Purging HIV-1 from Latent Reservoirs Using Human Methyltransferase Inhibitors

S Samer[1], LB Giron[1], MS Arif[1], T Oshiro[2], MC Sucupira[1], A Duarte[2] and RS Diaz[1]

[1]*Retrovirology Laboratory, Paulista school of Medicine, Federal University of São Paulo, Brazil*

[2]*Laboratory of Investigation in Dermatology and Immunodeficiencies – LIM 56, Department of Dermatology, School of Medicine – University of São Paulo – Brazil*

Corresponding author - **Ricardo S. Diaz**

E-mail: **rsdiaz@catg.com.br**

Phone +55 11 991090445; Fax +55 11 55712130

Rua Pedro de Toledo, 669 – 6 andar - 04039-032 São Paulo – SP – Brazil

Background

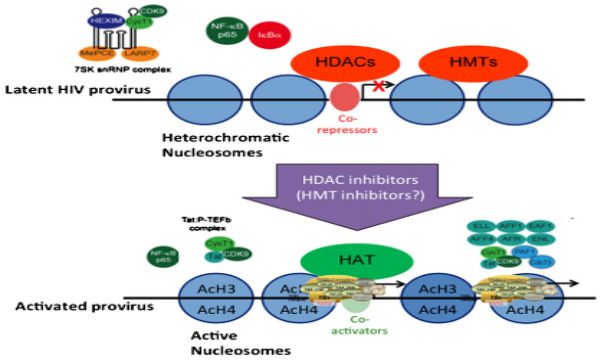
Transcriptional shutdown and multistep formation of restrictive chromatin at long terminal repeat (LTR) are two interconnected events leading to the latent stage of HIV-1 provirus[1]. At the level of chromatin, entry of HIV-1 into the latency requires recruitment of histone deacetylase type 1, histone methyltransferase Suv39H1 and heterochromatin protein HP1 to the chromatin around HIV-1 LTR[2]. So, Histone methylation is one of the most robust histone modifications, with central role in conferring epigenetic control to the chromatin template[3]. Latent HIV proviruses are silenced as a result of deacetylation and methylation of histones located at the LTR [4] Thus the chromatin remodeling plays a major role in chromatin-mediated repression or expression of the HIV-1 promoter. Here, we evaluated the potential of histone methyltransferase inhibitors (HMTIs) namely Chaetocin and BIX-01294 in reactivating HIV-1 from latency. Induction through Chaetocin is associated with loss of histone H3 lysine 9 (H3K9) trimethylation at the LTR promoter and a corresponding increase in H3K9 acetylation [5]. BIX-01294, a diazepin-quinazolin-amine derivative, functions as a specific inhibitor of G9a in an uncompetitive manner with S-adenosylmethionine by binding the G9a SET catalytic domain [6].

Methodology

We used CD8+ T-cells depleted peripheral blood mononuclear cells (PBMCs) isolated from 15 HIV+ HAART-treated patients with undetectable viral load for a period over 4 years. CD4 nadir of the patients varied from 50 to 836 (median=265; mean=340) cells/µl. We measured HIV-1 recovery in ex-vivo cell cultures first activated by PHA for one day and then treated with chaetocin and BIX-01294 and cultivated in RPMI medium supplemented with IL-2 and fetal bovine serum while CD8+ T-cells depleted PBMCs activated with PHA only and then cultivated in RPMI medium supplemented with IL-2 and fetal bovine serum were used as a control samples.

Results

HMTIs induced purging in 11 out of 15 subjects. Second day after treatment with the drugs, culture supernatants were tested for viral load using qPCR and the results revealed HIV-1 emergence from day 3rd -day 29th (median 09 days) with viral load from 2.2 log10 to 6.0 log10 (median of 5.7). To find a correlation between PBMC proviral load and culture positivity, qPCR was done. Proviral load varied from 28.51 to 515.90 (median=91; mean=144.21). The results showed that culture positivity is independent of proviral load, CD4+T cell nadir, time of viral load below detection limits and antiretroviral scheme (Table 1 and Figure 1). Thus the absence of virus replication could possibly be because of host restriction factors, inactivated mutations in the genome or transcriptional interference.



(David M. Knipe et al, 2013, Virology 141–156)

Subject	C-0		C-1		C-2		C-3		C-4		C-5		C-6		C-7		C-8		C-9	
ID	Day	VL	Day	VL	Day	VL	Day	VL	Day	VL	Day	VL	Day	VL	Day	VL	Day	VL	Day	VL
S-01	0	<50	3	164	7	1454	10	683												
S-02	0	<50	3	<50	8	<50	11	<50	14	<50	17	<50	21	<50	24	<50	28	<50		
S-03	0	<50	2	<50	7	<50	10	<50	15	<50	18	<50	22	<50	25	<50	29	253	31	6167
S-04	0	<50	4	<50	7	763	10	1141	14	264										
S-05	0	<50	3	165	7	8950	10	1665	14	454										
S-06	0	<50	3	<50	7	<50	10	<50	14	<50	17	<50	21	<50						
S-07	0	<50	4	195823	7	>500000	11	>500000												
S-08	0	<50	4	239	7	2419	13	1029												
S-09	0	<50	4	<50	6	280	10	829	14	1662	17	25673								
S-10	0	<50	4	<50	6	<50	10	<50	14	<50	17	<50								
S-11	0	<50	4	<50	7	<50	10	<50	14	<50	16	89	21	4032	24	5226	28	39169		
S-12	0	<50	4	<50	7	2575	10	16122	14	49746										
S-13	0	<50	4	<50	7	<50	9	<50	14	<50	17	<50	21	<50						
S-14	0	<50	3	<50	7	8529	10	503704	14	1000000										
S-15	0	<50	3	<50	7	<50	10	<50	14	<50	17	<50	21	<50	25	<50	30	<50		

Table 1: Showing the viral load of the culture supernatants. Subjects with viral rebound after the treatment with drugs are highlighted.
C = Supernatant collection; VL = Viral load (copies/ml)

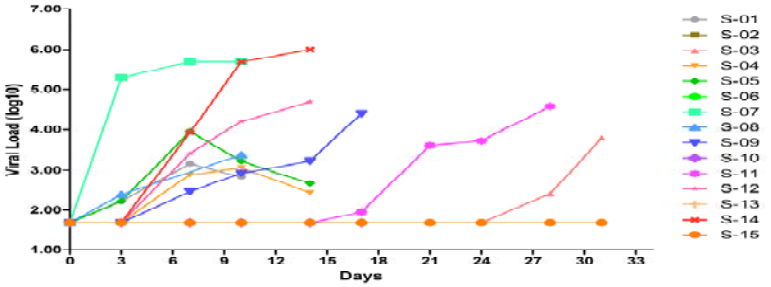


Figure 1: Graph showing the Log10 viral load (copies/ml) of the culture supernatant.

Conclusion

As part of an attempt to HIV eradication in human hosts, it would be important to overcome HIV latency which is a continuous threat. Even when the circulating virus is cleared, the threat remains and is thus one of the major obstacles towards the sterilizing cure of HIV-1. We showed here that these non-administrable HMTIs may provide a therapy to purge the dormant HIV-1 from reservoirs possibly in combination with other chromatin remodeling drugs. Therefore, clinical grade HMTIs should be synthesized or screened and evaluated to exploit their HIV reactivation potential along with potent HAART.

References

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ID	Proviral load (copies/10^6 cells)	Nadir CD4 (cells/µl)	Viral Culture	Gender	Age (yrs)	HIV Diagnosis	VL (copies/ml) before treatment	ART	Treatment initiation	Current CD4 (cells/µl)	Current VL (copies/ml)
S01	277.7	178	POS	M	52	2002	54000	TDF/3TC/ATV-R	2002	450	<50
S-02	50.3	505	POS	M	46	2010	3300	TDF/3TC/EFV	2010	505	<50
S-03	44.0	220	POS	M	51	1997	15000	K/EFV	2000	513	<50
S-04	49.0	460	POS	M	54	2005	278000	TDF/3TC/EFV	2005	1230	<50
S-05	268.0	213	POS	M	43	1995	136000	AZT/3TC/NFV, BIOEFV, BIO/IDV-R/EFV, TDF/3TC/K/EFV, TDF/3TC/K	1998	592	<50
S-06	91.0	354	NEG	M	30	2005	12000	TDF/3TC/ATV-R	2010	919	<50
S-07	42.0	836	POS	M	34	2010	62027	TDF/3TC/EFV	2011	1155	<50
S-08	106.3	259	POS	M	46	2006	375000	TDF/3TC/EFV	2007	581	<50
S-09	28.5	685	POS	M	41	2007	57800	TDF/3TC/EFV	2011	1010	<50
S-10	0.0	450	POS	M	54	2005	33000	TDF/3TC/EFV	2005	999	<50
S-11	515.9	50	POS	M	52	2000	99000	ABV/3TC/ATV-R	2001	552	<50
S-12	63.0	265	POS	M	48	2005	56000	ABV/3TC/FPV-R	2009	889	<50
S-13	0.0	330	NEG	M	42	2000	30000	TDF/3TC/ATV=R	2008	650	<50
S-14	98.0	200	POS	M	42	1998	79000	TDF/3TC/K	2009	590	<50
S-15	241.0	100	NEG	M	46	2000	55000	TDF/3TC/FPV-R	2006	652	<50

Table 2: Demographic, virologic and immunologic characteristics of enrolled individuals.

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