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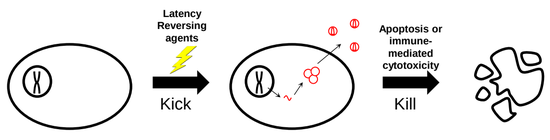
Reversal of HIV-1 latency by CD4+ T-cell activation results in clonal expansion and sustained production of infectious virus in a subset of cells

John Bui[1,2], Elias Halvas[1], Elizabeth Fyne[1], Michele Sobolewski[1], Diana Koontz[1], Mary F. Kearney[3], Wei Shao[4], Feiyu Hong[1], John W. Mellors[1]

[1]University of Pittsburgh, Pittsburgh, PA; [2]Howard Hughes Medical Institute, Bethesda, MD; [3]National Cancer Institute (NCI), Frederick, MD; [4]Leidos, Frederick, MD
Correspondence to John Bui University of Pittsburgh, 3550 Terrace St, Scaife Hall, suite S813, Pittsburgh, PA 15261; JOB74@pitt.edu

Background

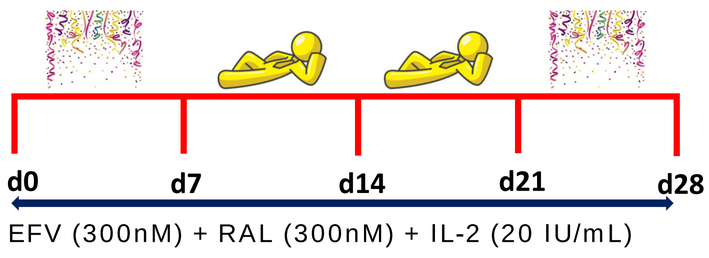
- The major barrier to an HIV-1 cure is the latent reservoir, which refers to stably infected cells in ART treated patients.[1]
- The "kick-and-kill" strategy, consisting of latency reversal followed by death of cells with activated proviruses, has been proposed as a means of eliminating the HIV-1 reservoir.[2]



- The most effective latency reversing agents are also potent T-cell activators.[3,4]
- Although rapid cell death is a common fate of activated HIV-infected T cells,[4,5] recent studies show that virus-producing cells can persist and expand *in vivo*.[6,7]
- The present study explores whether activation of CD4+ T-cells from chronically suppressed HIV-infected donors can lead to clonal expansion of proviruses rather than their elimination.

Methods

- Experiments were performed in five chronically-suppressed HIV-1 infected individuals
- Total CD4+ T-cells (tCD4) were isolated from large volume blood-draw and stimulated with PMA/ionomycin (50/500 ng/mL) between days 0-7 and days 21-28.
- Experiments were also performed with peripheral blood mononuclear cells (PBMC) in donors 2 and 5.



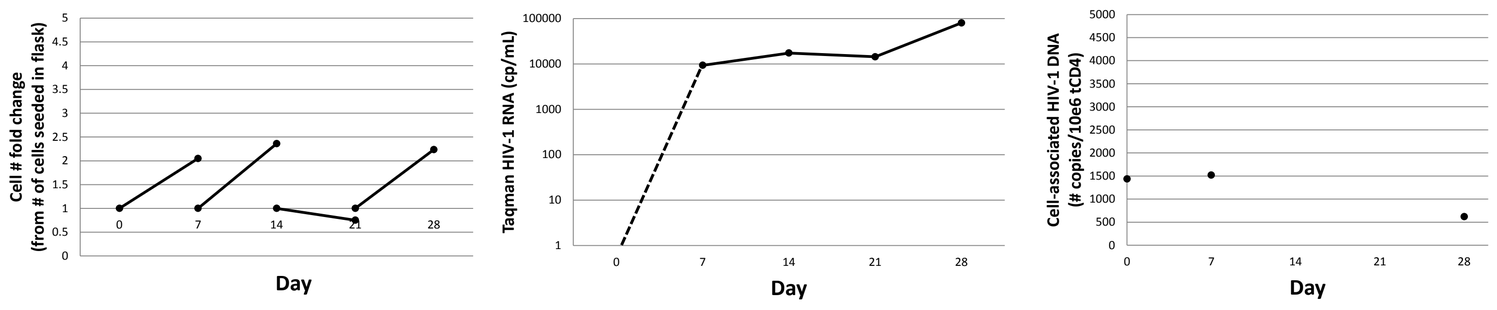
- Replication-competence of virions produced from total CD4+ T-cells in donor 2 was determined by the viral outgrowth assay.
- HIV-1 RNA in cell-free supernatants was quantified by qRT-PCR using the AmpliPrep/COBAS TaqMan assay.
- HIV-1 cell-associated DNA (CAD) was quantified by in-house qPCR
- Single Genome Sequencing (SGS) was performed to characterize proviruses and virion RNA.

Results

Table 1. Donor Clinical Characteristics.

Donor	Age	Race	Gender	Years infected	Years suppressed	Current CD4	Pre-ART VL	Nadir CD4
1	42	African American	Male	20	2	524	--	210
2	56	African American	Female	25	14	1505	366,200	410
3	59	African American	Male	22	18	1023	117,068	314
4	52	Caucasian	Male	27	18	585	99,985	153
5	57	African American	Female	25	15	--	13,048	13

Figure 1. Representative Data of Cell Number, Virion RNA, and Cell-Associated DNA from Donor 2.



- In all experiments, HIV-1 RNA levels in supernatant increased following initial stimulation, decreased during the rest period, and increased again with restimulation.
- Cell-associated HIV-1 DNA levels did not show a consistent pattern of change.

Figures 2-8 display representative phylogenetic trees constructed for donor 2. Each figure displays a different fate of stimulated proviruses.
Legend for Figures 2-8: ● = day 0 CAD; ▲ = day 7 CAD; ■ = day 28 CAD; ▲ = day 7 supe; ▽ = day 14 supe; ◇ = day 21 supe; ■ = day 28 supe; * = replication-competent, determined by viral outgrowth assay

Figure 2. *In vivo* clonal expansion.

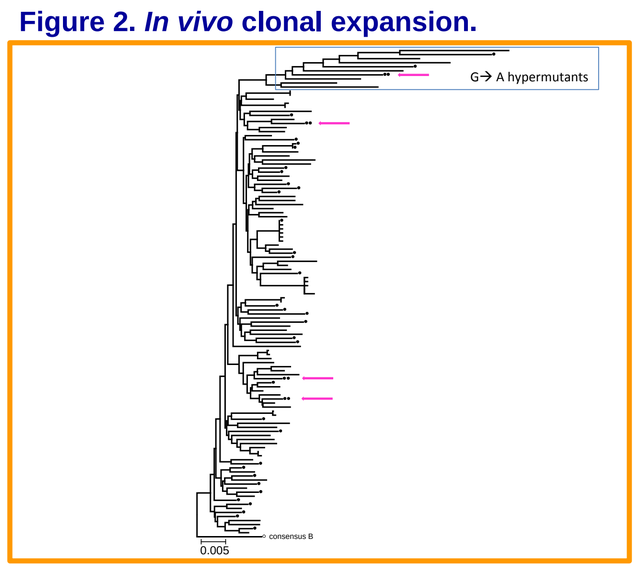


Figure 3. *In vitro* clonal expansion.

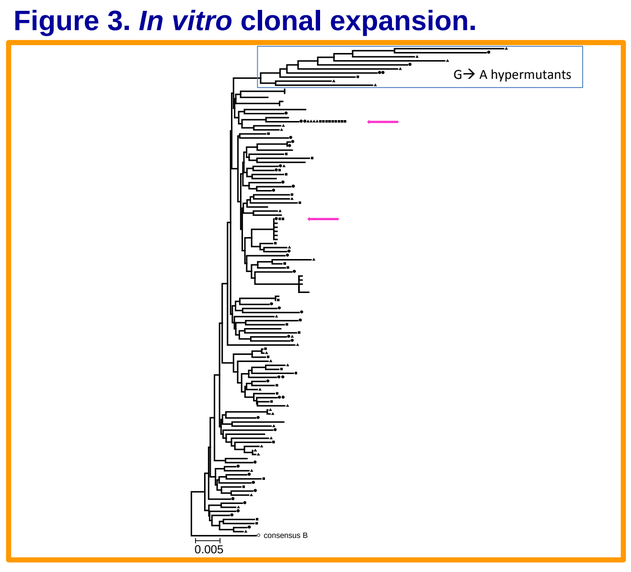


Figure 4. Virions detected only following the first stimulation.

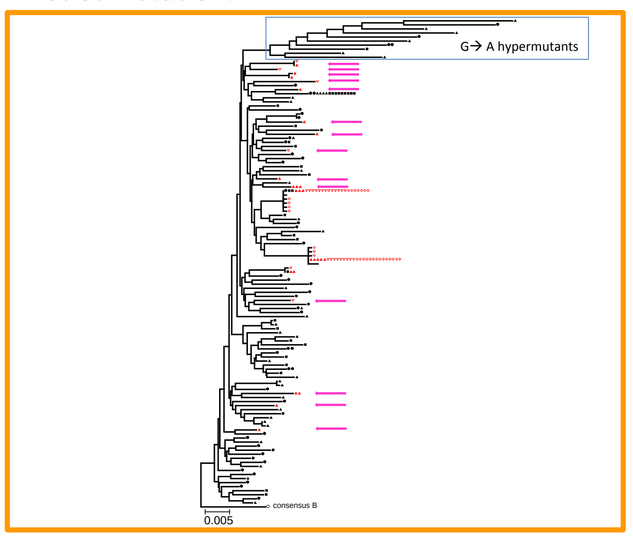


Figure 5. New virions detected following the second stimulation.

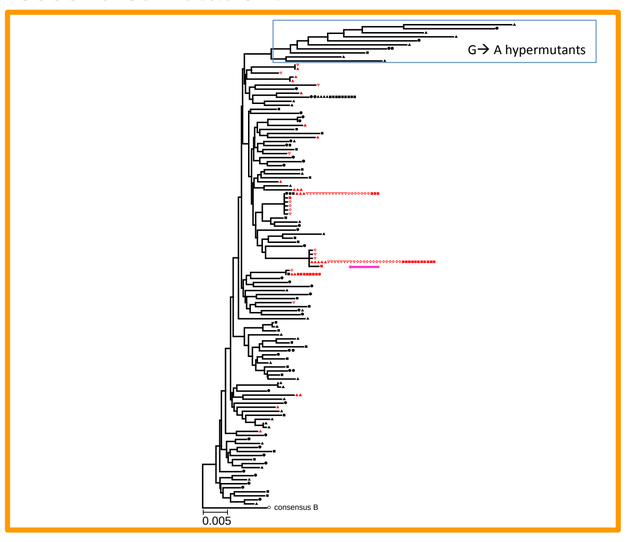


Figure 6. Virus production following both stimulations.

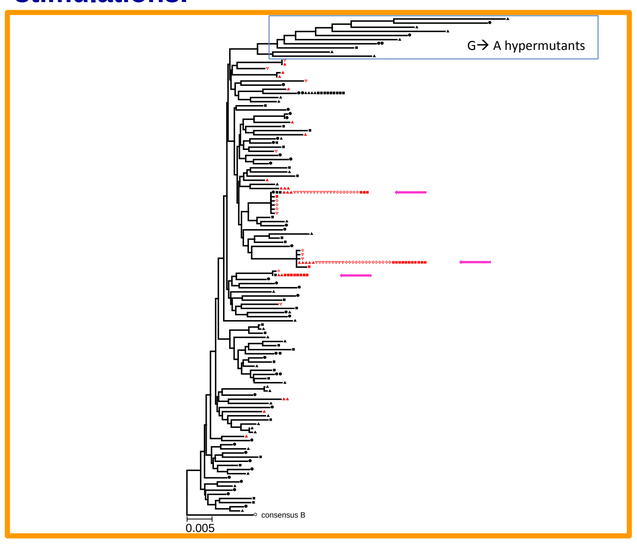


Figure 7. No virus production with either stimulation.

Figure 8. *In vitro* clonal expansion of non-producing proviruses.

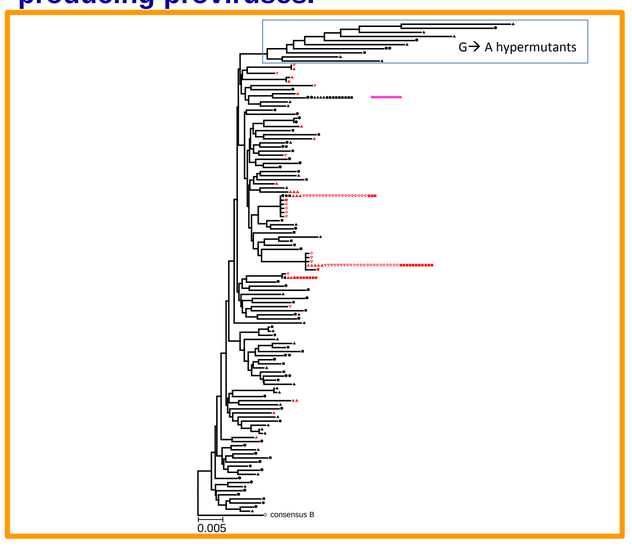
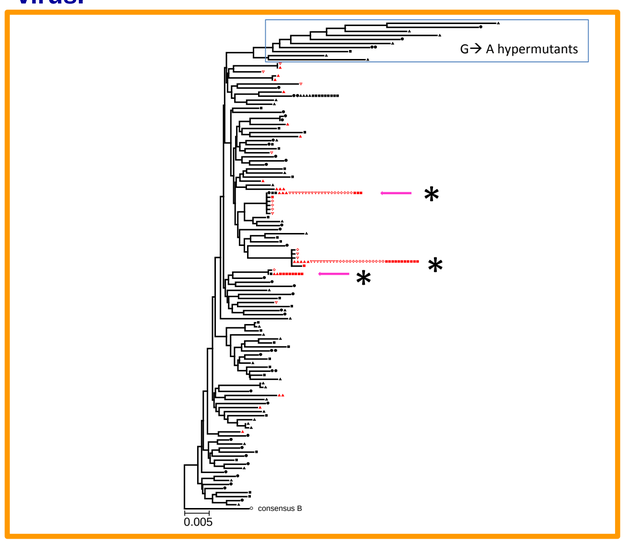


Figure 9. *In vitro* clonal expansion of producing proviruses, including replication-competent virus.



Conclusions

Table 2. Results Summary. ■ = not observed

		Donor 1 tCD4	Donor 2 tCD4	Donor 2 tCD4 (repeat)	Donor 2 PBMC	Donor 3 tCD4	Donor 4 tCD4	Donor 5 tCD4	Donor 5 PBMC
% of unique produced virions	Virions produced only after first stimulation	80.0%	77.8%	88.5%	55.6%	60.0%	65.2%	83.3%	50.0%
	Virions produced only after second stimulation	8.0%	5.6%	3.8%	22.2%	20.0%	21.7%	16.7%	50.0%
	Virions produced with both stimulations	12.0%	16.7%	7.7%	22.2%	20.0%	13.0%	0.0%	0.0%
% of unique proviruses	In vivo clonal expansion	1.1%	4.4%	2.5%	2.4%	3.7%	7.9%	0.0%	6.1%
	Proliferation of non-inducible proviruses	8.7%	1.1%	6.3%	6.1%	9.9%	5.3%	10.5%	3.0%
	Proliferation of proviruses producing virions	1.1%	2.2%	0.0%	0.0%	1.2%	1.3%	1.8%	3.0%

- SGS reveals complex proviral dynamics after cell activation:
  - Many proviruses do not produce virus, consistent with the high frequency of defective proviral genomes.[8]
  - A subset of proviruses show no virion production following repeat stimulation, suggesting death of cells containing that proviruses.
  - New proviruses can be expressed with repeat stimulation, consistent with previous studies.[8]
  - A subset of proviruses are expressed with both stimulations.
  - Non-producing proviruses can proliferate.
  - Inducible proviruses can persist and proliferate, including those that are replication-competent.
- **Reversal of HIV-1 latency by CD4+ T cell activation results in multiple outcomes of proviruses, including clonal expansion of proviruses that can produce infectious virions.**
- These findings underscore the complexity of eliminating HIV reservoirs and the need for **strategies to kill virus-producing cells before they can proliferate.**

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Acknowledgements

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