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Supplementary data

We tested the reactivation activity of GIBH-LRA002 in primary CD4+ T lymphocytes from four chronic HIV-1 patients, who have received prolonged HAART therapy several years and achieved an effective control of viral replication in plasma. Table 1 showed those patients' information.

Table.1 Patient information

Patient Number	Patient ID	Sex	Age (year)	ART start time (year)	ART regimen	CD4 count before ART(/ul)	CD4 count after ART (/ul)	Viral load (copies/ml)
#1	XYX	M	55	2015/4/17	TDF+3TC+EFV	385	455	<20
#2	YKM	M	39	2015/5/19	TDF+3TC+EFV	272	605	<20
#3	ZHW	M	51	2011/3/1	AZT+3TC+NVP	194	357	<20
#4	CJ	M	35	2015/7/1	TDF+3TC+EFV	314	454	<20

Experimental procedures

Chemicals

All the synthetic methods for compounds used in this article can be found our recent published work [10].

Cells and reagents

Jurkat-Lat HIV-1 full length clone A10.6 cells (harboring a single copy of latent HIV-1 LTR and one copy of GFP gene under the HIV-1 LTR control) were obtained from NIH AIDS Reagent Program and cultured in RPMI1640 medium with 10% fetal bovine serum (FBS) and 1% Pen/strep in a 37°C incubator containing 5% CO_2. For reactivation of HIV-1, Jurkat-Latcells were treated with

PMA (Sigma), Sodium Valproate (VPA, Sigma), and different compounds for 24h. HIV-1 reactivation efficacy was quantified by GFP expression using flow cytometry and the data were analyzed using FlowJo Software.Macaques' PBMCs were obtained from SIV-infected macaques which bred at the Experimental Animal Center of Guangzhou Institutes of Biomedicine and Health (GIBH, Guangzhou, China). HIV-1 patients' PBMCs were obtained from the Eighth People's Hospital of Guangzhou, China. Those cells were cultured in RPMI1640 medium with 10% FBS、1% Pen/strep、2mM L-Glutamine and 1mM Sodium Pyruvate.

CD4+ T Cell sorting, reactivation, RNA extraction and Nested real-time PCR assay for cell-associated-RNA detection

HIV patients' or SIV-infected macaques' PBMCs were isolated by standard FicollHypaque density gradient centrifugation. CD4+ T cells were sorted using a human CD4+ T cell isolation kit or non-human primate CD4+ T cell isolation kit (MiltenyiBiotec) following the standard magnetic cell sorting protocol, and then the purified CD4+T cells were counted and suspended in 200ul of medium in 96-well U-bottom plate. Usually each well has 0.5 to 2 million of CD4+ T cells. After 24 hours of stimulation with different compounds, cell-associated-RNA was extracted according to the manufacturer's protocol (Qiagen, RNeasy Mini Kit), and eluted into 30ul of RNase-free water. Then 13ul of RNA was immediately reverse transcribed following the standard process (BIO-RAD, transcription supermix for RT-qPCR). For nested real-time

PCR, cell associated cDNA or serial dilutions of cDNA standards, were directly subjected to two rounds of PCR amplification. In this study, two kinds of cell associated RNA were detected, including msRNA (multiply spliced transcripts of HIV/SIV) and usRNA (unspliced transcripts of HIV/SIV). For detection of msRNA, three primers crossing Tat and Rev for semi-nested real-time PCR were designed because of the sequence specificity of detection region. The sequence of primers performed in this experiment are listed in Table 2. The first-round PCR was performed as the followed protocol: 94℃ for 5 min, followed by 20 cycles of 94℃ for 30s, 55℃ for 15s, 72℃ for 20s, and the reaction system is 20ul of PCR mix containing 2ul of cDNA and 1U of rTaq, 250nM concentration of both primers, 250uM concentration of dNTP and relevant buffer. Then 2ul diluted product of first-round PCR was used as a template for second-round PCR, and added to 20ul reaction system containing 10ul of 2× qPCR mix (Vazyme),250nM concentration of second-round PCR primers and 150nM concentration of qPCR probe. The second-round Real-time PCR were performed as follows: 95℃ for 5min, followed by 50 cycles of 95℃ for 30s and 60℃ for 30s.

Table 2. Primers used in cell-associated RNA detection

Primer or probe		Type	Sequence(5'-3')
SIV msRNA	SIV MS-1F	Sense	GAAGAAGAACTCCGAAAAAGG
	SIV MS-R	Anti-sense	GCTGTTGCCACCGCC
	SIV MS-2F	Sense	CTAATACATCTTCTGCATCAAAC
	SIV MS-Fam	probe	FAM-ATATCCAACAGGACCCGGC-TAMRA
SIV usRNA	SIV US-1F	Sense	AAATACTTTCGGTCTTAGCTCCATT
	SIV US-1R	Anti-sense	TAATTTCCTCCTCTGCCGCTAG
	SIV US-2F	Sense	CATTAGTGCCAACAGGCTCAGA
	SIV US-2R	Anti-sense	GTTGGTCTACTTGTTTTTGGCATAG
	SIV US-Cy5	probe	Cy5-CTGTTGTTCCTGTTTCCACCACTAGGTGTC-BHQ2
HIV msRNA	HIV MS-1F	Sense	CTTAGGCATCTCCTATGGCAGGAA
	HIV MS-R	Anti-sense	GGATCTGTCTCTGTCTCTCTCTCCACC
	HIV MS-2F	Sense	ACAGTCAGACTCATCAAGTTTCTCTATCAAAGCA
	HIV MS-FAM	probe	FAM-TTCCTTCGGGCCTGTCGGGTCCC-BHQ1
HIV usRNA	HIV US-F	Sense	TCAGCCCAGAAGTAATACCCATGT
	HIV US-1R	Anti-sense	TGCTATGTCAGTTCCCCTTGGTTCTCT
	HIV US-2R	Anti-sense	CACTGTGTTTAGCATGGTGTTT
	HIV US-Cy5	probe	Cy5-ATTATCAGAAGGAGCCACCCCACAAGA-BHQ2

T cell activationby FACS

Heathy macaques' PBMCs were isolated using Ficoll-Hypaque solution, and incubated with GIBH-LRA002for 24 hours or 5 days. A cocktail of phorbol 12-myristate 13-acetate (PMA) and ionomycin was used as a positive control for T cell activation.Then, the cellswerecollected and stained with monoclonal antibodies. Samples were detected by aAccuri C6 (BD), and the data were analyzed using FlowJo Software.

Data analysis

Flow cytometry software analysis was performed using FlowJo 7.6 (Tree Star Inc.). Graphical representations were generated with GraphPad Prism 5.01 (GraphPad Software Inc., La Jolla, CA). Two-tailed P values were calculated

for all analyses, and differences were considered statistically significant when P values were 0.05.