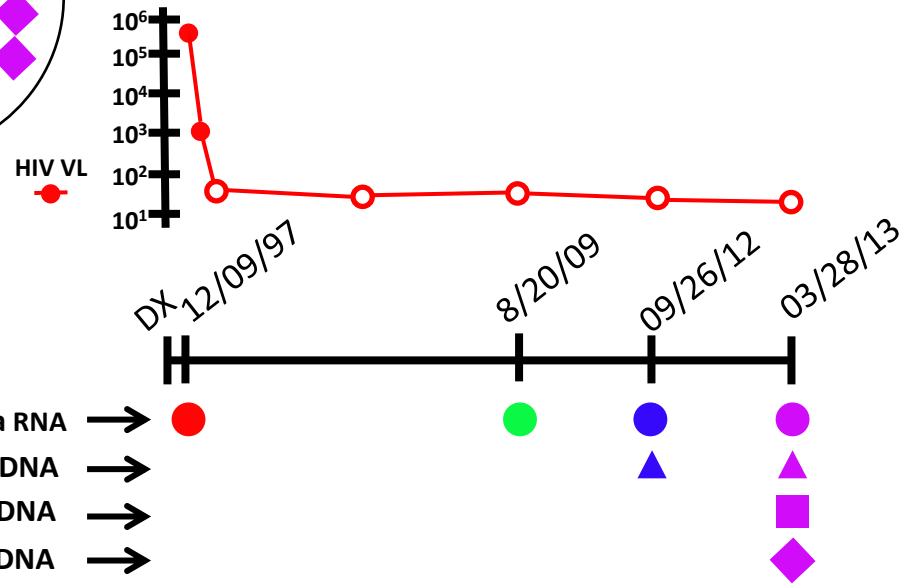
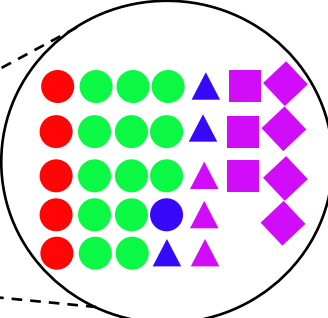
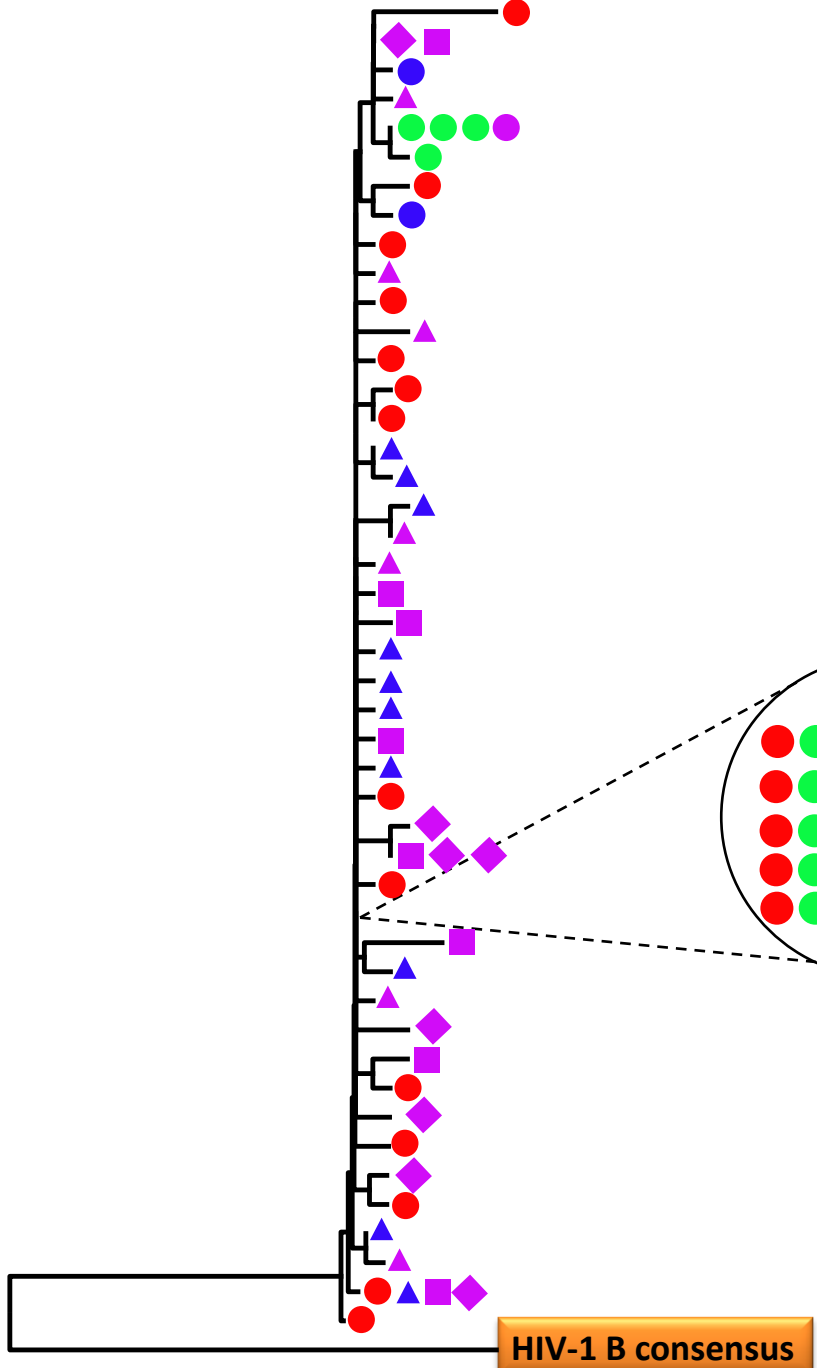


Patient 3: Prolonged HIV Suppression After Early cART



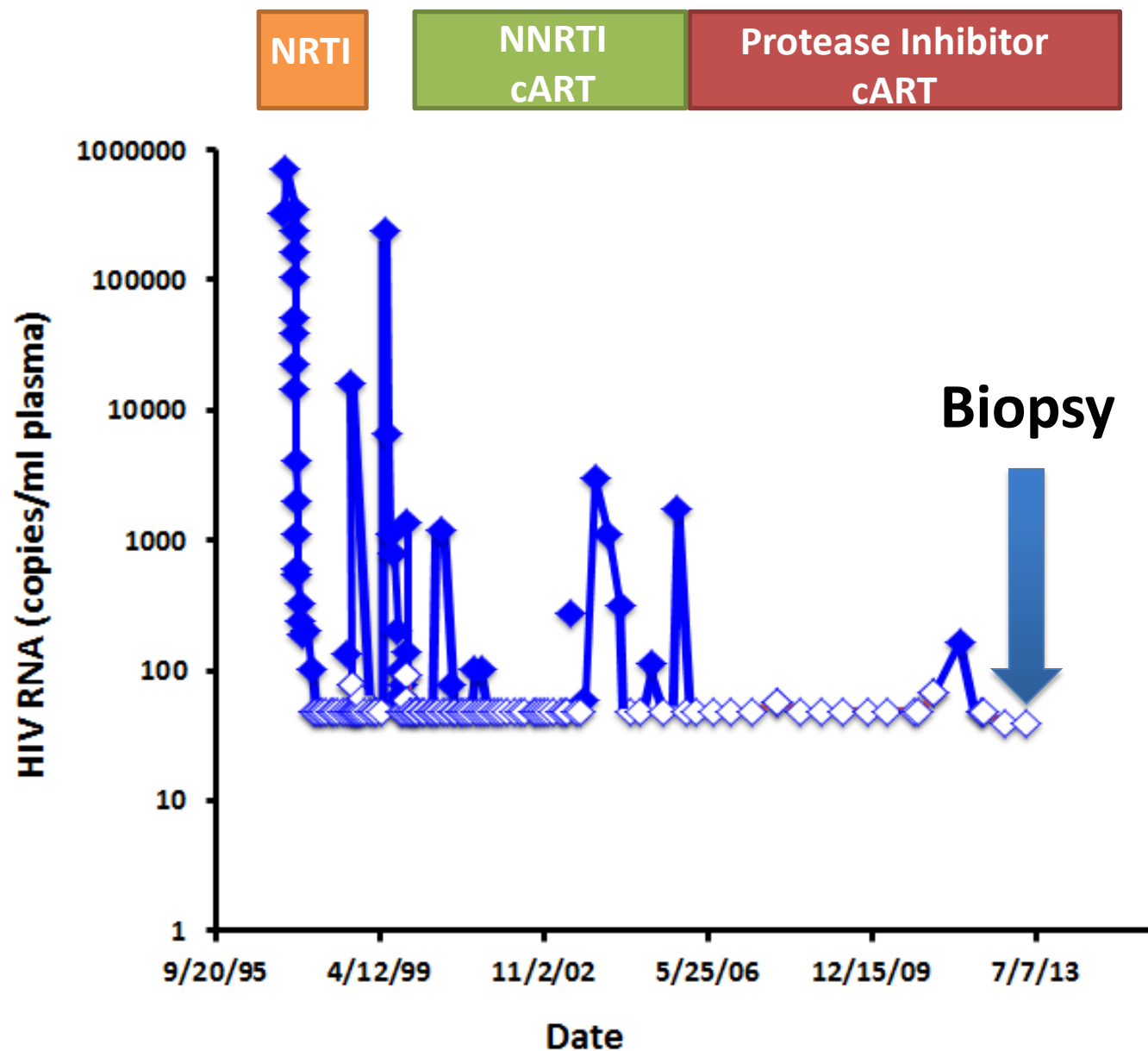
Pt 3: Phylogenetic Analysis

**NO Divergence of HIV
after 14 years**



0.005

Patient 4: Prolonged Suppression After Prior Rebound

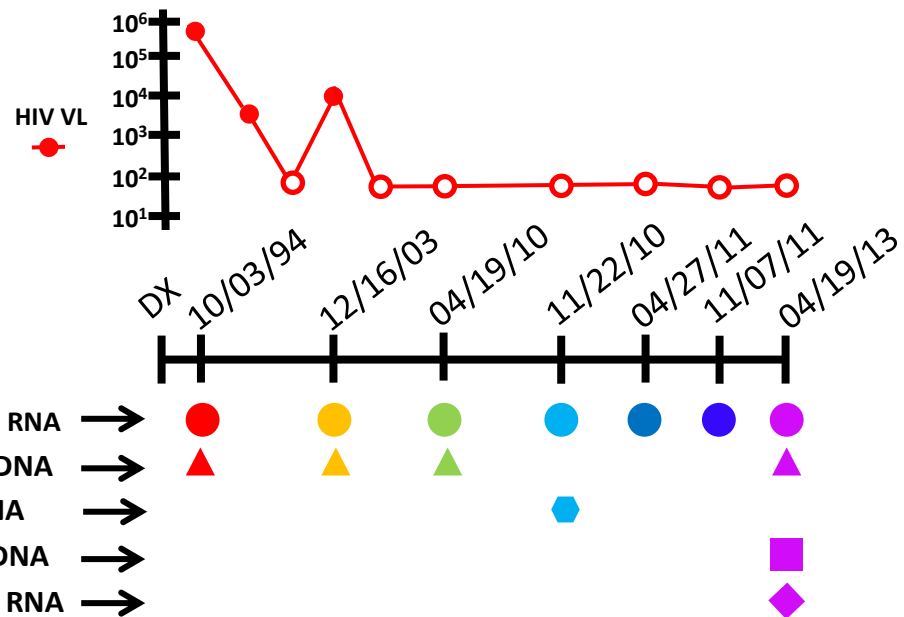
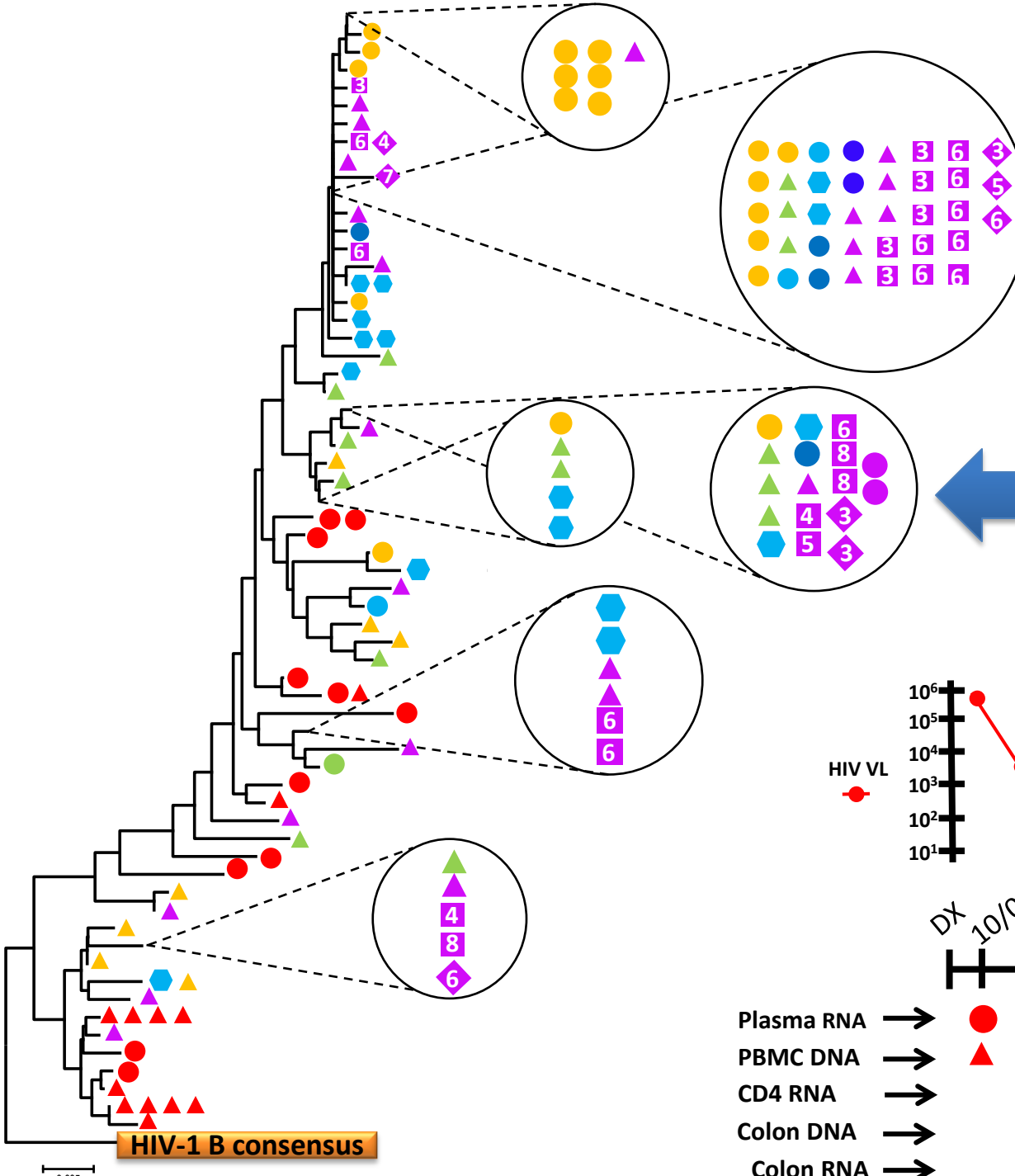


HIV DNA and RNA are present in all pinches

Colon Biopsy #	CCR5 cell equivalents	Total DNA copies	DNA copies per 10 ⁶ cells	Total RNA copies	RNA copies per 10 ⁶ cells
#1	6,678,235	323	48	141	21
#2	6,506,082	740	114	251	39
#3	10,794,788	403	37	208	19
#4	9,145,729	313	34	208	23
#5	9,948,699	260	26	167	17
#6	10,103,124	410	41	83	8
#7	8,970,346	243	27	237	26
#8	10,699,057	224	21	347	32

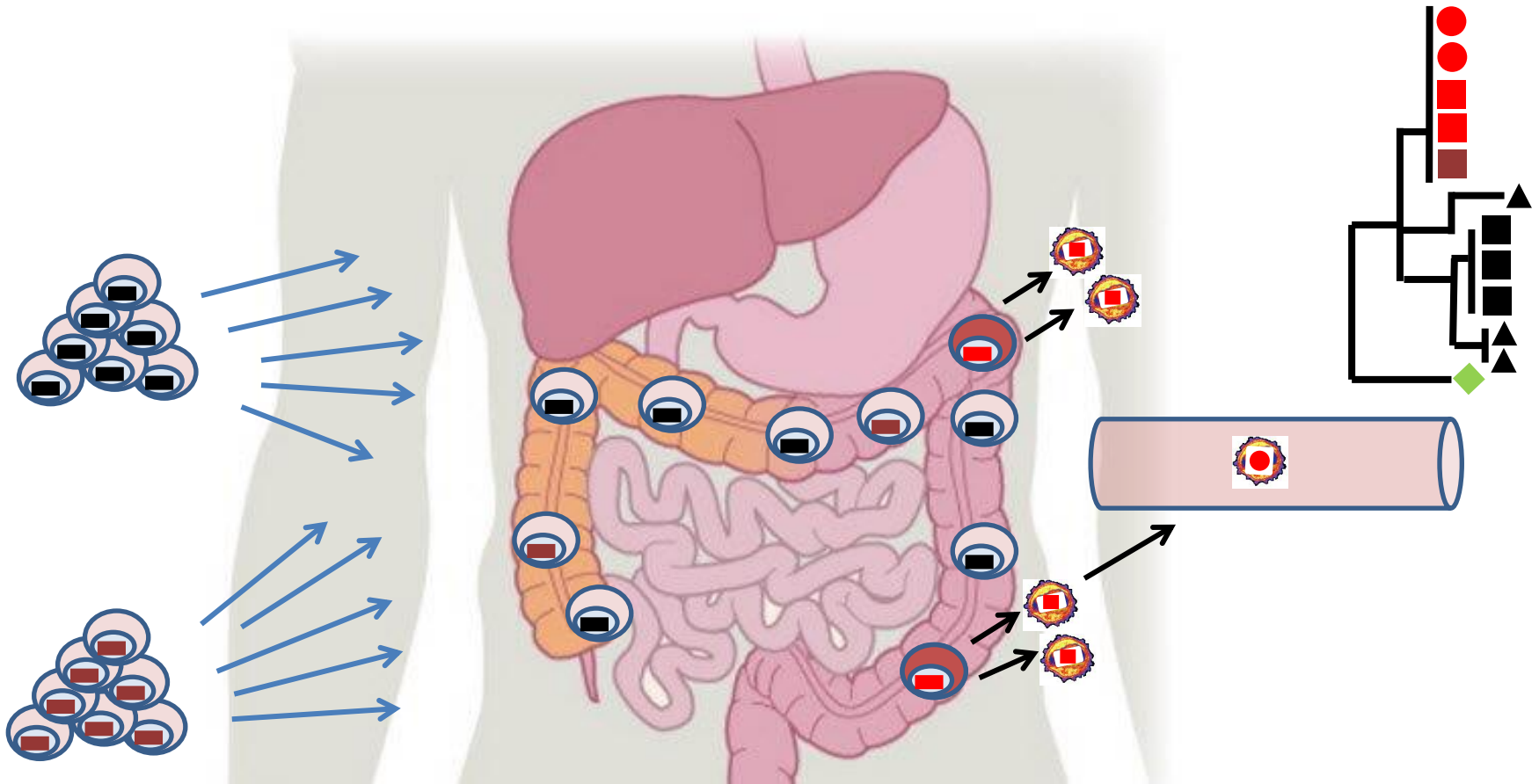
Results: Pt 4

Distinct sets of identical HIV sequences in plasma and colon RNA



Summary

- An estimated 100 million HIV infected cells are present in colonic mucosa despite prolonged cART
- Clonally expanded infected cells are distributed across the GALT and NOT anatomically restricted
- Cells in GALT can produce HIV that can contribute to residual viremia (●)



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Cheryl Chairez

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Patient Participants