Therapeutic options for LLV number of drugs vs drug potency

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Conflict of interests

- Consultancy fees from Mylan, Janssen and ViiV
- Speakers honoraria from ViiV, Gilead, MSD

Residual viremia and LLV: therapeutic options

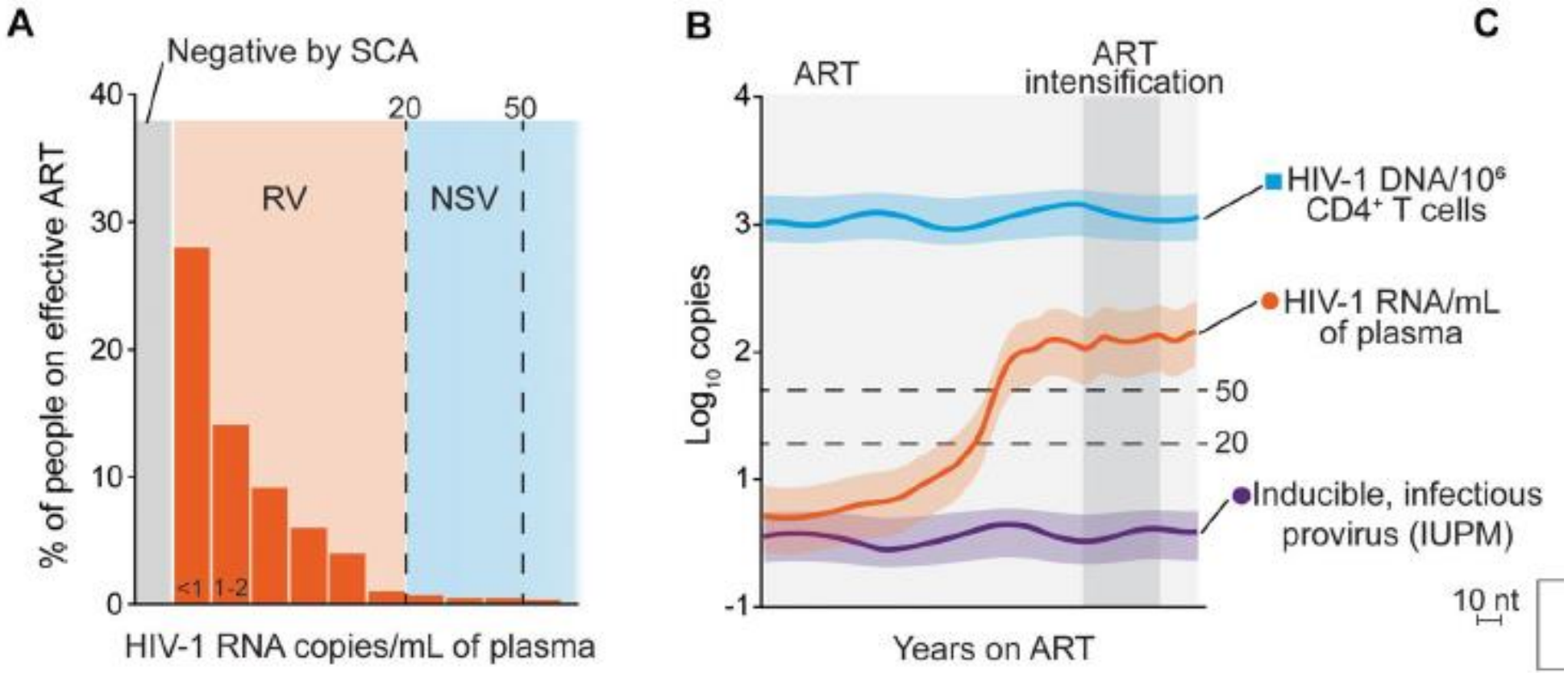
- The root mechanisms of RV have been one of the most debated topics in HIV-1 biology: is RV caused by ongoing viral replication, or is it the result of virus production from a pool of persistent cells infected before ART?
- Understanding which of these two mechanisms contributes to RV and reservoir maintenance has profound implications, as it dictates whether the development of curative strategies should focus on **antiretrovirals with better potency and tissue penetration**, or the elimination of latently infected cells

Current HIV/AIDS Reports (2023) 20:428–439

Viral replication on ART due to low **drug penetration** and exclusion of immune cells in anatomical sanctuary sites suggest that some residual low-level viral replication on ART cannot be definitively ruled out.

- lymph nodes
- cerebrospinal fluid
- adipose tissue
- gut-associated lymphoid tissue (GALT)
- urethra

Front Microbiol. 2019 Oct 15;10:2383.



Current HIV/AIDS Reports (2023) 20:428–439

LLV definitions and applications in clinical practice

- Antiretroviral therapy (ART) halts HIV-1 replication resulting in the rapid reduction of plasma viral load to undetectable levels. Some people with HIV-1 (PWH) experience viral load above limit of detection of clinical assays (20-50 copies/mL) even after years of full suppression. The following **definitions** can help to frame viremia based on its magnitude, temporal pattern, and clinical implications.
- **Low-level viremia** (LLV): HIV-1 plasma viral load above the limit of quantification of clinical assays (20-50 copies/mL), usually between **20-500 copies/mL.**
- **Viral blip**: an episode of **LLV** that is preceded and **followed by suppression** below the quantification limit.
- **Persistent LLV**: At least **two consecutive episodes of LLV**. In some individuals LLV can be persistent-intermittent, with episodic viral load below the limit of quantification and/or detection.
- **Very low-level viremia (VLLV):** HIV-1 plasma viral load **detected** by clinical assays but **below their limit of quantification** cutoffs.
- **Residual viremia**: cryptic viral load present in most individuals on ART, between **1-10 copies/mL**, is detected only by ultrasensitive assays.
- **Virologic failure**: repeated HIV-1 plasma viral load of **>200 copies/mL after >6m of ART**.

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Non suppressible viremia

The term **NSV** shifts the focus on the fact that this viremia, regardless of the amount of virus measured by clinical assays, is **not due to viral replication and cannot be further decreased by modification or intensification of ART**.

How frequent is this problem in clinical practice?

1 in 250 individuals on effective ART experiences NSV

J Clin Invest. 2020;130:5847.
J Clin Invest. 2023;133:e165245



Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy

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In HIV-1-infected individuals on currently recommended antiretroviral therapy (ART), viremia is reduced to <50 copies of HIV-1 RNA per milliliter, but low-level residual viremia appears to persist over the lifetimes of most infected individuals. There is controversy over whether the residual viremia results from ongoing cycles of viral replication. To address this question, we conducted 2 prospective studies to assess the effect of ART intensification with an additional potent drug on residual viremia in 9 HIV-1-infected individuals on successful ART. By using an HIV-1 RNA assay with single-copy sensitivity, we found that levels of viremia were not reduced by ART intensification with any of 3 different antiretroviral drugs (efavirenz, lopinavir/ritonavir, or atazanavir/ritonavir). The lack of response was not associated with the presence of drug-resistant virus or suboptimal drug concentrations. Our results suggest that residual viremia is not the product of ongoing, complete cycles of viral replication, but rather of virus output from stable reservoirs of infection.

Proc Natl Acad Sci U S A. 2009 9;106(23):9403-8.

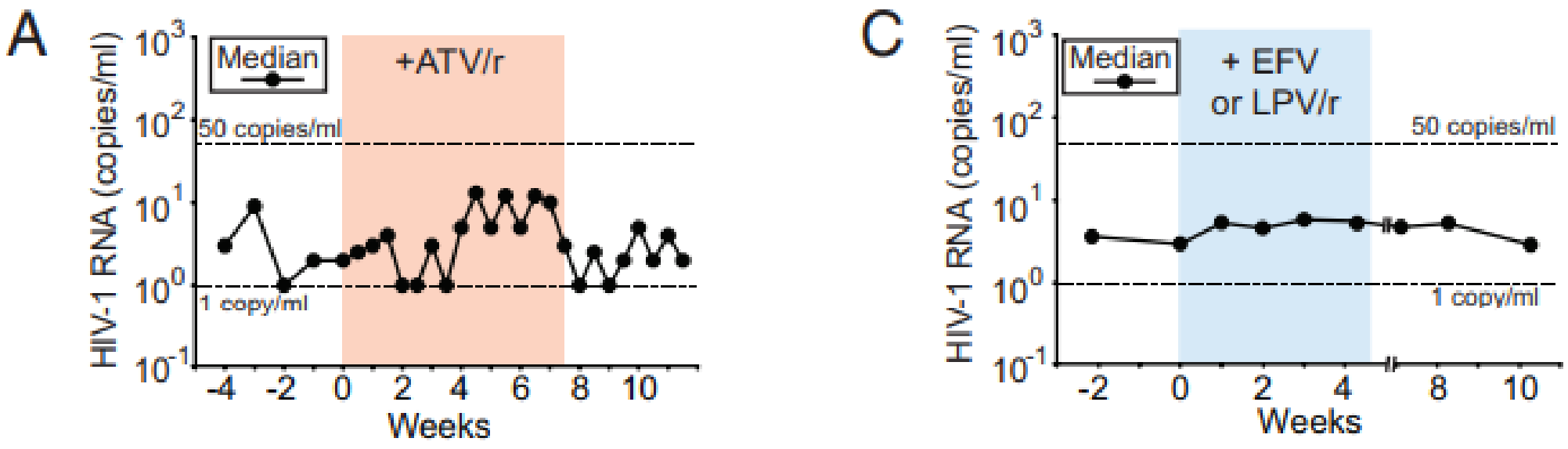
Table 1. Demographic factors and clinical characteristics

Patient ID	Age	Gender	Race	HIV-1 risk factor	CD4 count (cells/μl)	Current ART regimen	Intensifying drug
JHH-3	48	M	White	MSM	438	AZT 300 mg/3TC 150 mg twice daily, EFV 600 mg once daily	ATV 300 mg/100mg RTV once daily
JHH-5	54	M	Black	Heterosexual	540	TDF 300 mg/FTC 300 mg once daily, EFV 600 mg once daily	ATV 300 mg/100mg RTV once daily
JHH-6	46	M	Black	MSM	367	TDF 300 mg/FTC 300 mg once daily, EFV 600 mg once daily	ATV 300 mg/100mg RTV once daily
JHH-7	46	M	Black	Unknown	590	AZT 300 mg/3TC 150 mg twice daily, EFV 600 mg once daily	ATV 300 mg/100mg RTV once daily
JHH-9	60	M	White	MSM	639	ABC 600 mg/3TC 300 mg once daily, EFV 600 mg once daily	ATV 300 mg/100mg RTV once daily
NIH-1	46	M	White	MSM	431	AZT 300 mg/3TC 150 mg twice daily, ATV 400 mg daily	EFV 600 mg daily*
NIH-2	46	F	White	IVDU	743	TDF 300 mg/FTC 200 mg once daily, NFV 1250 mg twice daily	EFV 600 mg daily
NIH-4	32	M	Hispanic	MSM	536	AZT 300 mg/3TC 150 mg twice daily, TDF 300 mg once daily, EFV 600 mg daily	LPV 600 mg/150 mg RTV twice daily
NIH-5	43	M	White	MSM	799	TDF 300 mg/FTC 300 mg once daily, NVP 200 mg twice daily	LPV 600 mg/150 mg RTV twice daily

ABC, abacavir; ART, antiretroviral therapy; ATV, atazanavir; AZT, zidovudine; EFV, efavirenz; FTC, emtricitabine; IVDU, intravenous drug use; MSM, men who have sex with men; LPV, lopinavir; NA, not applicable; NIH, National Institutes of Health; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine

*ATV was changed to 300 mg once daily, 100 mg RTV was added, as recommended (30).

Proc Natl Acad Sci U S A. 2009 9;106(23):9403-8.



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Published in final edited form as:
Clin Infect Dis. 2010 March 15; 50(6): 912–919. doi:10.1086/650749.

Short-course raltegravir intensification does not reduce persistent low-level viremia in patients with HIV-1 suppression during receipt of combination antiretroviral therapy

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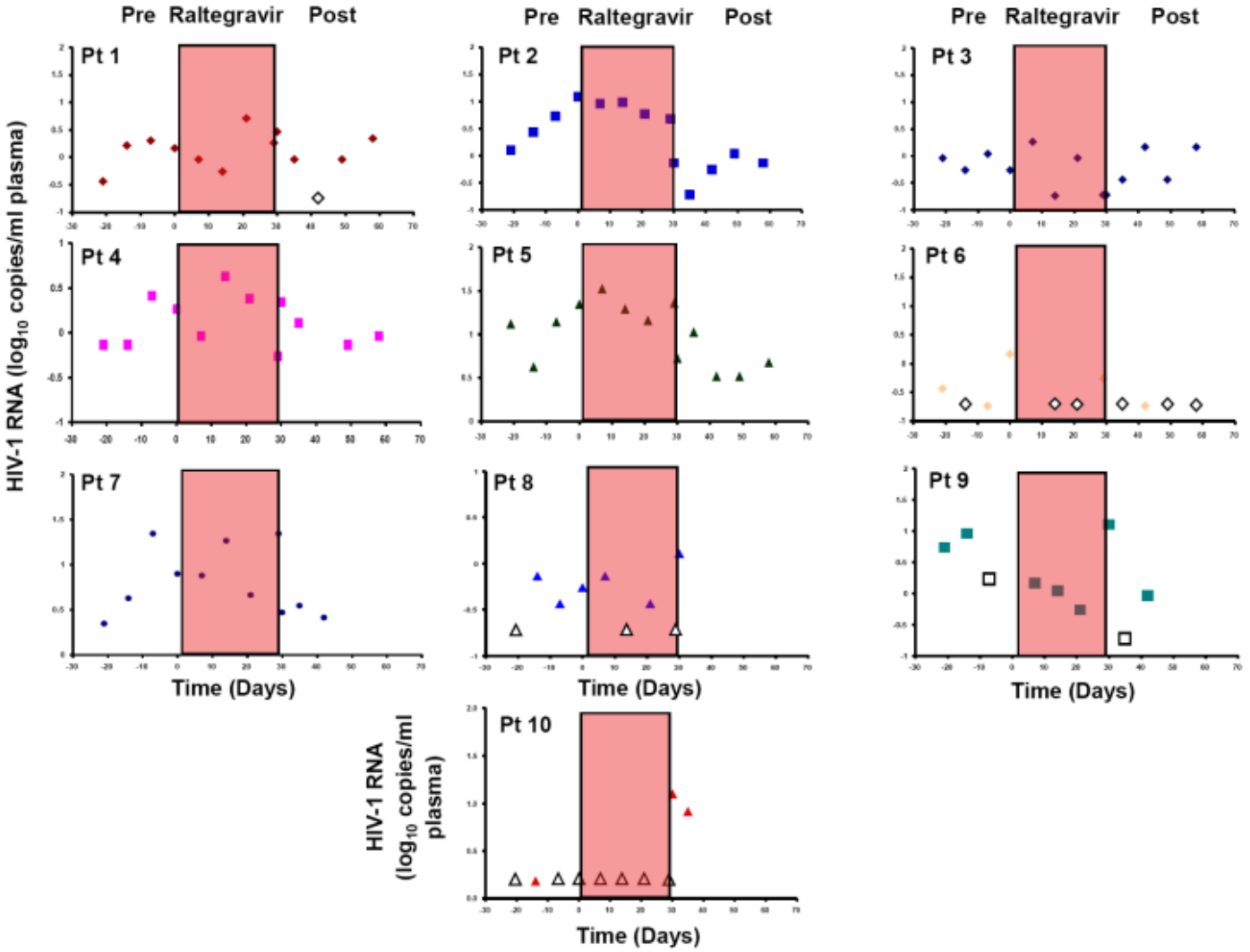
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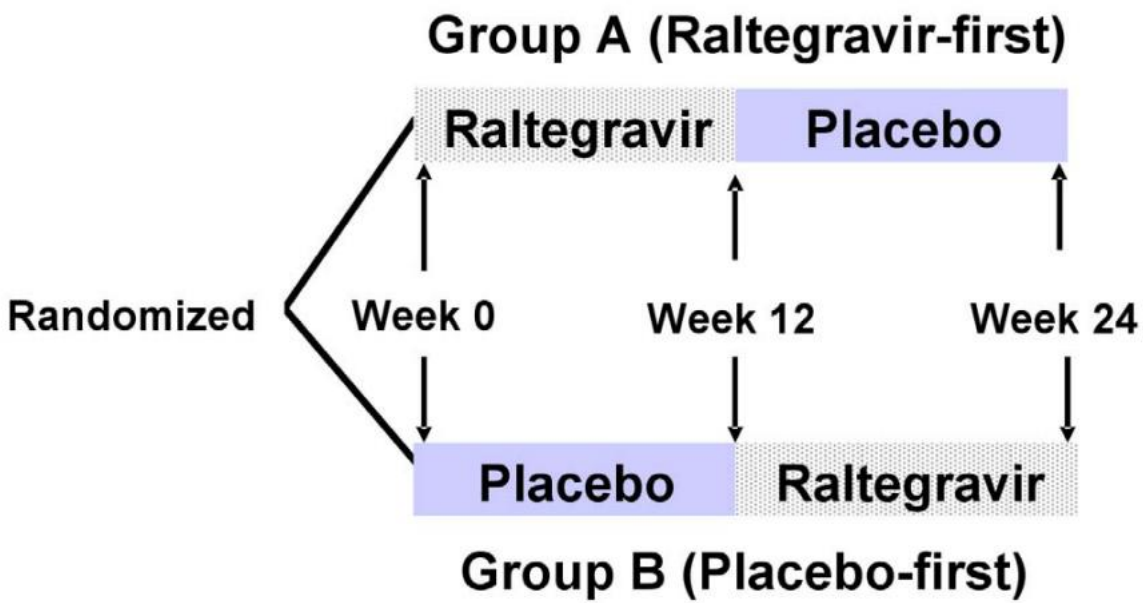
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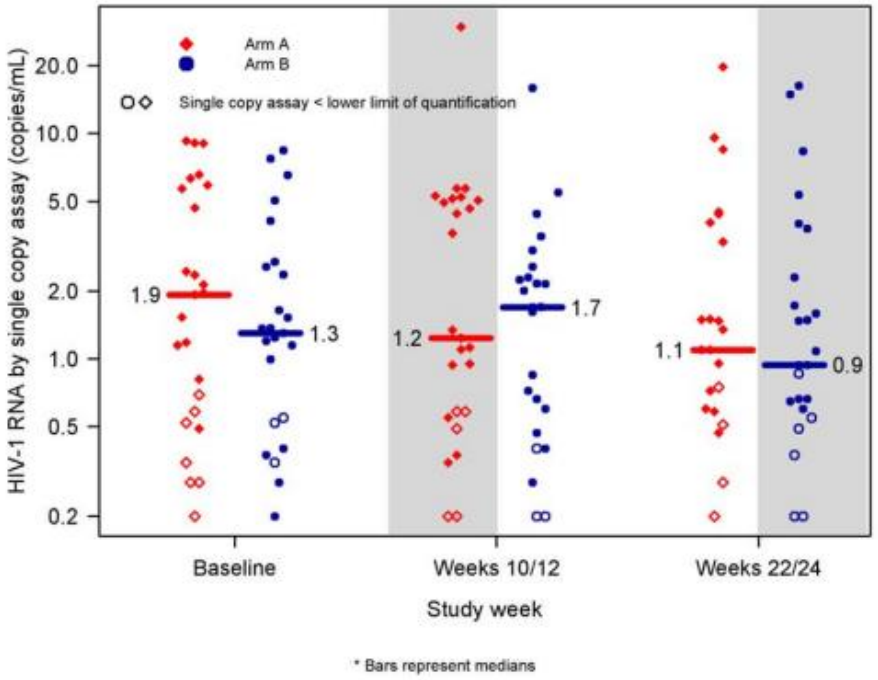
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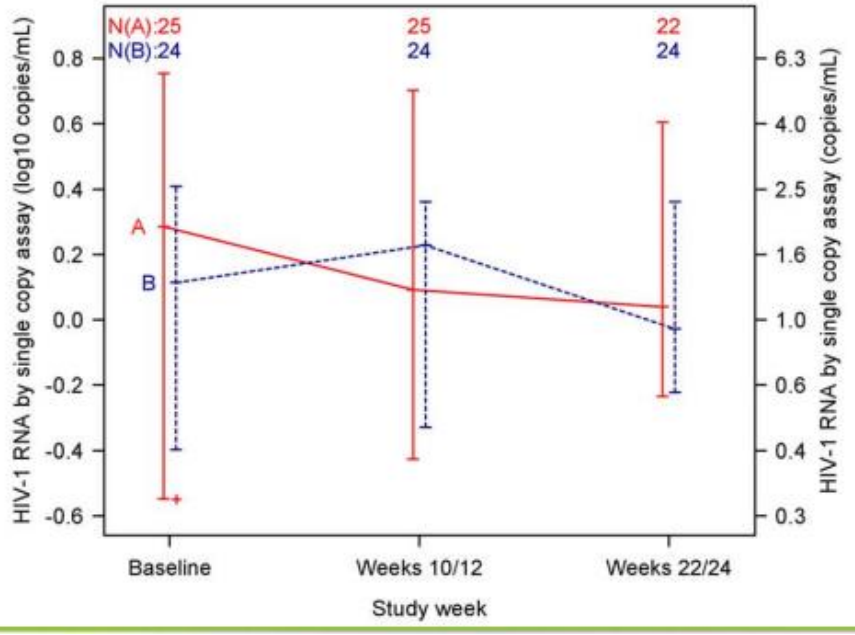
The Effect of Raltegravir Intensification on Low-level Residual Viremia in HIV-Infected Patients on Antiretroviral Therapy: A Randomized Controlled Trial

Rajesh T. Gandhi[1]*, Lu Zheng[2], Ronald J. Bosch[2], Ellen S. Chan[2], David M. Margolis[3], Sarah Read[4], Beatrice Kallungal[5], Sarah Palmer[6], Kathy Medvik[7], Michael M. Lederman[7], Nadia Alatrakchi[8], Jeffrey M. Jacobson[9], Ann Wiegand[10], Mary Kearney[10], John M. Coffin[11], John W. Mellors[12], Joseph J. Eron[3], on behalf of the AIDS Clinical Trials Group A5244 team[¶]

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PLoS Med. 2010 Aug 10;7(8):e1000321.

JCI The Journal of Clinical Investigation
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HIV-1 viremia not suppressible by antiretroviral therapy can originate from large T cell clones producing infectious virus

- At the time of referral, the **median plasma HIV-1 RNA was 87 copies/mL** (range 43–197 copies/mL), despite **1 or 2 prior regimen changes** in 5 of the 8 donors **during the duration of the nonsuppressible viremia**.
- Drug susceptibilities of the plasma viruses, predicted by **sequence analysis**, showed **no evidence of drug resistance** to each donor's current ART regimen
- **Drug concentrations** for all donors were **within the expected therapeutic range** based on target trough concentrations
- **Longitudinal analyses** of the viral sequences in all donors showed **no evidence of virus evolution** over time, indicating that these viruses originated from a stable, nonevolving reservoir of infected cells

J Clin Invest. 2020;130(11):5847–5857.

Table 2. Immunologic and virologic characteristics of donors referred for nonsuppressible viremia

Donor ID	Pre-ART Plasma HIV-1 RNA (cps/mL)	Nadir CD4⁺ T Cells (cells/mm³)	Current CD4⁺ T Cells (cells/mm³)	Plasma HIV-1 RNA at Referral (cps/mL)[A]	HIV-1 DNA (cps/10⁶ PBMCs)[B]	Cell-Associated HIV-1 RNA (cps/10⁶ PBMCs)[B]	IUPM[C]
R-09	97,000	105	380	197	1,533	139	18.1
C-03	16,700,000	10	416	62	2,505	1,162	1.4
C-02	30,375	286	1,022	184	373	29	0.1
F-07	117,068	314	1,023	52	1,603	1,112	3.8
K-01	147,189	133	533	68	1,383	74	0.6
P-08	604,000	172	444	106	1,056	382	0.4
T-05	197,826	299	1,105	113	650	109	0.4
A-06	1,877,100	251	831	43	1,825	630	0.2
Median	**172,506**	**212**	**682**	**87**	**1,458**	**261**	**0.5**

[A]Plasma HIV-1 RNA copies/mL at time of referral determined by FDA-approved Roche CAP/CTM v2.0 or Abbott M2000 platforms. [B]HIV-1 DNA and cell-associated RNA copies/million peripheral blood mononuclear cells (PMBCs) measured by quantitative polymerase chain reaction (22). [C]Infectious units per million (IUPM) total CD4⁺ T cells by quantitative viral outgrowth assay (qVOA) (23, 24).

J Clin Invest. 2020;130(11):5847–5857.

- The important implications of these findings for clinical management are that **changes in the antiretroviral drug regimen** or efforts to **enhance drug adherence** are **unlikely to change the viremia** that is being **produced by infected cell clones**, which are not affected by current antiretroviral drugs.
- Drugs that **block virion production from cells** that are already infected would be expected to lower viremia, but such agents are not currently available.
- HIV-1 **protease inhibitors** that are available **block virion maturation** into infectious particles, but **do not reduce virion production** from cells that are already infected

J Clin Invest. 2020;130(11):5847–5857.

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Clonally expanded HIV-1 proviruses with 5′-leader defects can give rise to nonsuppressible residual viremia

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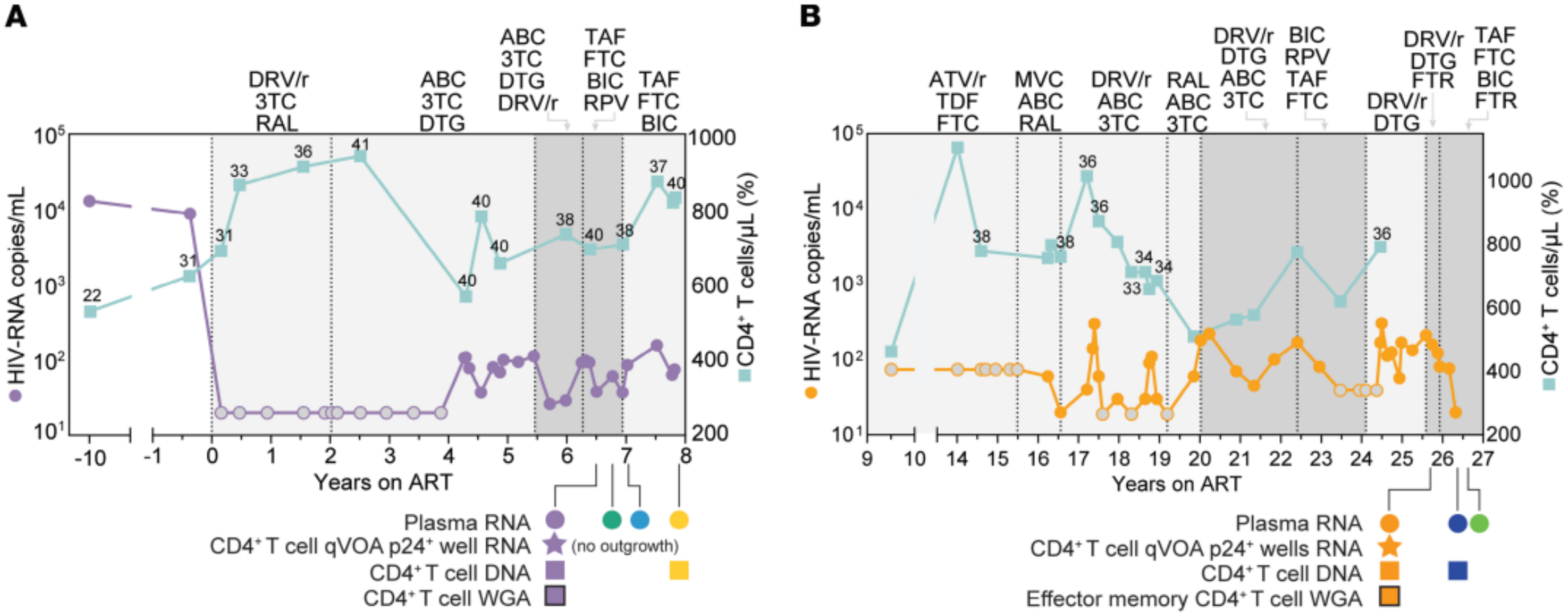
BACKGROUND. Antiretroviral therapy (ART) halts HIV-1 replication, decreasing viremia to below the detection limit of clinical assays. However, some individuals experience persistent nonsuppressible viremia (NSV) originating from CD4+ T cell clones carrying infectious proviruses. Defective proviruses represent over 90% of all proviruses persisting during ART and can express viral genes, but whether they can cause NSV and complicate ART management is unknown.

METHODS. We undertook an in-depth characterization of proviruses causing NSV in 4 study participants with optimal adherence and no drug resistance. We investigated the impact of the observed defects on 5′-leader RNA properties, virus infectivity, and gene expression. Integration-site specific assays were used to track these proviruses over time and among cell subsets.

RESULTS. Clones carrying proviruses with 5′-leader defects can cause persistent NSV up to approximately 10^3 copies/mL. These proviruses had small, often identical deletions or point mutations involving the major splicing donor (MSD) site and showed partially reduced RNA dimerization and nucleocapsid binding. Nevertheless, they were inducible and produced noninfectious virions containing viral RNA, but lacking envelope.

CONCLUSION. These findings show that proviruses with 5′-leader defects in CD4+ T cell clones can give rise to NSV, affecting clinical care. Sequencing of the 5′-leader can help in understanding failure to completely suppress viremia.

FUNDING. Office of the NIH Director and National Institute of Dental and Craniofacial Research, NIH; Howard Hughes Medical Institute; Johns Hopkins University Center for AIDS Research; National Institute for Allergy and Infectious Diseases (NIAID), NIH, to the PAVE, BEAT-HIV, and DARE Martin Delaney collaboratories.



J Clin Invest. 2023;133(6):e165245

- **CARD-8** => The protein encoded by this gene belongs to the caspase recruitment domain (CARD)-containing family of proteins, which are involved in pathways leading to activation of caspases or nuclear factor kappa-B (NFKB).
- Recent discoveries showed that the inflammasome molecule **CARD8 can sense HIV-1 protease activity** in virus producing T cells and macrophages.
- HIV-1 eludes this innate mechanism because protease is mostly active only after viral particles leave the cell.
- However, some nonnucleoside reverse transcriptase inhibitors (**NNRTIs**) that have been used for two decades to treat HIV-1, such as **efavirenz** and **rilpivirine**, have recently been shown to **induce intracellular Gag-Pol dimerization and premature protease activation**, **triggering the CARD8 inflammasome**, and **death of HIV-1-producing cells via pyroptosis**.
- In the context of RV and NSV, the use of NNRTIs or other **molecules inducing CARD8-sensing of HIV-1** could represent a promising strategy to **eliminate infected cells** contributing to persistent viremia and accelerate reservoir decay.

Science. 2021 Mar 19;371(6535):eabe1707.
J Virol. 2022;96:e0219821.
Sci Transl Med. 2023;15:eabn2038.

Table 2 Clinical management of detectable viremia on ART

Machanism underlying detectable viremia on ART	Causes	Consequences	Management
Viral replication (ongoing new infection events)	Suboptimal adherence	Viral evolution	Adherence counselling
	Reduced ART concentration due to drug-to-drug interactions or limited absorbsion	Drug resistance	Therapeutic drug monitoring
		Increase in viremia and virological failure	HIV-1 genotyping from plasma***
		$CD4^+$ T cells loss	ART regimen optimization
	Drug resistance	Risk of transmission	
Viral expression from infected cells (virus production)	Latency reversal resulting in continuous virus production	No viral evolution	If all of the above not suggestive for viral replication:
		No drug resistance to current regimen	Monitoring of viremia over time
		Intermittent or stable viremia for months/years	Short course ART intensification
	Clonal expansion of cells carrying intact or defective proviruses	Stable $CD4^+$ T cells	***Characterization of virus in plasma with ultrasensitive assays***
		Potential higher inflammation	

***Usually possible only with HIV-1 RNA levels above 400 copies/mL

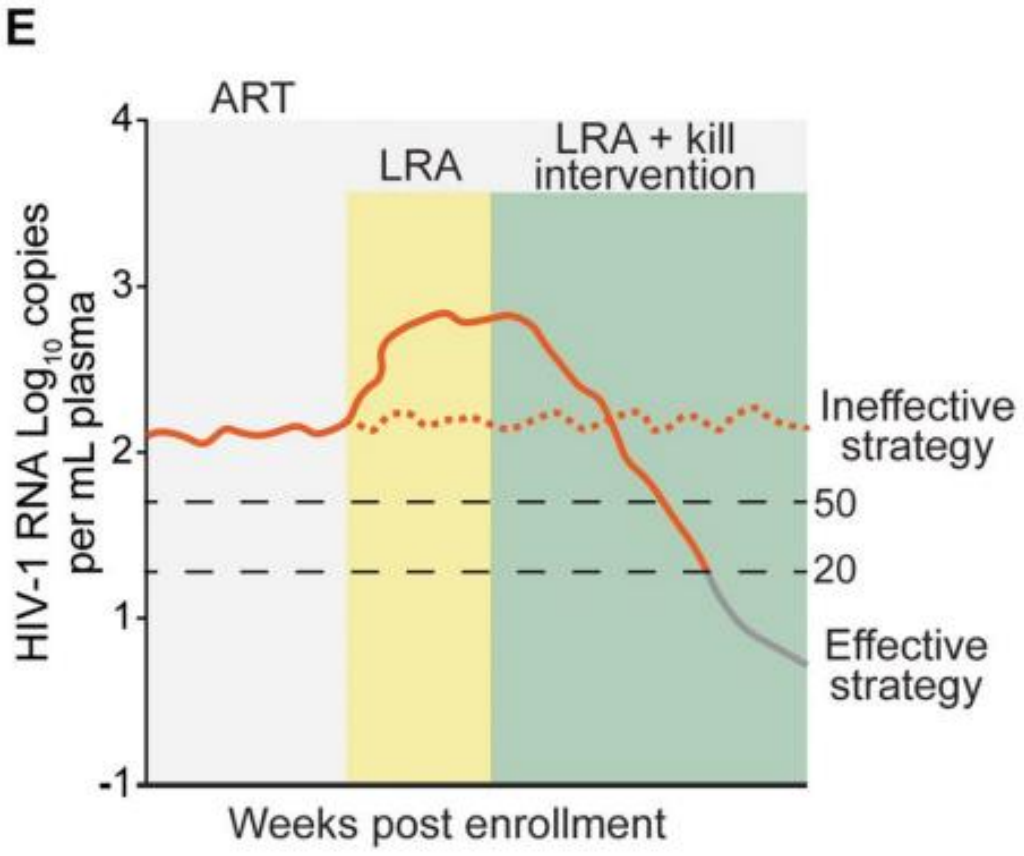
Current HIV/AIDS Reports (2023) 20:428–439

New drug options for NSV?

- **Fostemsavir** has no effect on virus production, it cannot reduce HIV-1 RNA expressed from persistent reservoirs.
- In a recent study, fostemsavir intensification in a participant with NSV did not decrease plasma viral load
- **Lenacapavir** is a new-in-class long-acting capsid inhibitor that blocks core disassembly, the interaction between capsid and host factors, viral assembly, and maturation
- At higher concentrations, lenacapavir inhibits capsid assembly and viral particle formation in vitro, which may reduce HIV-1 RNA in plasma.
- Future studies should investigate whether ART intensification with lenacapavir could be a successful strategy to treat individuals with NSV.

J Clin Invest. 2023;133:e165245.
N Engl J Med. 2022;386:1793–803.

Schematic of the potential response of a study participant with NSV to experimental interventions aimed at enhancing virus production and/or elimination of infected cells causing NSV



Cui prodest?

Current HIV/AIDS Reports (2023) 20:428–439

Non-suppressible viraemia during HIV-1 therapy: a challenge for clinicians

Andrés Esteban-Cantos, Rocío Montejano*, Adriana Pinto-Martínez, Javier Rodríguez-Centeno, Federico Pulido†, José R Arribas†*

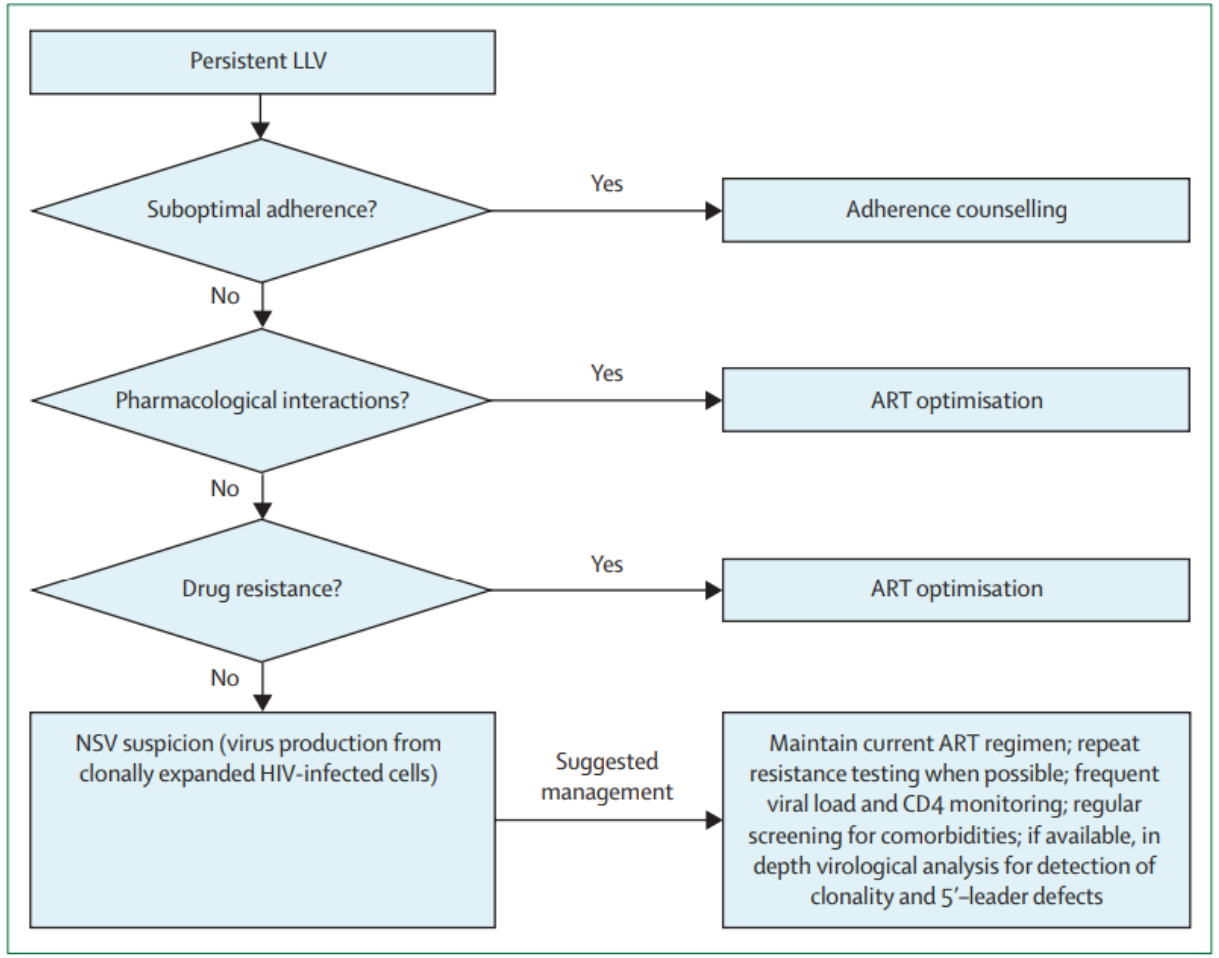


Figure: **Clinical management recommendations for patients with persistent LLV and suspected of NSV**
ART=antiretroviral therapy. LLV=low-level viremia. NSV=non-suppressible viremia.

Lancet HIV. 2024 Apr 8:S2352-3018(24)00063-8

Conclusions

- NSV rather than LLV as clinical definition
- Short course treatment intensification required to confirm NSV
- No evidence of benefit of intensification

Thank you for your attention