

11TH EDITION

DECEMBER 10 - 13, 2024

HIV PERSISTENCETM DURING THERAPY

Reservoirs & Eradication Strategies Workshop



Welcome to day 2

w w w . h i v - p e r s i s t e n c e . c o m

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DECEMBER 10-13, 2024

HIV PERSISTENCE DURING THERAPY

Reservoirs & Eradication Strategies Workshop



Proviral gene expression and quantitation of the latent HIV-1 reservoir

Tokameh MAHMOUDI

Erasmus MC, Rotterdam, The Netherlands

www.hiv-persistence.com

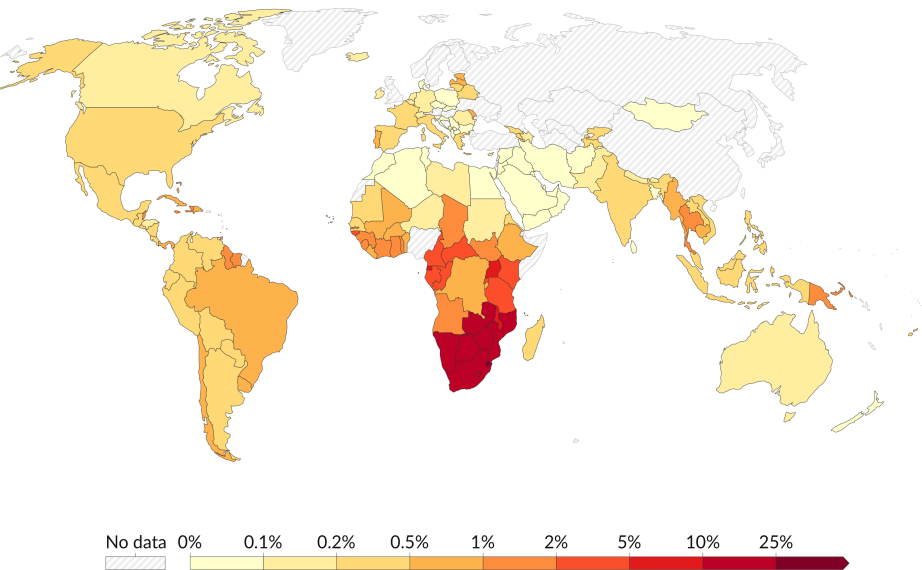
CONFLICTS OF INTEREST

Competing Interest Statement:

Tokameh Mahmoudi is co-inventor on patent applications EP23183103 filed by the Erasmus MC related to SQuHIVLa and US patent No. 11,980,613 related to BAF inhibitor compounds.

TM has a financial relationship in the form of research funding with Gilead, Viiv and MSD

Global Burden of HIV infection

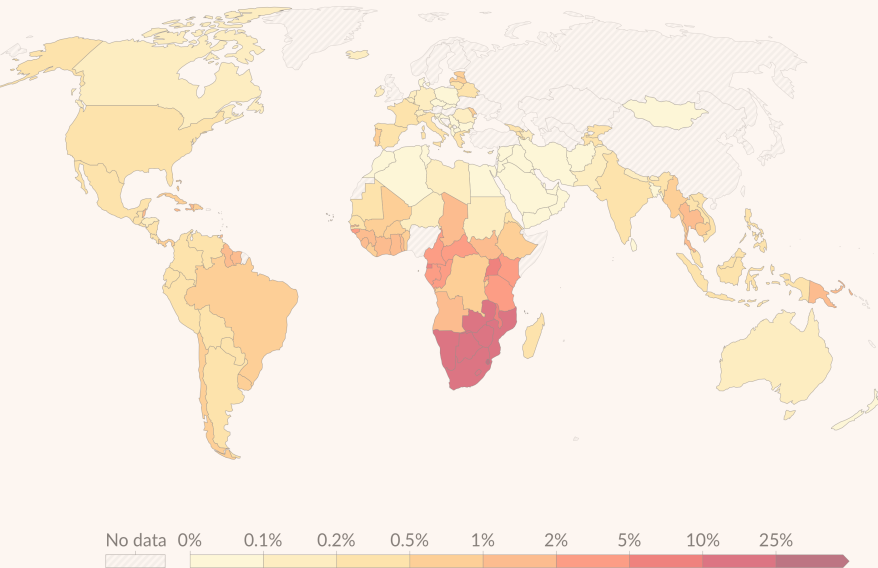


40 million
people living with HIV

Data source: UNAIDS (2023)

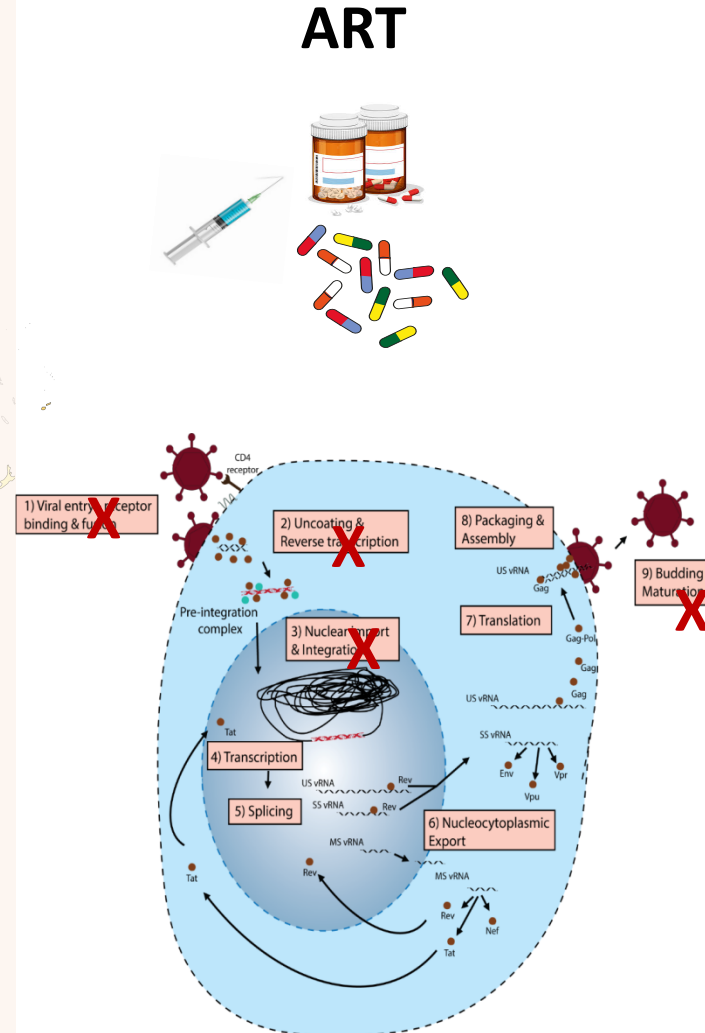
Antiretroviral therapy (ART) suppresses but does not cure HIV

Global Burden of HIV infection



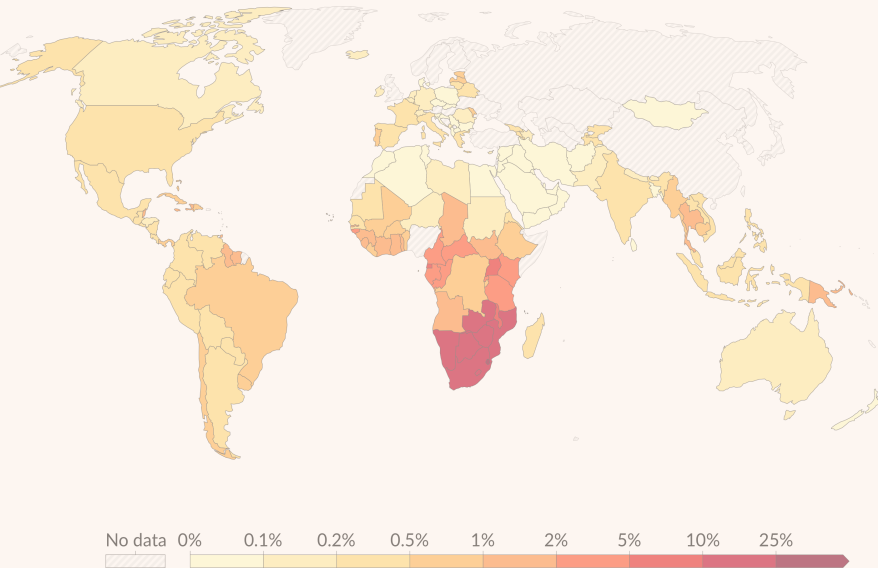
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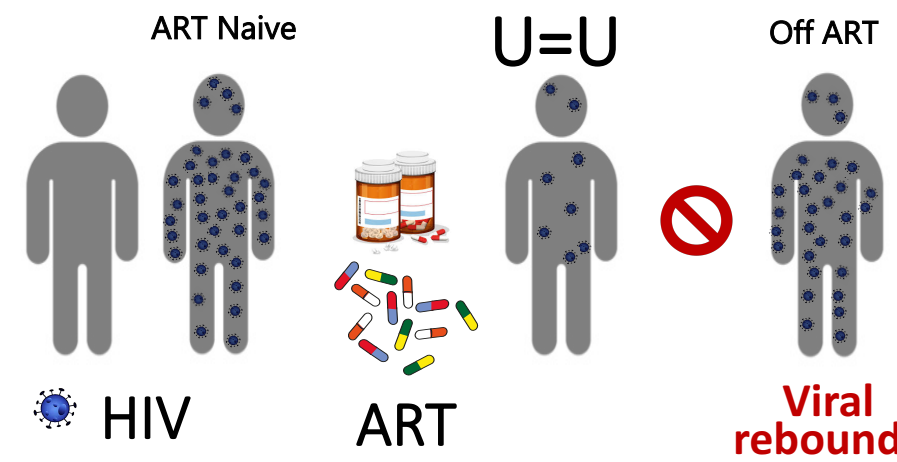
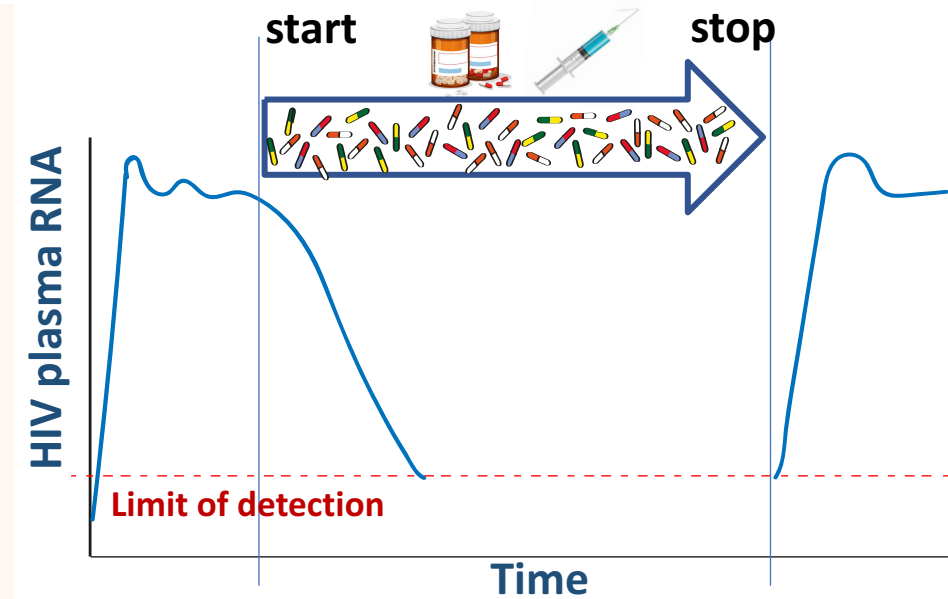
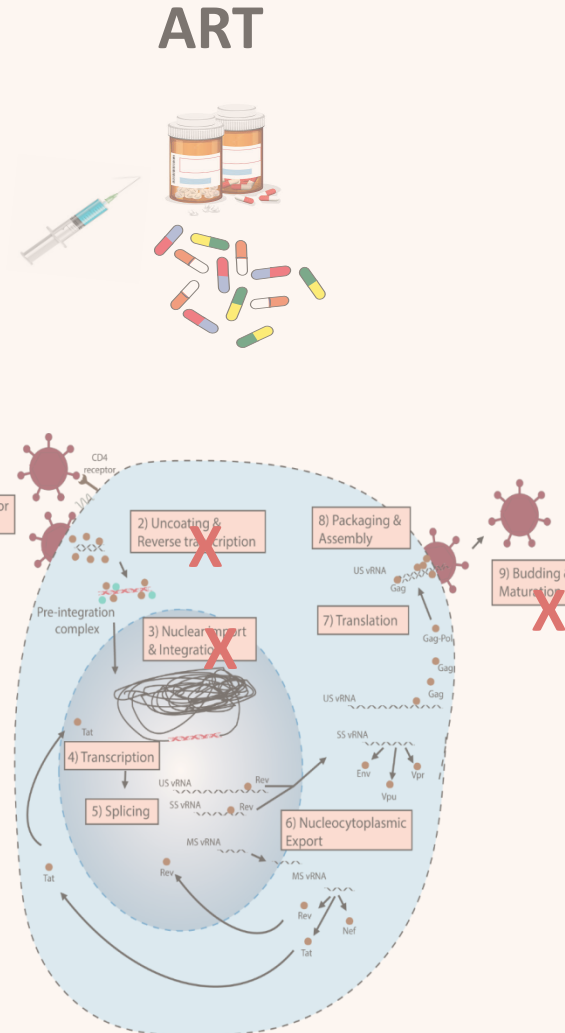
Antiretroviral therapy (ART) suppresses but does not cure HIV

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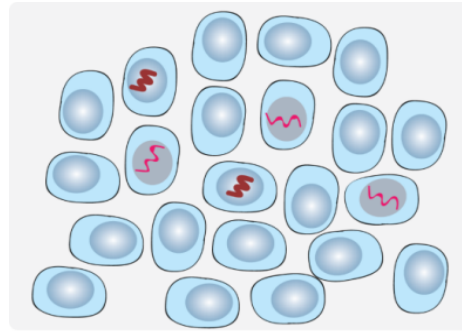


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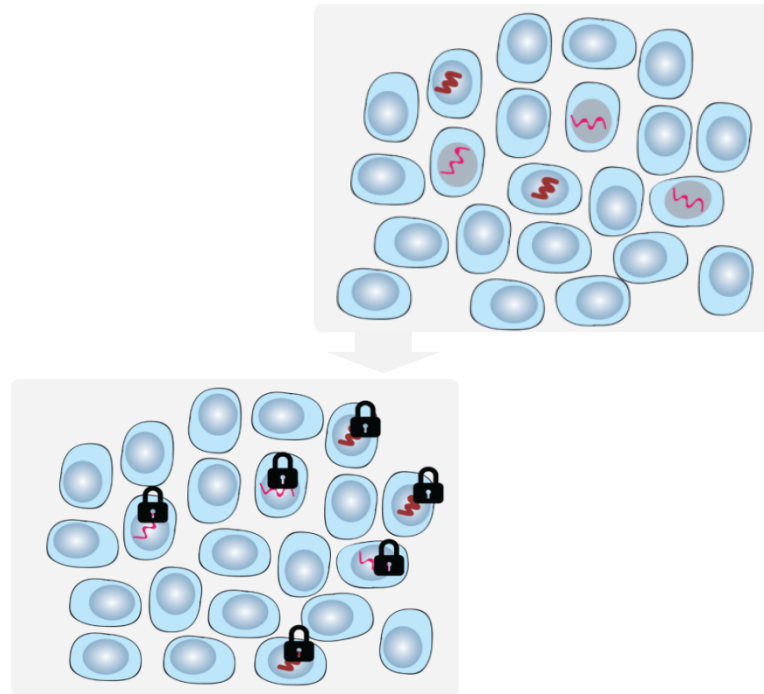
Pharmacological strategies toward HIV-1 cure



Pharmacological strategies toward HIV-1 cure

1. Silence the reservoir

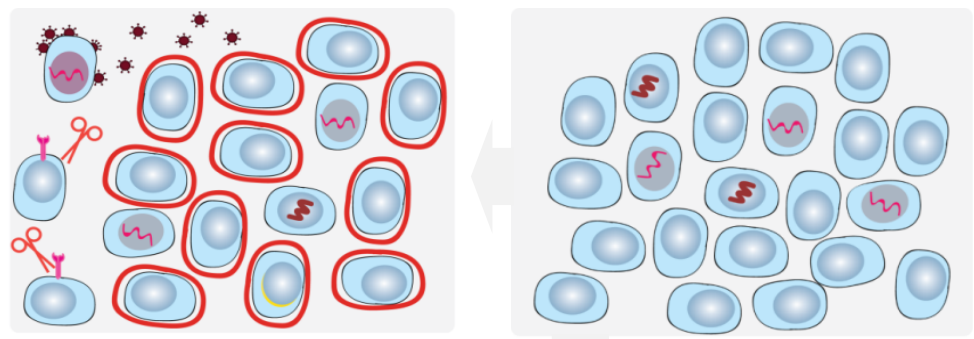
“Block and lock”



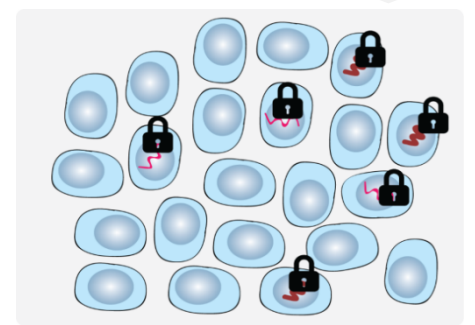
Pharmacological strategies toward HIV-1 cure

- 1. Silence the reservoir
- 2. Control viral rebound

- CCR5 editing
generate
"resistant" cells

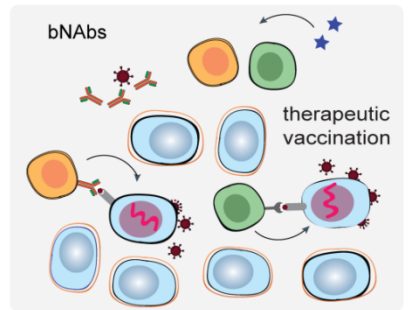


"Block and lock"



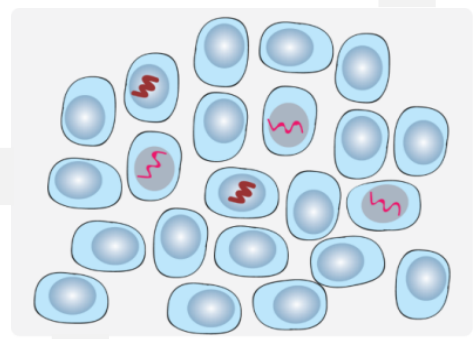
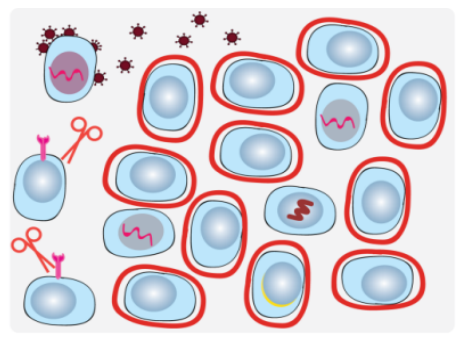
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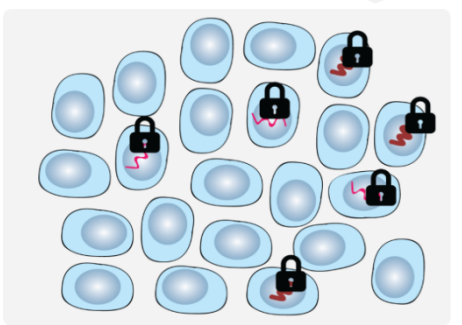


- bNAbs,
therapeutic
vaccines

- CCR5 editing
generate
"resistant" cells



"Block and lock"



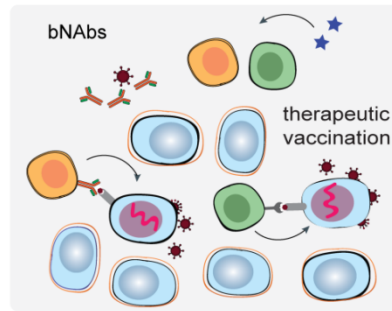
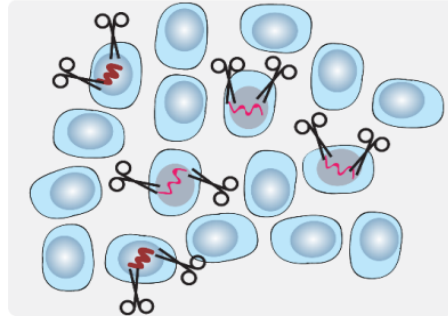
Pharmacological strategies toward HIV-1 cure

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2. Control viral rebound

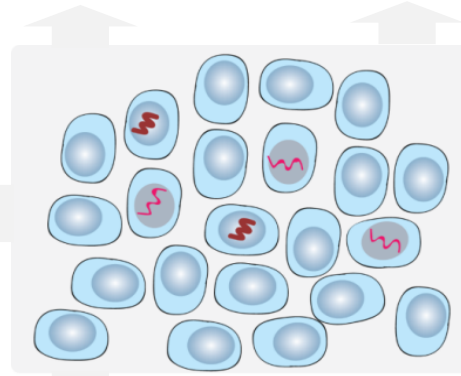
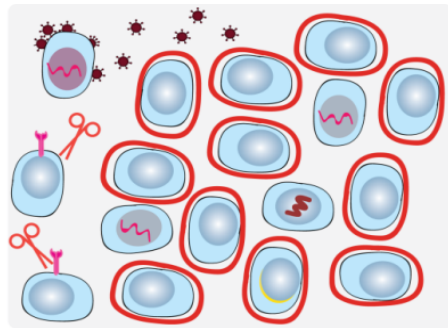
3. reduce the reservoir

- gene editing
CRISPR-Cas9/
nucleases

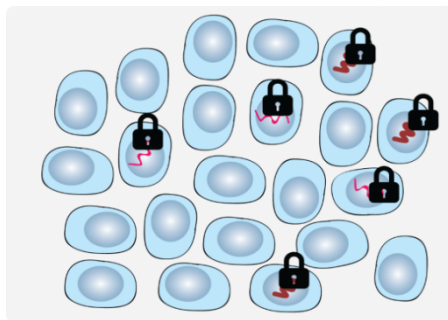


- bNAb's,
therapeutic
vaccines

- CCR5 editing
pool of
"resistant" cells



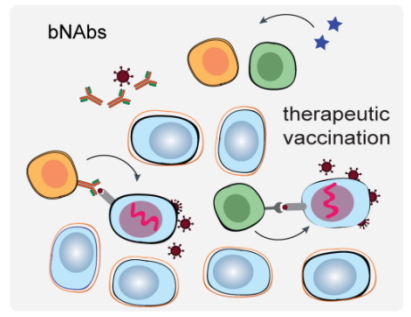
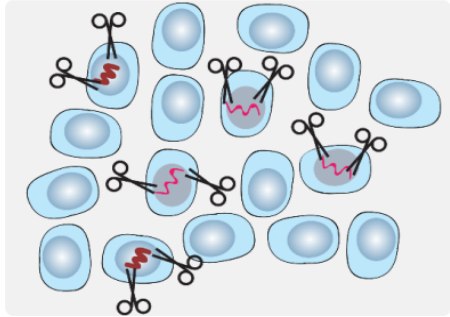
"Block and lock"



Pharmacological strategies toward HIV-1 cure

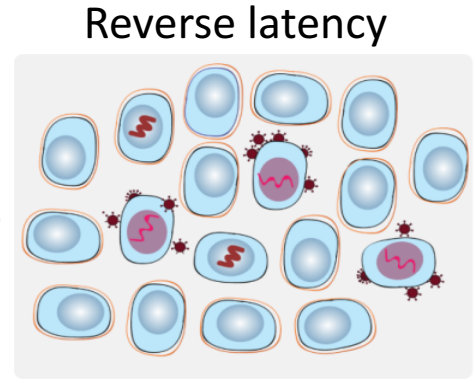
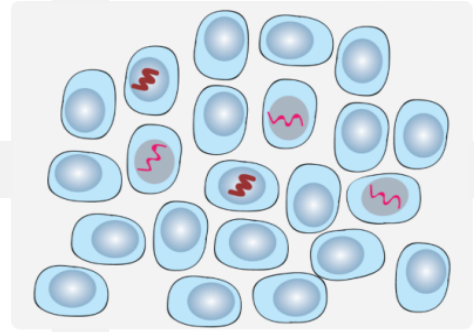
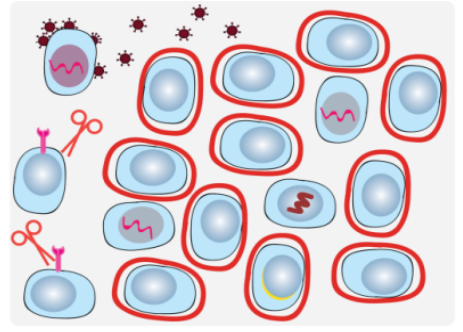
- 1. Silence the reservoir
- 2. Control viral rebound
- 3. reduce the reservoir

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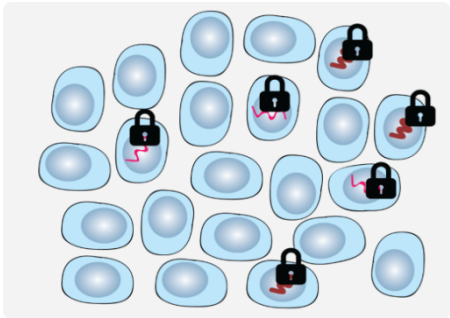


- bNAbs,
therapeutic
vaccines

- CCR5 editing
pool of
"resistant" cells



"Block and lock"



"Shock
and kill"

Pharmacological strategies toward HIV-1 cure

1. Silence the reservoir

- gene editing
 CRISPR-Cas9/
 nucleases

2. Control viral rebound

3. reduce the reservoir

- bNAbs,
 therapeutic
 vaccines

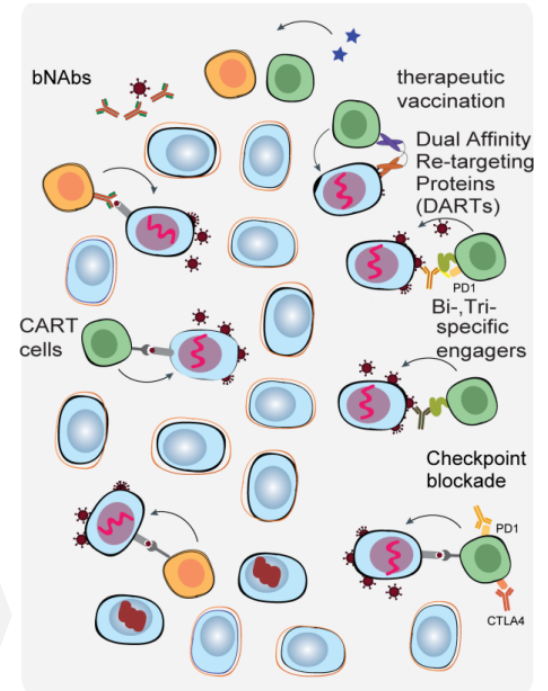
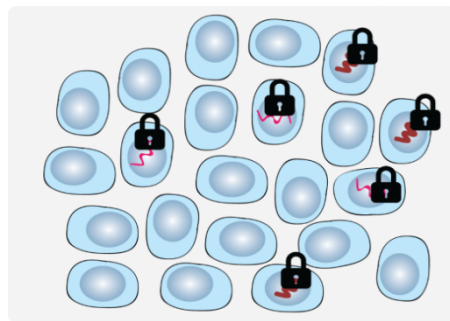
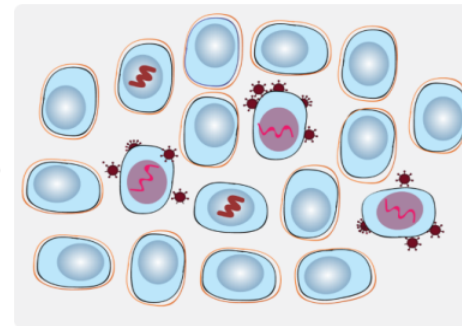
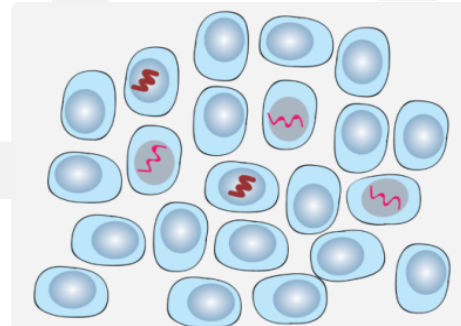
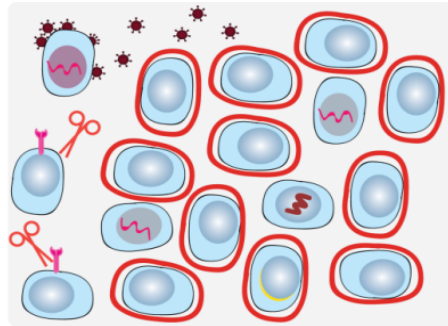
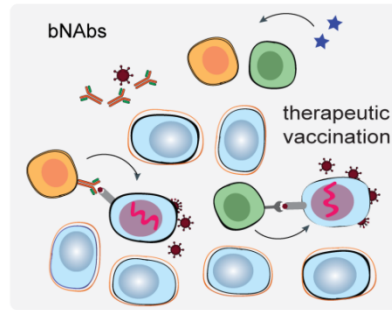
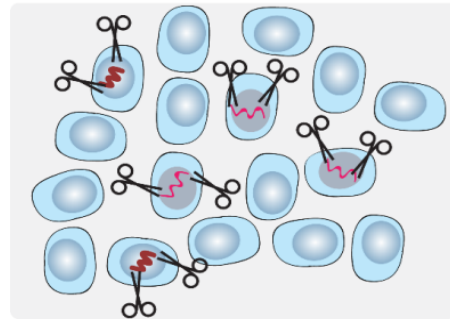
- CCR5 editing
 pool of
 "resistant" cells

Reverse latency

"Block and lock"

Induce cell killing

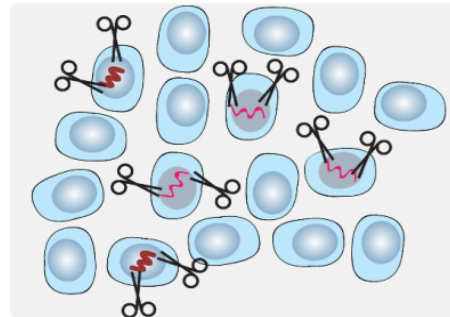
"Shock
 and kill"



Pharmacological strategies toward HIV-1 cure

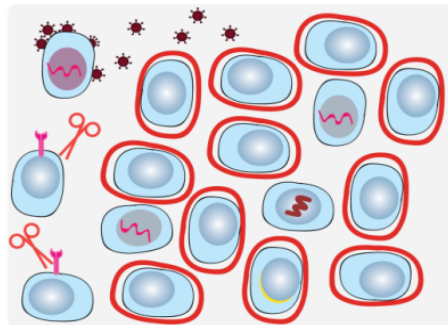
1. Silence the reservoir

- gene editing
 CRISPR-Cas9/
 nucleases

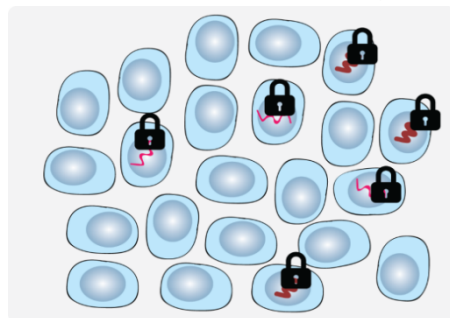


2. Control viral rebound

- CCR5 editing
 pool of
 "resistant" cells

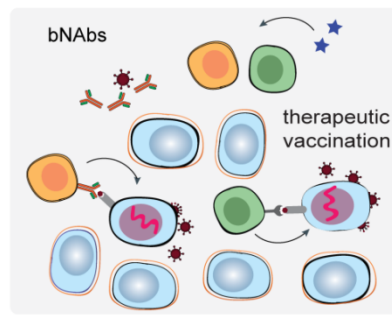


"Block and lock"

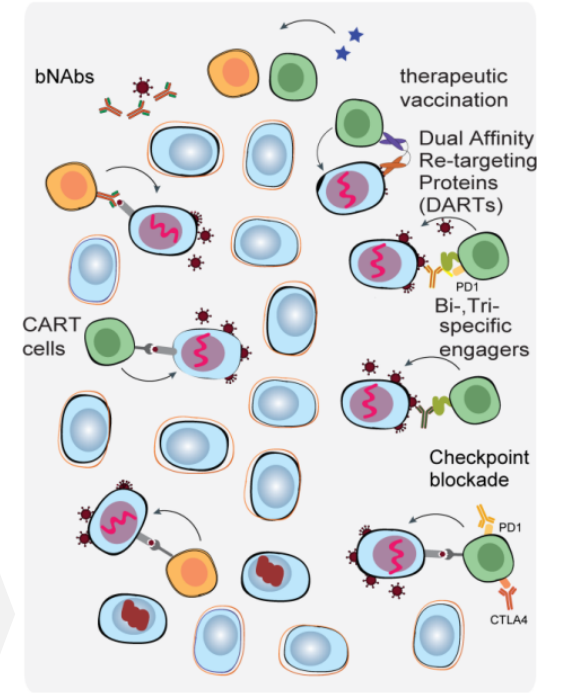
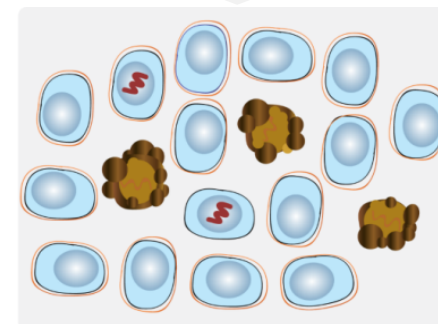
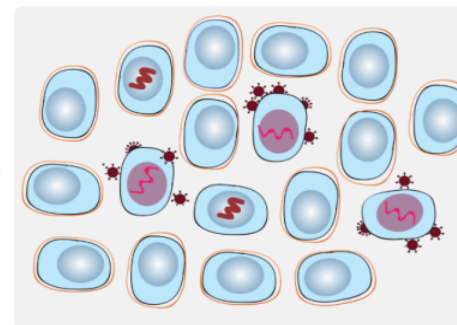


3. reduce the reservoir

- bNAbs,
 therapeutic
 vaccines



Reverse latency



Induce cell killing

"Shock and kill"

Induce cell death

Reverse latency = remove blocks in HIV-1 gene expression

Epigenetic regulation of HIV-1 latency: focus on polycomb group (PcG) proteins

Sheraz Khan, Mazhar Iqbal, Muhammad Tariq, Shahid M. Baig & Wasim Abbas

Clinical Epigenetics 10, Article number: 14 (2018) | [Cite this article](#)

4988 Accesses | 24 Citations | 5 Altmetric | [Metrics](#)

HIV RESERVOIRS: FROM PATHOGENESIS TO DRUG DEVELOPMENT: EDITED BY ROBERT F. SILICIANO AND

Histone deacetylase inhibitors and HIV latency

Margolis, David M

Author Information

Current Opinion in HIV and AIDS 6(1):p 25-29, January 2011. | DOI: 10.1097/COH.0b013e328341

JOURNAL OF VIROLOGY, Sept. 2011, p. 9078-9089
 0022-538X/11/311812-08 doi:10.1128/JVI.01836-11
 Copyright © 2011, American Society for Microbiology. All Rights Reserved.

Epigenetic Silencing of HIV-1 by the Histone H3 Methyltransferase Enhancer of Zeste 2^V Contributes to HIV-1 Latency

Julia Friedman,¹ Won-Kyung Cho,¹ Chung K. Chu,² Kara S. Keedy,³ Nancie M. Margolis,³ and Jonathan Karn^{1,4*}

Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, OH; Pharmacy, The University of Georgia, Athens, Georgia 30602; and Departments of Microbiology, Medicine, and Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

PLOS BIOLOGY

OPEN ACCESS PEER-REVIEWED
 RESEARCH ARTICLE

Repressive LTR Nucleosome Positioning by the BAF Complex Is Required for HIV Latency

Haleh Rafati, Maribel Parra, Shweta Hakre, Yuri Moshkin, Eric Verdin, Tokameh Mahmoudi

Published: November 29, 2011 | <https://doi.org/10.1371/journal.pbio.1001206>

SCIENCE ADVANCES | RESEARCH ARTICLE

BIOCHEMISTRY

Enhancing HIV-1 latency reversal through regulation of the elongating RNA Pol II pause-release by a small-molecule disruptor of PAF1C

Shimaa H. A. Soliman,¹ William J. Cisneros,^{2,3} Marta Iwanaszko,¹ Yuki Aoi,¹ Sheetal Ganesan,¹ Miriam Walter,² Jacob M. Zeidner,¹ Rama K. Mishra,¹ Eun-Young Kim,² Steven M. Wolinsky,² Judd F. Hultquist,^{2,3} Ali Shilatifard^{1*}

Research Article | 1 May 2018

SUN2 Modulates HIV-1 Infection and Latency through Association with Lamin A/C To Maintain the Repressive Chromatin

Authors: Wei-Wei Sun, Shi Jiao, Li Sun, Zhaocai Zhou, Xia Jin, Jian-Hua Wang | [AUTHORS INFO & AFFILIATIONS](#)



Combined noncanonical NF-κB agonism and targeted BET bromodomain inhibition reverse HIV latency ex vivo

Shane D. Falcinelli, ... , Nancie M. Archin, David M. Margolis

Cell Host & Microbe

Volume 21, Issue 5, 10 May 2017, Pages 569-579.e6

Article

SMYD2-Mediated Histone Methylation Contributes to HIV-1 Latency

Daniela Boehm,^{1,2} Mark Jeng,^{1,2} Gregory Camus,^{1,2} Andrea Gramatica,^{1,2,3} Roland Schwarzer,^{1,2,3} Jeffrey R. Johnson,^{1,4} Philip A. Hull,^{1,2} Mauricio Montano,^{1,2,3} Noaki Sakone,^{1,5} Sara Pagnani,^{1,2} Robert Godin,⁵ Steven G. Deeks,² Nevan J. Krogan,^{1,4} Warner C. Greene,^{1,2,3} Melanie Ott^{1,2,7} *

HSF1 inhibition attenuates HIV-1 latency reversal mediated by several candidate LRAs In Vitro and Ex Vivo

Andrew Timmons,¹ Emily Fray,¹ Mithra Kumar,¹ Fengting Wu,¹ Weiwei Dai,¹ Cynthia Korin Bullen,¹ Peggy Kim,¹ Carrie Hetzel,¹ Chao Yang,¹ Subul Beg,¹ Jun Lai,¹ Joel L. Pomerantz,¹ Steven A. Yukl,¹ Janet D. Siliciano,¹ and Robert F. Siliciano^{1,2,3,4} *

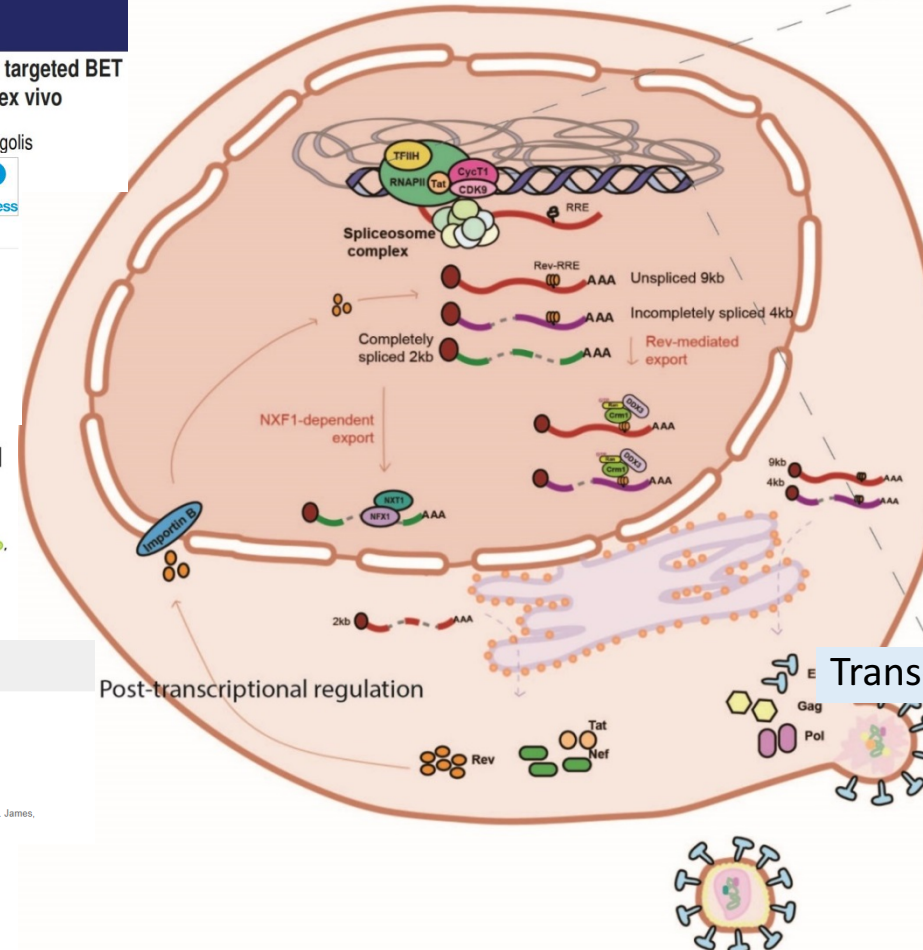
PLOS PATHOGENS

OPEN ACCESS PEER-REVIEWED
 RESEARCH ARTICLE

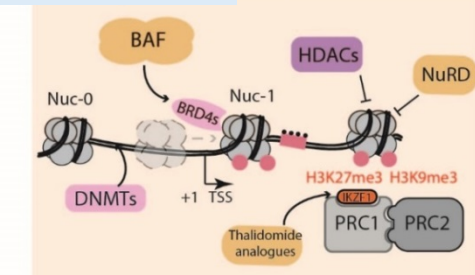
Epigenomic characterization of latent HIV infection identifies latency regulating transcription factors

Stuart R. Jefferys, Samuel D. Burgos, Jackson J. Peterson, Sara R. Sellitsky, Anne-Marie W. Turner, Lindsey I. James, Yi-Hsuan Tsai, Alisha R. Coffey, David M. Margolis, Joel Parker, Edward P. Browne

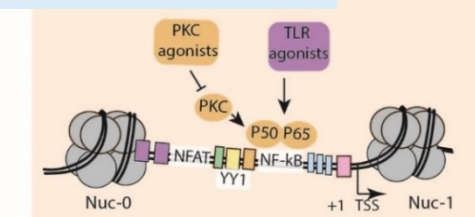
f t in e



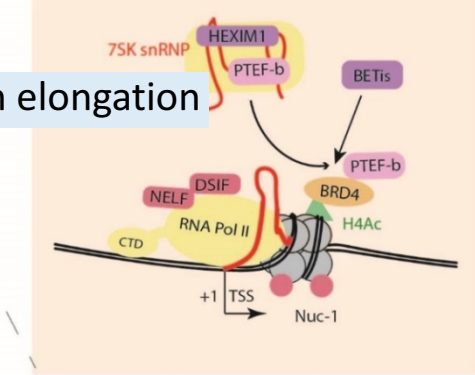
Chromatin de-repression



TF-mediated activation



Transcription elongation



Focus on chromatin de-repression, trxn initiation, elongation

Article | Open access | Published: 30 April 2021
Selective cell death in HIV-1-infected cells inhibitors leads to depletion of the inducible

Shringar Rao, Cynthia Lungu, Raquel Crespo, Thijs H. Steijaert, Alicja Gorska, Rob A. B. Prins, Wilfred van Ijcken, Yvonne M. Mueller, Jercoen J. A. van Kampen, Annel Katsikis, Charles A. B. Roucher, Casper Rokx, Rob A. Gruters & Tokameh Mahmou

Nature Communications 12, Article number | 7529 Accesses | 23 Citations
The Splice of Life: Does RNA Processing Have a Role in HIV-1 Persistence?

by Alexander O. Pasternak and Ben Berkhout

RESEARCH ARTICLE
Human splice factors contribute to latent HIV infection in primary cell models and blood CD4⁺ T cells from ART-treated individuals

Sara Moron-Lopez^{1,2}, Sushama Telwatte^{1,2}, Indra Sarabia³, Emilie Battivelli⁴, Mauricio Montano⁵, Amanda B. Macedo², Dvir Aran^{6,7}, Atul J. Butte^{8,9}, R. Brad Jones⁶, Alberto Bosque^{6,7}, Eric Verdin¹⁰, Warner C. Greene^{1,3}, Joseph K. Wong^{1,2}, Steven A. Yuki^{1,2,*}

The RNA-Binding Proteins SRP14 and HMGB3 Control HIV-1 Tat mRNA Processing and Translation During HIV-1 Latency

Georges Khoury^{1†}, Michelle Y. Lee^{1†}, Sri H. Ramarathinam², James McMahon³, Anthony W. Purcell², Secondo Sonza¹, Sharon R. Lewin^{3,4,5} and Damian F. J. Purcell^{1*}

HIV

HIV latency in isolated patient CD4⁺ T cells may be due to blocks in HIV transcriptional elongation, completion, and splicing

Steven A. Yuki^{1*}, Philipp Kaiser¹, Peggy Kim, Sushama Telwatte, Sunil K. Joshi², Mai Vu, Harry Lampiris, Joseph K. Wong

Posttranscriptional Regulation of HIV-1 Gene Expression during Replication and Reactivation from Latency by Nuclear Matrix Protein MATR3

Authors: Ambra Sarracino, Lavina Gharu, Anna Kula, Alexander O. Pasternak, Veronique Avettand-Fenoel, Christine Rouzioux, Maryana Bardina, Stéphane De Wit, Monsef Benkirane, Ben Berkhout, Carine Van Lint, Alessandro Marcello | AUTHORS INFO & AFFILIATIONS

Nuclear Retention of Multiply Spliced HIV CD4⁺ T Cells

Kara G Lassen, Kasra X Ramyar, Justin R Bailey, Yan Zhou, Robert F Siliciano

Published: July 7, 2006 • https://doi.org/10.1371/journal.ppat.0020068

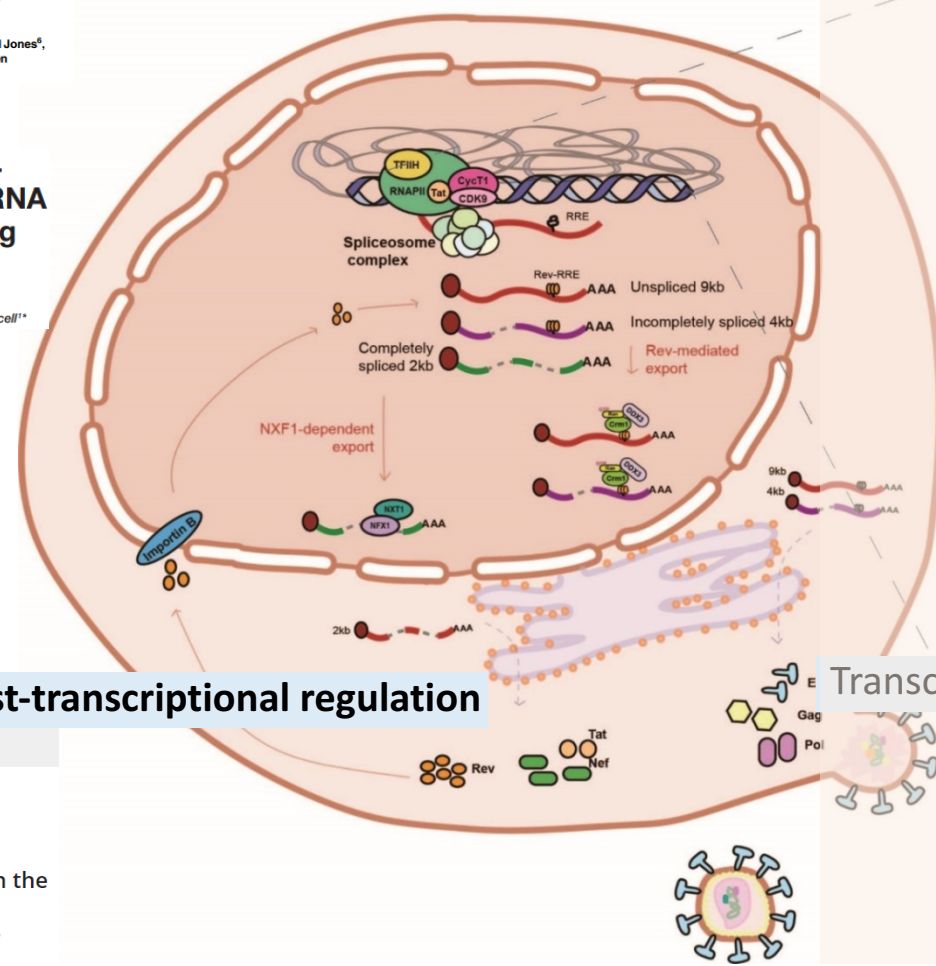
Gut and blood differ in constitutive blocks to HIV transcription, suggesting tissue-specific differences in the mechanisms that govern HIV latency

Sushama Telwatte, Sulggi Lee, Ma Somsouk, Hiroyu Hatano, Christopher Baker, Philipp Kaiser, Peggy Kim, Tsui-Hua Chen, Jeffrey Milush, Peter W. Hunt, Steven G. Deeks, Joseph K. Wong, Steven A. Yuki

Published: November 15, 2018 • https://doi.org/10.1371/journal.ppat.1007357

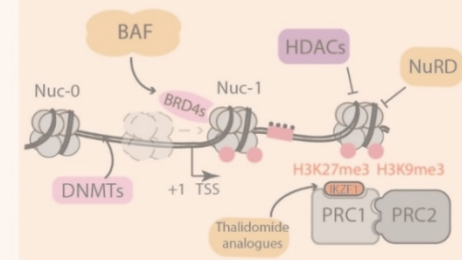
Single-cell analysis of HIV-1 transcriptional activity reveals expression of proviruses in expanded clones during ART

Ann Wiegand, Jonathan Spindler, Eelyu F. Hong, and Mary F. Kearney | Authors Info & Affiliations

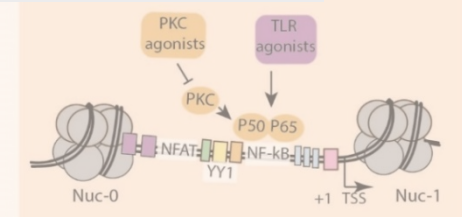


Post-transcriptional regulation

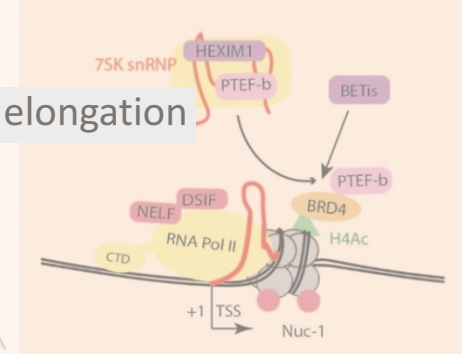
Chromatin de-repression



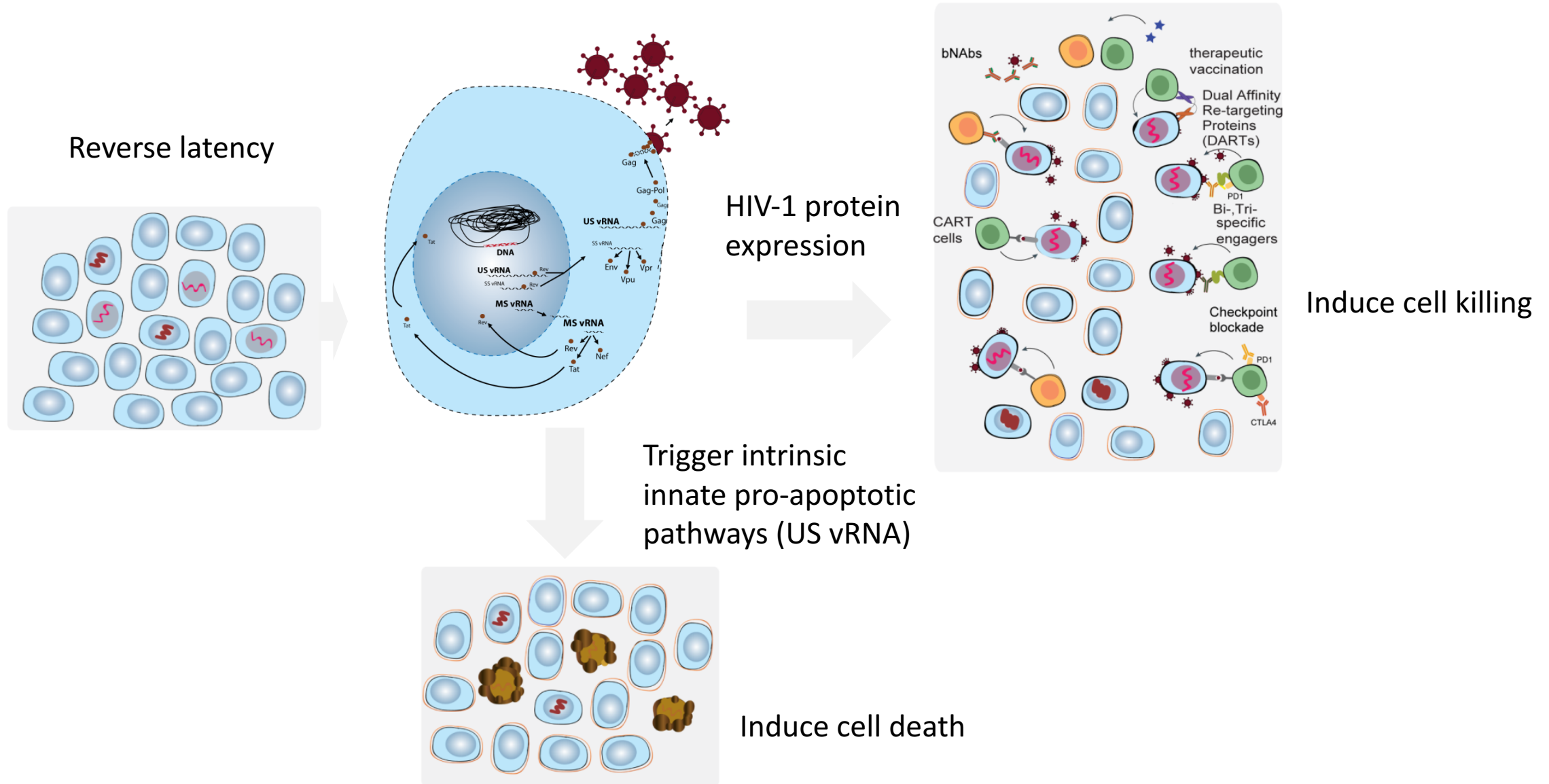
TF-mediated activation



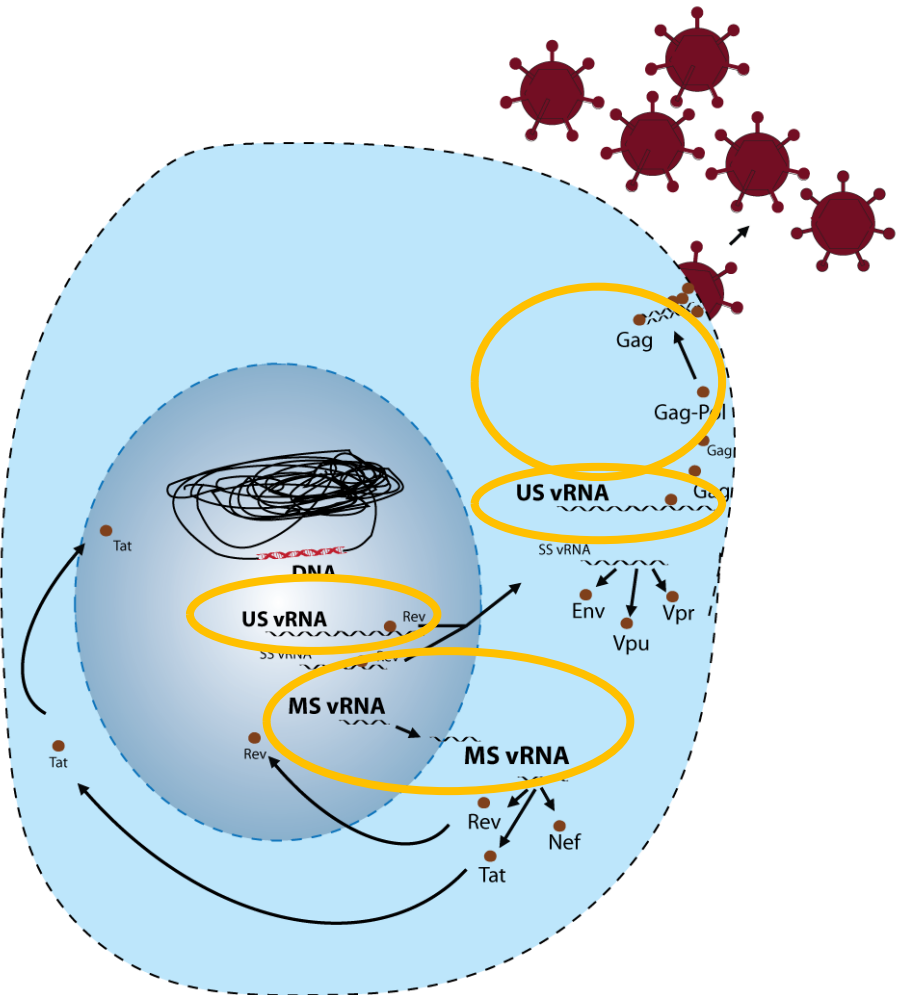
Transcription elongation



Regulation of vRNA processing to impact ratio of splice variants, protein expression



Tools to quantify and distinguish between vRNA transcripts



RT-PCR/RT-ddPCR - US vRNA
 - MS vRNA

TILDA - MS vRNA

Single cell level
 FISH-Flow
 HIV-Flow - Viral RNA
 and proteins

IVRA - Intact vRNA

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

HIV
HIV latency in isolated patient CD4⁺ T cells may be due to blocks in HIV transcriptional elongation, completion, and splicing

Steven A. Yuki,^a Philipp Kalsner,^a Peggy Kim, Sushama Telwatte, Sunil K. Joshi,^a Mai Vu, Harry Lampiris, Joseph K. Wong



Original Article
 A Novel Assay to Measure the Magnitude of the Inducible Viral Reservoir in HIV-infected Individuals

Francesco Andrea Procopio^a, Rémi Fromentin^a, Deanna A. Kulpa^a, Jessica H. Brehm^a, Anne-Gaëlle Bebin^a, Matthew C. Strain^b, Douglas D. Richman^b, Una O'Doherty^c, Sarah Palmer^{d,e}, Frederick M. Hecht^f, Rebecca Hoh^g, Richard J.O. Barnard^h, Michael D. Miller^h, Daria J. Hazuda^h, Steven G. Deeks^f, Rafick-Pierre Sékaly^{a,g}, Nicolas Chomont^{a,g,g}

PROTOCOL

Multiparametric characterization of rare HIV-infected cells using an RNA-flow FISH technique

Amy E Baxter^{1,2}, Julia Niessl^{1,2}, Rémi Fromentin¹, Jonathan Richard¹, Filippos Porichis^{3,6}, Marta Massanella¹, Nathalie Brassard¹, Nirmin Alsahafi^{1,4}, Jean-Pierre Routy⁵, Andrés Finzi^{1,4}, Nicolas Chomont¹, & Daniel E Kaufmann^{1,2}

RESEARCH ARTICLE
 Single-cell characterization and quantification of translation-competent viral reservoirs in treated and untreated HIV infection

Marion Pardons¹, Amy E. Baxter^{1*}, Marta Massanella^{1*}, Amélie Pagliuzza², Rémi Fromentin², Caroline Dufour¹, Louise Leyre², Jean-Pierre Routy², Daniel E. Kaufmann^{2,4}, Nicolas Chomont^{1,2*}



GENOME REPLICATION AND REGULATION OF VIRAL GENE EXPRESSION



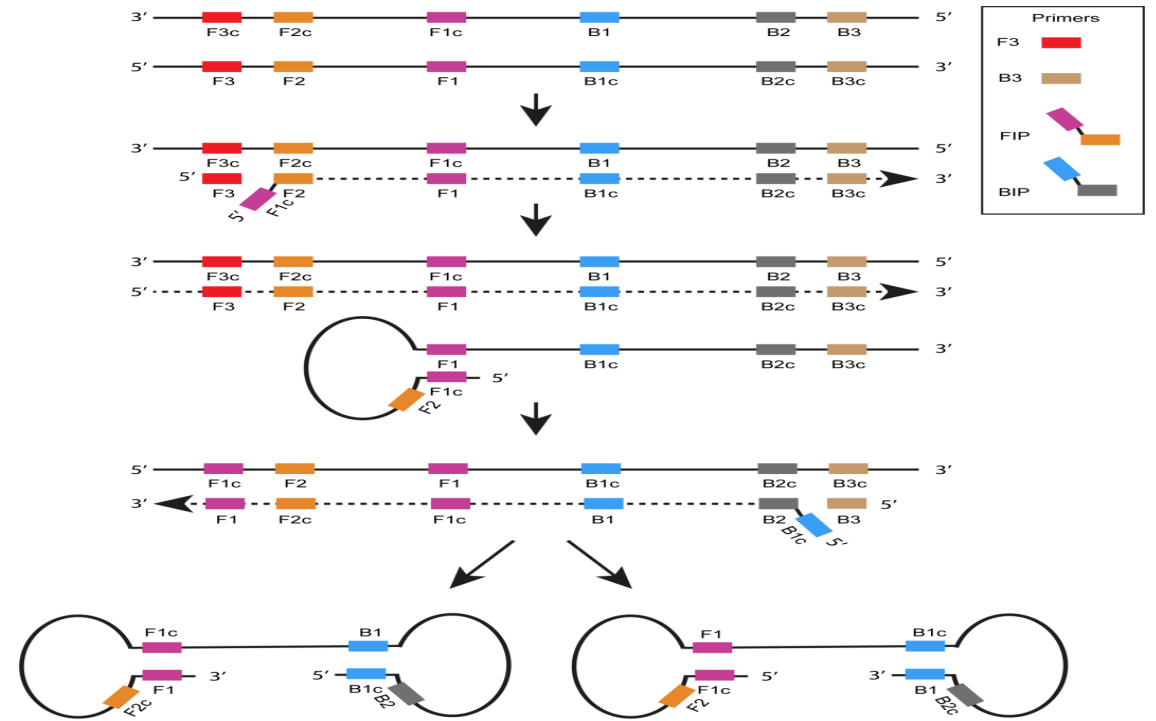
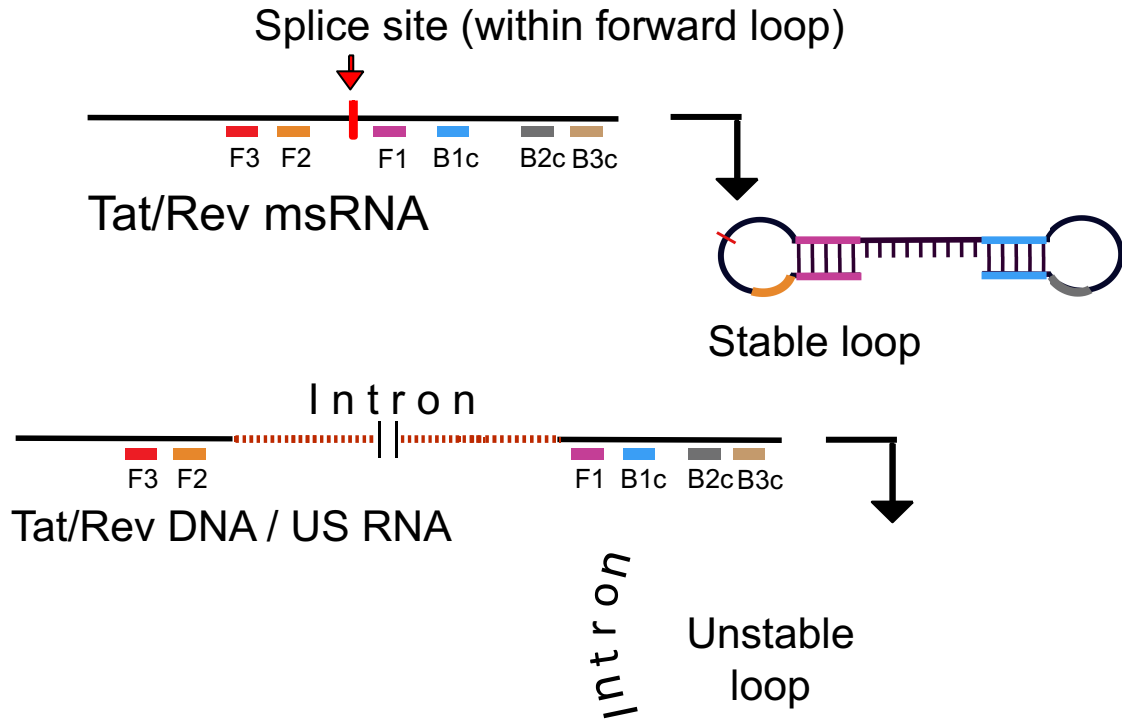
New Assay Reveals Vast Excess of Defective over Intact HIV-1 Transcripts in Antiretroviral Therapy-Suppressed Individuals

Holly Anne Martin,^{a,b} Gayatri Nikhila Kadiyala,^{a,b} Sushama Telwatte,^{a,b} Adam Wedrychowski,^{a,b} Tsui-Hua Chen,^b Sara Moron-Lopez,^{a,b} Doug Arneson,^a Rebecca Hoh,^a Steven Deeks,^a Joseph Wong,^{a,b} Steven A. Yuki^{a,b}



Tonmoy Hossain
 PhD student

1. Specific detection and amplification of HIV-1 msRNA by RT-LAMP

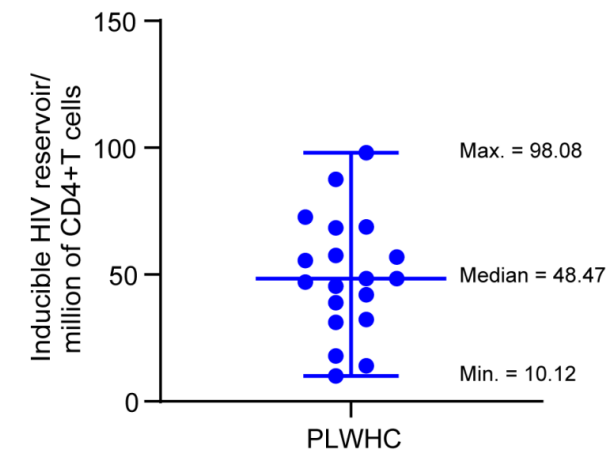
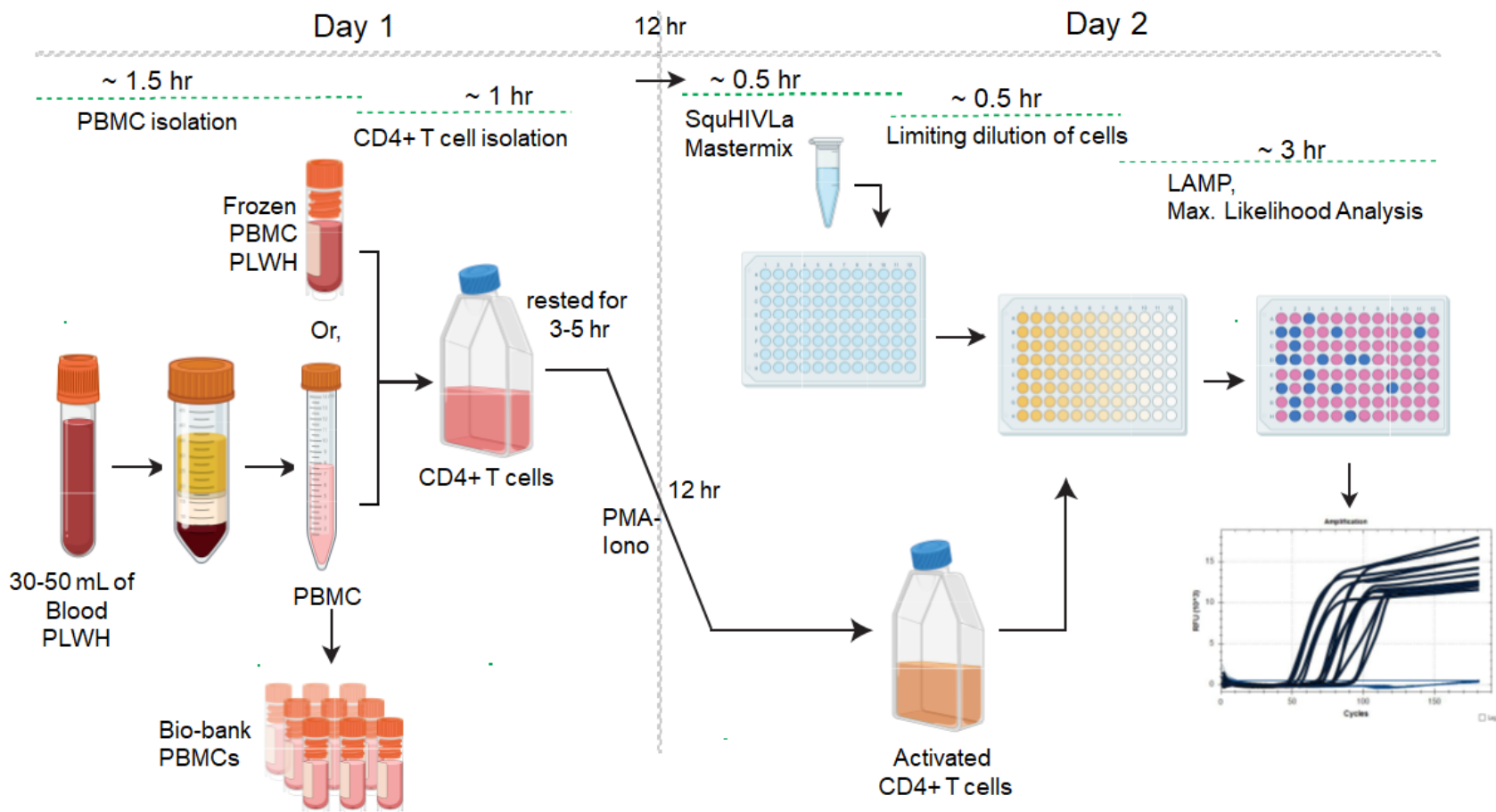


Isothermal exponential amplification



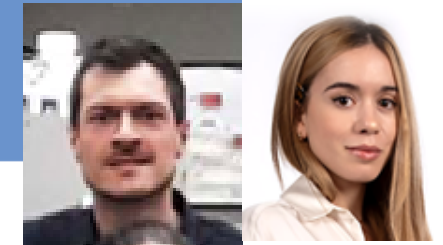
Tonmoy Hossain
 PhD student

2. Quantitation of number of HIV-1 msRNA+ cell / million CD4+ T cells by limiting dilution



PWH-C, HPP cohort

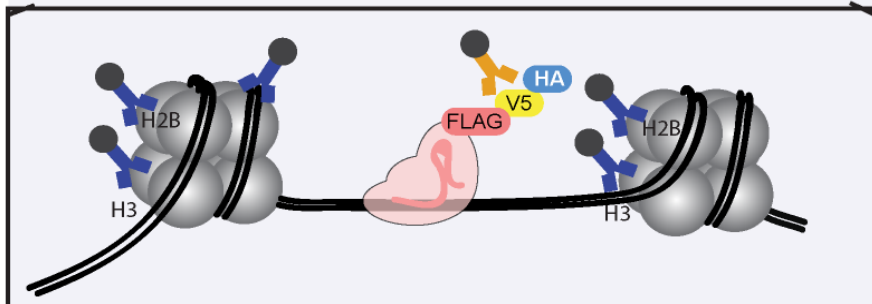
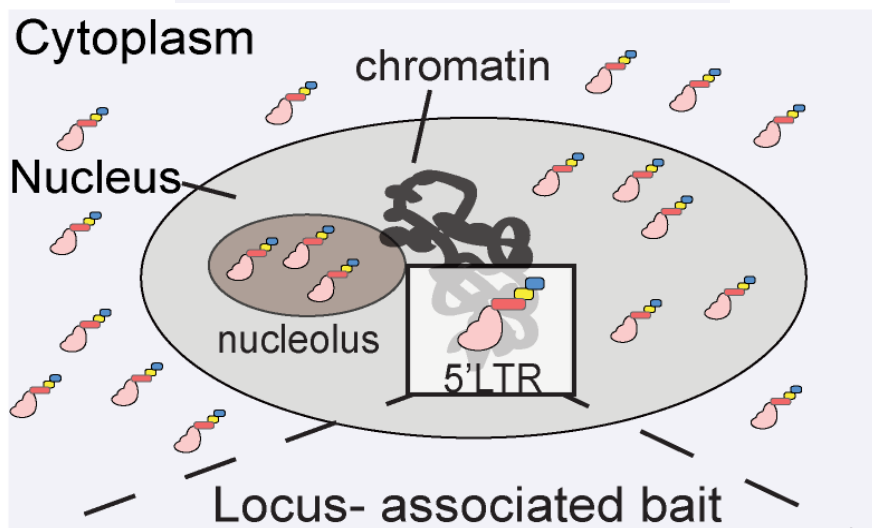
What is bound to the latent HIV-1 LTR nuc-1?



Enrico Ne
 PhD student

Raquel Crespo
 PhD student

Catchet-MS (dCas9 targeted chromatin and histone enrichment coupled to mass Spec)

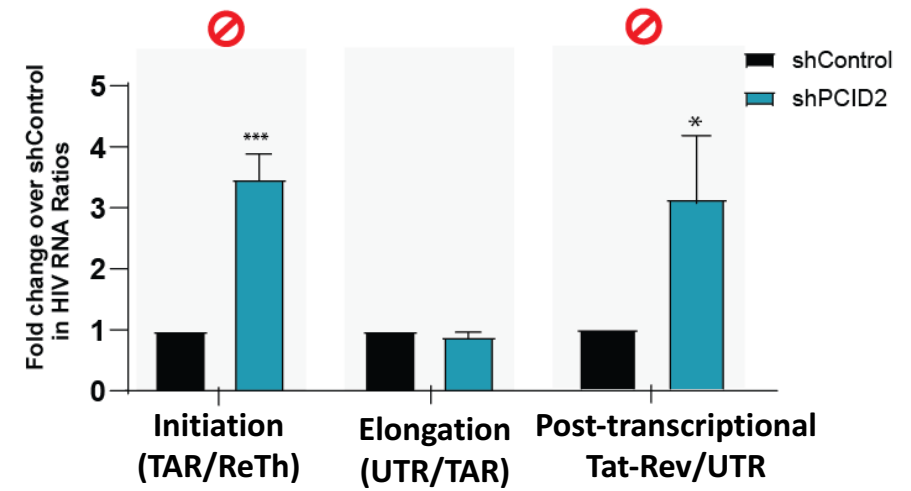
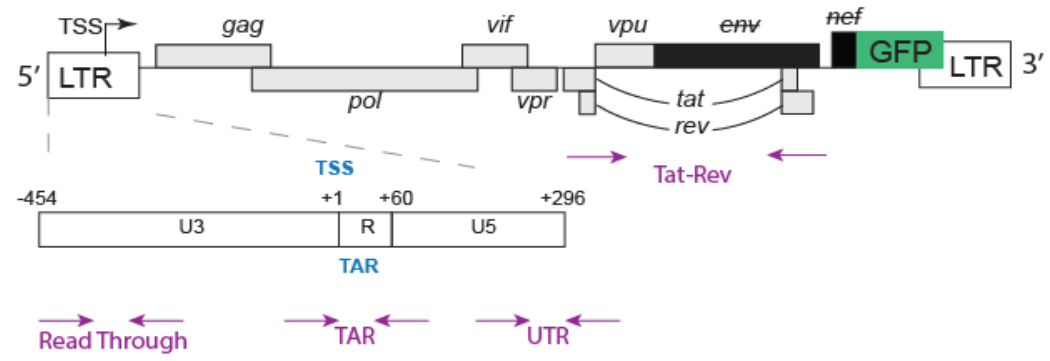
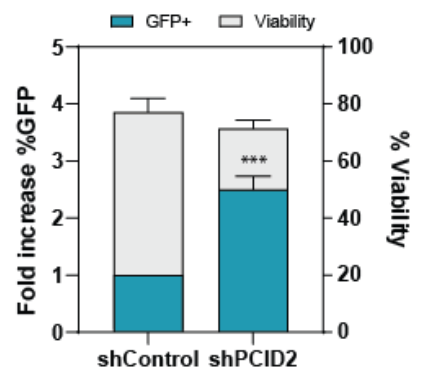
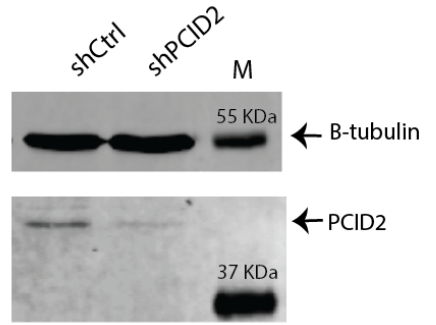


			-PMA	+PMA	Known HIV repressors
DNA associated	Chromatin remodeling	SIN3A			Jean et al., 2017
		SMARCA5			
		SUZ12			Matsuda et al., 2015
	Structural	CHD1			Gallastegui et al., 2011
		HP1BP3			
		CBX8			Khan et al., 2018
	Transcription factor	PDS5B			
		PDS5A			
		RCC1			
		ZNF346			
IKZF1					
HMG20A					
RFX5					
POU2F1				Goffin et al., 2005	
NFRKB					
YY1				Coull et al., 2000	
RNA associated	PFY1				
	PUF60				
	SLBP				
	RNGTT			Chiu et al., 2001	
	CDC73				
	FAM103A1				
	SRRT				
	AQR				
	CDK5				
	DDX39A				
	PCID2				
	FAM50A				
	RNASEH2B				
	UBE2I				
	FAM32A				
	DKC1			Zhao et al., 2016	
	DDX24			Ma et al., 2008	
	CRNKL1			Xiao et al., 2020	
	DHX16				
	PNN				
SUPT16H			Huang et al., 2015		
TCEA1					

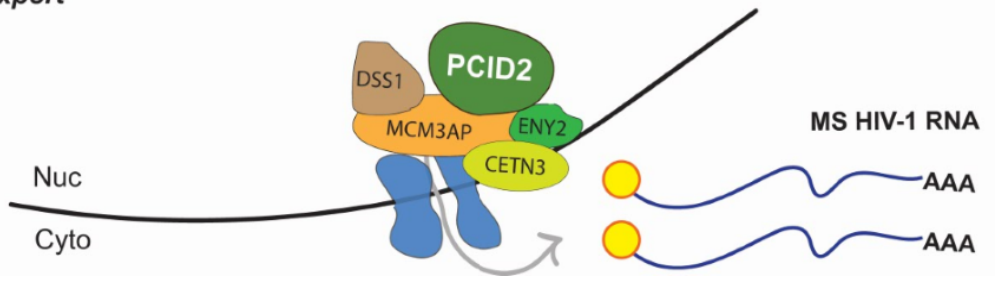
PCID2 dysregulates HIV-1 transcription and viral RNA processing



Raquel Crespo
 PhD student

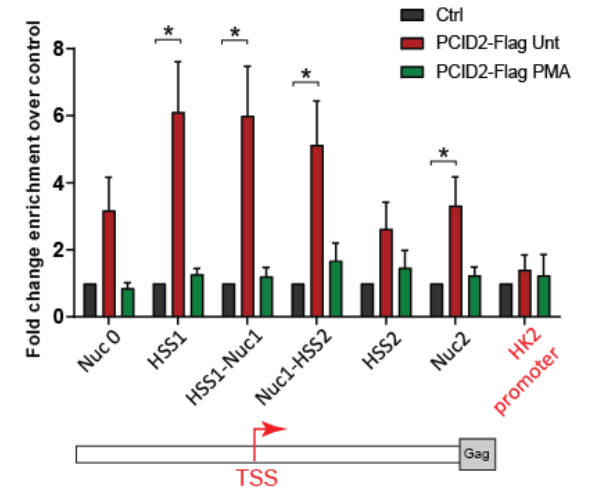
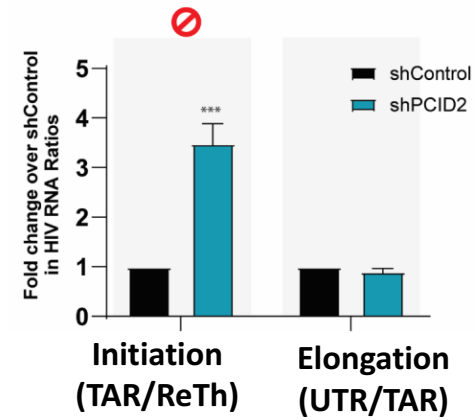
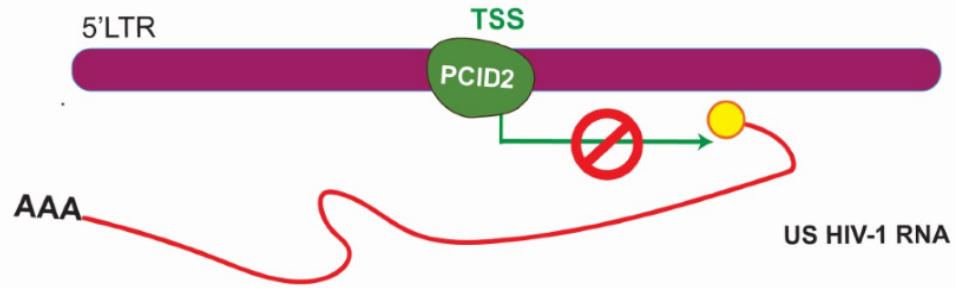


3. Export

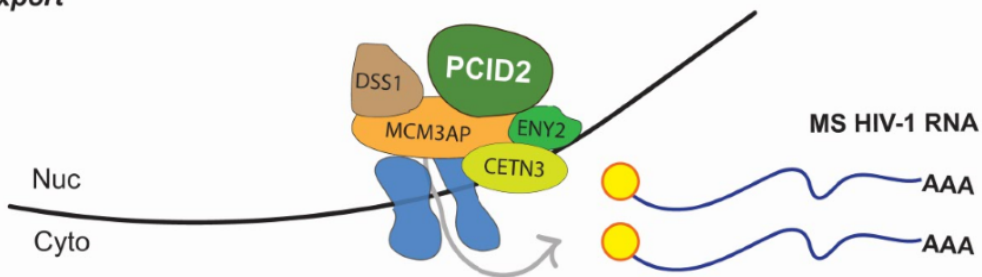


PCID2 dysregulates transcription and viral RNA processing to promote HIV-1 latency

1. Transcription initiation

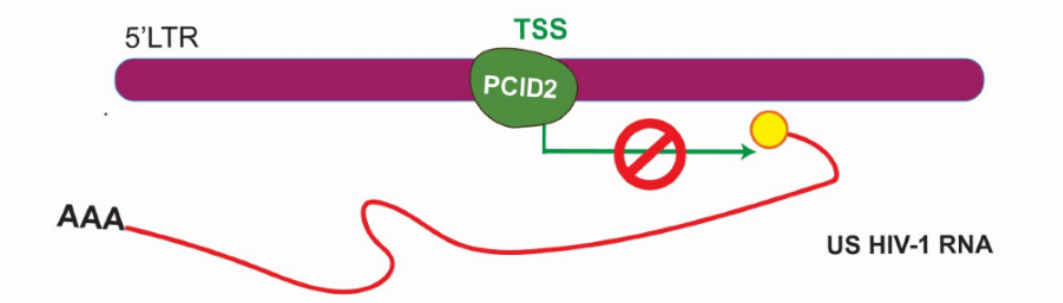


3. Export

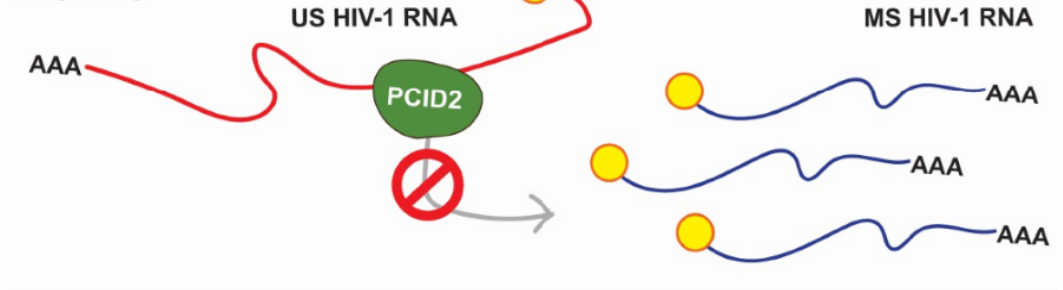


PCID2 dysregulates transcription and viral RNA processing to promote HIV-1 latency

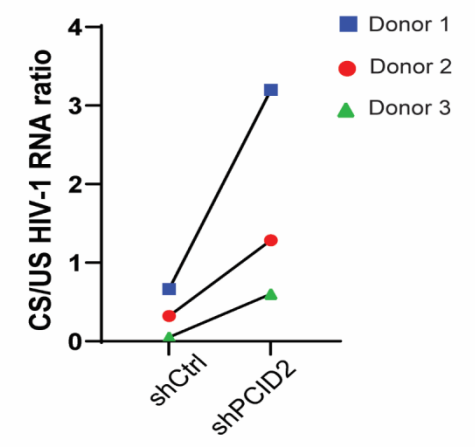
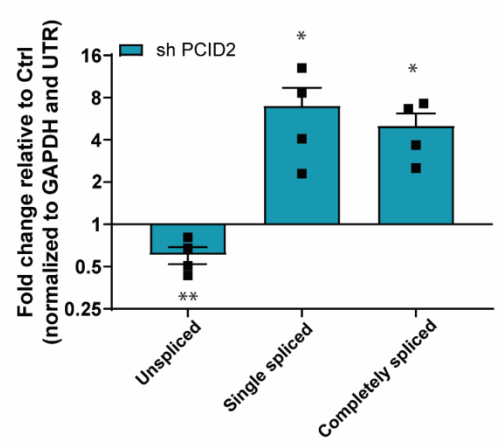
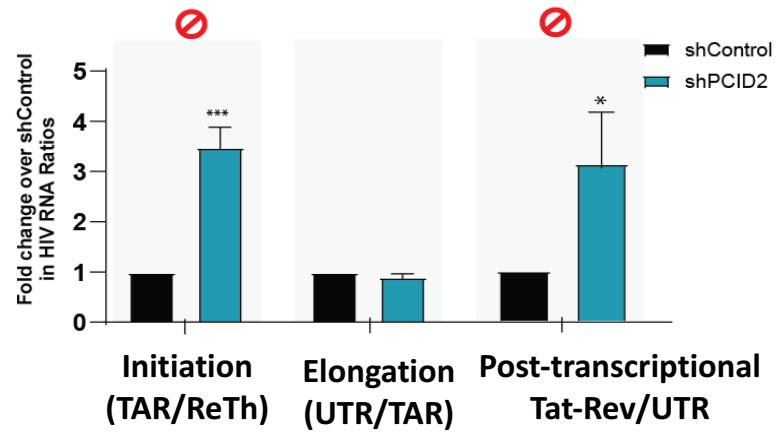
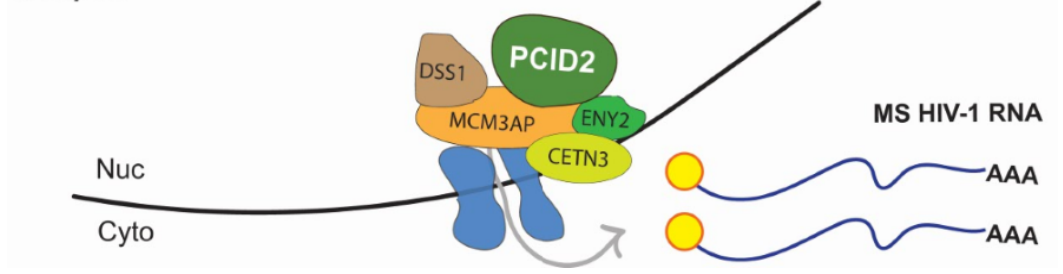
1. Transcription initiation



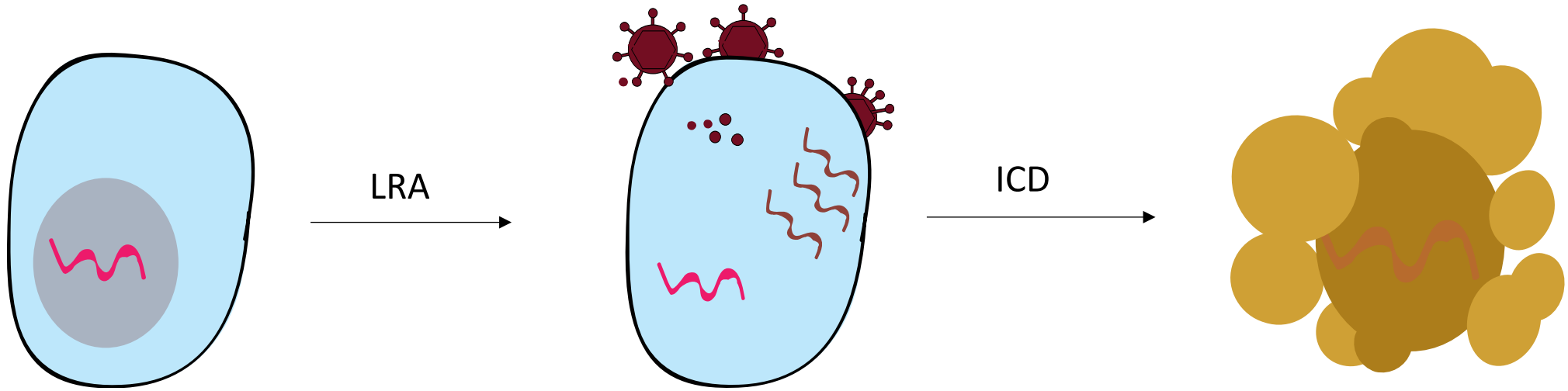
2. Splicing



3. Export



Inducing cell death (ICD) in HIV-1 infected cells, including transcription-competent defective proviruses



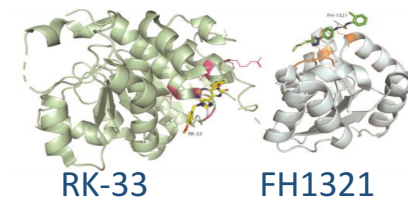
- Activated innate antiviral signalling pathways
- Pro-apoptotic



DDX3 inhibition: reverses latency, impairs vRNA nucleocytoplasmic export



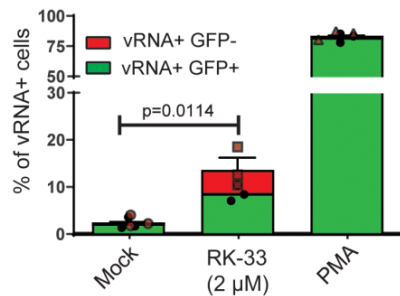
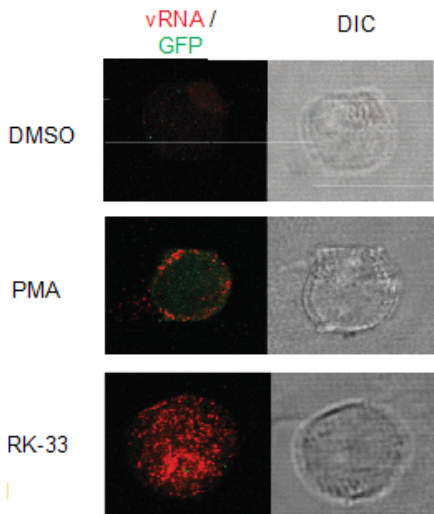
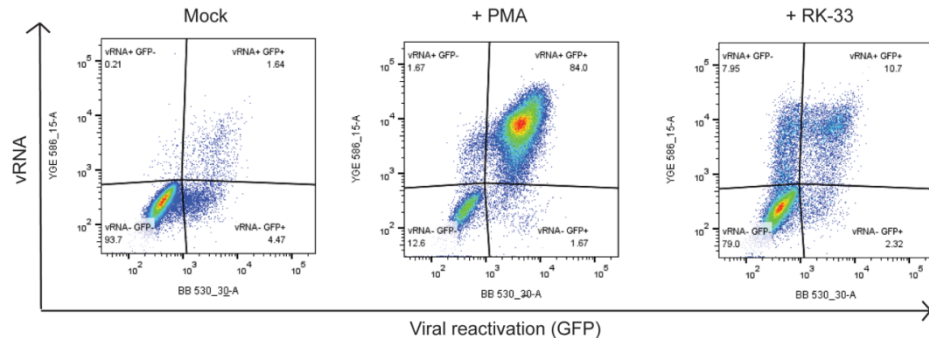
Shringar Rao
 Post-doc



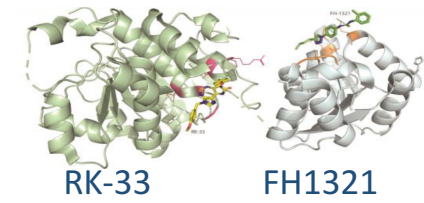
➤ pharmacological DDX3 Inhibitors:

DDX3 implicated in HIV-1 RNA metabolism, transport, innate sensing, apoptosis

FISH-Flow

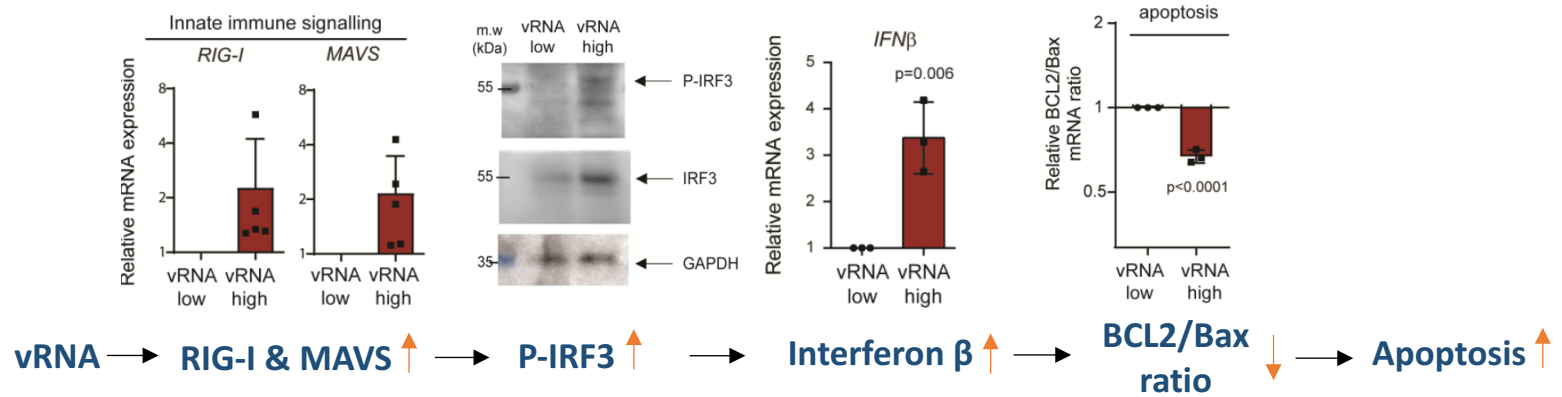
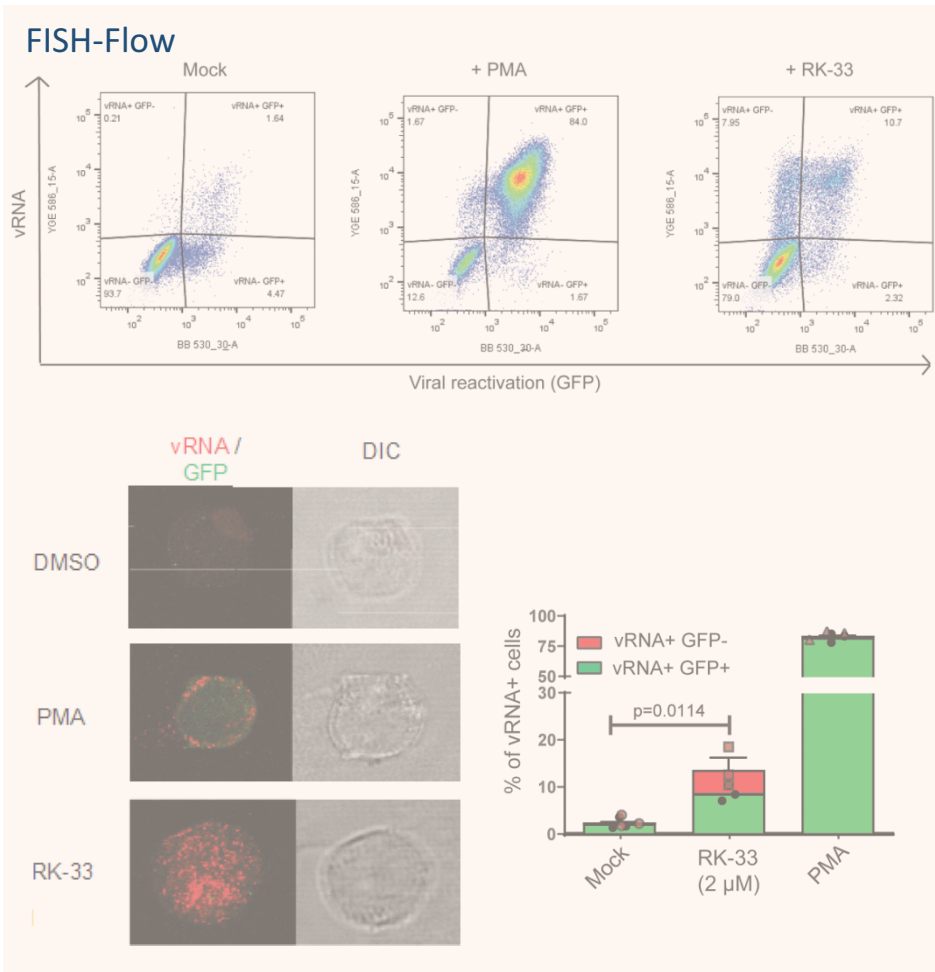


DDX3 inhibition: vRNA expressing cells are pro-apoptotic



DDX3 implicated in HIV-1 RNA metabolism, transport, innate sensing, apoptosis

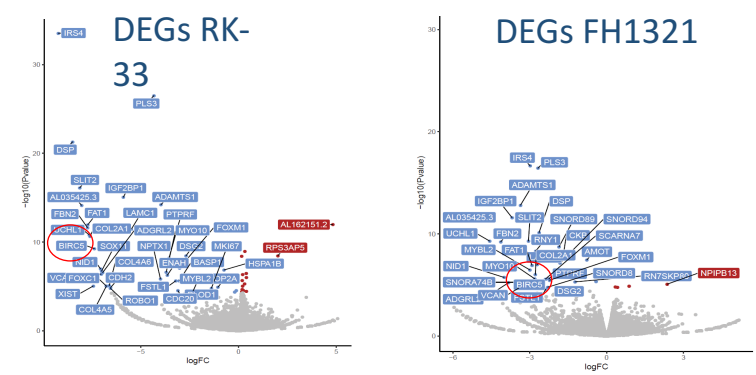
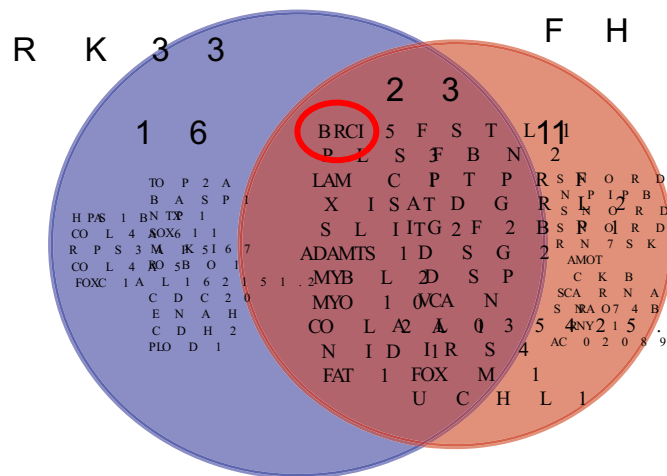
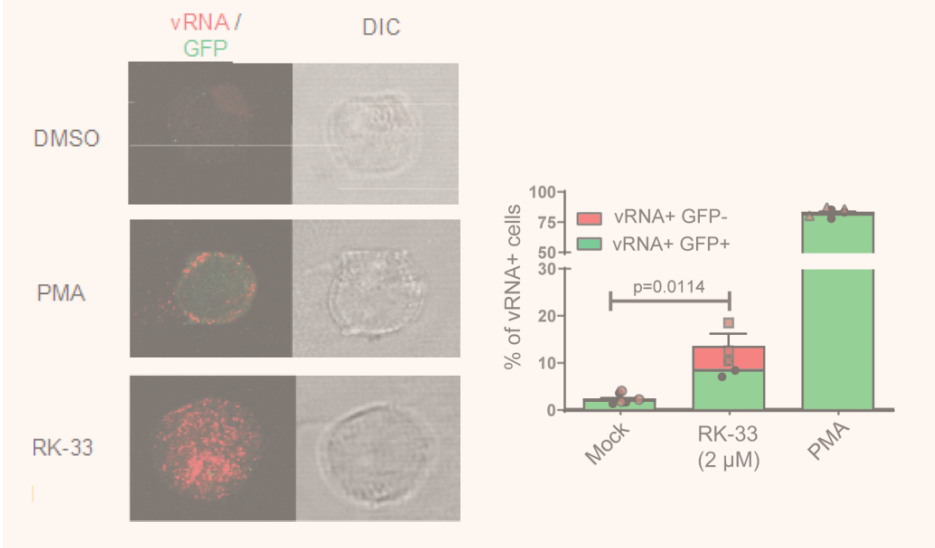
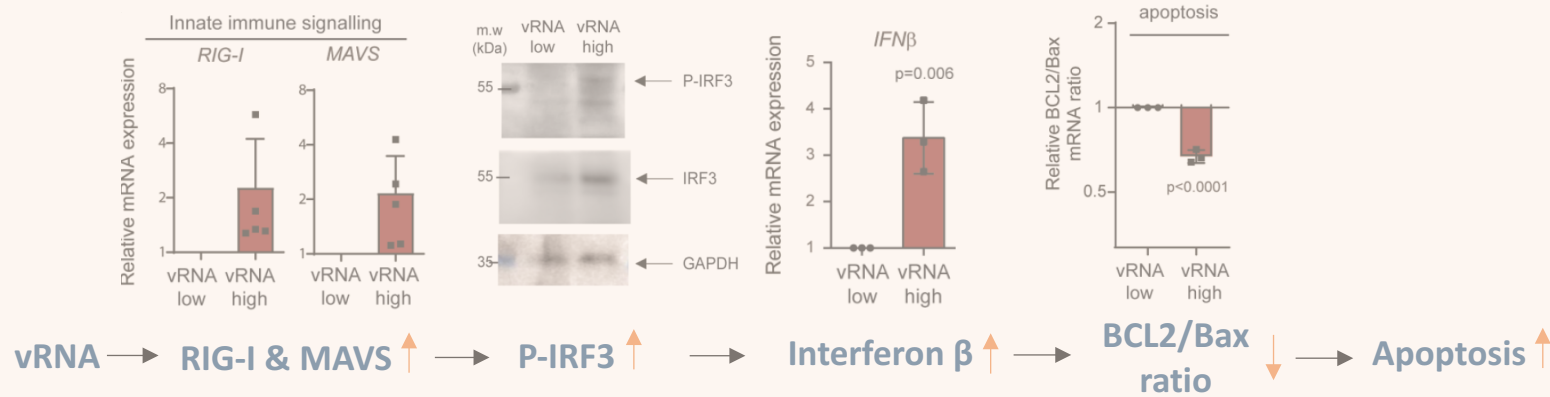
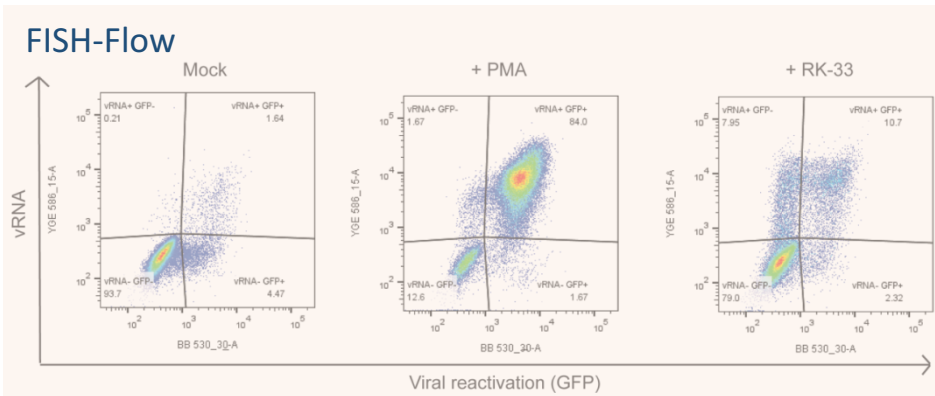
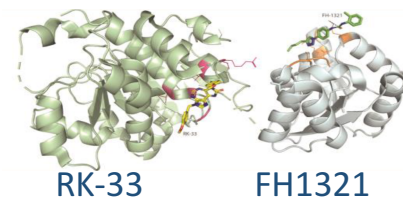
➤ pharmacological DDX3 Inhibitors:



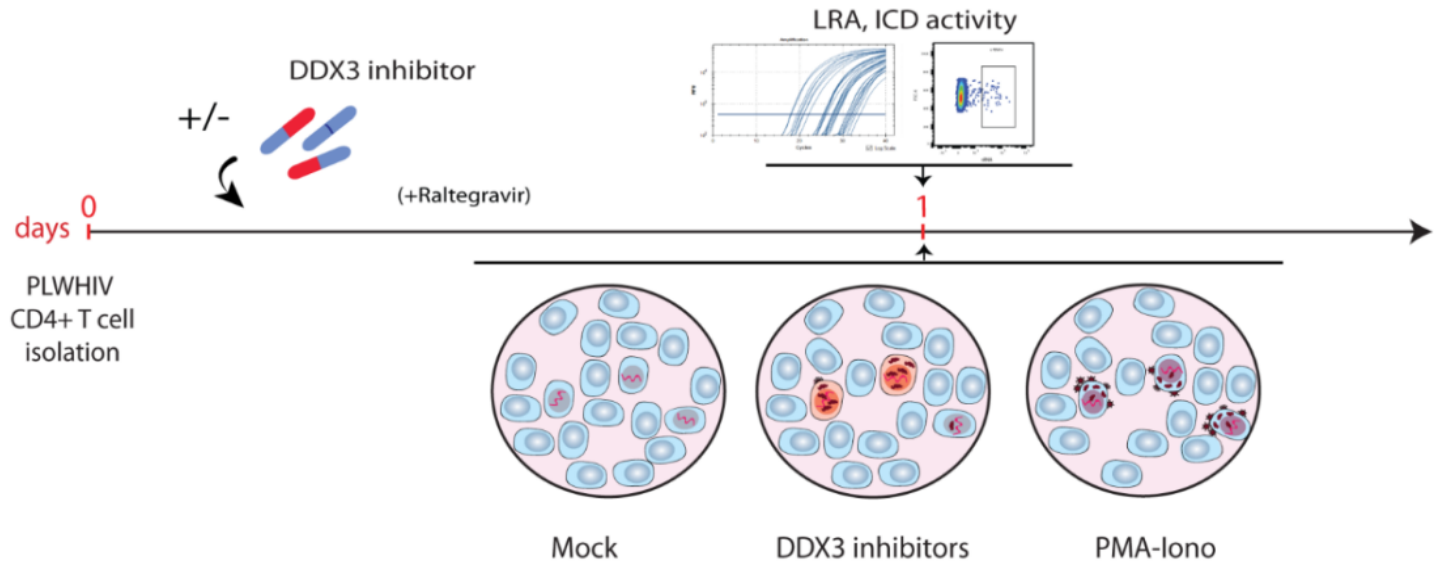
DDX3 inhibition: BIRC5 (Survivin) is downregulated in primary CD4+ T cells

DDX3 implicated in HIV-1 RNA metabolism, transport, innate sensing, apoptosis

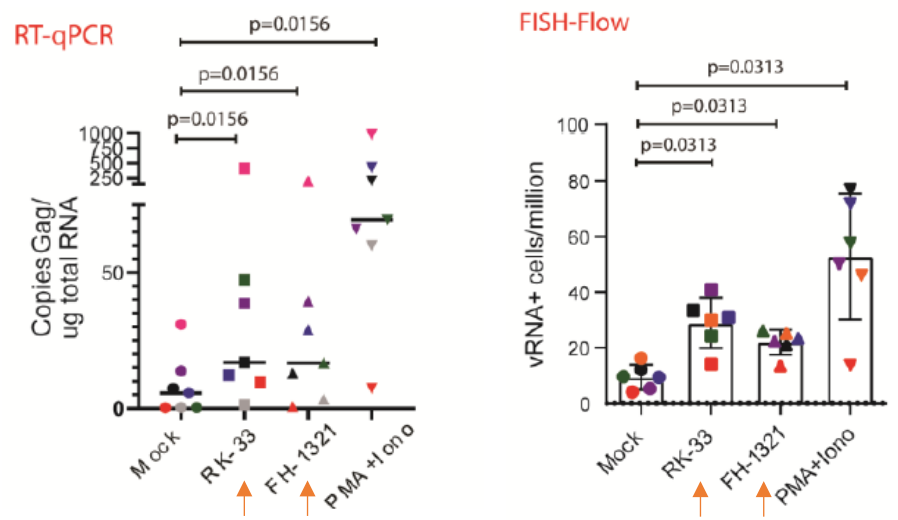
➤ pharmacological DDX3 Inhibitors:



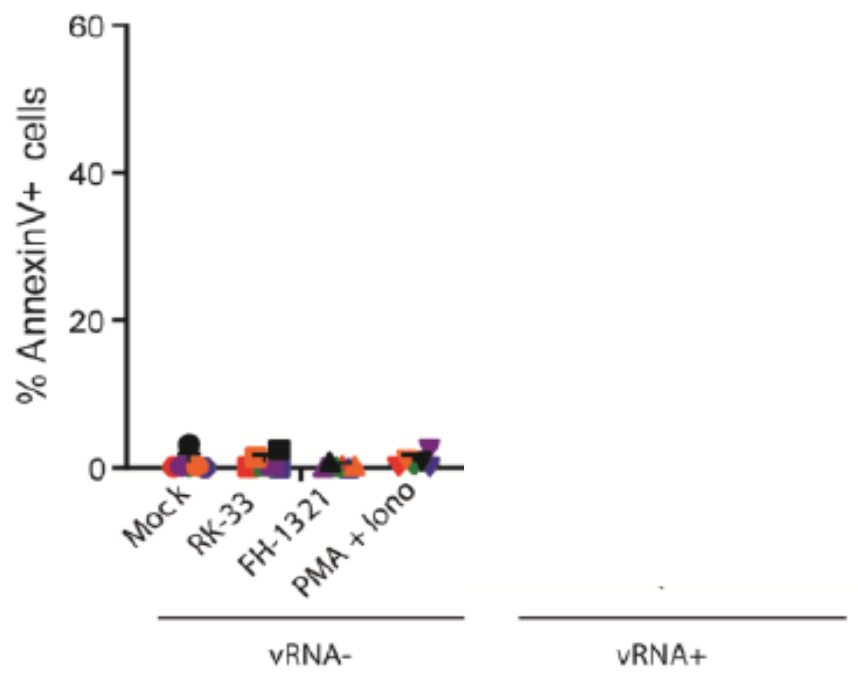
DDX3 inhibitors induce LRA-ICD activity ex vivo in CD4+ T cells from PWH



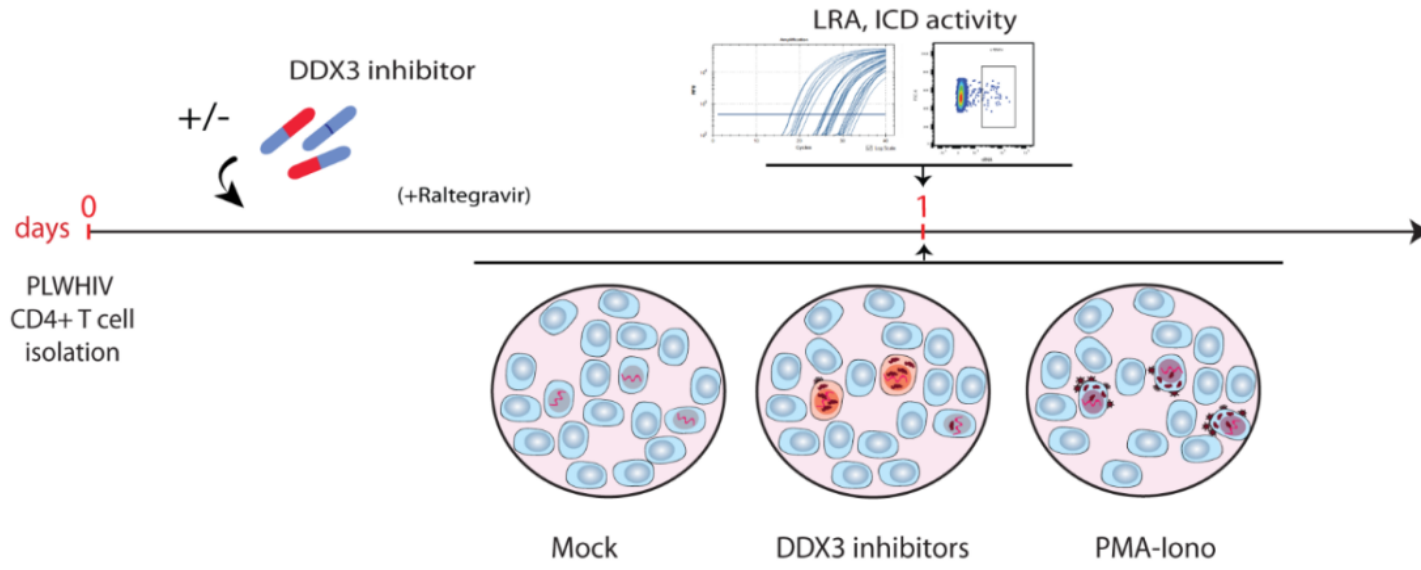
Latency reversal:



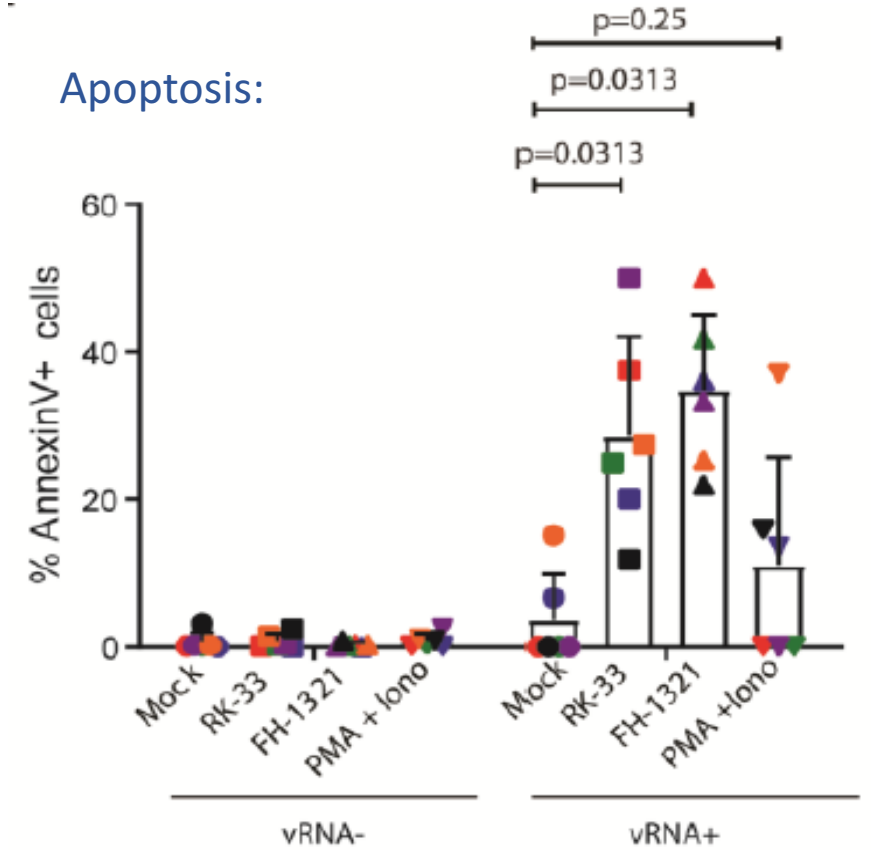
Apoptosis:



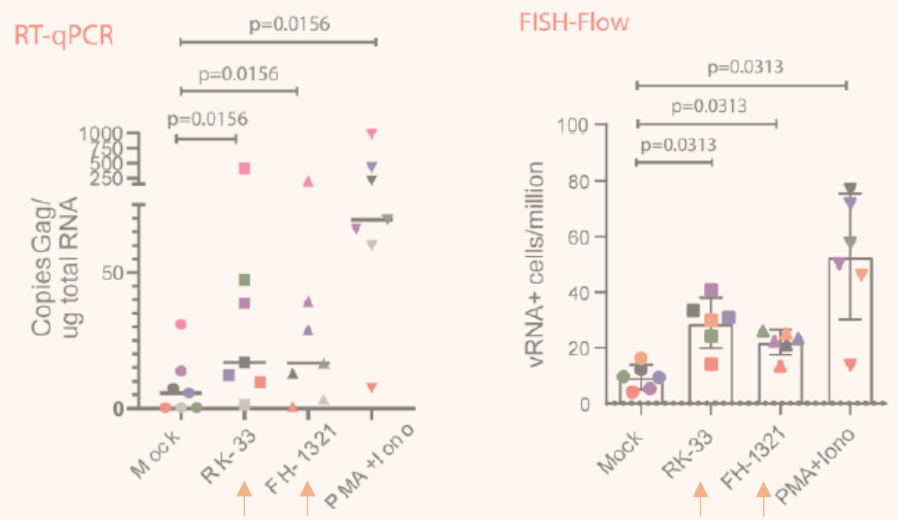
DDX3 inhibitors induce LRA-ICD activity ex vivo in CD4+ T cells from PWH



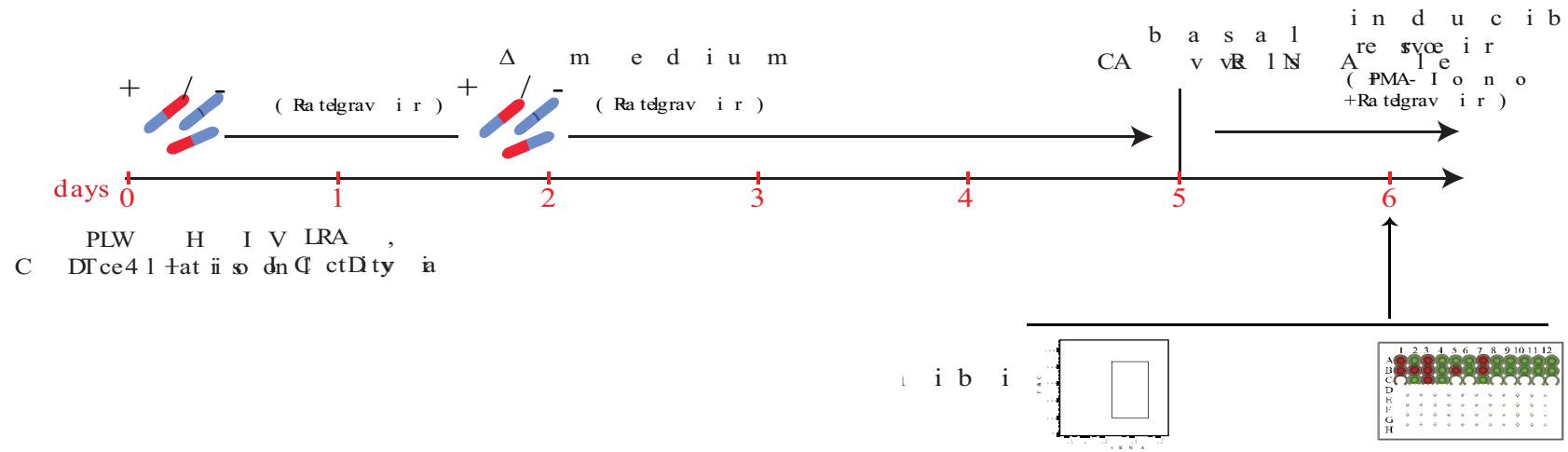
Apoptosis:



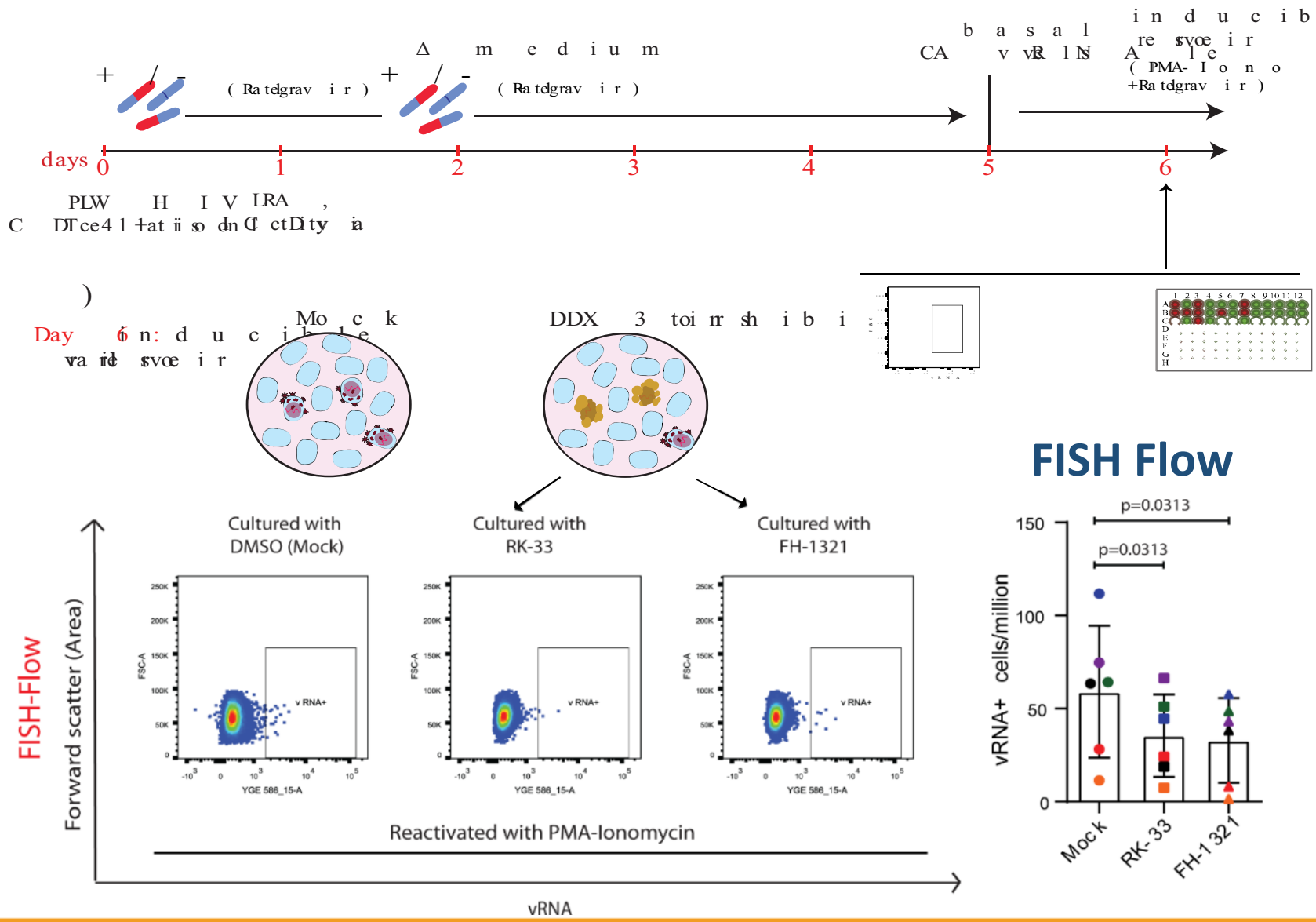
Latency reversal:



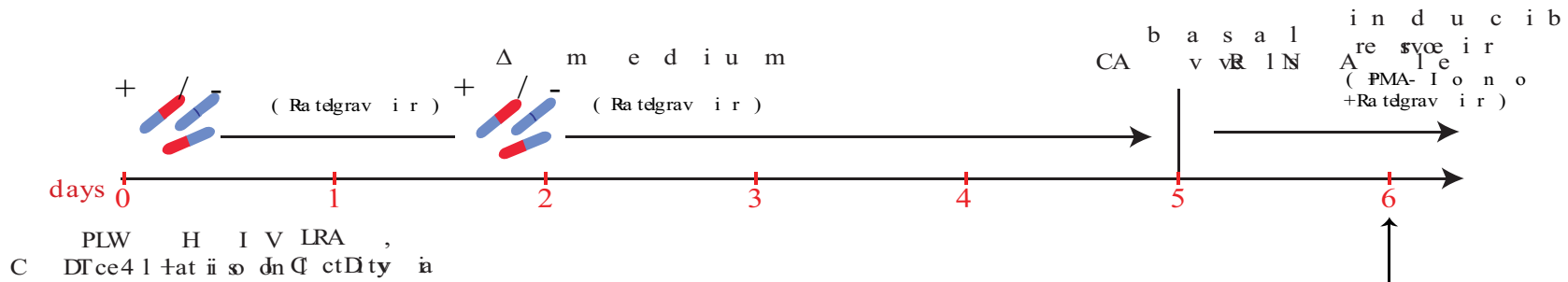
DDX3 inhibitors: reduce the inducible reservoir in cells from PWH ex vivo



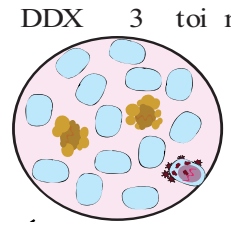
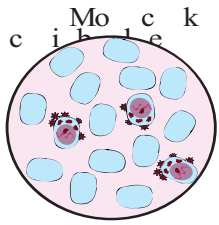
DDX3 inhibitors: reduce the inducible reservoir in cells from PWH ex vivo



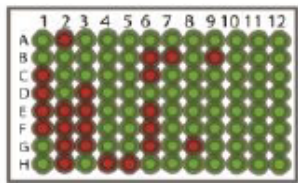
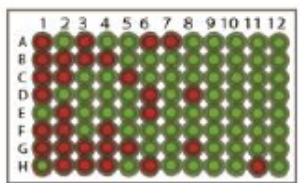
DDX3 inhibitors: reduce the inducible reservoir in cells from PWH ex vivo



Day 6: inducible reservoir



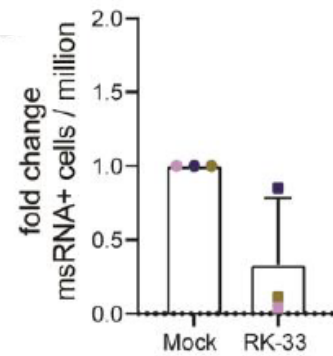
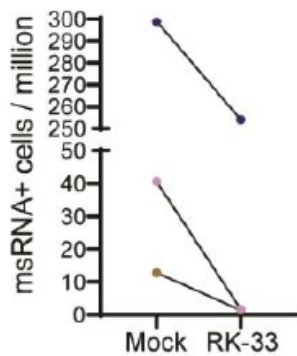
TILDA



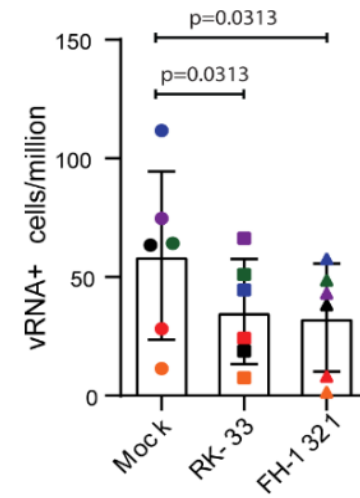
Positive Negative

Mock

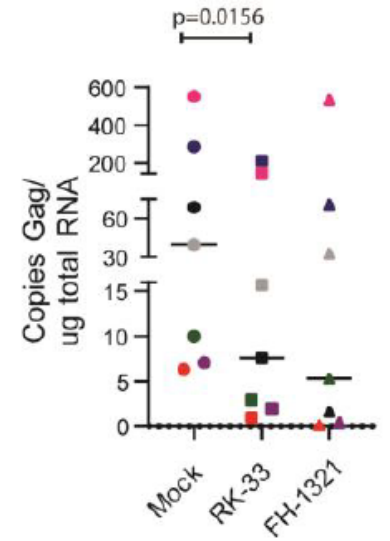
RK-33



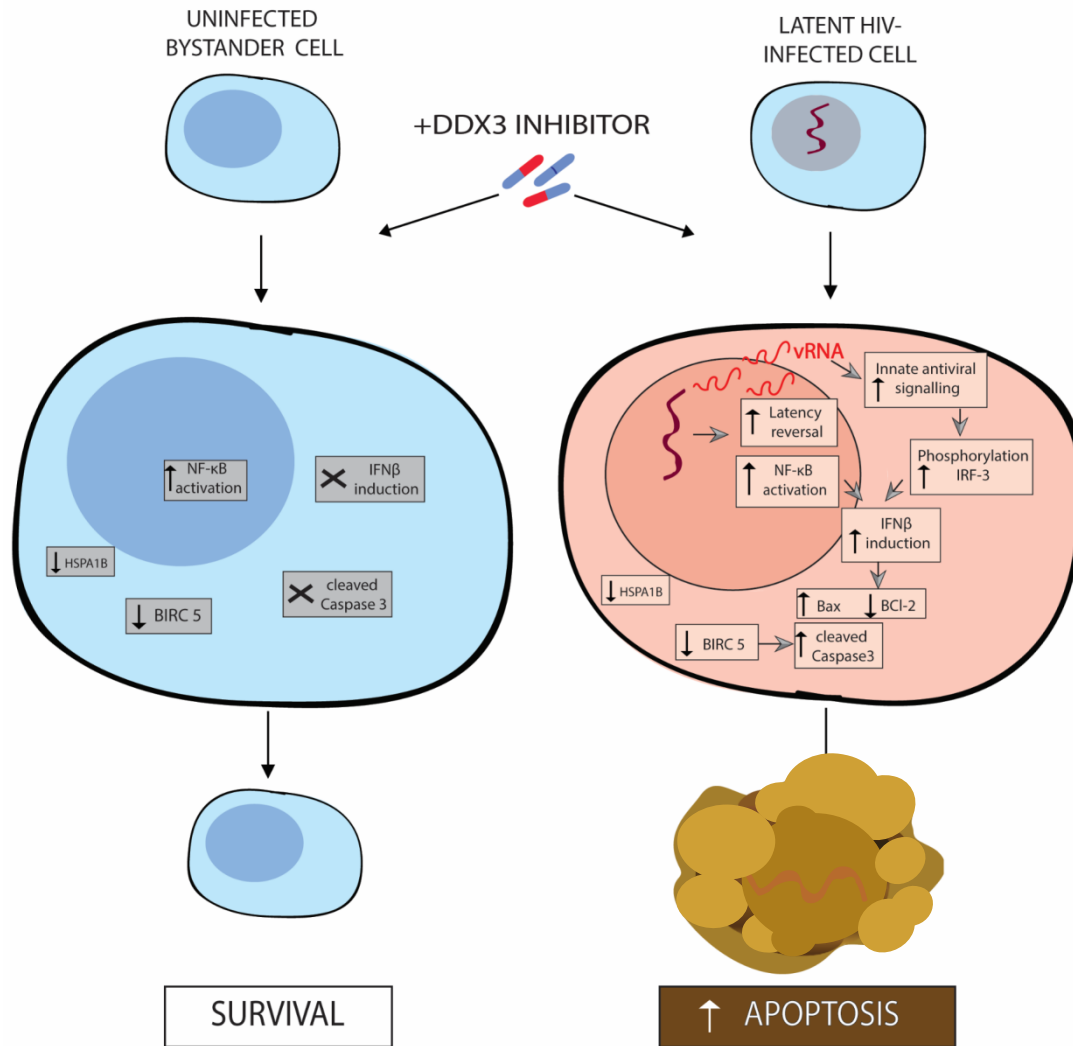
FISH Flow



CA HIV-1 vRNA



Selective killing of HIV infected reservoir cells



- US HIV-1 vRNA triggers innate pathways
- Anti-apoptotic gene downregulated
- Transcription-competent defective proviruses can also be targeted

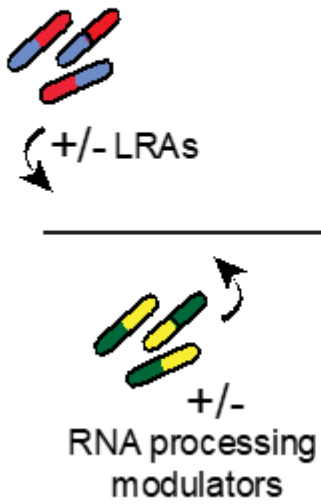
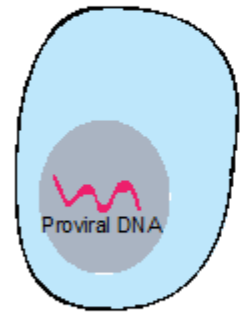
Modulating vRNA splicing to increase innate immune sensing and apoptosis of HIV-infected cells



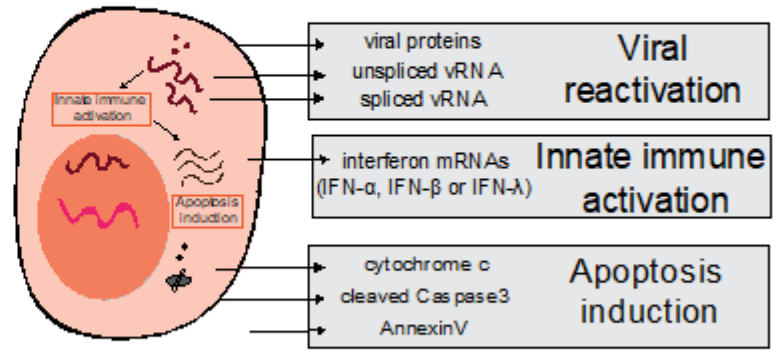
Shringar Rao
PI

Liset de Vries
PhD student

Latent
HIV-infected
cell

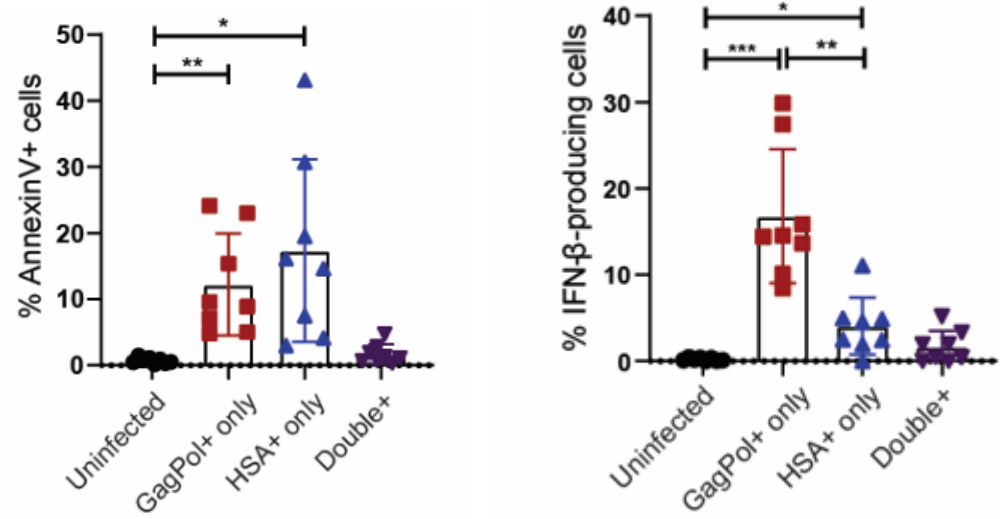
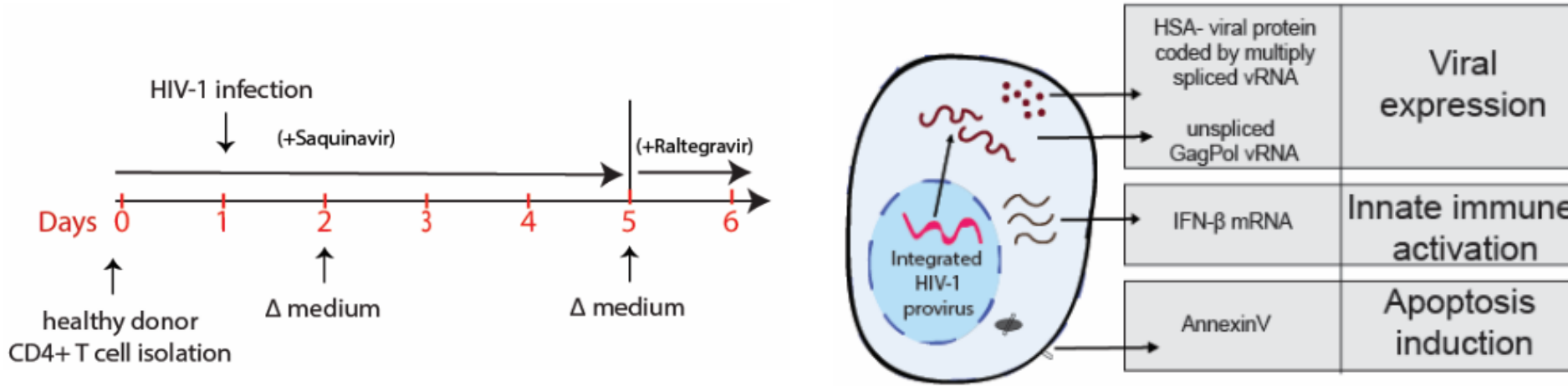


Multiplexed single cell technology (FISH-flow)
Simultaneous detection of:



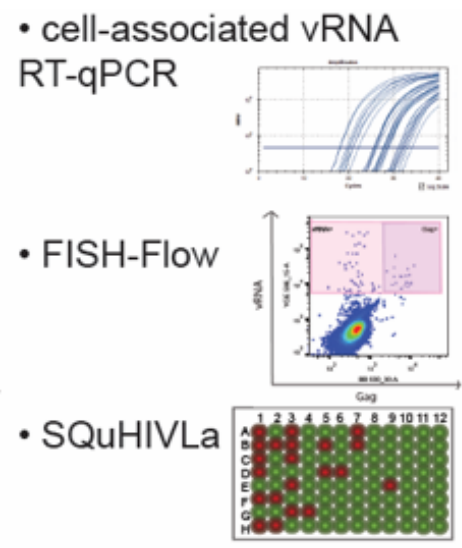
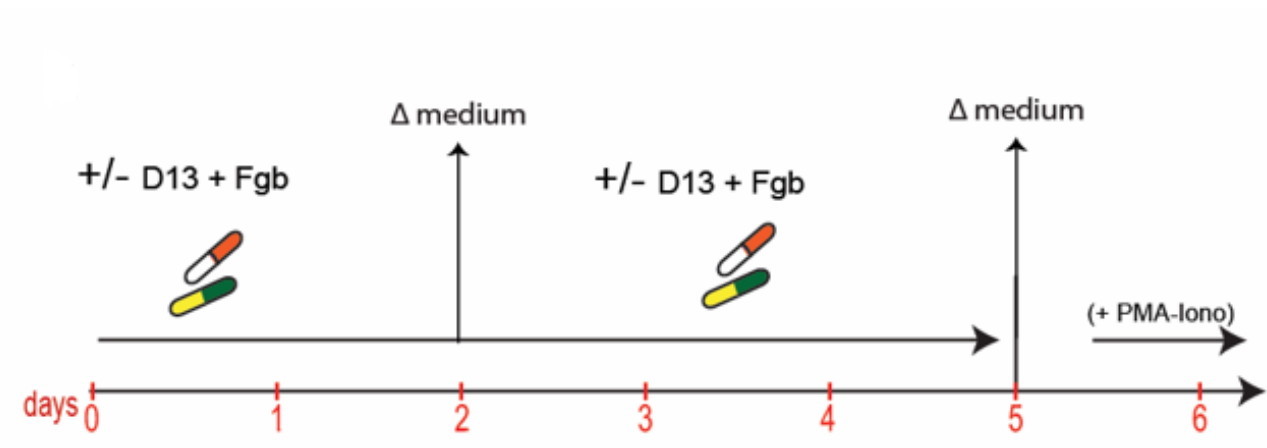
- viral proteins
 - unspliced vRNA
 - spliced vRNA
- Viral reactivation**
- interferon mRNAs (IFN- α , IFN- β or IFN- λ)
- Innate immune activation**
- cytochrome c
 - cleaved Caspase 3
 - Annexin V
- Apoptosis induction**

US (Gag-pol+) vRNA is immunogenic (increased IFN β expression)

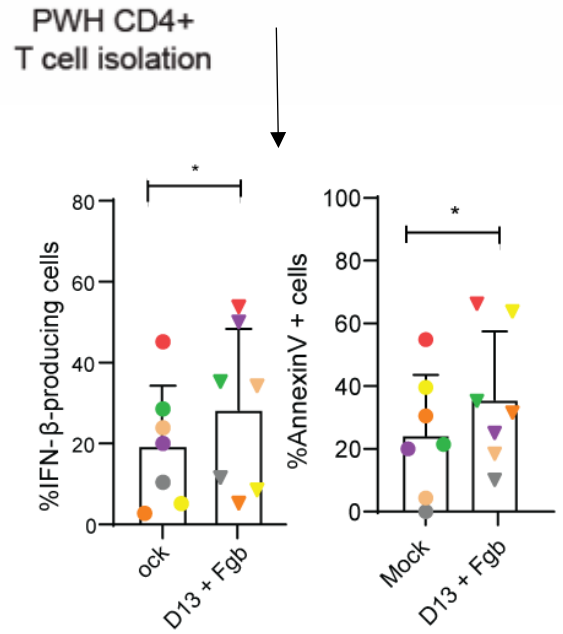


US vRNA is more immunogenic

Targeting splicing to trigger innate immune signalling and apoptosis



Filgotinib = vRNA splicing inhibitor
D13 = LRA (BAF inhibitor)
 Marian et al, Cell Chem Biol. 2018



US vRNA (both defective and intact)

Rao et al. submitted

The Journal of Clinical Investigation

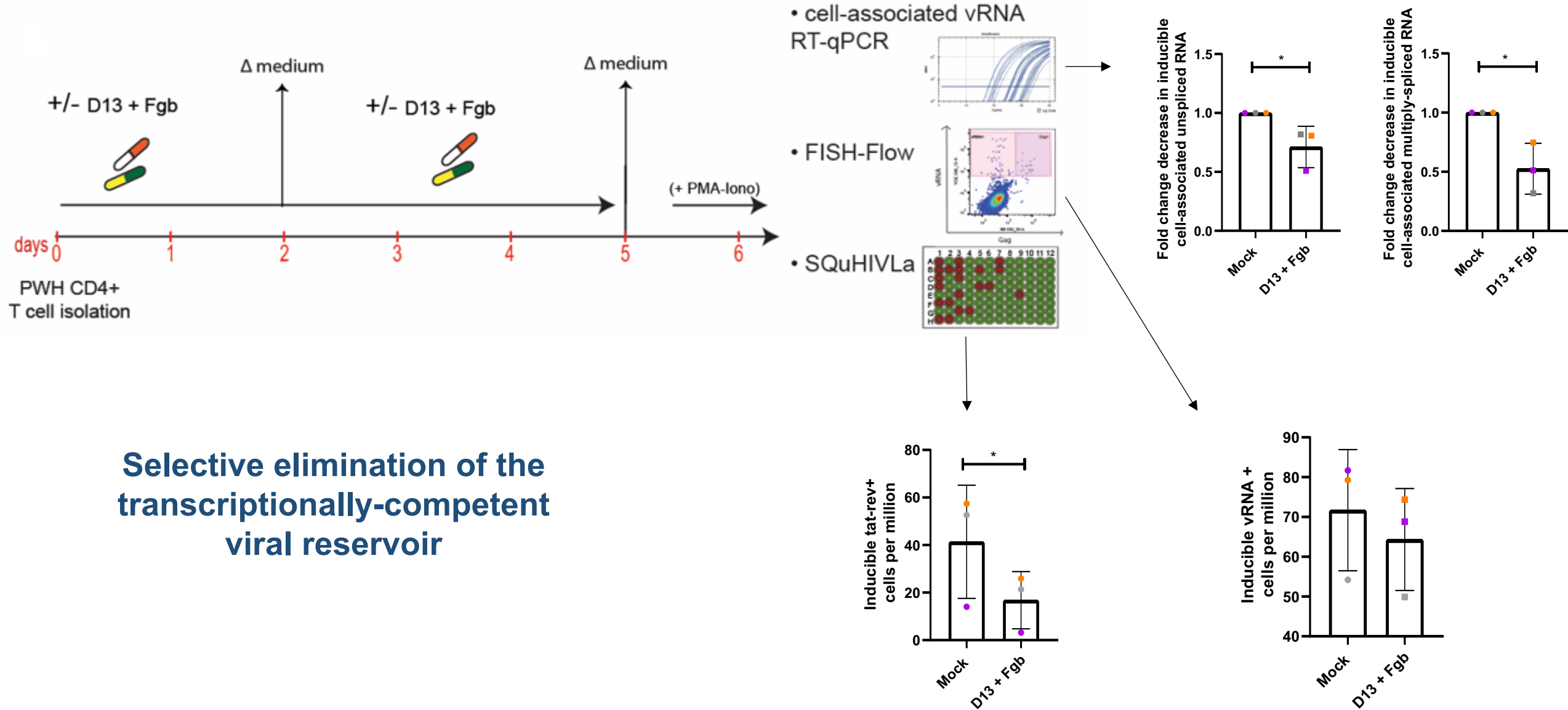
RESEARCH ARTICLE

Filgotinib suppresses HIV-1-driven gene transcription by inhibiting HIV-1 splicing and T cell activation

Yang-Hui Jimmy Yeh,¹ Katharine M. Jenike,² Rachela M. Calvi,³ Jennifer Chiarella,³ Rebecca Hoh,⁴ Steven G. Deeks,⁴ and Ya-Chi Ho¹

¹Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, Connecticut, USA. ²Human Genetics PhD Program, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. ³Department of Neurology, Yale University School of Medicine, New Haven, Connecticut, USA. ⁴Department of Medicine, UCSF, San Francisco, California, USA.

Reduction in the size of the inducible transcriptionally competent viral reservoir

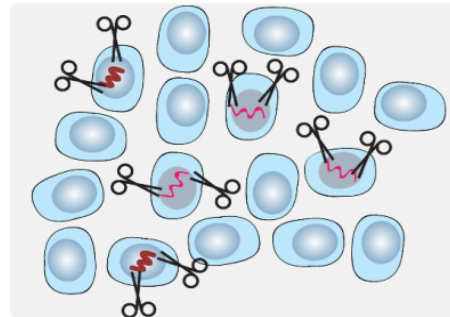


Selective elimination of the transcriptionally-competent viral reservoir

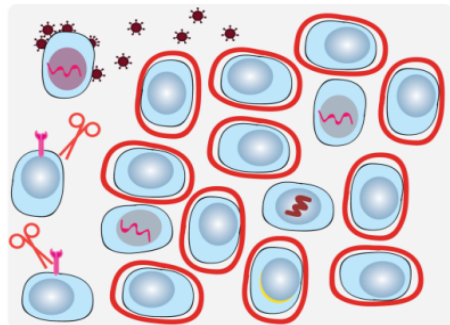
Pharmacological strategies toward HIV-1 cure

1. Silence the reservoir

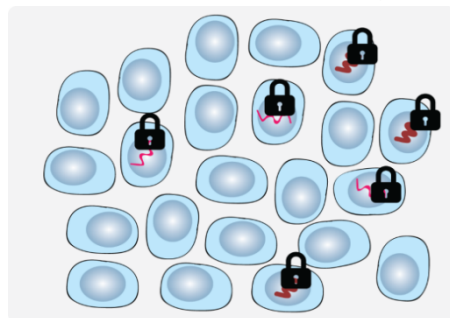
- gene editing
 CRISPR-Cas9/
 nucleases



- CCR5 editing
 pool of
 "resistant" cells

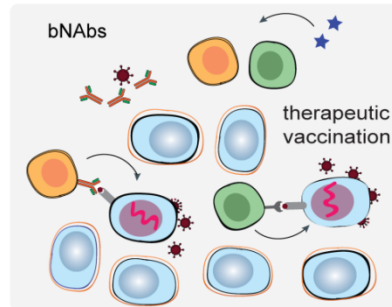


"Block and lock"



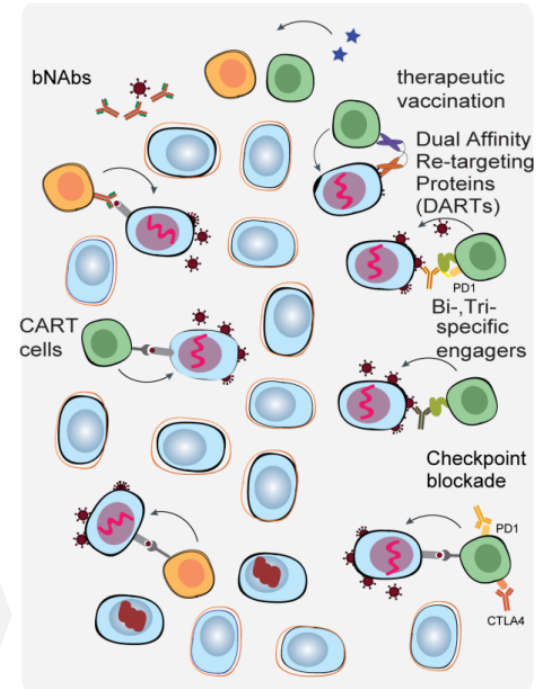
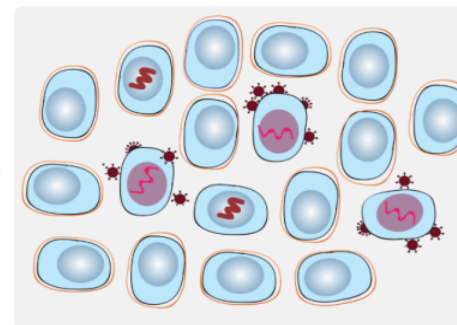
2. Control viral rebound

3. reduce the reservoir



- bNAbs,
 therapeutic
 vaccines

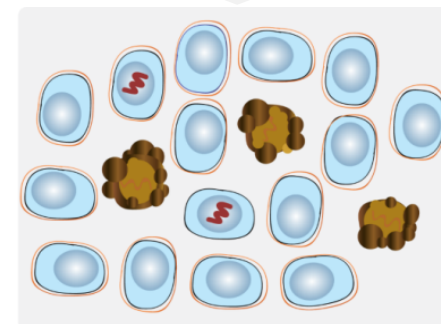
Reverse latency



Induce cell killing

"Shock
 and kill"

Induce cell death

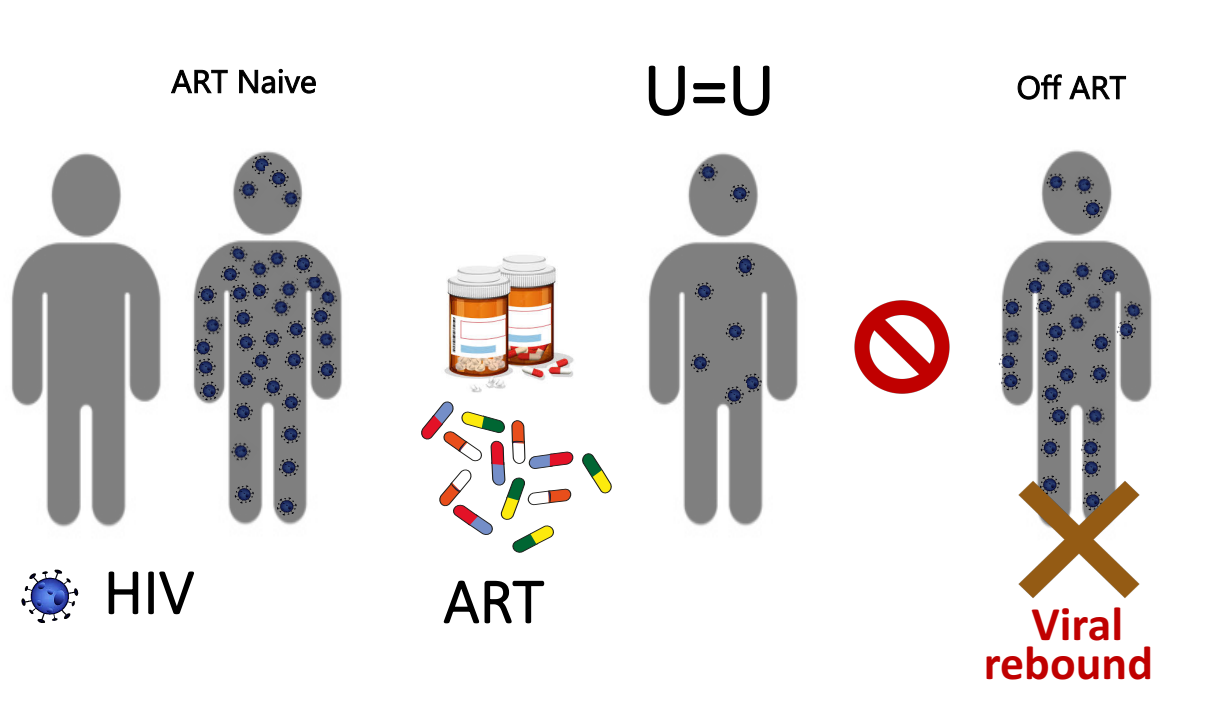
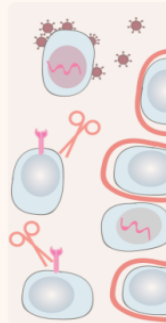


Acute Treatment Interruption (ATI)

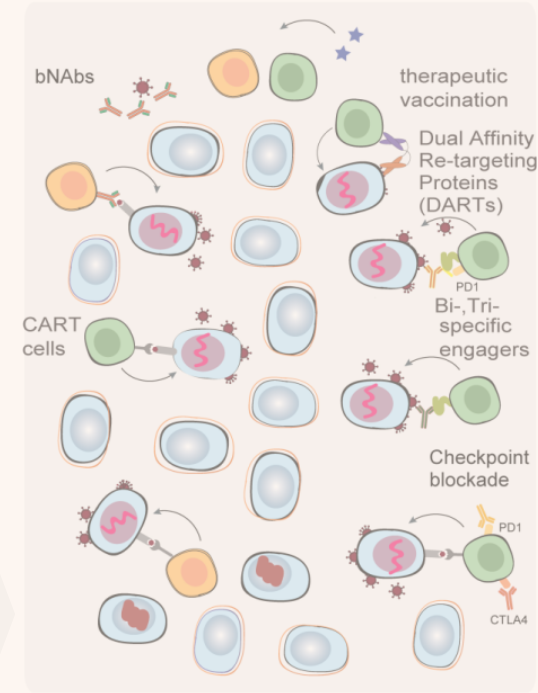
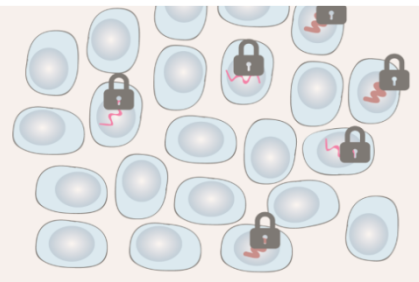
used to test effectiveness of cure intervention

- gene editing
 CRISPR-Cas9/
 nucleases

- CCR5 editing
 pool of
 "resistant" cells



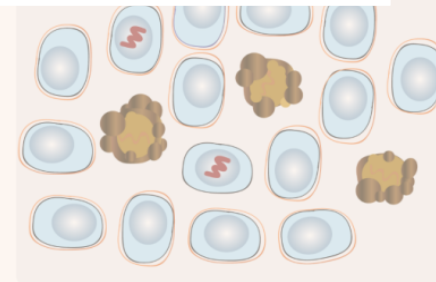
"Block and lock"



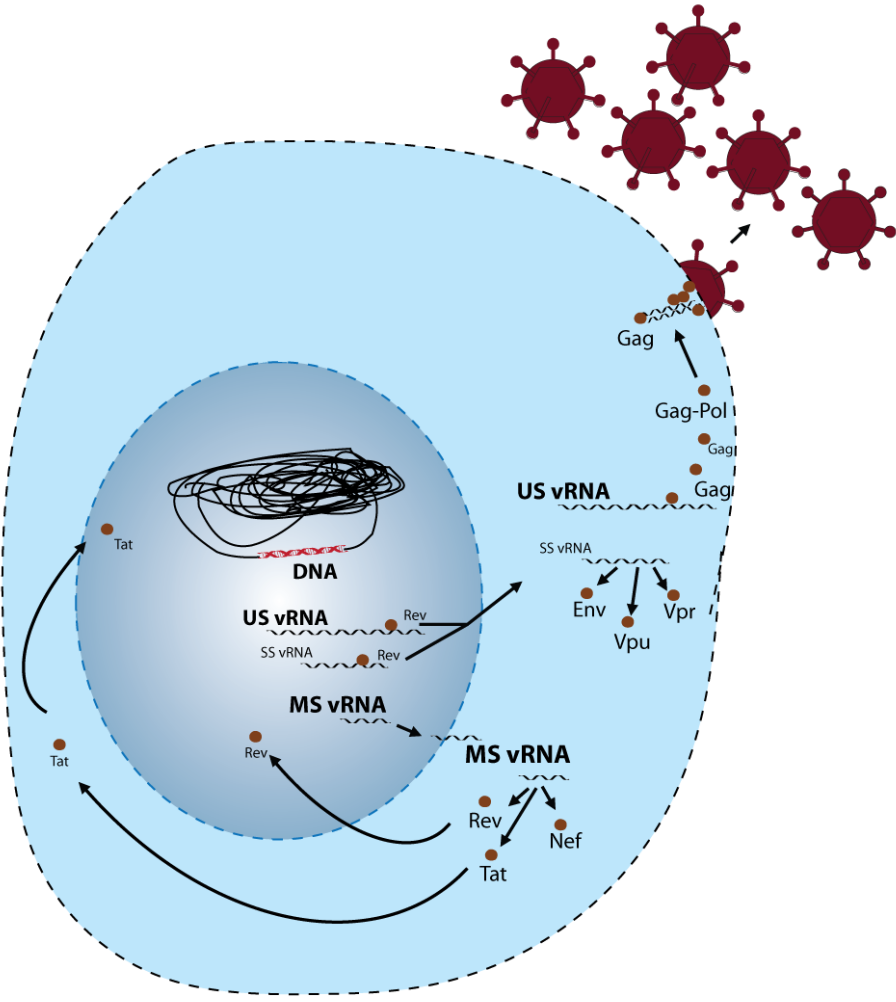
Induce cell killing

"Shock and kill"

Induce cell death

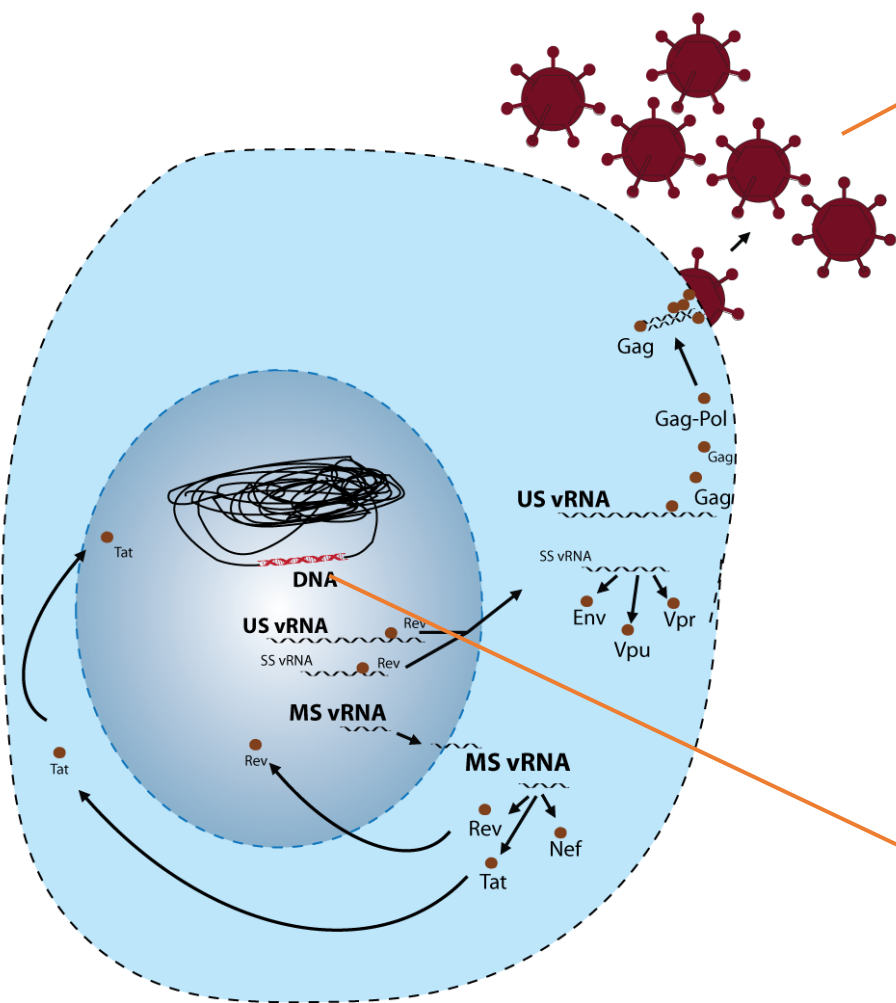


How/ if/ when to do ATI's? How do we measure the size of the functional reservoir?



- How long should ART be interrupted?
- How small should reservoir be?
- Can we predict and prevent rebound? Biomarkers?
- **How do we measure the functional reservoir?**
At what level? DNA? RNA? Protein? Replication?

How do we measure the size of the replication competent reservoir?



QVOA: quantitative viral outgrowth assay

- Underestimation? – sub-optimal induction, propagation

RT-PCR/RT-ddPCR	- US vRNA - MS vRNA
TILDA SQuHIVLa	- MS vRNA
IVRA	- Intact vRNA
FISH-Flow HIV-Flow VIPSPOT SIMOA	- Viral RNA and proteins p24

IPDA: Intact proviral DNA assay

multiplexed versions, quadruplex qPCR and next-gen seq

- Overestimation?
- not all intact proviruses are replication competent

What is the long term impact of ATI's?

- What happens to the reservoir after viral rebound at the different molecular levels?
- How do the quantitation tools compare?

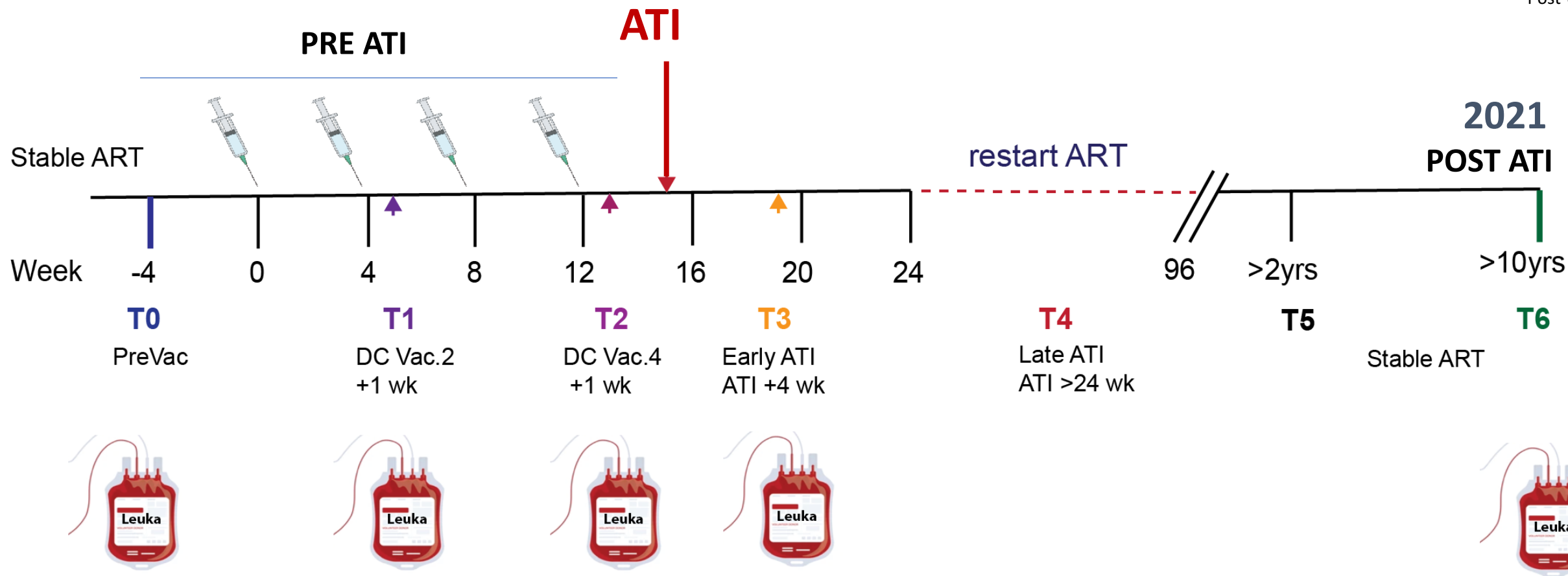
DC-TRN trial Erasmus MC 2006



Cynthia Lungu
Post-doc

Netherlands Clinical trial registry No. NTR2198

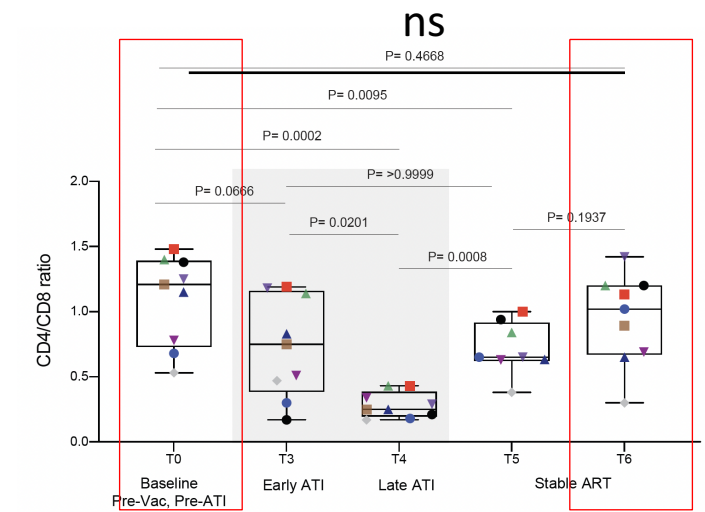
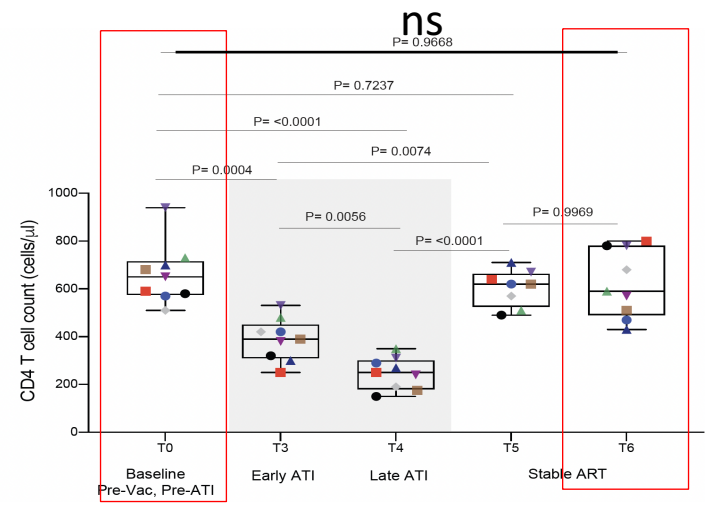
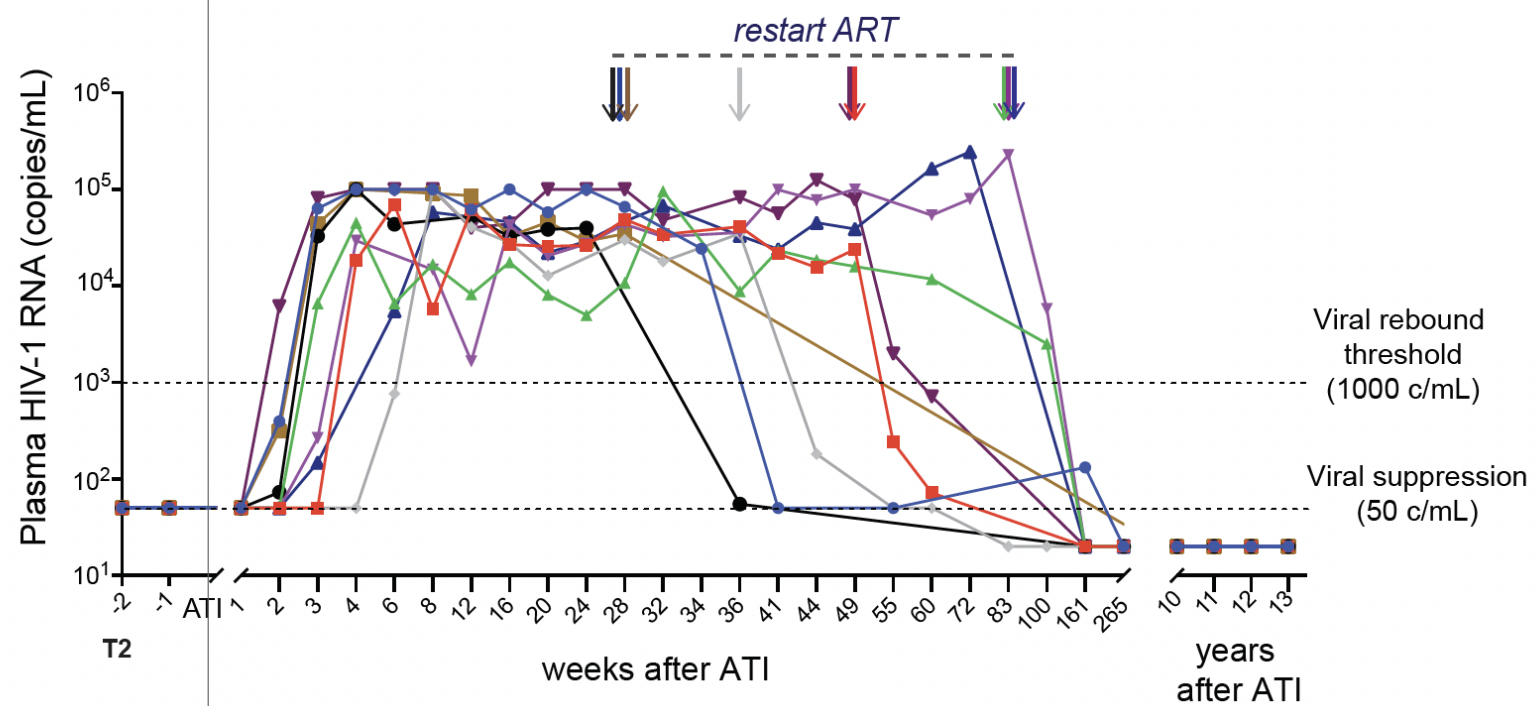
Allard, S.D., et al. *Clin Immunol* **142**, 252-268 (2012).



Lungu C, Hossain T, Crespo R et al., (in preparation)

Analytical treatment interruption after autologous dendritic cell vaccination reshapes the functional HIV-1 reservoir

Failed intervention: rebound, then viral re-suppression with ART and immune reconstitution



What is happening to the viral reservoir at the molecular level?

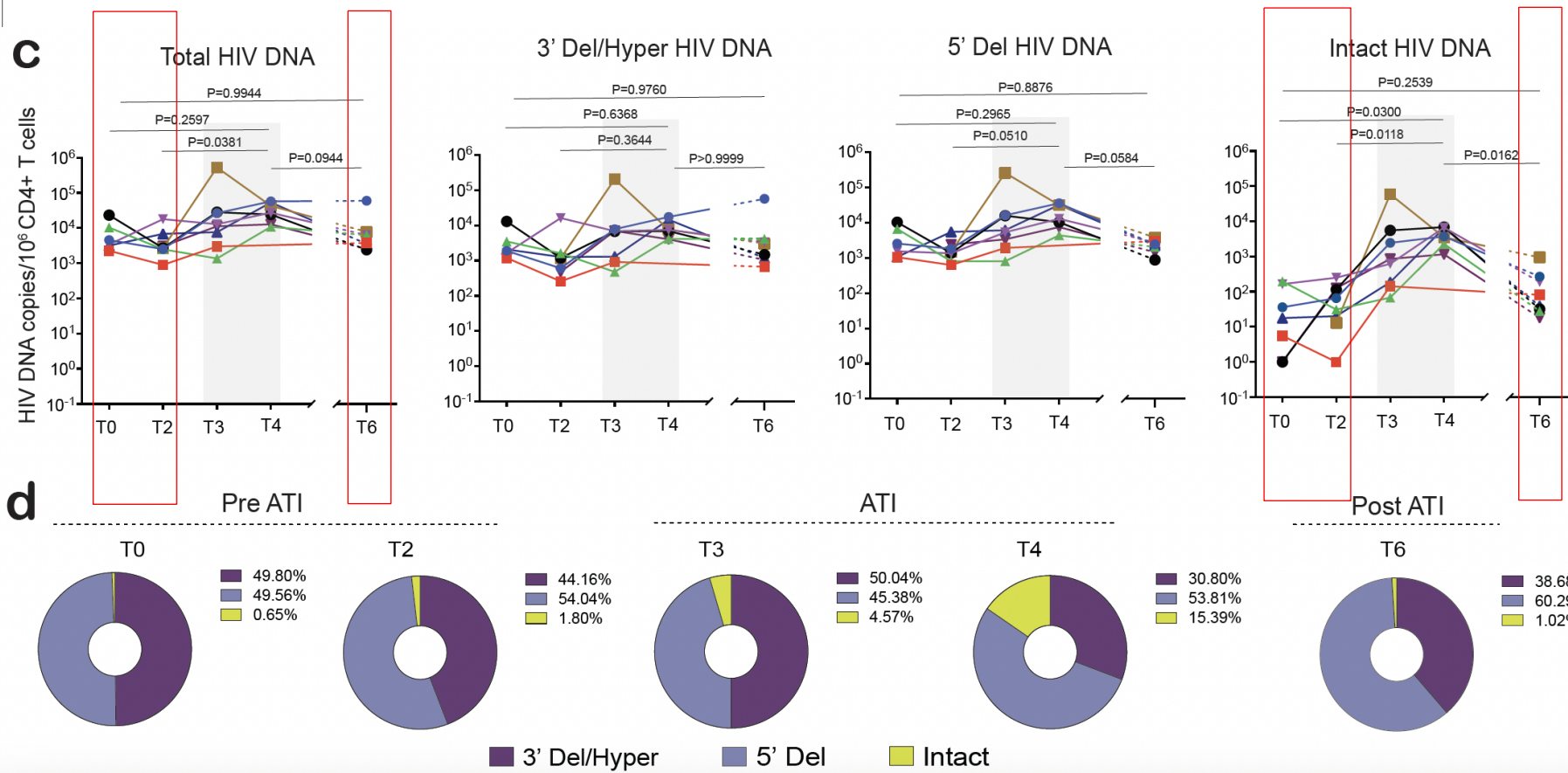
Lungu C, Hossain T, Crespo R et al., (in preparation)
 Analytical treatment interruption after autologous dendritic cell vaccination reshapes the functional HIV-1 reservoir

Intact proviral DNA

– no significant change in reservoir pre vs post ATI

T0, T1, T2 = pre-ATI

T6 = post ATI and > 10 year after re-suppression

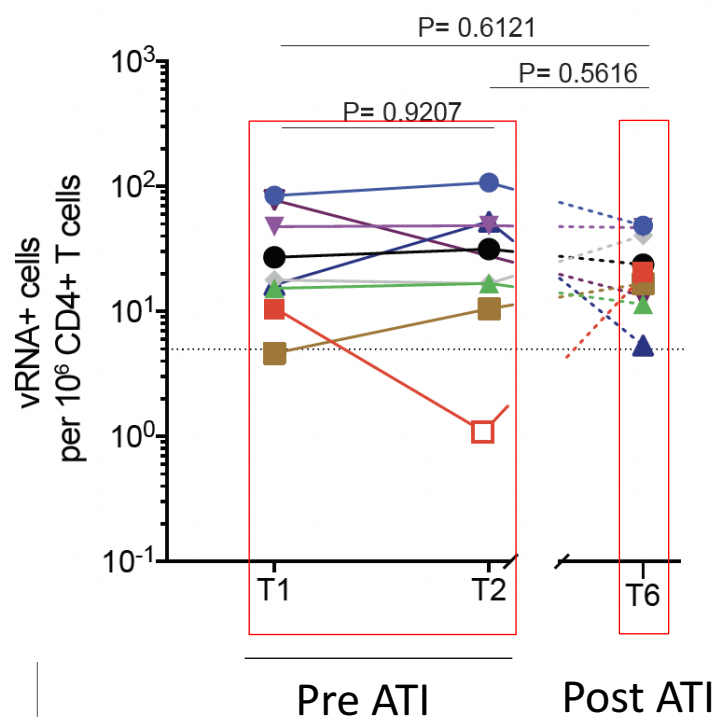


US HIV RNA and Gag protein (FISH-Flow)

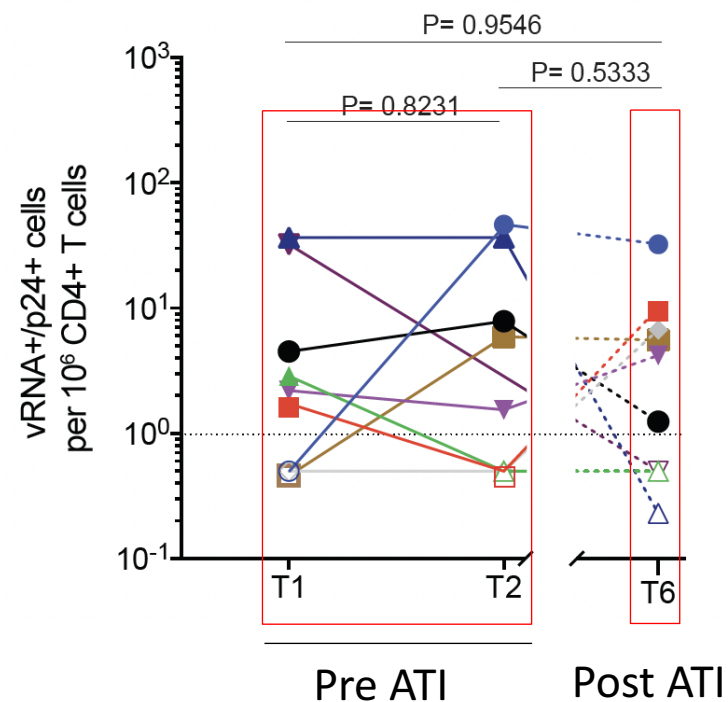
– no significant change in reservoir pre vs post ATI

T0, T1, T2 = pre-ATI
 T6 = post ATI and > 10 year after re-suppression

Unspliced (gagpol) HIV RNA



Unspliced (gagpol) HIV RNA/ p24



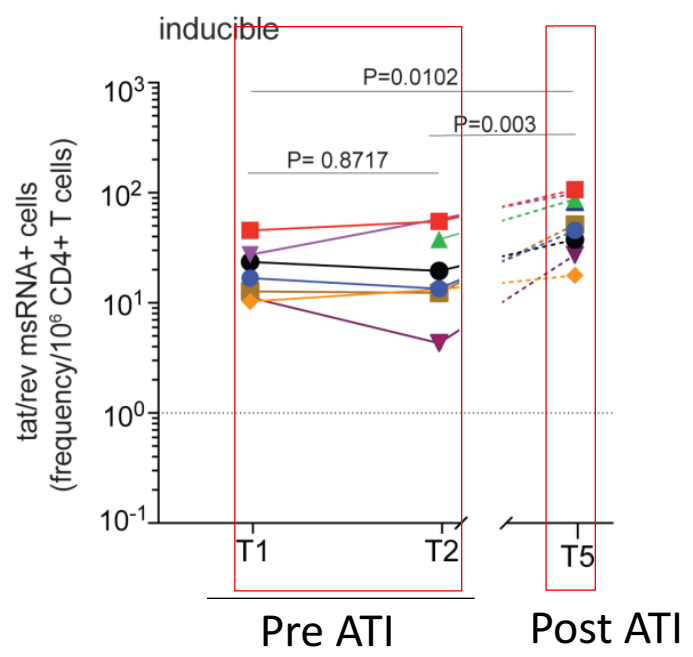
Significant increase in frequency of cells inducibly expressing tat/rev ms HIV RNA in all 9 participants post intervention-ATI > 10 year after re-suppression

T0, T1, T2 = pre-ATI

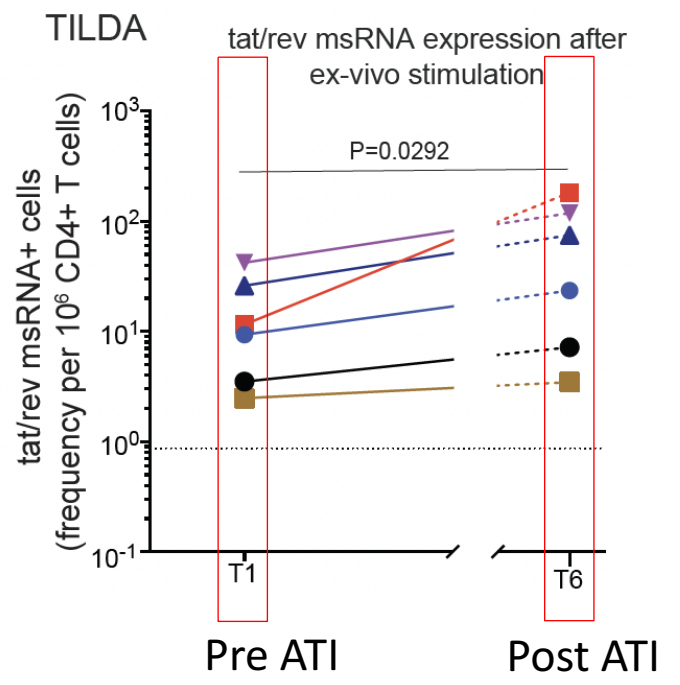
T6 = post ATI and > 10 year after re-suppression

SQuHIVLa

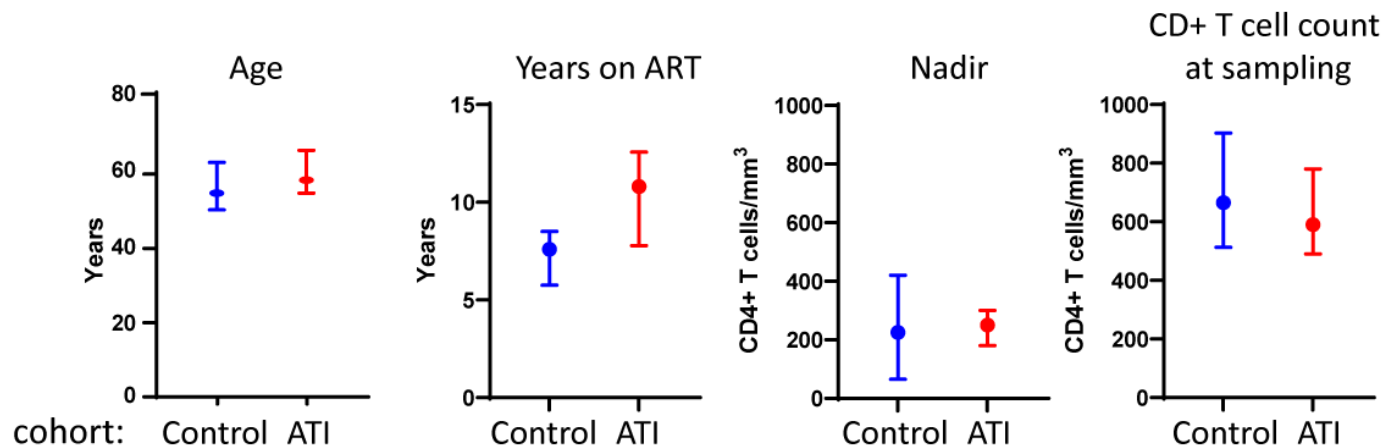
(Hossain et al, Communications Medicine 2024)



TILDA

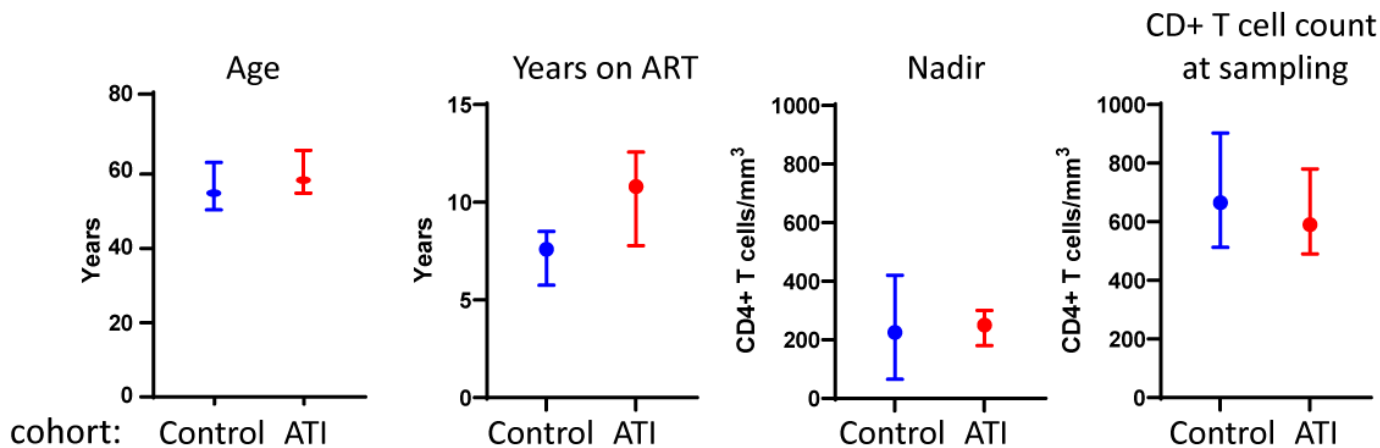


T0, T1, T2 = pre-ATI
T6 = post ATI and
> 10 year after re-suppression

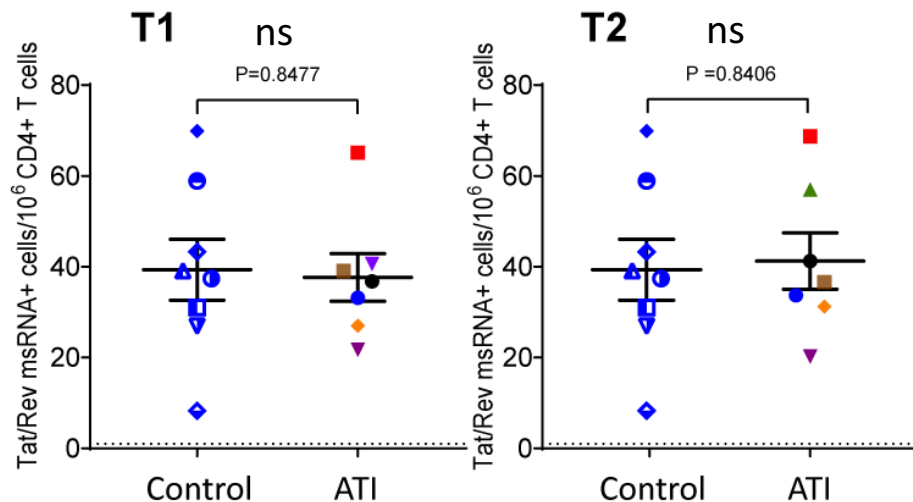


Comparable inducible reservoirs between control and ATI cohort before ATI

T0, T1, T2 = pre-ATI
 T6 = post ATI and
 > 10 year after re-suppression



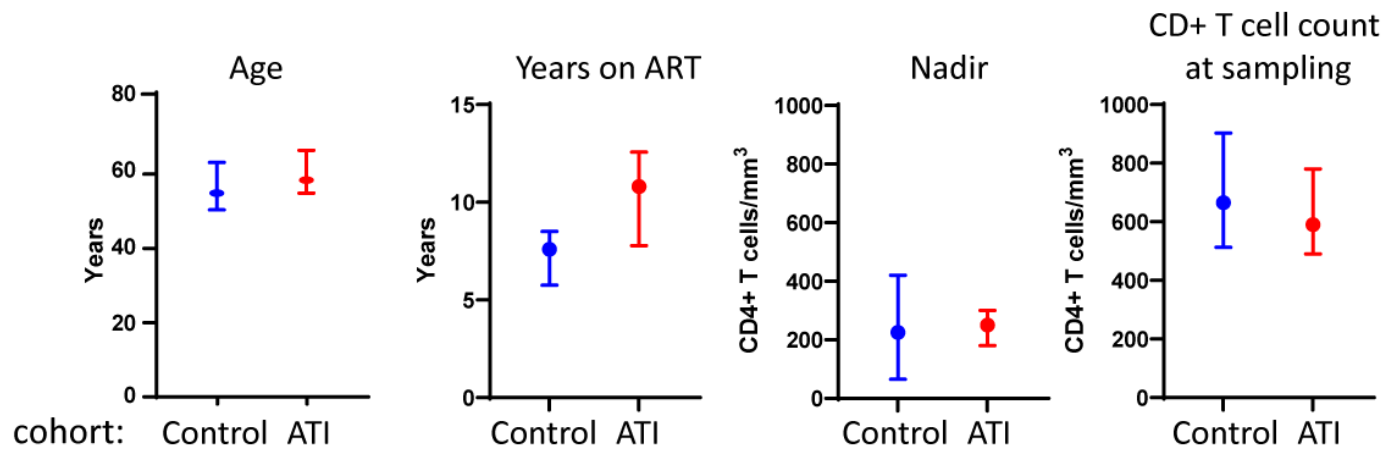
Pre ATI



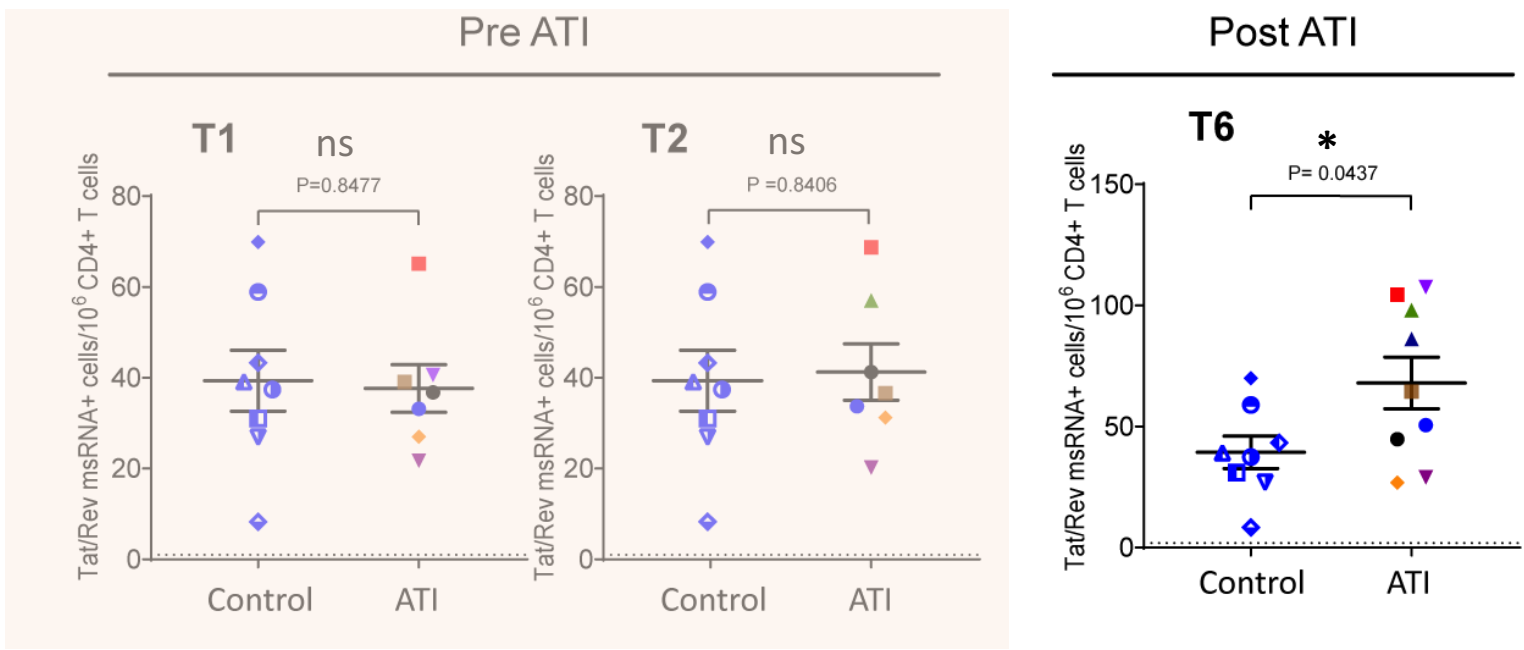
SQuHIVLa

Higher inducible reservoir in ATI cohort participants > 10 years after re-suppression than in matched control cohort

T0, T1, T2 = pre-ATI
 T6 = post ATI and
 > 10 year after re-suppression



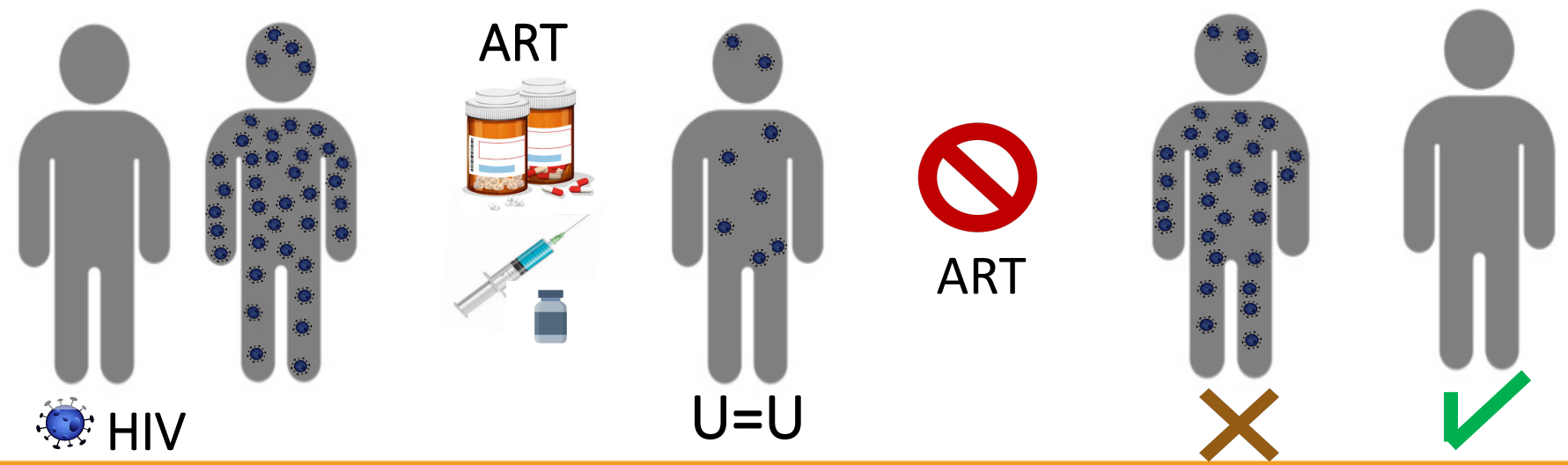
SQuHIVLa



Considerations: What is the long term impact of ATI's?

- On Reservoir – size (reseeding), integration site, functionality
 - Vigilant sampling and monitoring using non-cell-intensive assays that reveal reservoir dynamics
- On Immune compartment – exhaustion, activation, immune capacity (function, proliferation)
- biomarkers of rebound: msRNA? Intact DNA? Immunological markers?

Richart, V., *et al.* High rate of long-term clinical events after antiretroviral therapy resumption in HIV-positive patients exposed to antiretroviral therapy interruption. *AIDS* **35**, 2463-2468 (2021).



Summary and perspective:

Role of RNA (in how we measure viral reservoirs):

- Critical to target and eliminate the HIV-1 transcription-competent reservoir, including and especially of defective proviruses:
 - continues to contribute to chronic inflammation and immune dysfunction!!
 - replication from intact proviruses efficiently targeted by ART
- Need to invest in tools for deeper characterization of reservoir (also RNA compartment) in people with non-B HIV-1 subtypes
 - smaller, more silent reservoirs?
 - block and lock, ICD approaches?

Concept of “treatment as cure” given success and promises of LA-ART:

In the era of LA-ART, treatment interruption can be considered an intervention

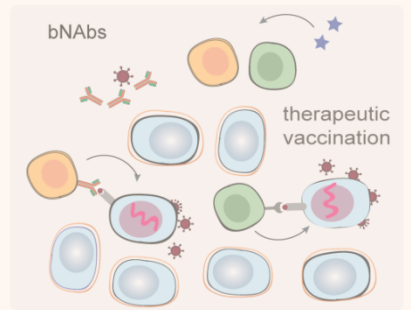
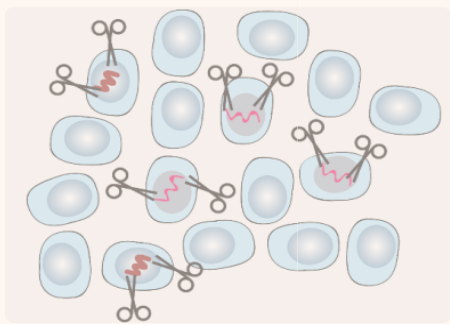
Pharmacological strategies toward HIV-1 cure

1. Silence the reservoir

2. Control viral rebound

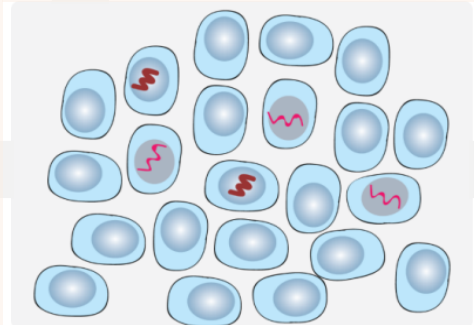
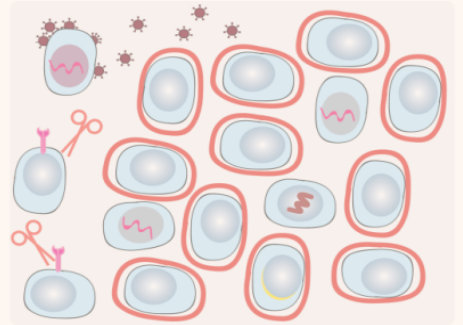
3. reduce the reservoir

- gene editing
 CRISPR-Cas9/
 nucleases

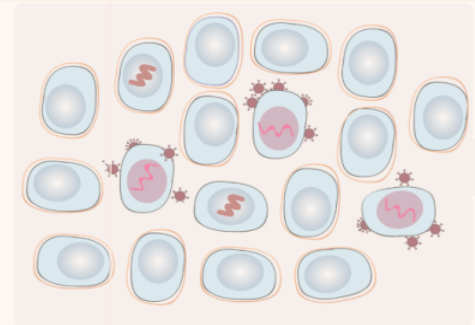


- bNAbs,
 therapeutic
 vaccines

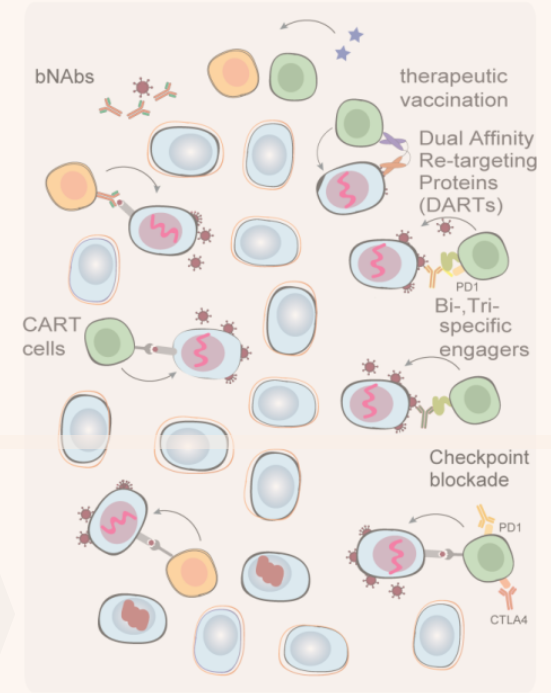
- CCR5 editing
 pool of
 "resistant" cells



LA-ART

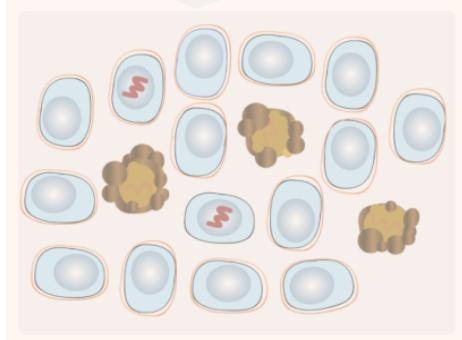
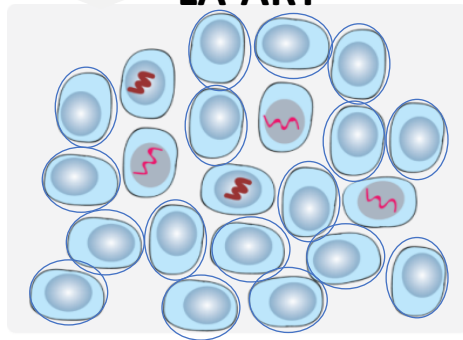
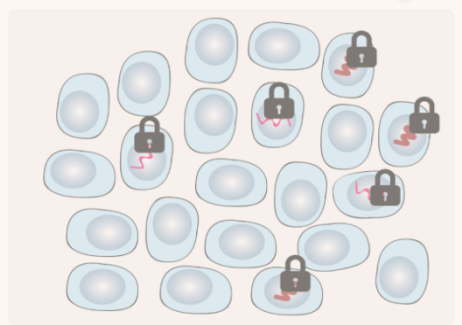


Reverse latency



Induce cell killing

"Block and lock"



"Shock
 and kill"

Induce cell death

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Council



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Biomedical Sciences Research Center

