IrsiCaixa
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New assays for assessing viral reservoirs

HOT TOPICS IN HIV

8th November 2022

FLS SCIENCE Fight Infections Foundation

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Associate Researcher

CONTEXT: Aiming a Functional Cure

- ✓ Total HIV eradication from the body is unlikely, instead a **functional cure** might be reached, which means control HIV in absence of antiretroviral treatment.

- ✓ Cases of natural or post-treatment control all have in common a small HIV reservoir size.

CONTEXT: Aiming a Functional Cure

- ✓ Total HIV eradication from the body is unlikely, instead a **functional cure** might be reached, which means control HIV in absence of antiretroviral treatment.

- ✓ Cases of natural or post-treatment control all have in common a small HIV reservoir size.

HIV cure intervention strategies

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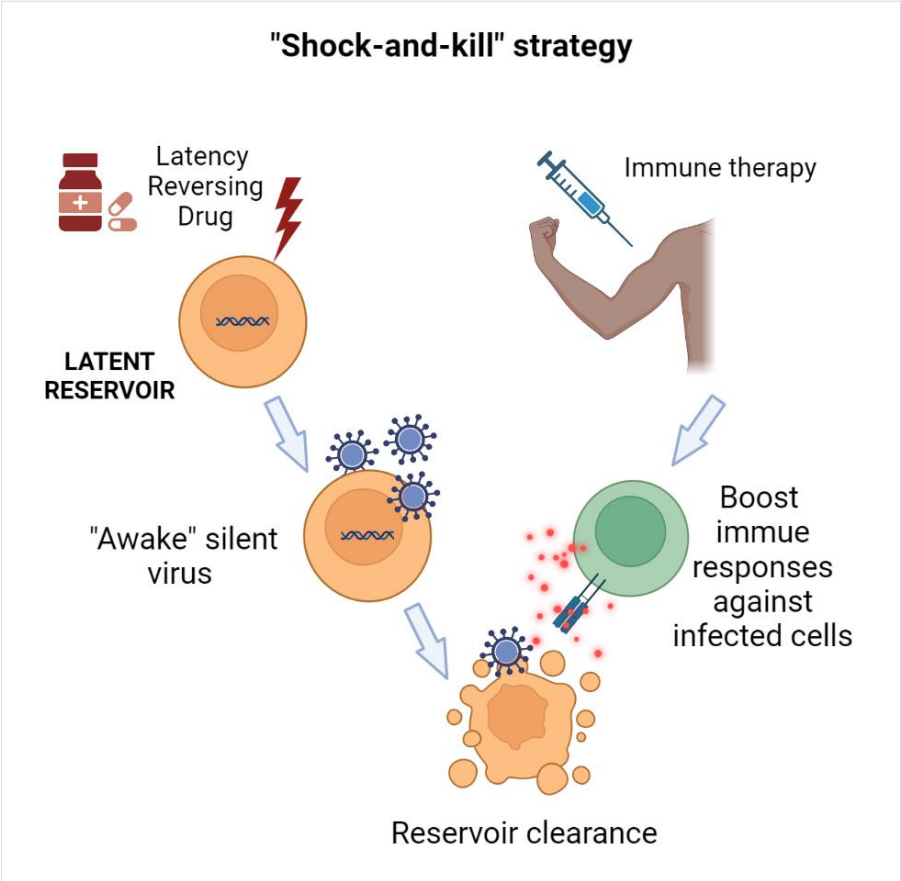

Evaluating the efficacy of clinical interventions

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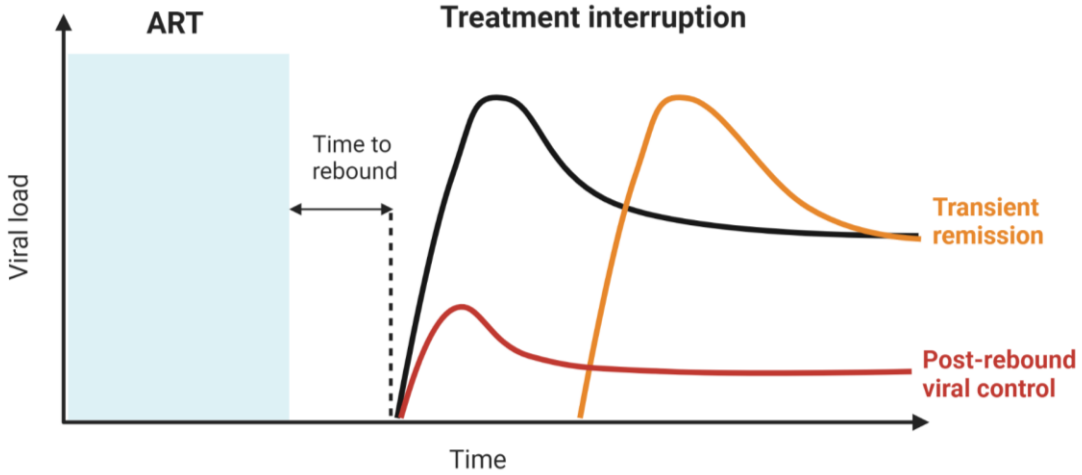
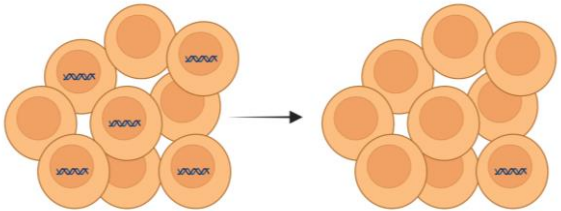
- Analytical Treatment Interruption:



Participants' risks
and psychological aspects
(deal with expectations vs. dissapointment)

- Surrogate Marker of Effective Virological Response:



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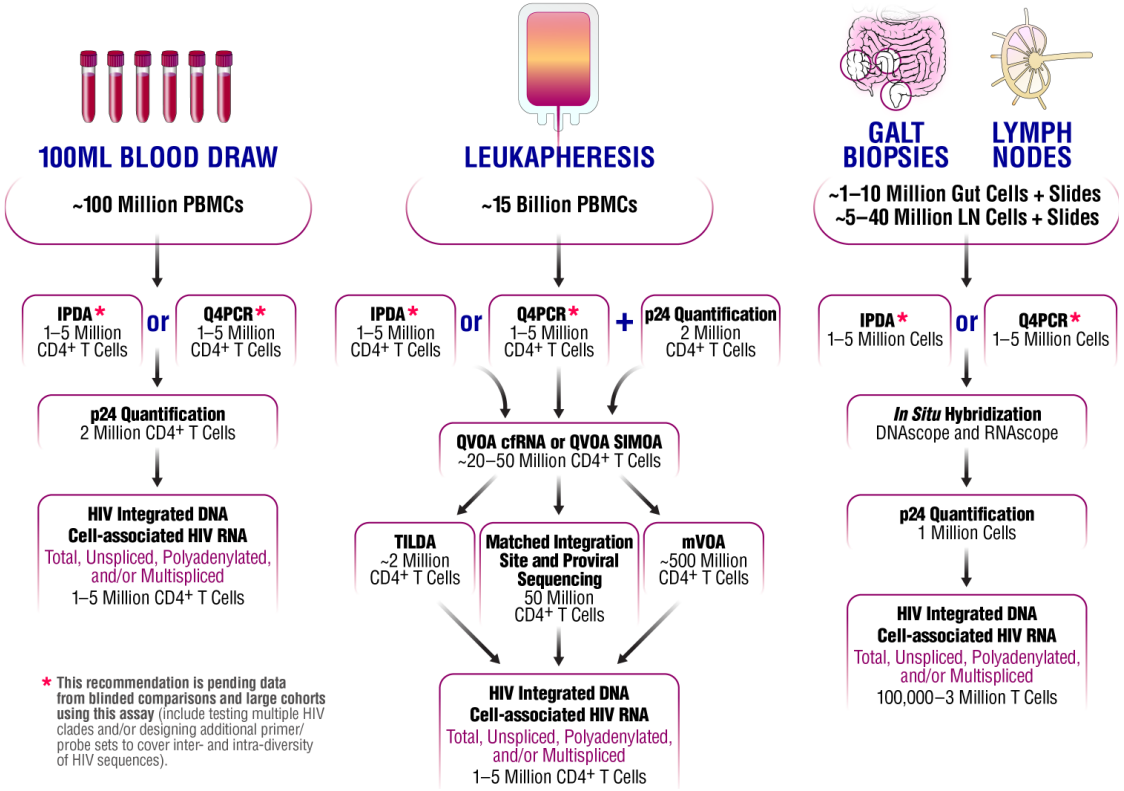
Recommendations for measuring HIV reservoir size in cure-directed clinical trials



Abdel-Mohsen *et al.* 2020 Sci Transl Med

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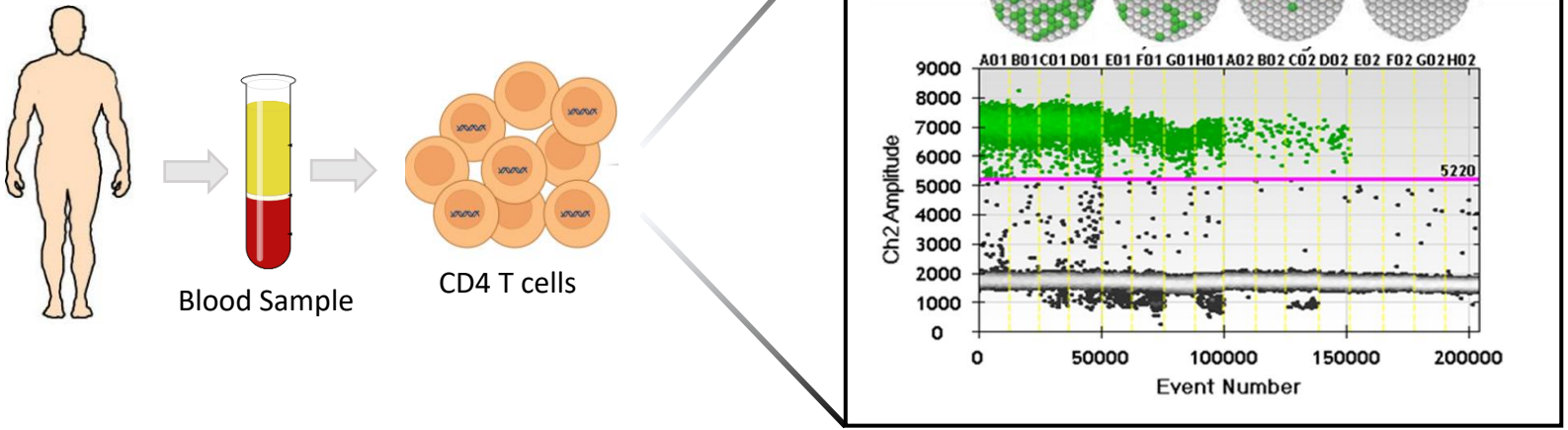
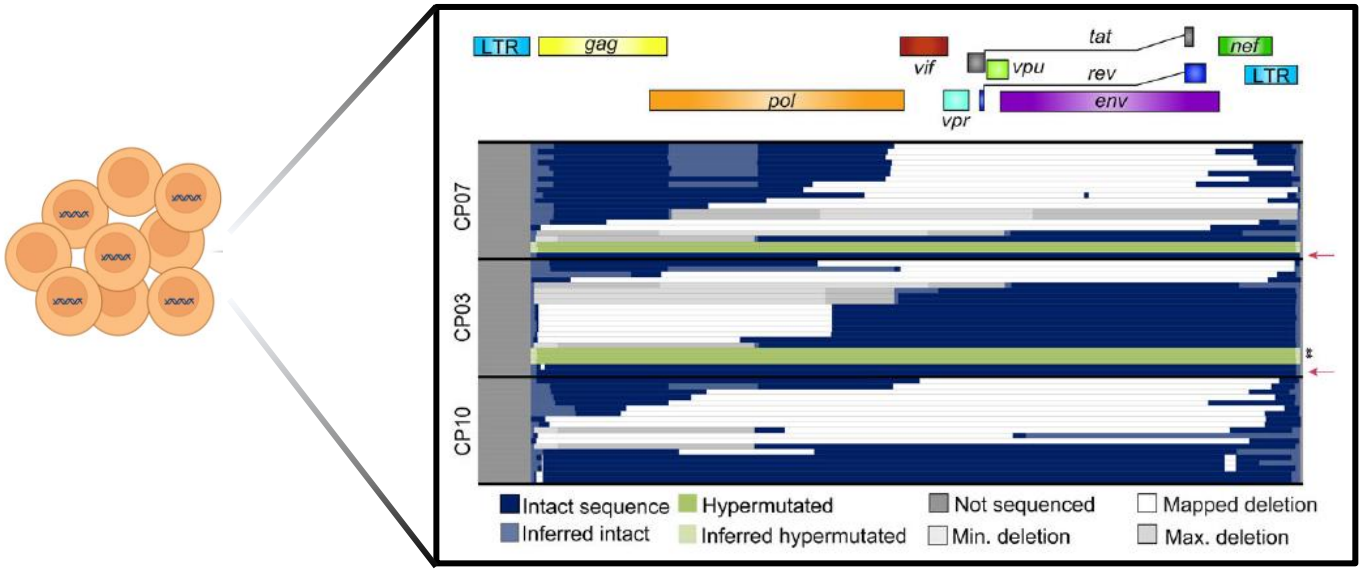
Total Proviral DNA measured by droplet digital PCR (ddPCR)



- ✓ Very sensitive and precise, and convenient for measuring the size of the viral reservoir in naural infection (heterogeneous among people with HIV)

Total Proviral DNA measured by droplet digital PCR (ddPCR)



Bruner et al, Nat Med, 2016

✓ About 98% of HIV sequences are defective !!
But sequencing all the viral repertoire is unaffordable in the clinical setting

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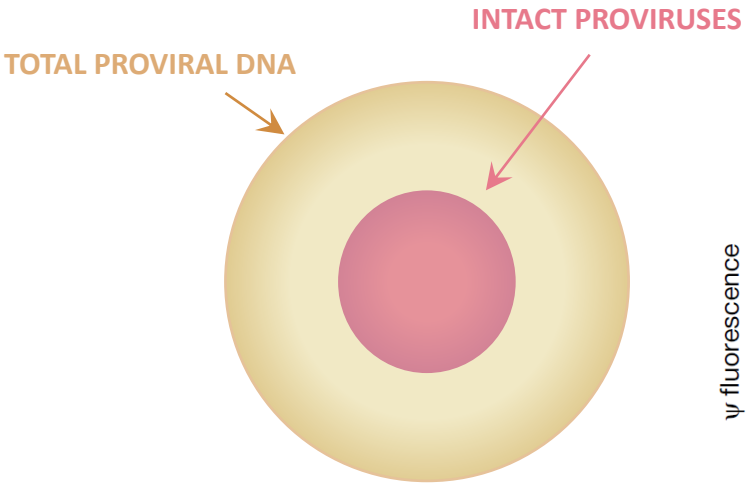
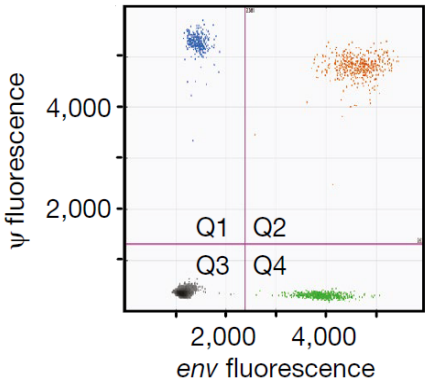
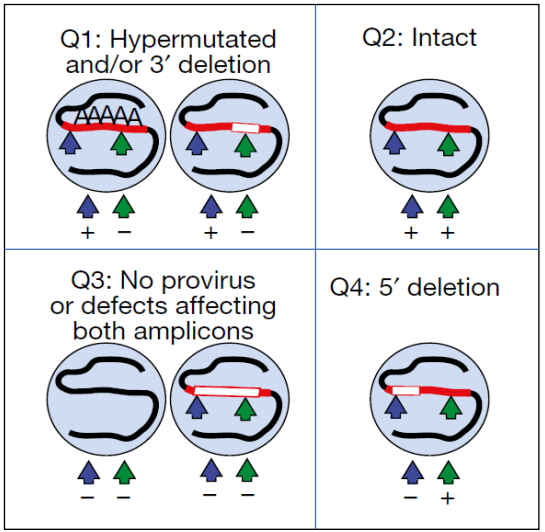
Intact Proviral DNA Assay (IPDA)

Bruner et al, Nature, 2019







- ✓ Many samples (30%) fail because of variability in the viral sequence
- ✓ Designed for Subtype B HIV-1

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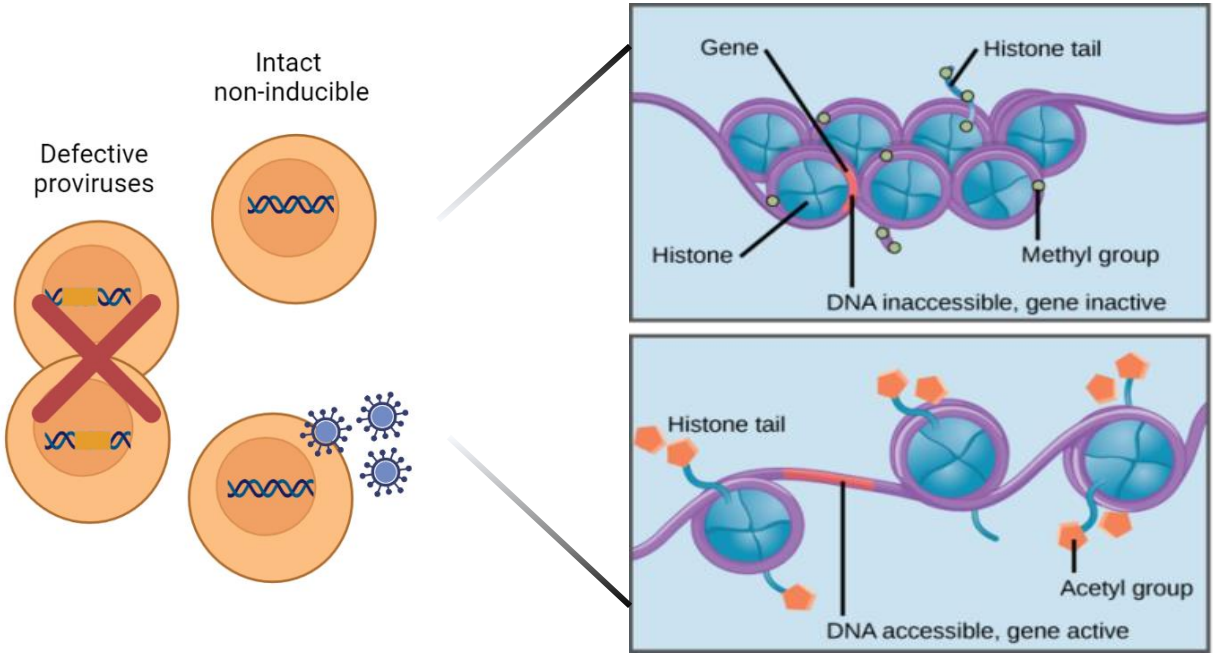
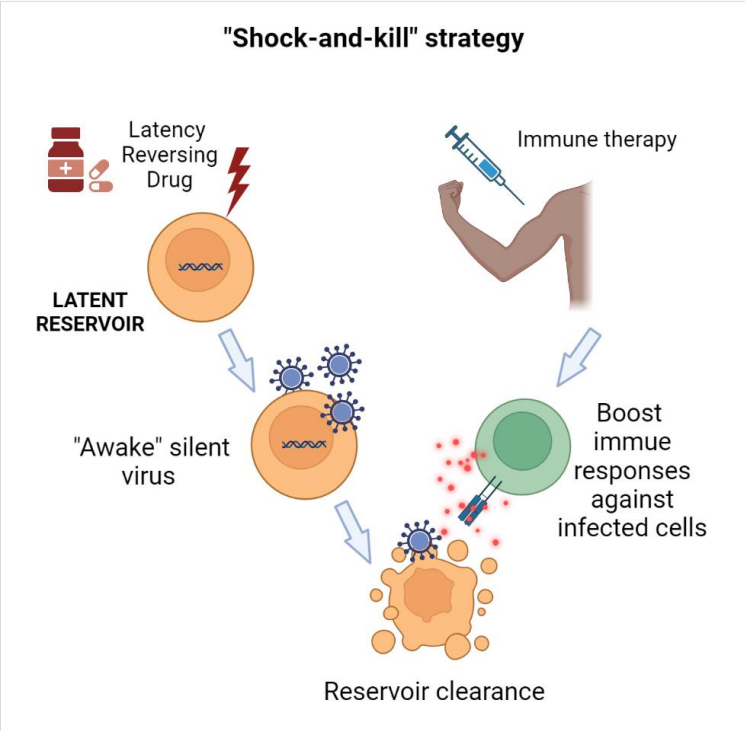
Intact Proviral DNA Assay (IPDA)

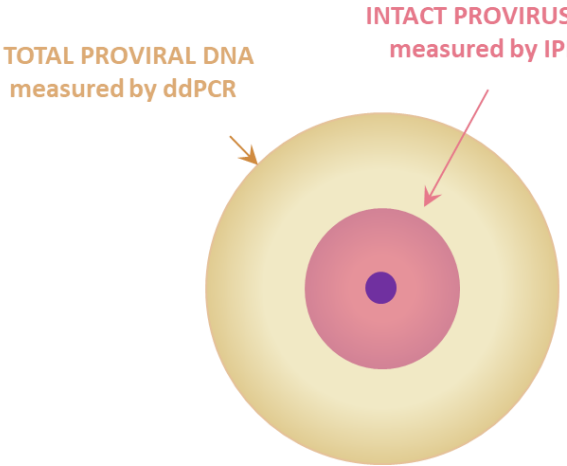


https://jackwestin.com/resources

- ✓ Most intact proviruses are non-inducible,
  sometimes because they are integrated in silent areas of the cell genome

Measuring the viral reservoir: Which is our target population?





✓ Poor results of previous shock-and-kill interventions…
Are we using the right tools to detect their effect?

Measuring the viral reservoir: Which is our target population?

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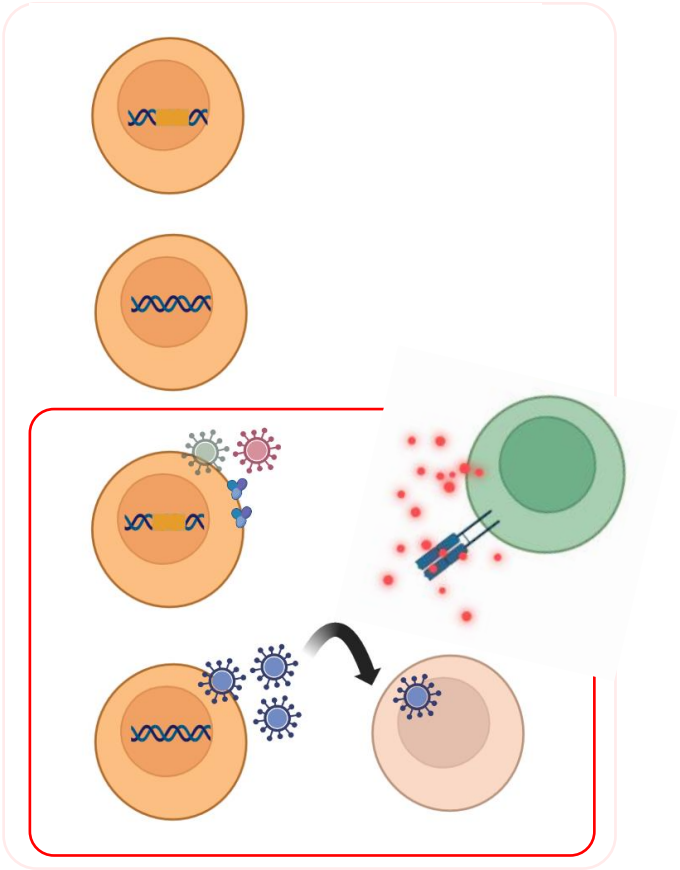
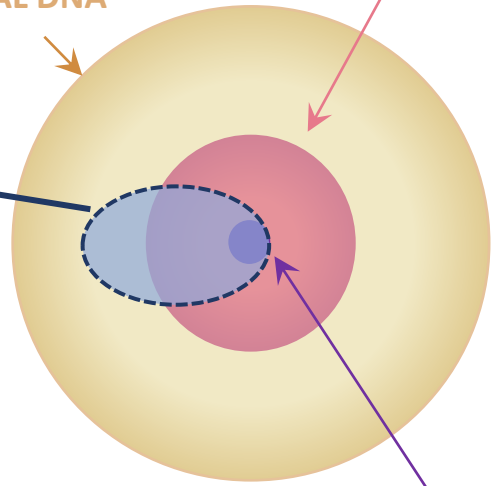


TOTAL PROVIRAL DNA

INTACT PROVIRUSES

FUNCTIONAL RESERVOIR

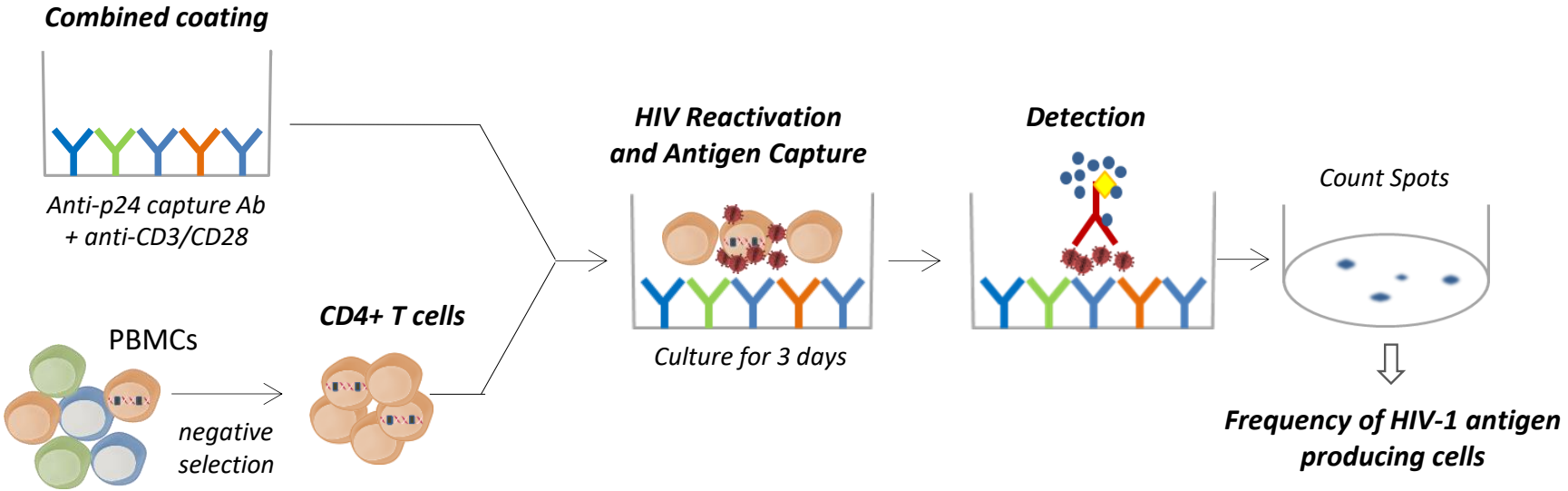
Production of viral proteins = viral antigens



Replication-competent Reservoir (qVOA)

Development of the novel VIP-SPOT assay

❖ Quantify the frequency of peripheral CD4+ T cells able to reactivate and produce viral proteins (p24).



Puertas MC, et al,. 2021. mBio 12:e00560-21.

Development of the novel VIP-SPOT assay

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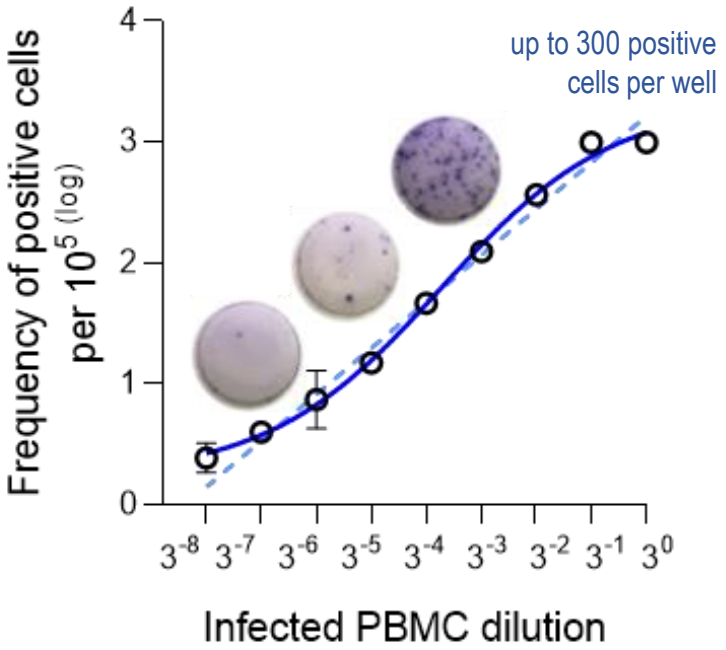

- ✓ Elispot-based assay : detection of viral proteins at the **single-cell level.**
- ✓ VIP-SPOT shows good **sensitivity and reproducibility** at the lower ranges (down to 1 in 10^5 cells per well)
- ✓ **Scalable** in terms of sample needs ($20x10^6$ criopreserved PBMCs), procedure format (96-well plates) and working time (2 days).

Puertas MC, et al,. 2021. mBio 12:e00560-21.

VIP-SPOT correlation with other measures of viral burden in untreated HIV-1 infection.

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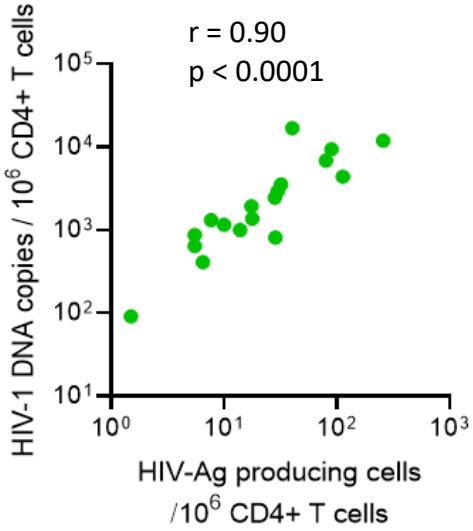
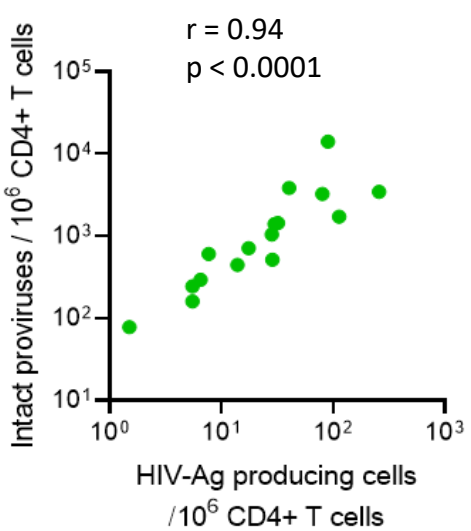
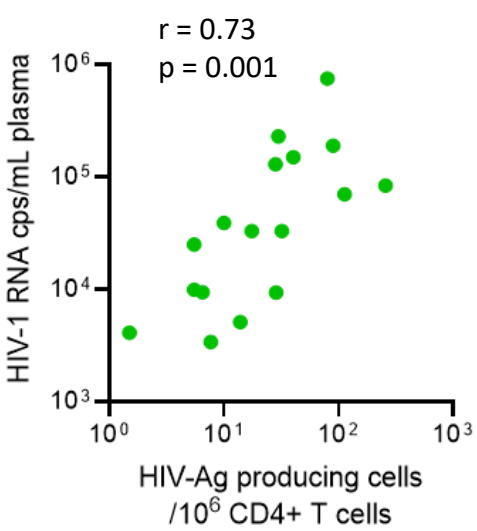
*N=18 viremic samples





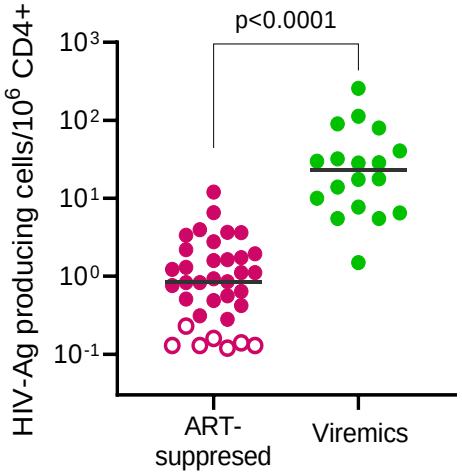


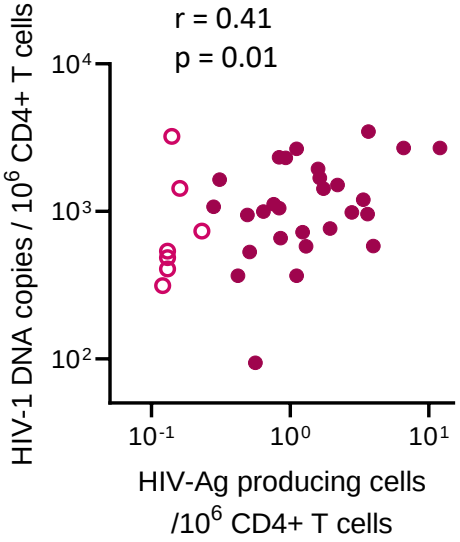
- VIP-SPOT correlates with other measures of viral burden in untreated HIV-1 infection

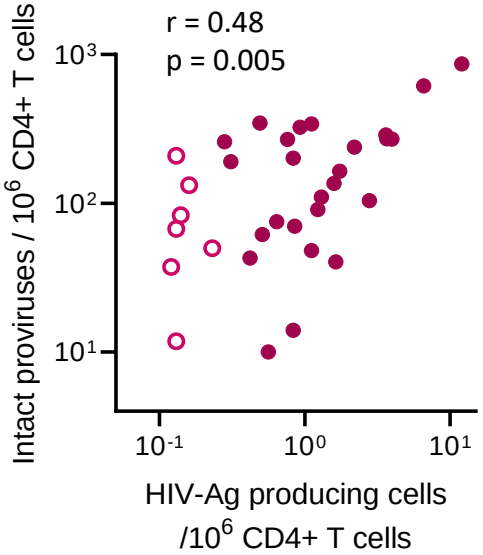
Puertas MC, et al,. 2021. mBio 12:e00560-21.

VIP-SPOT correlation with other measures of viral reservoir on suppressive ART.

*N=35 ART suppressed



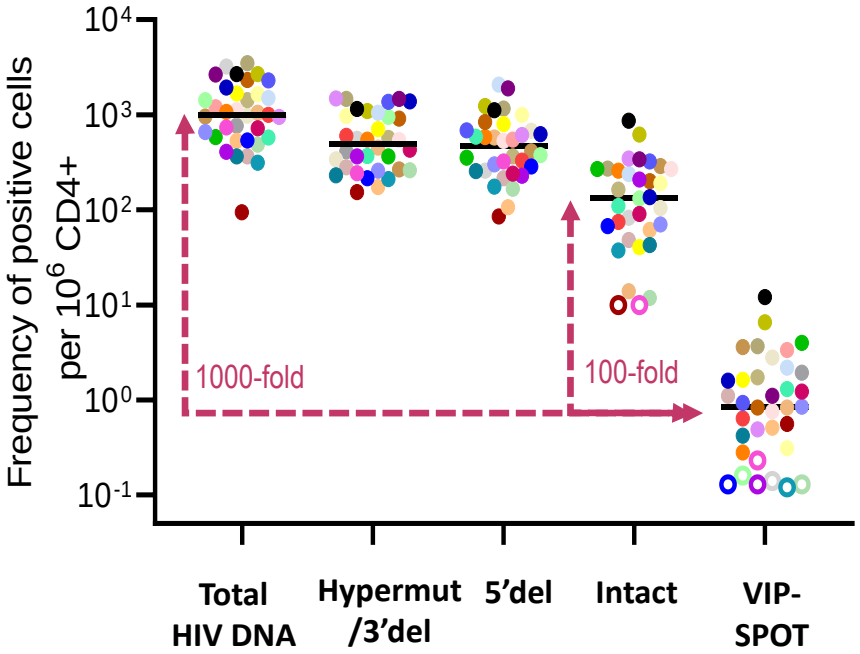




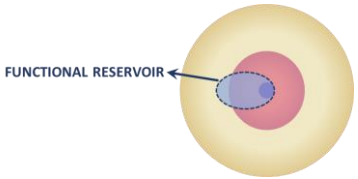
- VIP-SPOT measures the inducible HIV antigen–producing reservoir and correlates with other measures of viral persistence during suppressive ART

Puertas MC, et al,. 2021. mBio 12:e00560-21.

VIP-SPOT correlation with other measures of viral reservoir on suppressive ART.



- The frequency of inducible p24-producing cells is 1000-fold lower than total HIV DNA and 100-fold lower than the intact proviral reservoir, thus indicating that viral reactivation is strongly restrained in long-lived reservoir cells.



Puertas MC, et al,. 2021. mBio 12:e00560-21.

The VIP-SPOT assay in the context of the eCLEAR Trial

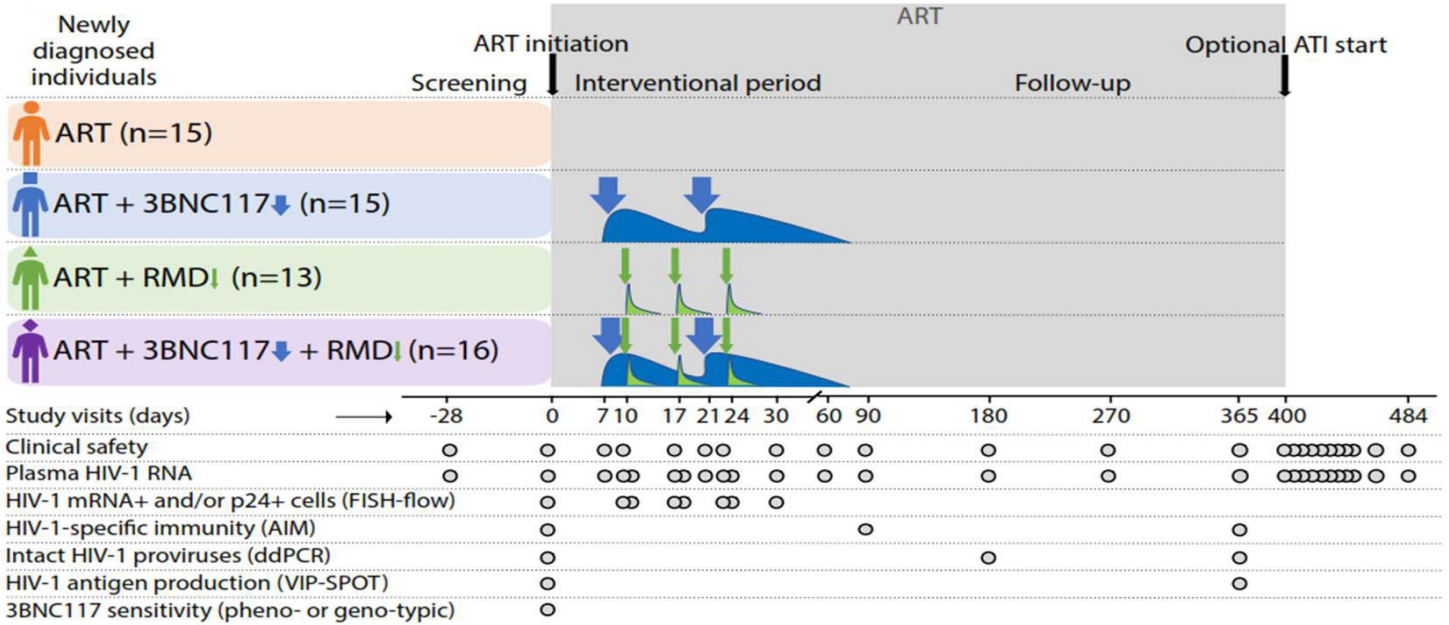
- Shock&kill intervention at the time of ART initiation

nature medicine

Article

https://doi.org/10.1038/s41591-022-02023-7

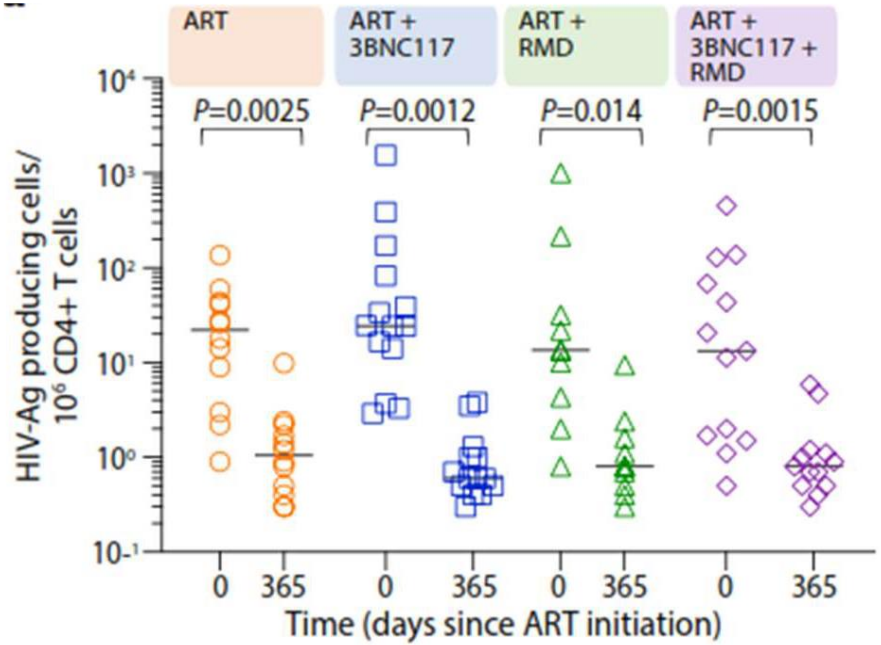
Early intervention with 3BNC117 and romidepsin at antiretroviral treatment initiation in people with HIV-1: a phase 1b/2a, randomized trial

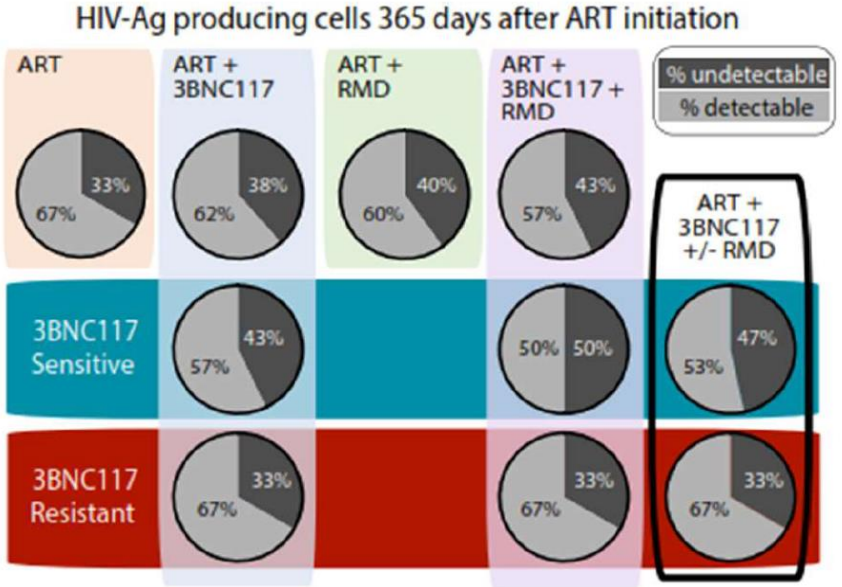


Gunst. et al, Nat Med, Oct 2022

Evaluating the *in vivo* clearance of HIV-1 infected cells by VIP-SPOT

✓ Longitudinal changes in the frequency of HIV-Ag producing cells along the study period.





Gunst. et al, Nat Med, Oct 2022

Performance of the VIP-SPOT assay with non-B subtypes

- ✓ Only half of the individuals (49%) had HIV-1 subtype B infection while the rest of the participants had a broad range of other HIV-1 subtypes and recombinant forms

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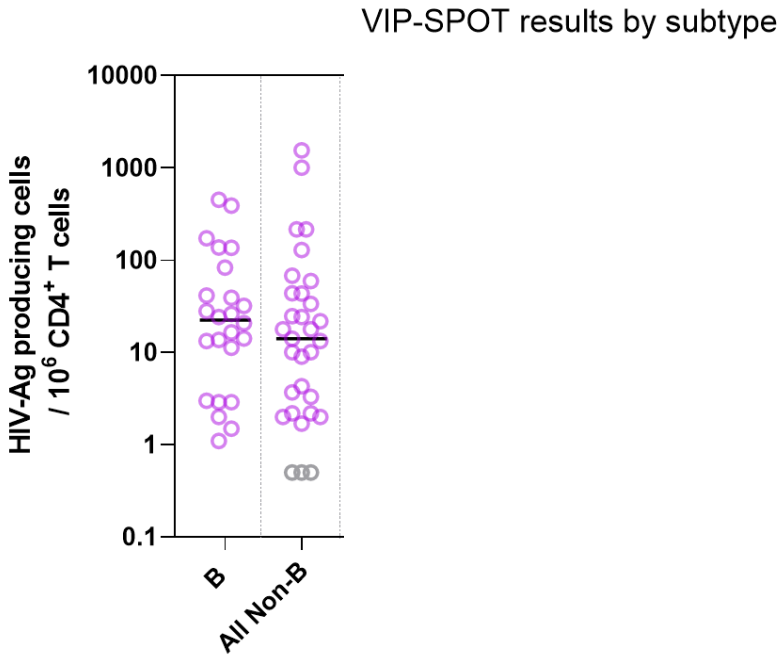

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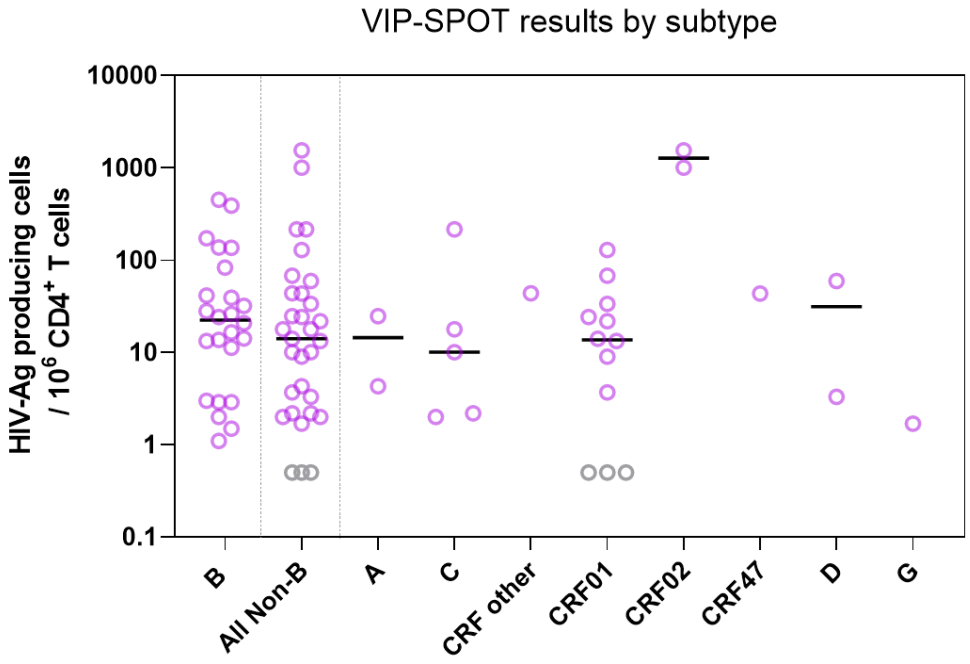
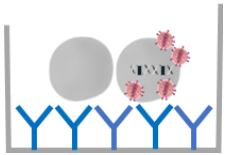
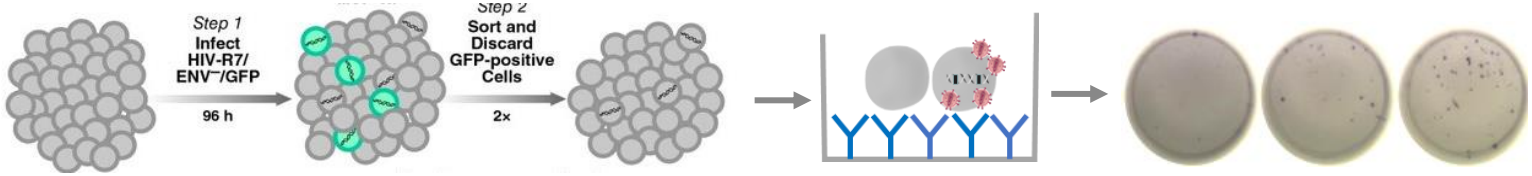


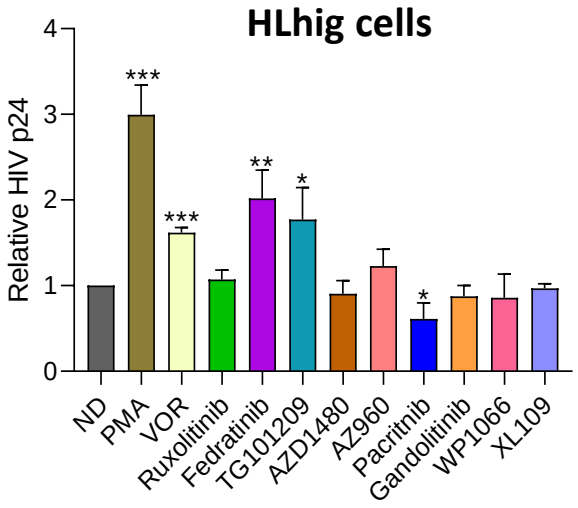
In vitro testing novel LRAs candidates: adapted VIP-SPOT assay

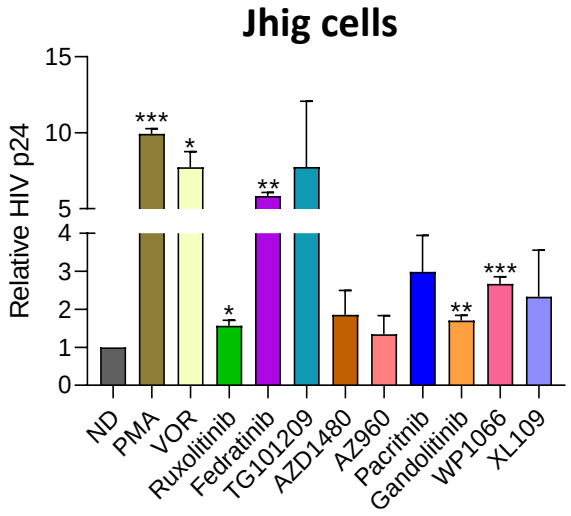
- Evaluation of viral reactivation



❖ Evaluation of viral reactivation with non-clonal latently infected cell models







➤ Two novel LRAs identified

Jude I. et al, Frontiers in Immunology *Sept 2022*

New assays for assessing viral reservoirs

- ✓ **Total HIV DNA and Intact proviral DNA** (IPDA) are convenient assays to measure the size of the viral reservoir in natural HIV infection, but most viral genomes are defective or non-inducible.

- ✓ **VIP-SPOT is a novel assay** to precisely quantify the frequency of infected cells that retain the ability to reverse HIV-1 from latency and to produce viral antigens.

- ✓ This assay might be specially useful for evaluation of **therapeutic strategies based on immune clearance**, since the **productive reservoir** is the specific population that is going to be targeted by these interventions.

- ✓ The VIP-SPOT assay is robust, **cross-clade sensitive**, and suitable for longitudinal and multicenter retrospective/prospective clinical trials.

- ✓ Adapted versions of the VIP-SPOT are useful for **evaluating the efficacy of latency-reversing agents** in cell lines and primary cells.

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GRIFOLS

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HOT TOPICS IN HIV

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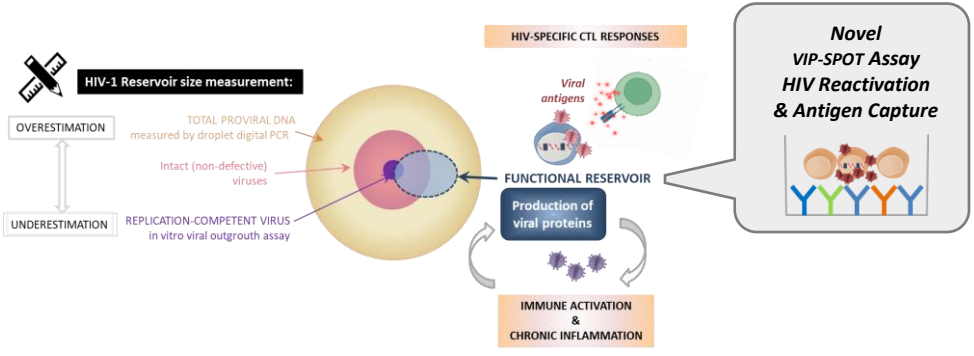
Ester Ballana

eCLEAR study group

VIP-SPOT: an Innovative Assay To Quantify the Productive HIV-1 Reservoir in the Monitoring of Cure Strategies



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- I-Na Lu
- Jesper Falkesgaard Højen
- The Clinical Research Unit
- Janne Martinsen
- Lene Svinth Jøhnke, and many more…

Lab for Molecular Immunology, The Rockefeller University
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- Yehuda Cohen
- Victor Ramos

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- Maryam Khan
- Amin Alamshah

King's College London/GSST
- Julie Fox

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- Dennis Copertino
- Winifffer Conce Alberto
- Adam Rackley Ward

The Brumme lab, Simon Fraser U
- Zabrina Brumme
- Natalie Kinloch

Celgene
- Anna Nielsson

.. and participants!

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