

Activation of an HIV-1 Reservoir During Hematopoietic Progenitor Cell Mobilization

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Introduction

- ACTG 285 evaluated the safety and efficacy of stem cell mobilization with G-CSF in HIV-1 infected subjects. ¹
- Eighteen HIV-1 infected persons received 10 µg/kg/day recombinant G-CSF (Filgrastim), for 7 days. On days 4 and 5 PBMC were harvested by leukapheresis. ¹
- For 9 of 18 subjects, plasma HIV-1 levels increased $> 0.6 \log_{10}$ above baseline between days 4 and 7 and returned to baseline by day 27. ²

¹ Schooley, *JID* 2000. ² Campbell, *Blood* 2000.

Objectives:

To investigate the source of increased HIV-1 replication during stem cell mobilization

- Analyze the genetic diversity of the G-CSF induced plasma virus quasispecies.
- Determine the relationship of G-CSF induced virus to pretreatment plasma and PBMC quasispecies virus.

Methods: subject characteristics

Subject*	Age (y)	CD4+ Count† (cells/uI)	HIV-1 RNA‡ (log ₁₀ copies/ml)	log ₁₀ HIV-1 RNA Viral Load Increase Above Baseline §	Antiretroviral Therapy□	Fraction Mono-phyletic§
61146	58	1270	<1.70	0.71	None	ND
61149	49	368	3.51	0.81	None	ND
610219	36	499	4.12	0.69	None	0/10
620745	37	798	3.66	1.05	None	6/10
61147	43	307	3.72	1.01	ZDV/3TC/SQV	5/10
610051	38	338	2.70	2.08	ZDV/3TC/IDV	7/10
620743	42	33	4.09	0.83	ZDV/3TC/SQV	0/10
61150	48	153	<1.70	1.11	D4T/3TC/SQV	ND
620495	38	230	<1.70	0.62	ZDV/3TC/IDV	ND

* All subjects were male

† Baseline values for CD4+ lymphocyte counts are averages of values on days -3 and 0

‡ Baseline plasma HIV-1 RNA values are the average of log₁₀ values on days -3 and 0

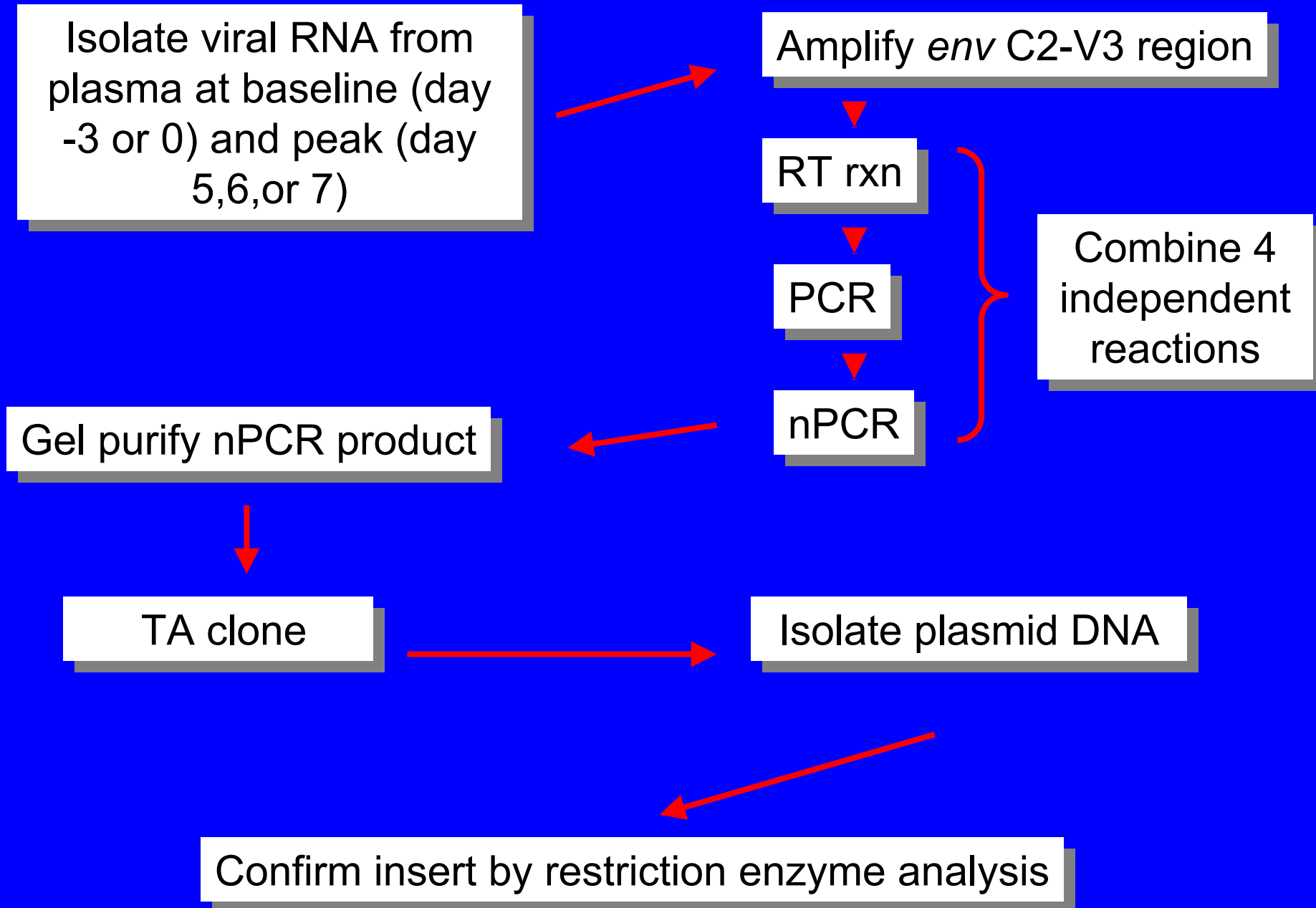
§ log₁₀ values equal viral load at peak minus viral load at baseline

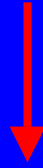
□ Antiretroviral treatment regimen for days -60 through 27

ZDV, zidovudine; 3TC, lamivudine; IDV, indinavir; SQV, saquinavir

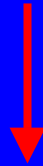
§ Fraction of post-G-CSF virus that grouped in unique phyletic clusters, ND = not done

Methods: virus to molecular clone

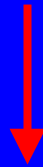




Obtain nucleotide sequence of 416 bp insert of 10 clones from pre and peak plasma samples

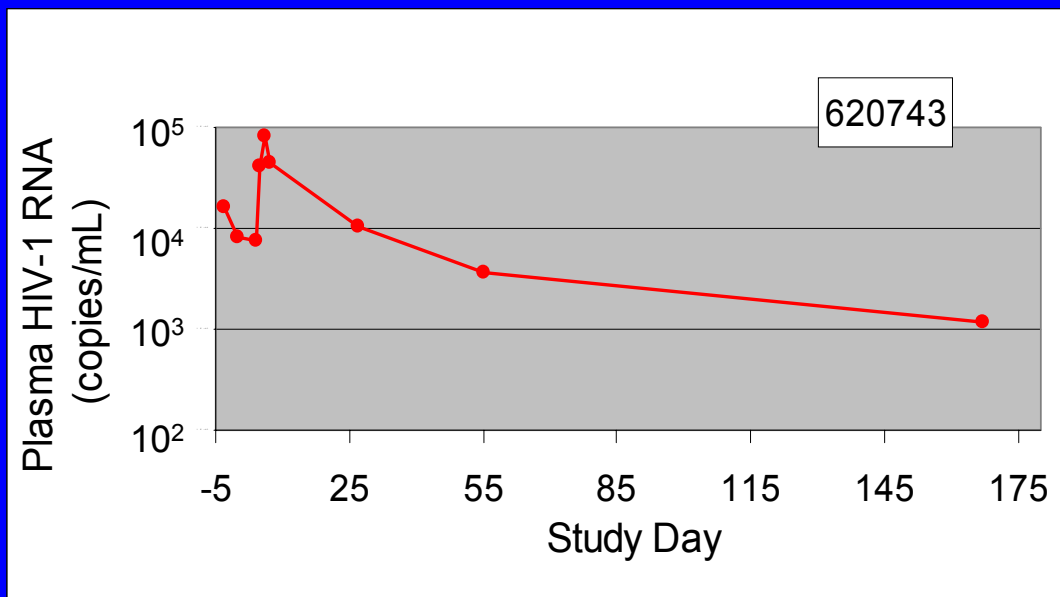
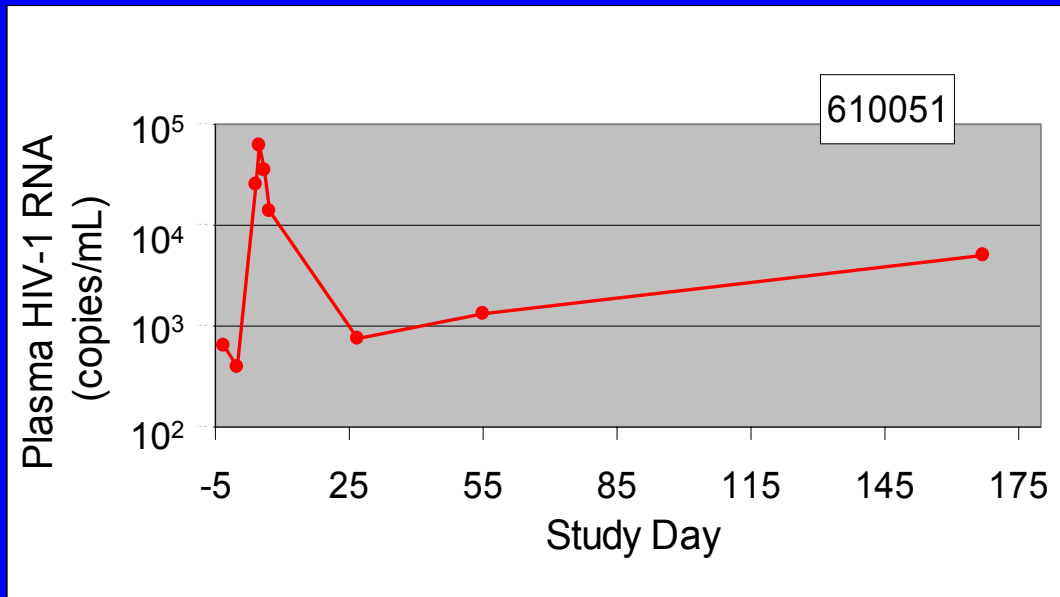


Edit and align sequences
(Sequencher 4.05, BioEdit 1.5, Clustal W)

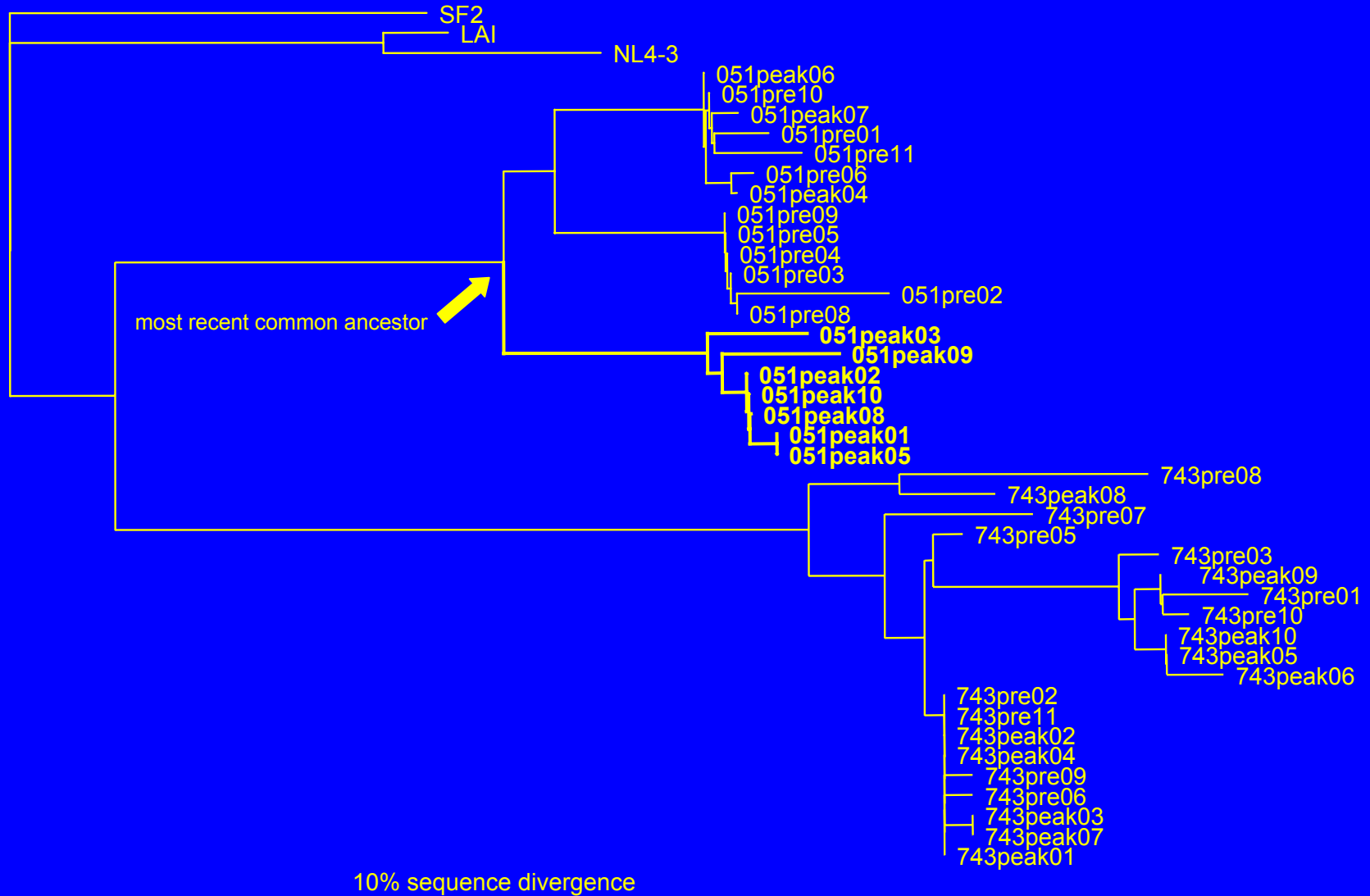


Determine genetic relationships by neighbor-joining methods (PHYLIP 3.5, TreeView 1.6.6)

Viral load change during stem cell mobilization: 2 subjects



Phylogenetic tree of pre G-CSF and peak G-CSF HIV quasispecies: 2 subjects



Characteristics of G-CSF induced HIV-1 in 3 subjects

Subject	Plasma HIV-1 RNA (copies/mL)				Fraction Monophyletic ⁵	Signature Nucleotides ⁶
	Baseline ¹	Maximum ²	Difference ³	Unique Virus ⁴		
620745	4633	51,378 (day 7)	46746	30827	37417	129T, 130G
610051	520	60,363 (day 5)	59844	42254	37447	224C, 230C, 238A, 239T, 292G, 343G, 351T, 354C
61147	4506	53,835 (day 6)	49329	26198	37386	258C, 273C, 289A, 290C

¹Average of plasma HIV-1 RNA on days -3 and 0. ²Peak plasma HIV-1 RNA. ³Difference between peak and baseline plasma HIV RNA. ⁴Amount of plasma HIV-1 RNA that is attributable to the unique monophyletic virus that appeared during G-CSF mobilization. Calculated as the product of the fraction monophyletic and the maximum plasma HIV RNA. ⁵Fraction of post-G-CSF virus that grouped in unique phyletic subclusters. ⁶Nucleotides present in all clones that were grouped in unique post-G-CSF monophyletic clusters, but not present in any of the baseline clones. Numbers indicate the nucleotide position in the CLUSTAL W alignment of cloned envelope fragments.

Conclusions

- The predicted V3 loop amino acid sequence for all genotypes (pre and peak) were consistent with CCR-5 co-receptor utilization.
- Stem cell mobilization with G-CSF caused a rapid change in plasma quasispecies composition in 3 of 5 subjects.
- Changed quasispecies composition was associated with a new majority virus (50-70%), not detected at baseline.