Westmead Millennium Institute for Medical Research

UCSF University of California San Francisco

Karolinska Institutet

THE UNIVERSITY OF SYDNEY

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Longitudinal analysis of infection frequencies and genetic makeup of intracellular HIV-1 from tissue compartments during long-term suppressive therapy

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IAS 2013 Towards an HIV Cure Symposium

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Background

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- **Persistent HIV can still be detected in plasma and cellular reservoirs even after several years of suppressive antiretroviral therapy**

- **Identifying the source and dynamics of persistent HIV in patients on suppressive therapy is an important step towards HIV eradication**

- **Understanding the stability of the latent reservoir in specific cells and tissues over time will also inform strategies aimed at purging these reservoirs for curative therapies**

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Objectives of the Study

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- **Use single-genome and single-proviral sequencing assays, to investigate the:**
  - **Source of persistent HIV-1**
    - **What cells are HIV reservoirs during suppressive therapy?**
  - **Dynamics of persistent HIV-1**
    - **Does low-level viral replication maintain HIV during suppressive therapy?**
  - **Stability of the latent reservoir**
    - **How do intracellular HIV-1 populations change over time?**
- **8 patients on suppressive antiretroviral therapy (4-12 years)**
  - **5 initiating therapy during acute/early infection (< 6months)**
  - **3 initiating therapy during chronic infection (> 1.5year)**
    - **Peripheral blood (PB)**
    - **Bone Marrow (BM)**
    - **Gut Associated Lymphoid Tissue (GALT)**
    - **Lymph Node Tissue (LN)**

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Study Design

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Initiation of cART

4 – 12 years

Treatment naive

HIV-1 < 75 copies/ml

6 months

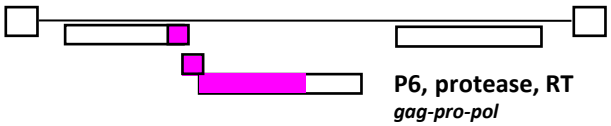
Plasma
1) Chronic:
1-3years

Plasma
2) Chronic:
1-6 months

Acute/early:
0-12 days

Time Point 1
PB
GALT
BM

Time Point 2
PB
GALT
LN



P6, protease, RT
gag-pro-pol

Cells Time Point 1:
Progenitor (CD34+ Lin-)
Late progenitor (CD34-, Lin-) (CD34+, Lin-)
Lymphoid cells (Lin+ CD4+)
Naïve (CD45RO-/CD27+)
Central/Transitional Memory (CD45RO+/CD27+)
Effector Memory (CD45RO+/CD27-)
Myeloid (CD3- /CD4lo)

Cells Time Point 2:
Naïve (CD45RO-/CCR7+/CD27+/CD57-)
Central Memory (CD45RO+/CD27+/CCR7+)
Transitional Memory (CD45RO+/CD27+/ CCR7-)
Effector Memory (CD45RO+/CD27-/ CCR7-)
Myeloid (CD3-/CD13+/CD14+)

5 patients treated during acute/early infection

3 patients treated during chronic infection

1. Investigate the source of persistent HIV-1
   - Characterization of HIV-1 in infected cells after long-term suppressive cART
2. Investigate the dynamics of persistent HIV-1
   - Compare pre-therapy HIV-1 populations to HIV-1 populations after long-term suppressive cART
3. Investigate the stability of the latent HIV-1 reservoir over time
   - Compare the longitudinal intracellular infection frequencies and genetic makeup of HIV-1 populations during long-term suppressive cART

Stable Latent HIV-1 Reservoir

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Peripheral blood

Patient	1	2	3	4	5	6	7	8	9	10
	Central/Transitional Memory (CD45RO+/CD27+)	Central Memory (CD45RO+/ CD27+/CCR7+)	Transitional Memory (CD45RO+/ CD27+/CCR7-)	MEAN (CM+TM)/2	Effector Memory (CD45RO+/CD27-)		Naive (CD45RO-/CD27+)		Myeloid (CD3-/CD4+)	
	Time Point 1	Time Point 2	Time Point 2	Time Point 2	Time Point 1	Time Point 2	Time Point 1	Time Point 2	Time Point 1	Time Point 2
	Estimated Frequency of Infection (%) *									
Initiated therapy during acute/early infection										
1	N/A	0.0239	0.0140	0.[illegible]90	N/A	0.1[illegible]1	0	0.[illegible]07	0	0.00002***
2	0.0229	0.0009	0.0119	0.[illegible]64	0.0089	0.0[illegible]7	0.0022	0.[illegible]01	0.0001**	0
3	0.0045	0.0016	0.0083	0.[illegible]50	0.0099	0.0[illegible]9	0.0003		0	0
4	0.0004	0.0001	0.0003	0.[illegible]02	0.0010	0.0[illegible]9	0.0001		0	0
5	0.0434	0.0338	0.0616	0.[illegible]77	0.0857	0.0[illegible]7	0.0023	0.[illegible]12	0.00005	0
Geometric Mean[illegible] Frequency of infection - Early infection										
	0.002%	0.0014	0.0025	0.002%	0.009%	0.006%	0.0006%	0.0002%	[illegible]04	0.000003
Initiated[illegible]herapy during chronic infect[illegible]n										
6		0.1594	0.0923	0.[illegible]	0.[illegible]	0.[illegible]	0.[illegible]	0.[illegible]	0.0003	0
7	X20	0.0240	0.1533	X30	X2	X13	X12[a]	0.[illegible]	0	0
8		0.0298	0.0529					0.[illegible]	0	0.00002
Geometric Mean** Frequency of Infection - Chr[illegible]ic infection										
	0.04%	0.0360	0.0800	0.06%	0.02%	0.08%	0.007%	0.[illegible]	[illegible]01	0.00001

X25[a] (0.005%)

* One cannot rule out the possibility of low but nonzero infection [illegible] cases where estimates were [illegible], as shown by the upper c[illegible]nfidence bounds.

** Numbers denotes the frequency of infection found in myeloid lysate w[illegible]h T-cell receptors present.

*** Cell lysate have not been analyzed for T-cell receptors

<4 fold <6 fold ≤4 fold

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Stable Latent HIV-1 Reservoir

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Gut Associated Lymphoid Tissue

Patient	1	2	3	4	5	6	7	8
	Central/Transitional Memory (CD45RO+ CD27+)		Effector Memory (CD45RO+ CD27-)		Naive (CD45RO- CD27+)		Myeloid (CD3- CD4+)	
	Time Point 1	Time Point 2	Time Point 1	Time Point 2	Time Point 1	Time Point 2	Time Point 1	Time Point 2
	Estimated Frequency of Infection (%)*							
Initiated therapy during acute/early infection								
1	0.0280	0.0373	0.0269	0.0104	N/A	0	0	0
2	0.0221	N/A	0.0390	N/A	0	N/A	0	N/A
3	0.0149	0	0.0304	0.0573	0	0	0	0
4	0	0	0.0132	0.0032	0	0	0	0
5	0.0019	0.0143	0.0228	0.0377	0.1365	0	0.0081*	0
Geometric Mean Frequency of infection - Early infection								
	0.008%	0.004%	0.02%	0.03%	0.0260	0	0.0024	
Initiated therapy during chronic infection								
6				0.0870	0.6975	1.6129	0.0297	0.0025**
7	X5	X5	X7[a]		0.0962	N/A	0.0277	N/A
8				X1.3	0	0	0.0223	0
Geometric Mean Frequency of Infection - Chronic infection								
	0.04%	0.02%	0.15%	0.04%	0.0520	0.1700	0.0260	

*Numbers denotes the frequency of infection found in myeloid lysate with T-cell receptors present.

**Cell lysate have not been analyzed for T-cell receptors

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<4 fold Geometric Mean

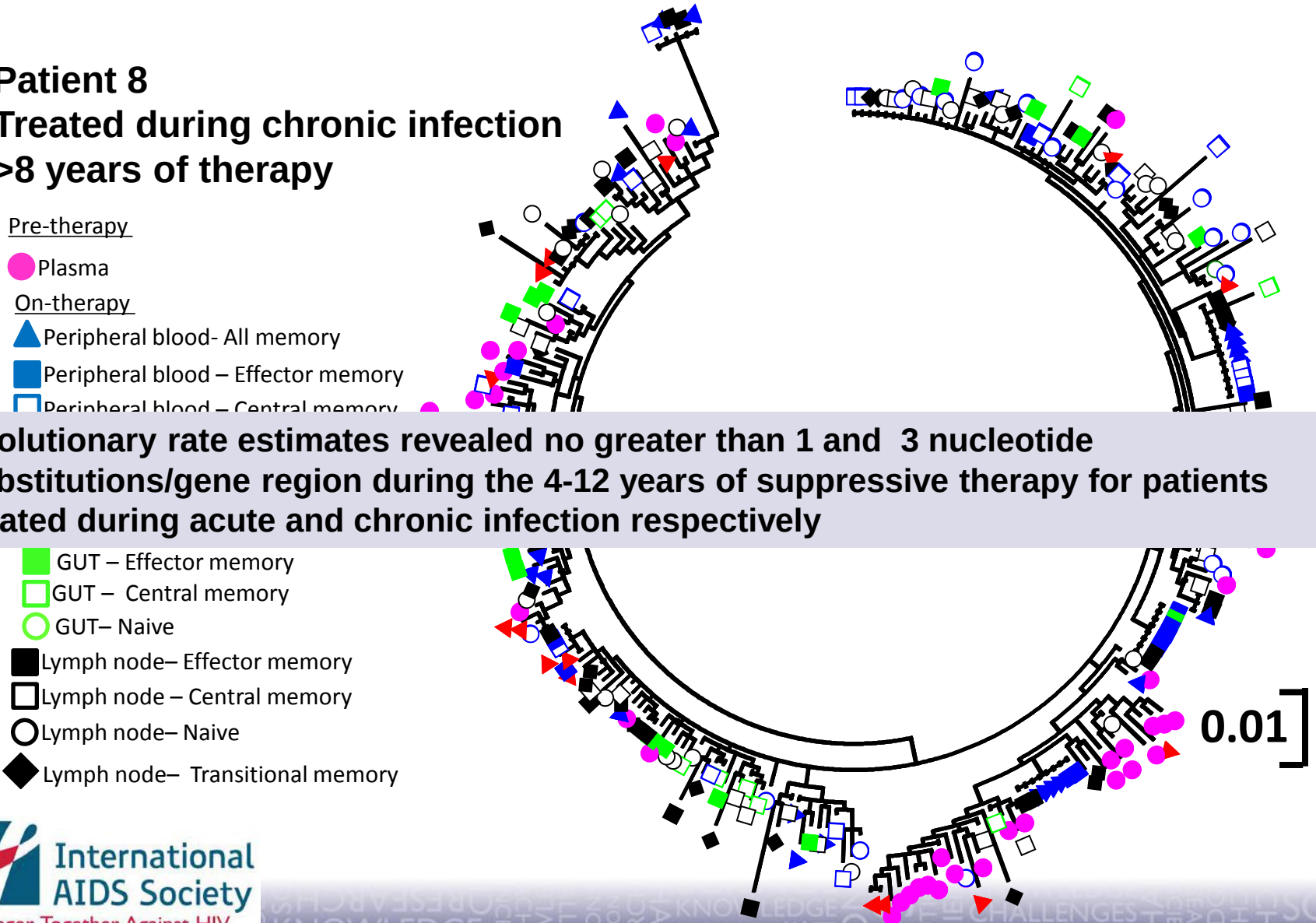
Percentage of the total viral reservoir contributed by different T cell subsets Time Point 1

1	2	3	4	5	6	7
Patient	Over all percent infection*	Lower 95% CI	Upper 95% CI	Central/Transitional Memory (% contribution)	Effector Memory (% contribution)	Naive (% contribution)
Peripheral blood						
1	N/A					
2	0.0093	0.0071	0.0110	80.6	4.40	15.0
3	0.0030	0.0023	0.0036	53.2	41.5	5.30
4	0.0003	0.0002	0.0005	63.0	27.8	9.20
5	0.0420	0.0320	0.0520	62.3	36.5	1.20
6	0.0330	0.0270	0.0390	45.6	0.49	53.9
7	0.0640	0.0540	0.0740	14.1	67.5	18.4
8	0.0290	0.0240	0.0350	75.1	21.5	3.30
Mean % contribution				**56.3**	**28.5**	**15.2**
GALT						
1	N/A					
2	0.0280	0.0150	0.0450	43.90	56.10	0.0
3	0.0190	0.0050	0.0350	55.20	44.80	0.0
4	0.0055	0.0000	0.0280	0.0	100.0	0.0
5	0.0079	0.0046	0.0110	18.20	71.20	10.60
6	0.1300	0.0980	0.1600	55.30	41.60	3.10
7	0.1100	0.0810	0.1400	4.80	93.50	1.70
8	0.0430	0.0300	0.0590	28.70	71.30	0.0
Mean % contribution				**29.4**	**68.4**	**2.2**

* Central and effector memory and naive T-cells combined

Phylogenetically indistinguishable HIV-1 sequences from plasma collected before initiation of cART and cells isolated after years of suppressive therapy

Patient 8
Treated during chronic infection
>8 years of therapy



Evolutionary rate estimates revealed no greater than 1 and 3 nucleotide substitutions/gene region during the 4-12 years of suppressive therapy for patients treated during acute and chronic infection respectively

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Phylogenetically indistinguishable HIV-1 sequences from plasma collected before initiation of cART and cells isolated after years of suppressive therapy

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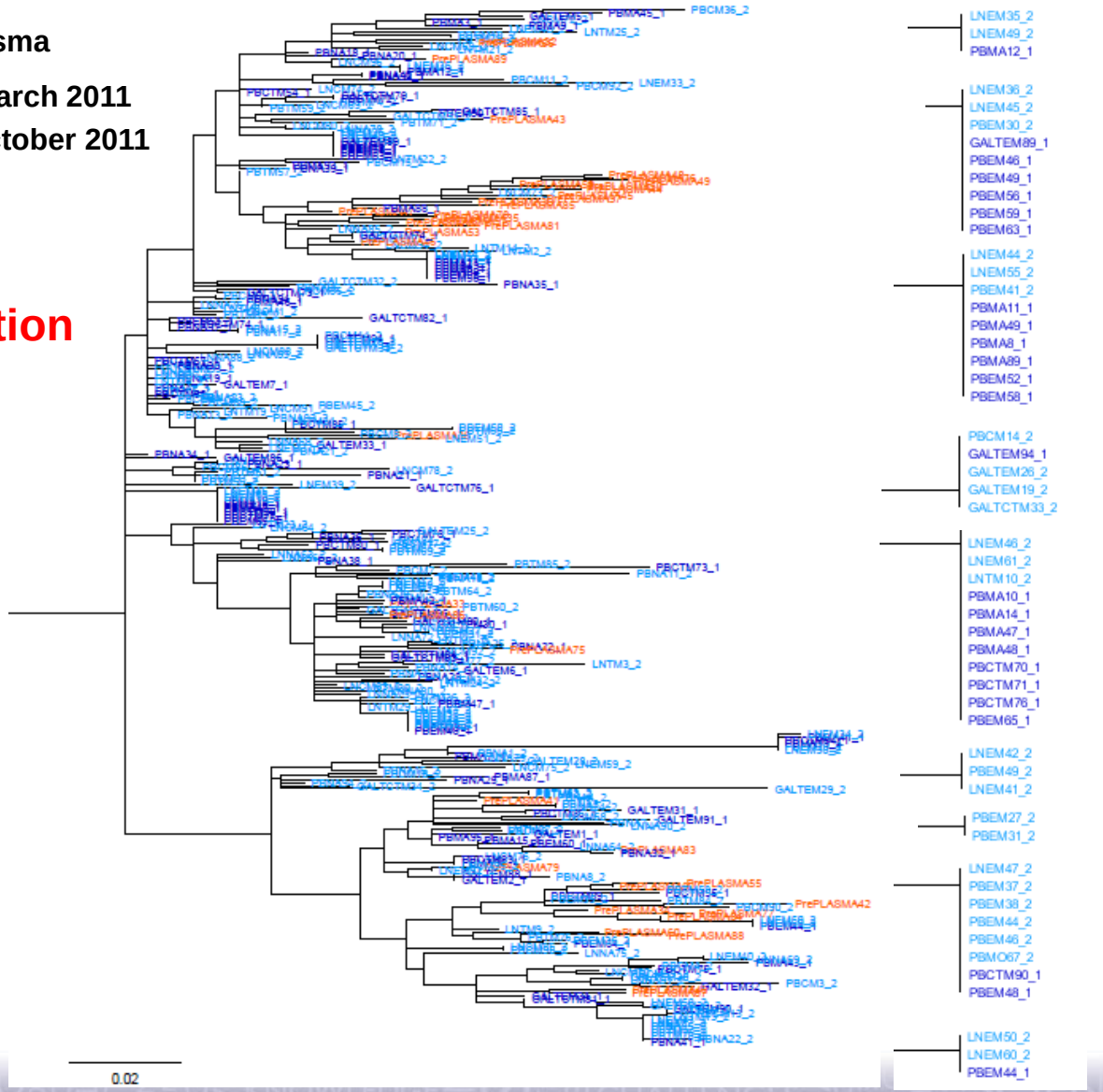
- HIV RNA sequences from pretherapy plasma
- Intracellular HIV DNA sequences from March 2011
- Intracellular HIV DNA sequences from October 2011

Patient 8

Time Point 1 and 2

Treated during chronic infection

>8 years of therapy



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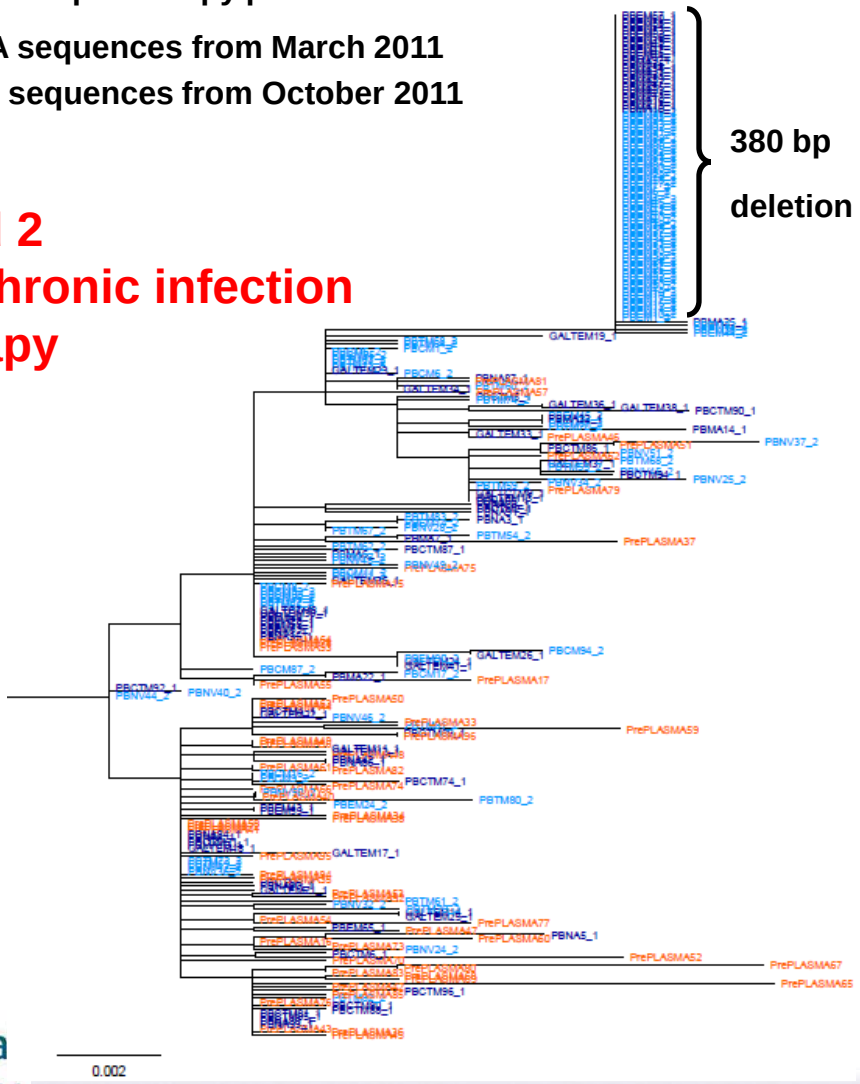
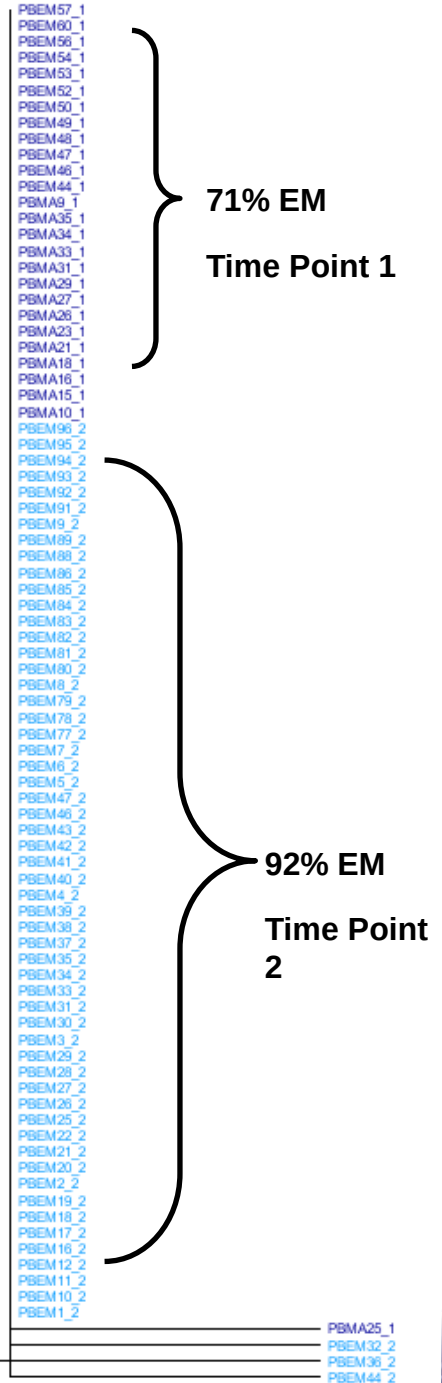
Phylogenetically indistinguishable HIV-1 sequences from plasma collected before initiation of cART and cells isolated after years of suppressive therapy

- HIV RNA sequences from pretherapy plasma
- Intracellular HIV DNA sequences from March 2011
- Intracellular HIV DNA sequences from October 2011

Patient 7
Time Point 1 and 2
Treated during chronic infection
>4 years of therapy





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Conclusions

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1. **The pool of HIV-infected resting memory CD4+ T cells did not change dramatically over 6 months in different tissue compartments.**

2. **This reflects a relatively stable HIV-infection frequency during suppressive therapy with the early initiation of effective therapy resulting in a lower reservoir size.**

3. **These longitudinal studies revealed the expansion of some HIV genetic populations and the contraction of others with little evidence of viral evolution. For example, a clonal species containing a 380bp deletion increased from 71% to 92% over 6 months in effector memory T cells of one patient but the overall infection frequency of these cells remained the same.**

4. **Taken together, these findings indicate that persistent HIV infection is maintained in an asynchronous manner across different cell types and that viral replication is not a major cause of persistent HIV infection.**

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to find a cure

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Patient Details

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Patient	Viral Load Pre Therapy (RNA copies/ml)	Viral Load On Therapy* (RNA copies/ml)	CD4+ T-cell Count Pre Therapy (cells/ul)	CD4+ T-cell Count On Therapy (cells/ul)	Lenght of infection (years)	Time on therapy (years)
Treated during acute/early infection						
1	88359	<40	165	470	8.6	8.5
2	44960	<40	494	1048	7.9	7.6
3	40930	<40	792	1279	12.8	12.5
4	3583	<40	648	867	6.7	6.6
5	118888	<40	243	571	4.3	4.3
Treated during chronic infection						
6	85999	<40	406	491	7.3	5.3
7	74117	<40	400	726	11.4	9.8
8	70510	<40	342	891	10.6	8.8

* Viral RNA levels at the time of the study

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Higher genetic diversity in cells isolated from patients treated during chronic infection compared to patients treated during acute/early infection

Genetic Diversity of HIV-1									
Patient		Pre-therapy	Time point 1- on therapy			Time Point 2- on therapy			
		Plasma	Overall	PB	GALT*	Overall	PB	GALT*	LN
Treated during early infection									
1	OP800	1.30%	1.2%	1.30%	1.00%	1.60%	1.60%	1.80%	N/A
2	OP875	0.20%	0.12%	0.11%	0.15%	0.40%	0.40%	N/A	N/A
3	OP268	0.05%	0.08%	0.09%	0.03%	0.10%	0.10%	0.019%	N/A
4	OP1383	0.12%	0.12%	0.11%	1 seq	0.10%	0.10%	N/A	0.10%
5	OP1559	0.10%	0.13%	0.12%	0.14%	0.20%	0.16%	0.61%	N/A
Treated during chronic infection									
6	OP1337	0.97%	0.78%	0.73%	0.86%	0.81%	0.82%	0.64%	N/A
7	OP443	0.98%	0.96%	0.92%	1.00%	0.81%	0.81%	N/A	N/A
8	OP548	1.21%	1.26%	1.27%	1.21%	1.31%	1.35%	1.52%	1.25%

* Few sequences from GALT were obtained.

N/A Not avaliable