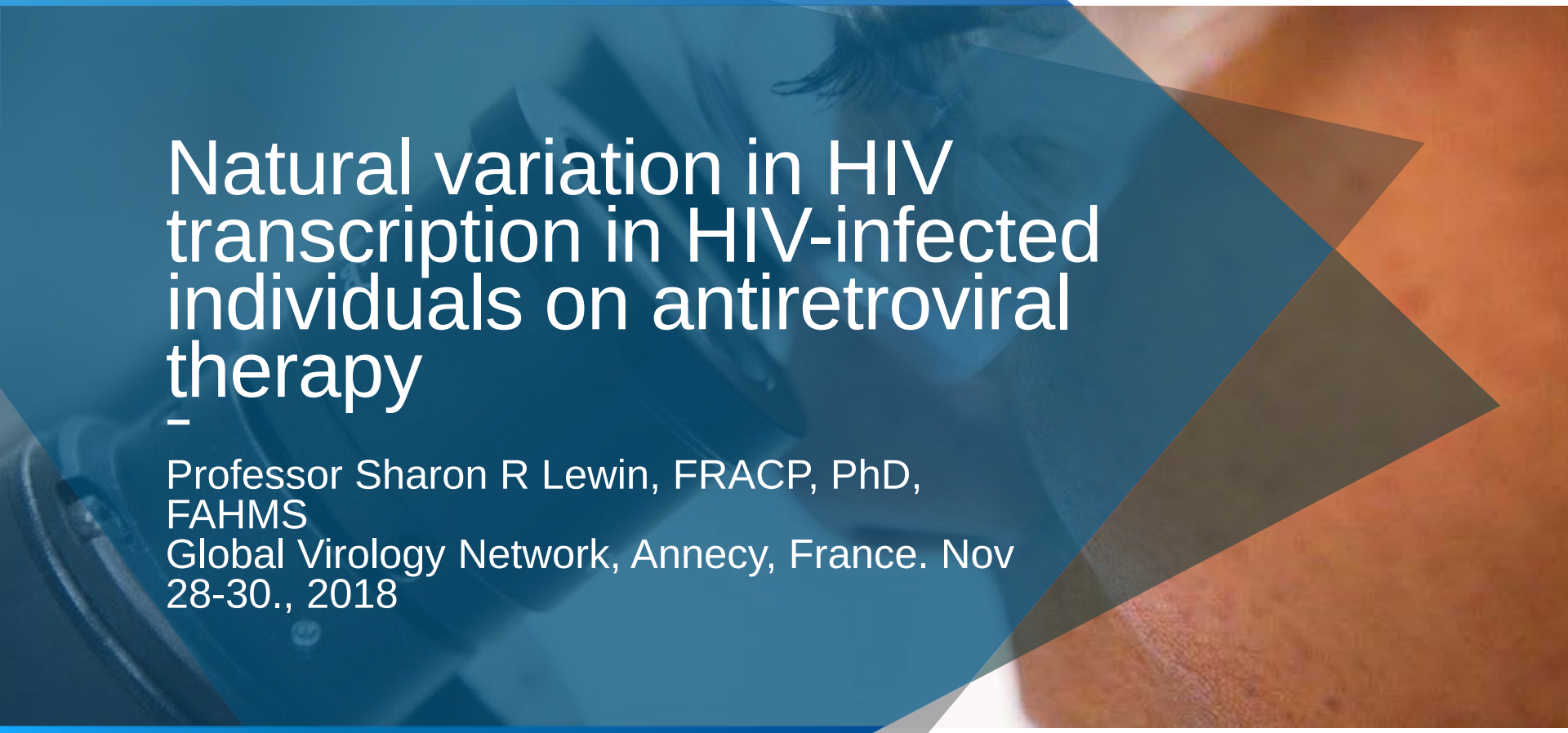
Natural variation in HIV transcription in HIV-infected individuals on antiretroviral therapy

Professor Sharon R Lewin, FRACP, PhD, FAHMS
Global Virology Network, Annecy, France. Nov 28-30., 2018



Doherty Institute
THE UNIVERSITY OF MELBOURNE
The Royal Melbourne Hospital
A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Newsdesk

Australia tackles HTLV-1

HTLV-1 research has long been under-funded, hampering research and control. A new Australian taskforce aims to change the situation at home and abroad. Karl Gruber reports.

CrossMark

Doctors raise alarm about ancient HTLV-1 virus: 'Prevalence is off the charts' in Australia

By Jacqueline Howard, CNN

Updated 0819 GMT (1619 HKT) May 8, 2018

The Hon. Greg Hunt MP
Minister for Health

The Hon. Ken Wyatt AM, MP
Minister for Aged Care
Minister for Indigenous Health

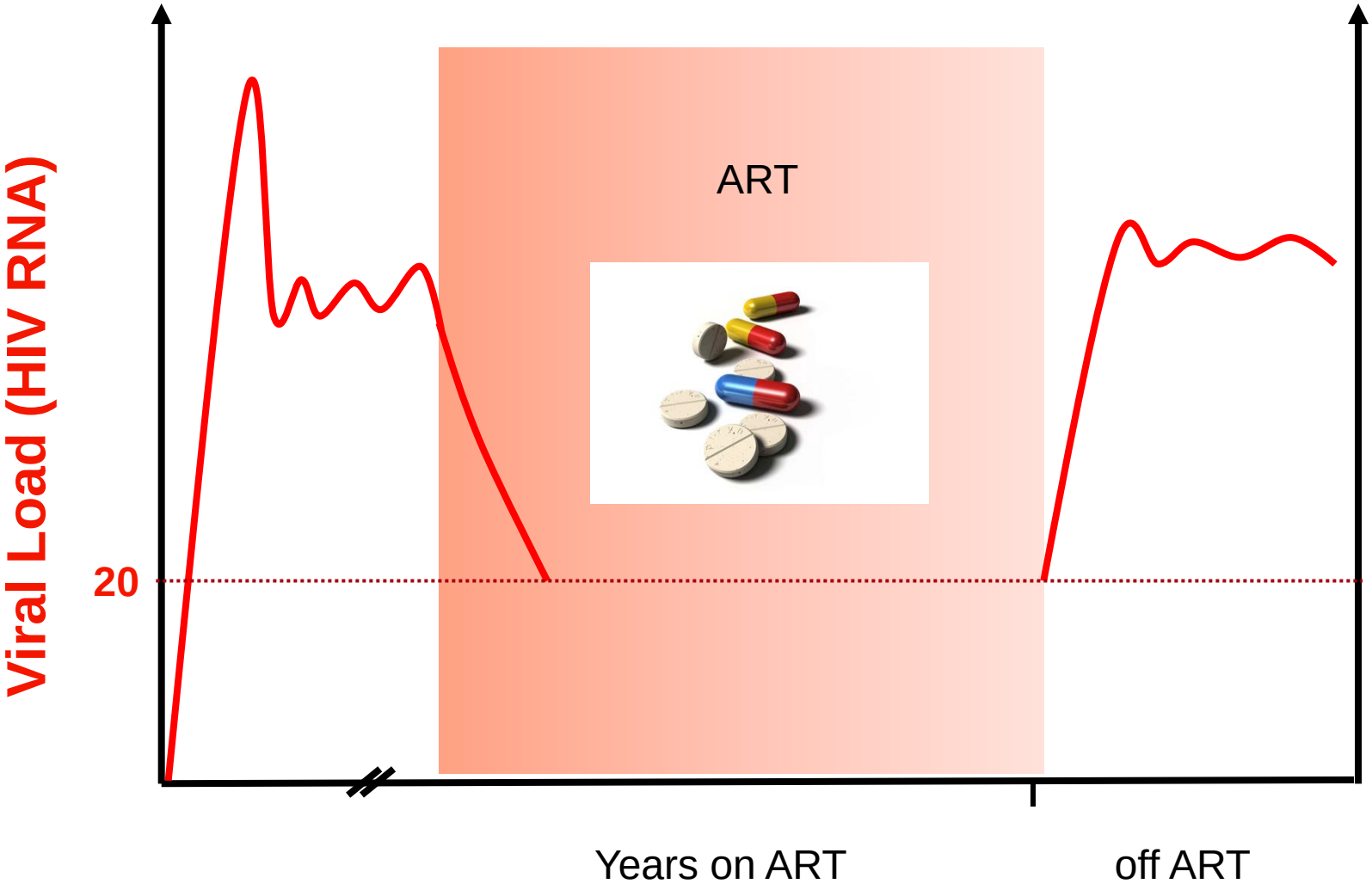
MEDIA RELEASE

25 May 2018

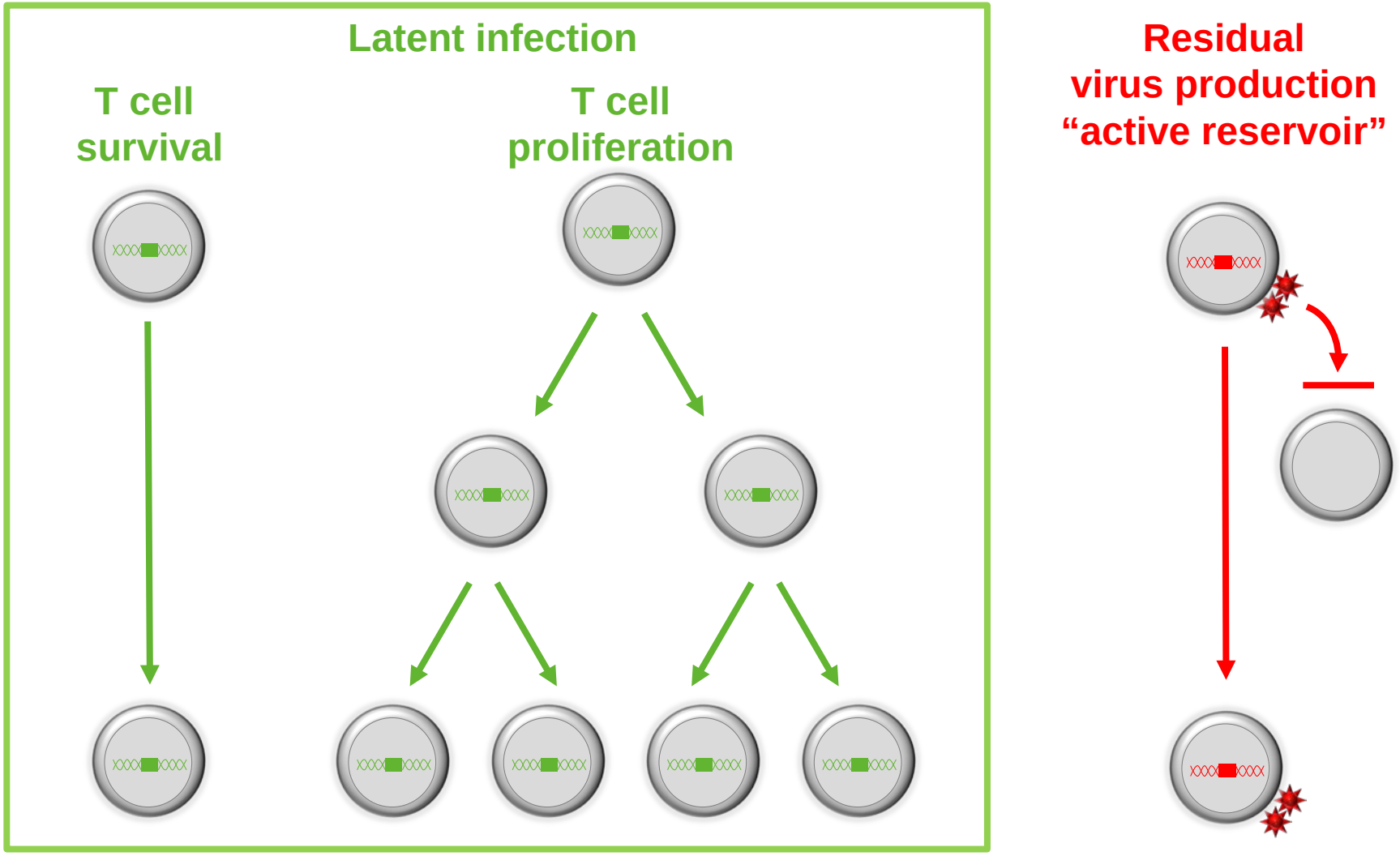
Funding to combat Human T-cell Lymphotropic Virus-1 in remote communities

The Turnbull Government will provide $8 million to form a taskforce, in collaboration with the states and territories, to combat Human T-cell Lymphotropic Virus-1 (HTLV-1) and other emerging communicable diseases in remote communities.

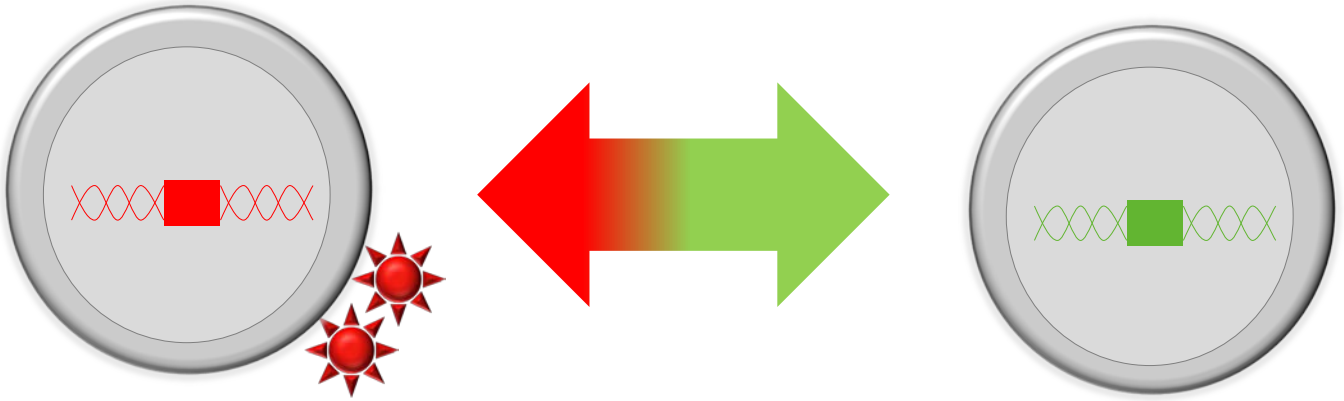
Rapid rebound of HIV when ART is stopped



HIV persists on ART in multiple forms



There is a spectrum of HIV transcriptional activity on ART: why?



Productive infection

DNA positive

RNA positive

HIV protein positive

DEATH

Latent infection

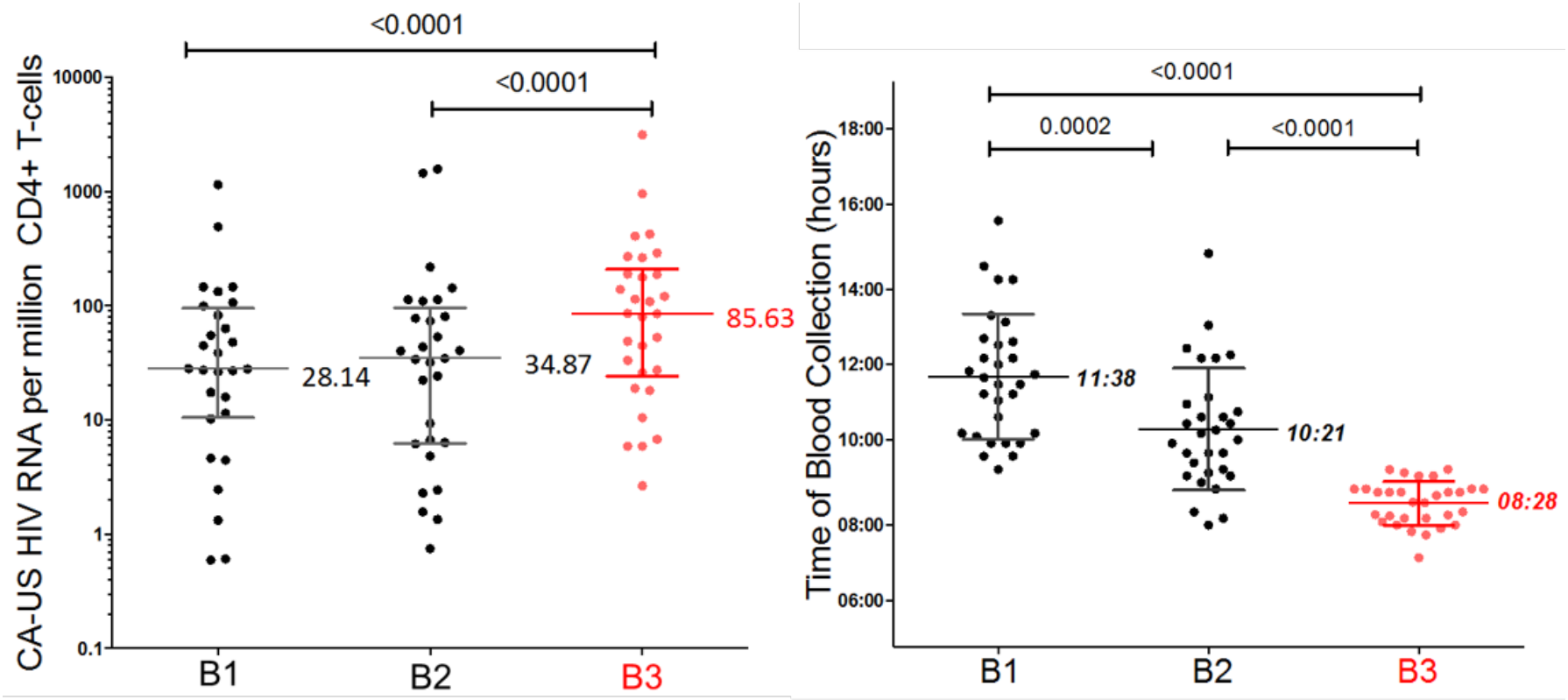
DNA positive

RNA negative

HIV protein negative

SURVIVAL

Variation in cell associated unspliced HIV RNA in HIV-infected individuals on ART

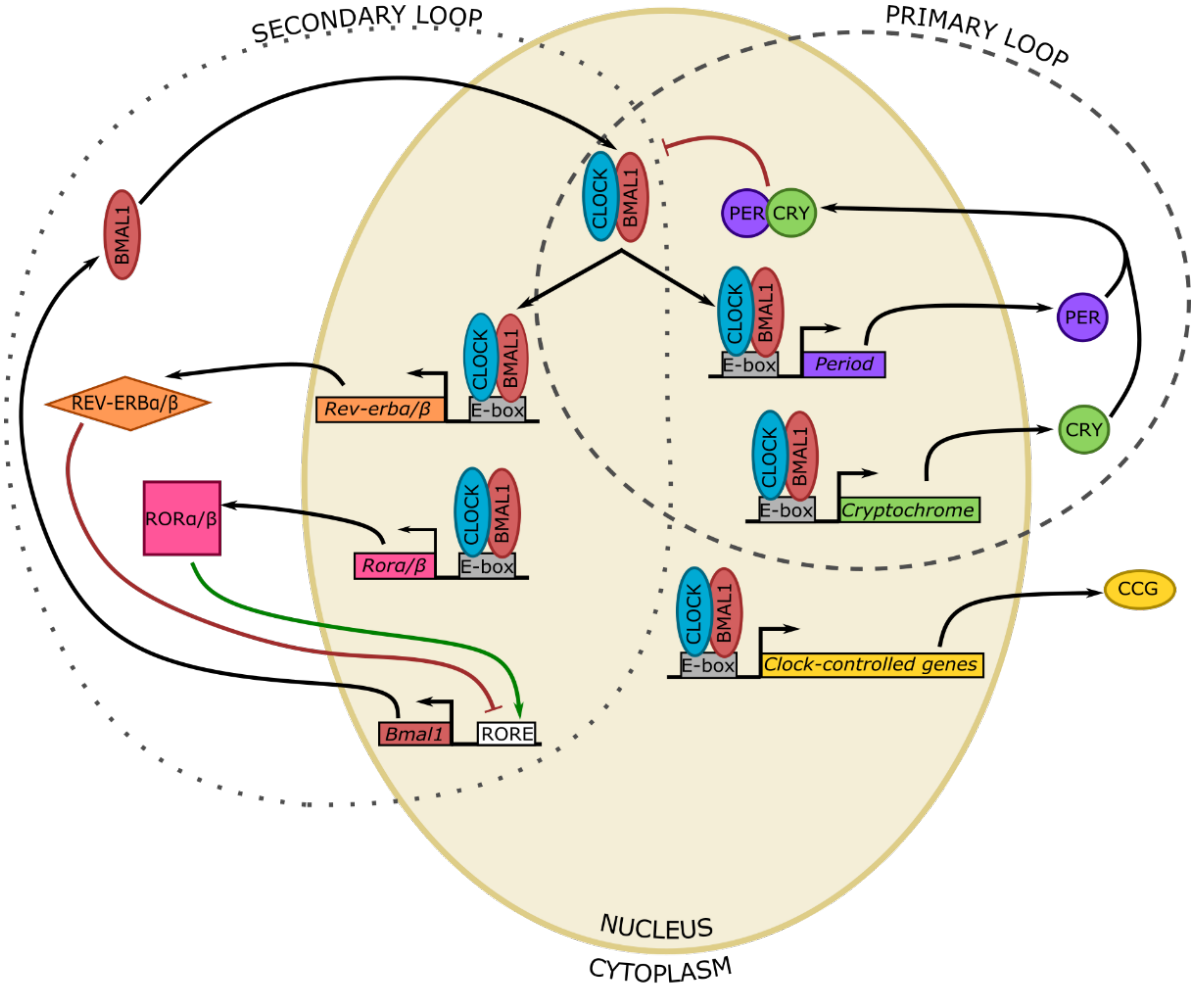


N=30 participants in a randomised clinical trial. B1 = screening; B2 = between screening and enrolment; B3 = day of administration of disulfiram

Elliott et al., Lancet HIV 2015

Does HIV transcription have a circadian rhythm?

Circadian Cycles



Nobel Prize 2017
Jeffrey Hall
Michael Rosbasch
Michael Young

- CLOCK and BMAL1 heterodimers bind to an E-box to drive transcription
- Control is mediated through *Per* and *Cry* genes

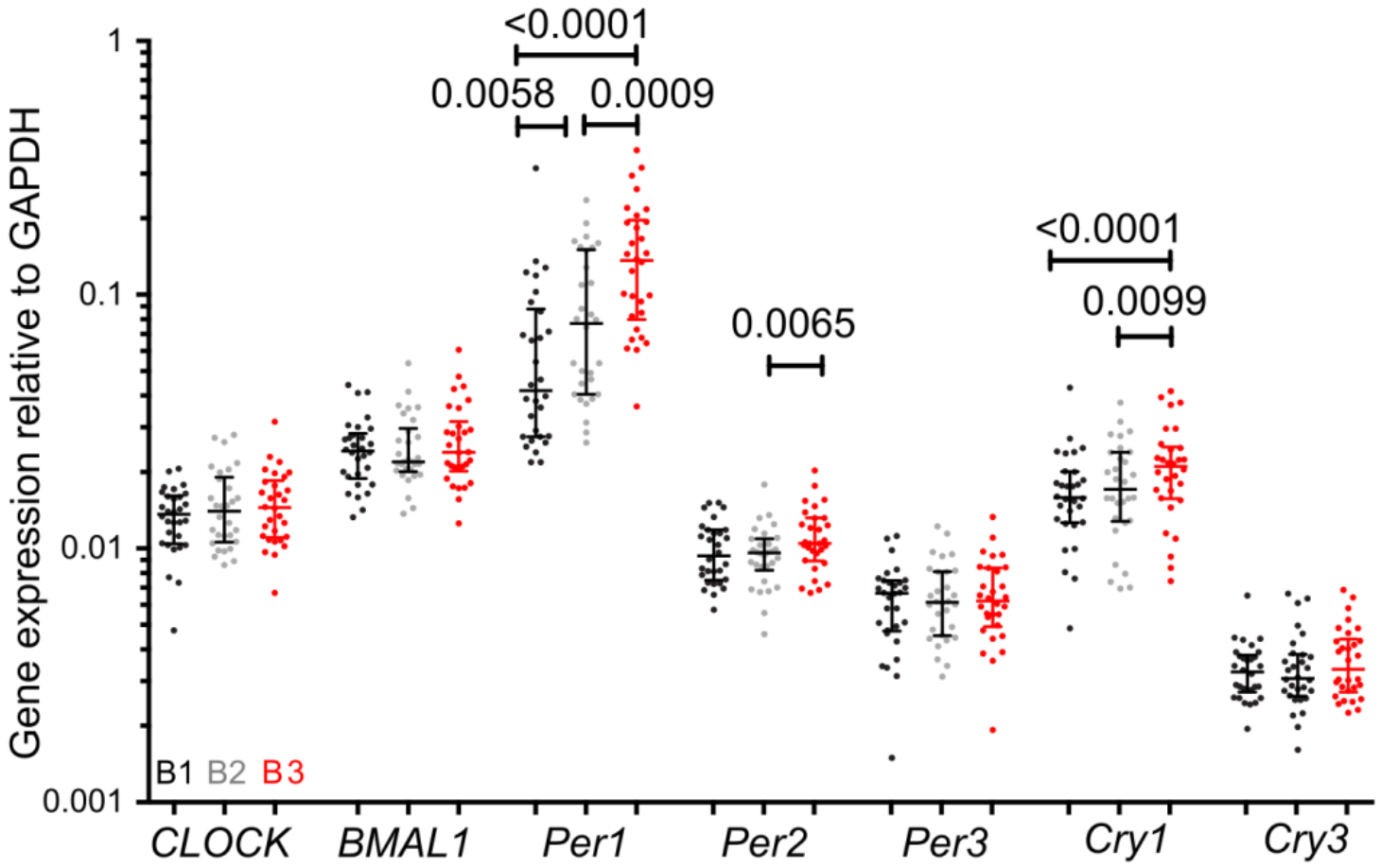
Gekakis et al., Science 1998; Hogenesch et al., PNAS 1998; Kume et al., Cell 1999; Jin et al., Cell 1999

Why would this be relevant to HIV transcription?

- The HIV LTR has 4 E-box binding sites with two flanking the TATA bindings site
- E-boxes are palindromic sequence motifs (CANNTG) for basic helix-loop-helix (bHLH) class of DNA-binding proteins
- Important for the regulation of transcription of multiple retroviruses including HIV-1 and HTLV-1
- CLOCK proteins have intrinsic histone acetyl transferase activity and can mediate chromatin re-modelling through histone acetylation
- In individuals off ART, plasma HIV RNA has circadian variation

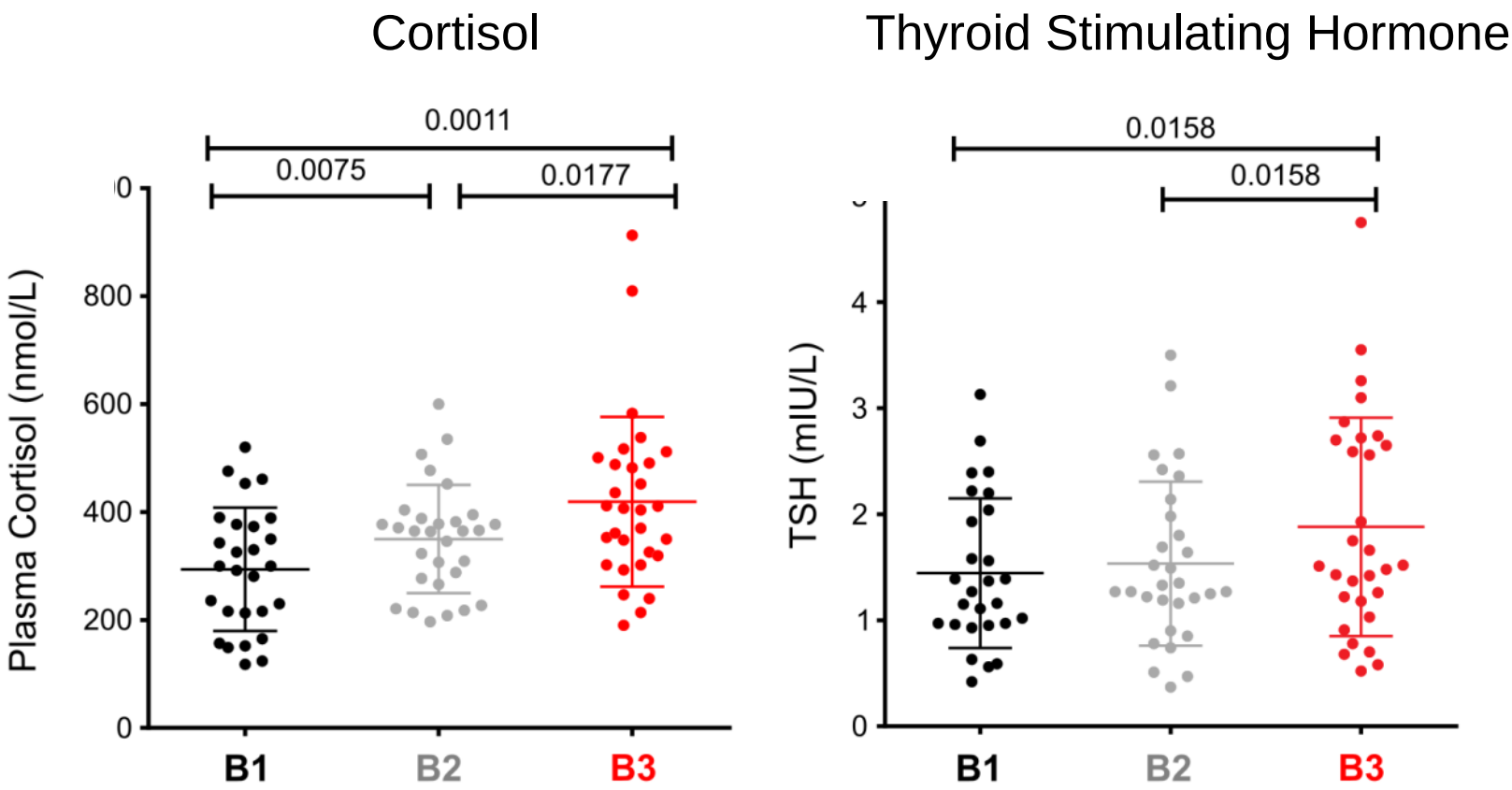
Terme et al., Retrovirology 2009; Ou et al., J Virol 1994; Doi et al., Cell 2006; Zeichner et al., Pathobiology 1996;

Visit related changes in expression of genes controlling circadian rhythms



Chang et al., AIDS 2018

Increased cortisol and thyroid stimulating hormone at B3



Measurement of T-cell numbers, T-cell subsets, activation markers and H3 acetylation showed no change at the three baseline timepoints

Chang et al., AIDS 2018

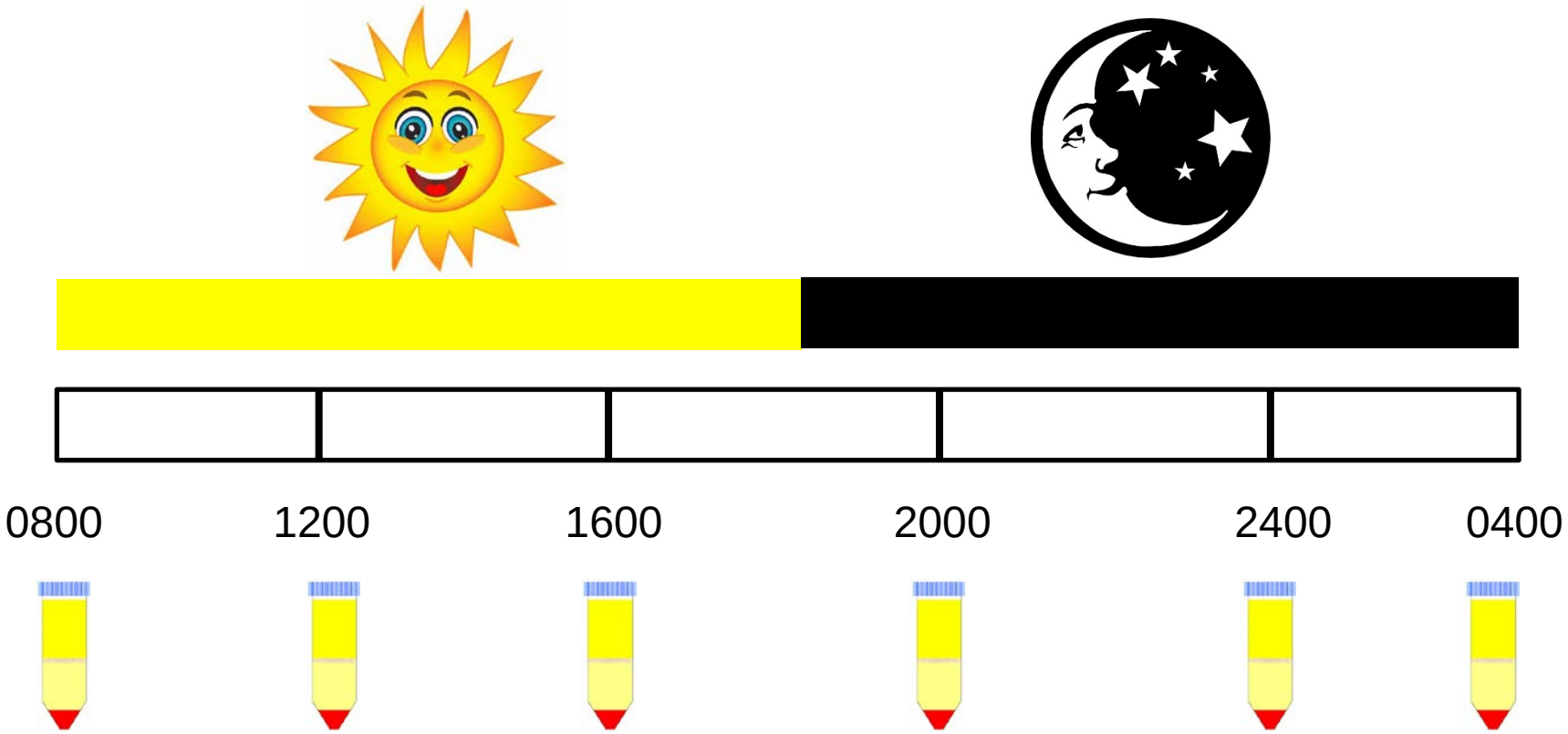
Major effect of “visit” and modest effect of “time” on unspliced HIV RNA

Parameters	Estimate	Standard Error	P-value
μ, Intercept	0.80	0.33	0.016
γ, Time of blood collection (per hour of day)	0.051	0.026	0.046
β_2, Visit 2	0.10	0.07	0.119
β_3, Visit 3	0.62	0.10	9×10^{-10}

Using a path analysis, we replaced the time of blood draw in the mixed effects model with the expression levels of the CLOCK-associated genes.

BMAL1 was the only gene that had a statistically significant effect on log CA-US HIV RNA, with an effect size of 8.508 (SE 3.777, p-value = 0.028).

Evaluation of changes in HIV transcription over 24 hours

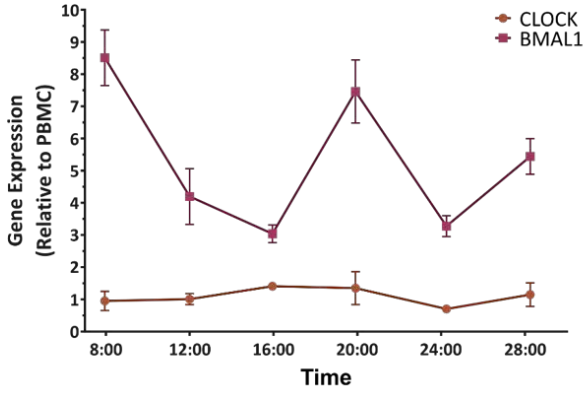


Prospective study of HIV-infected individuals on ART (n=17). Strict attention to lights, diet and other stimulants (University of Wisconsin - Madison)

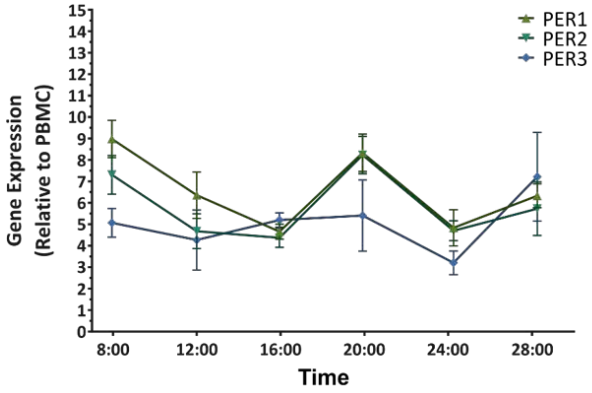
Circadian variation of HIV RNA over a 24 hour period in individuals on ART

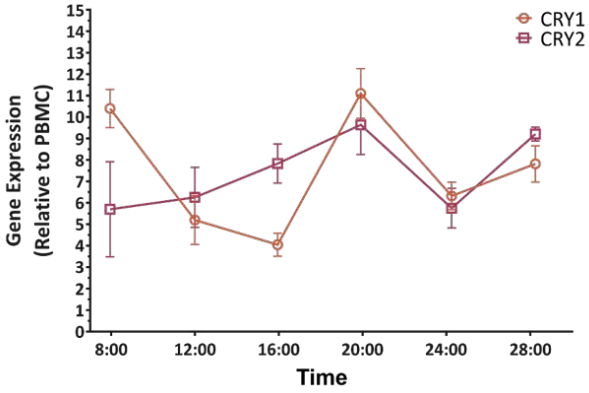
Participant 1312

Circadian Activators

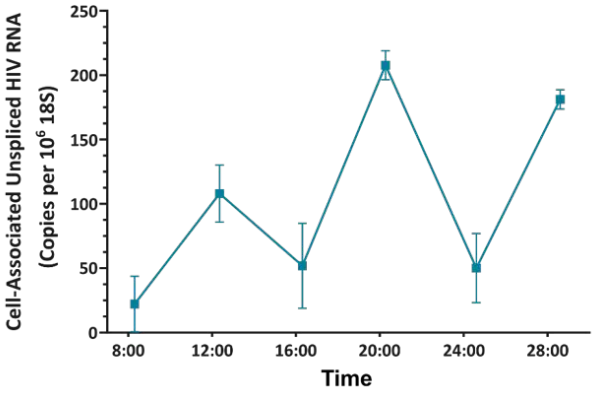


Circadian Repressor

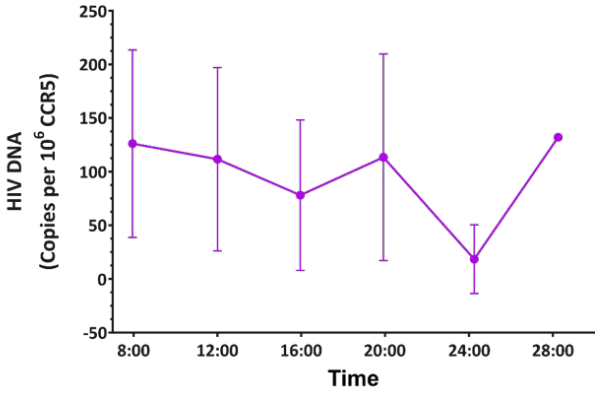




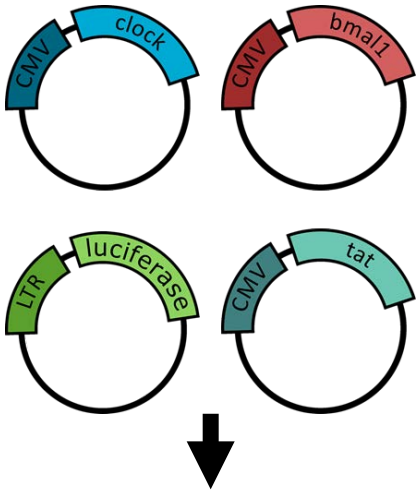
US HIV RNA



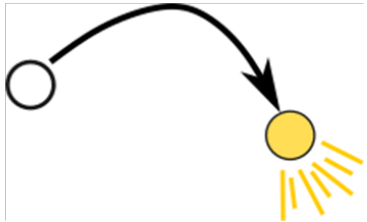
HIV DNA

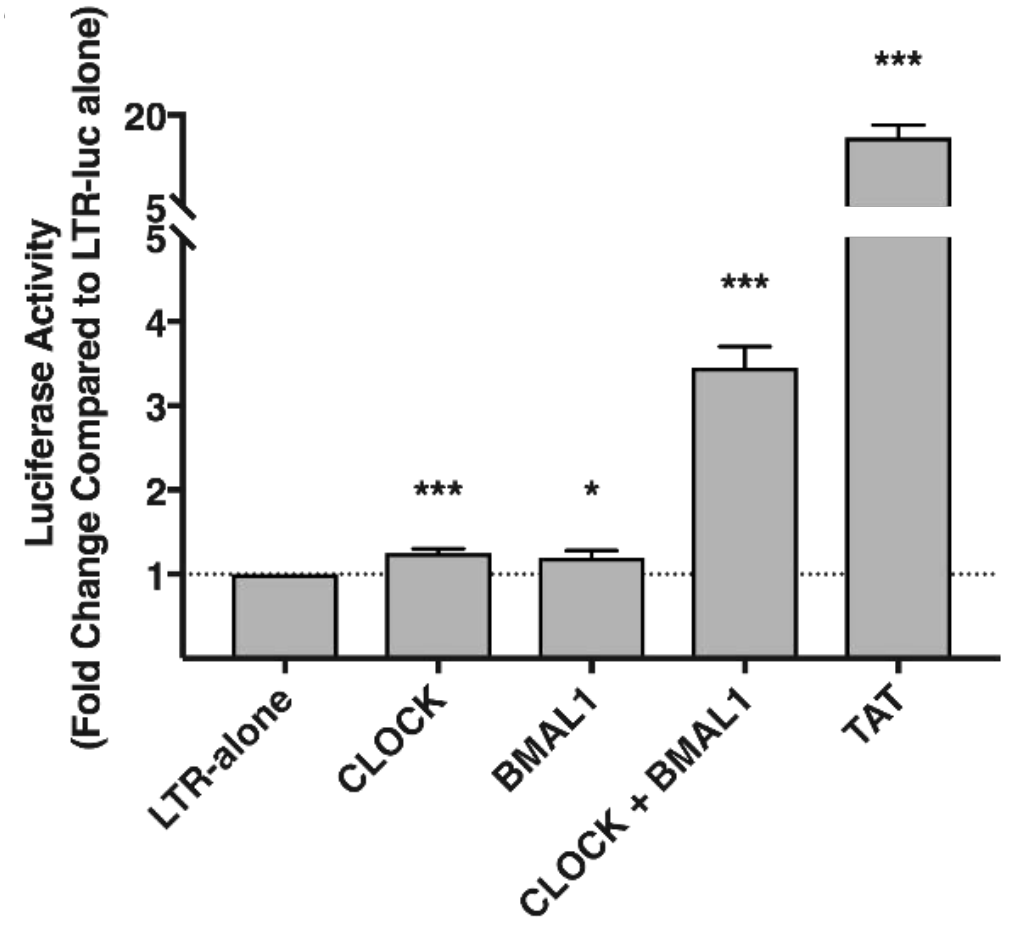


CLOCK/BMAL-1 together increase HIV transcription



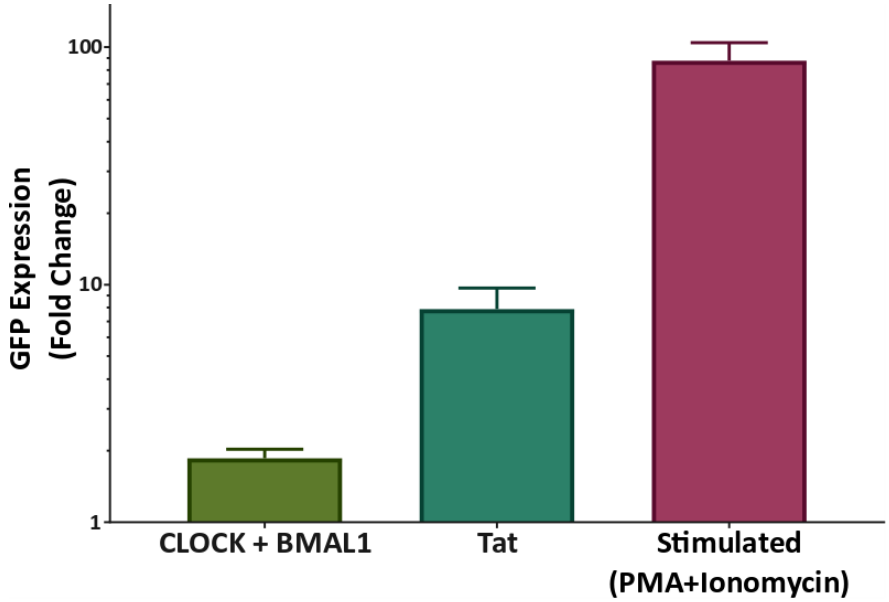
Hek 293T

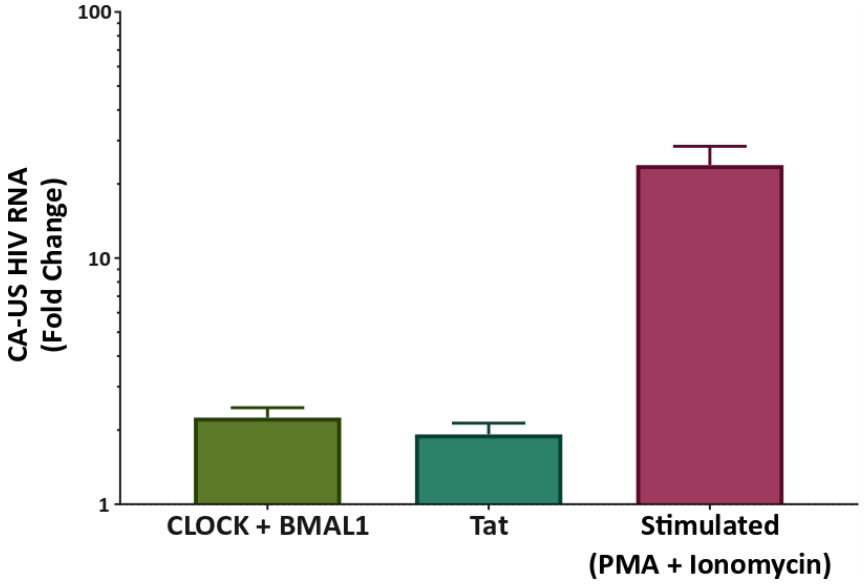




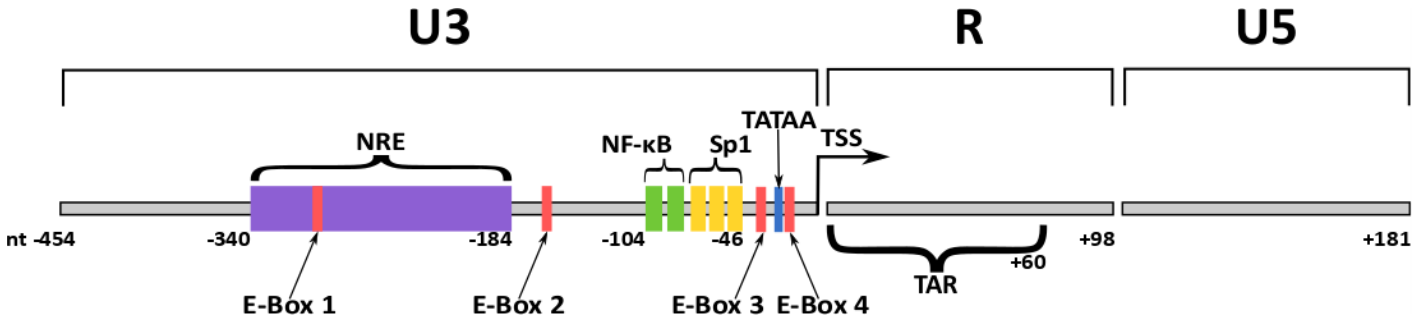
Chang et al., AIDS 2018

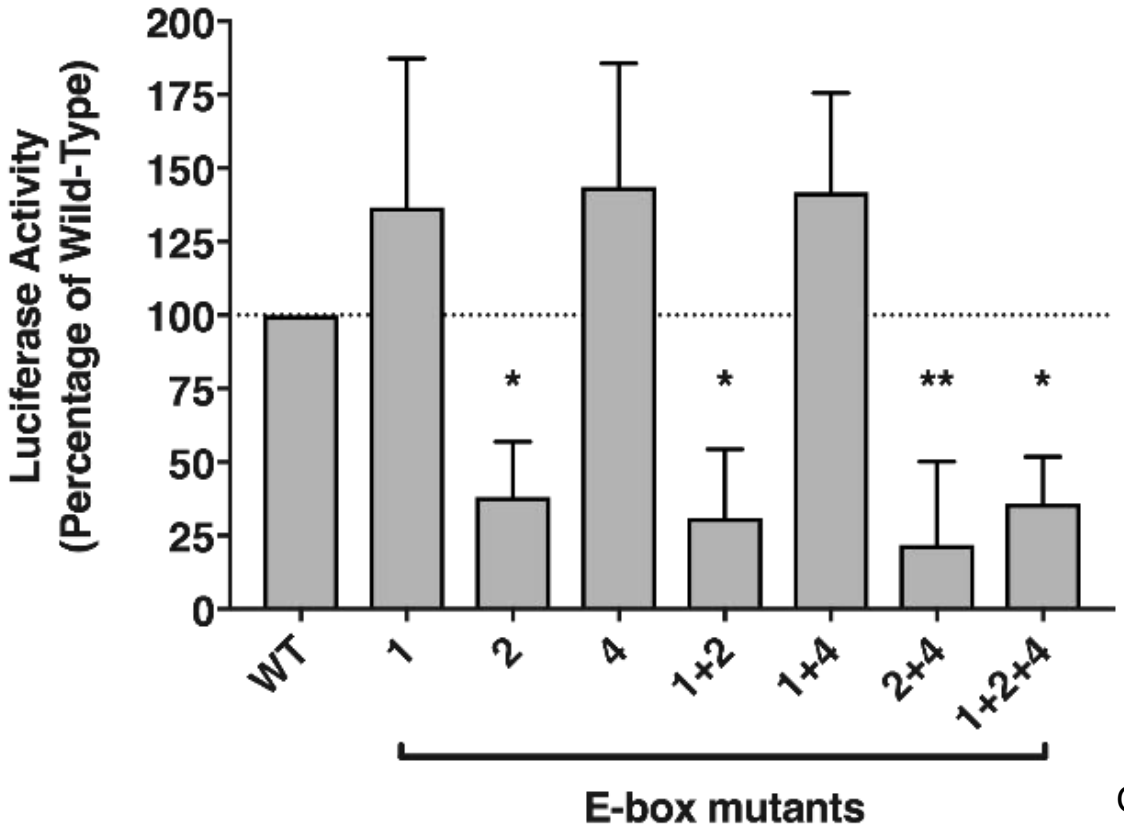
Increase in HIV transcription initiation with CLOCK/BMAL-1 in J-Lat cell line





E-box2 is required for CLOCK/BMAL-1 mediated HIV transcription





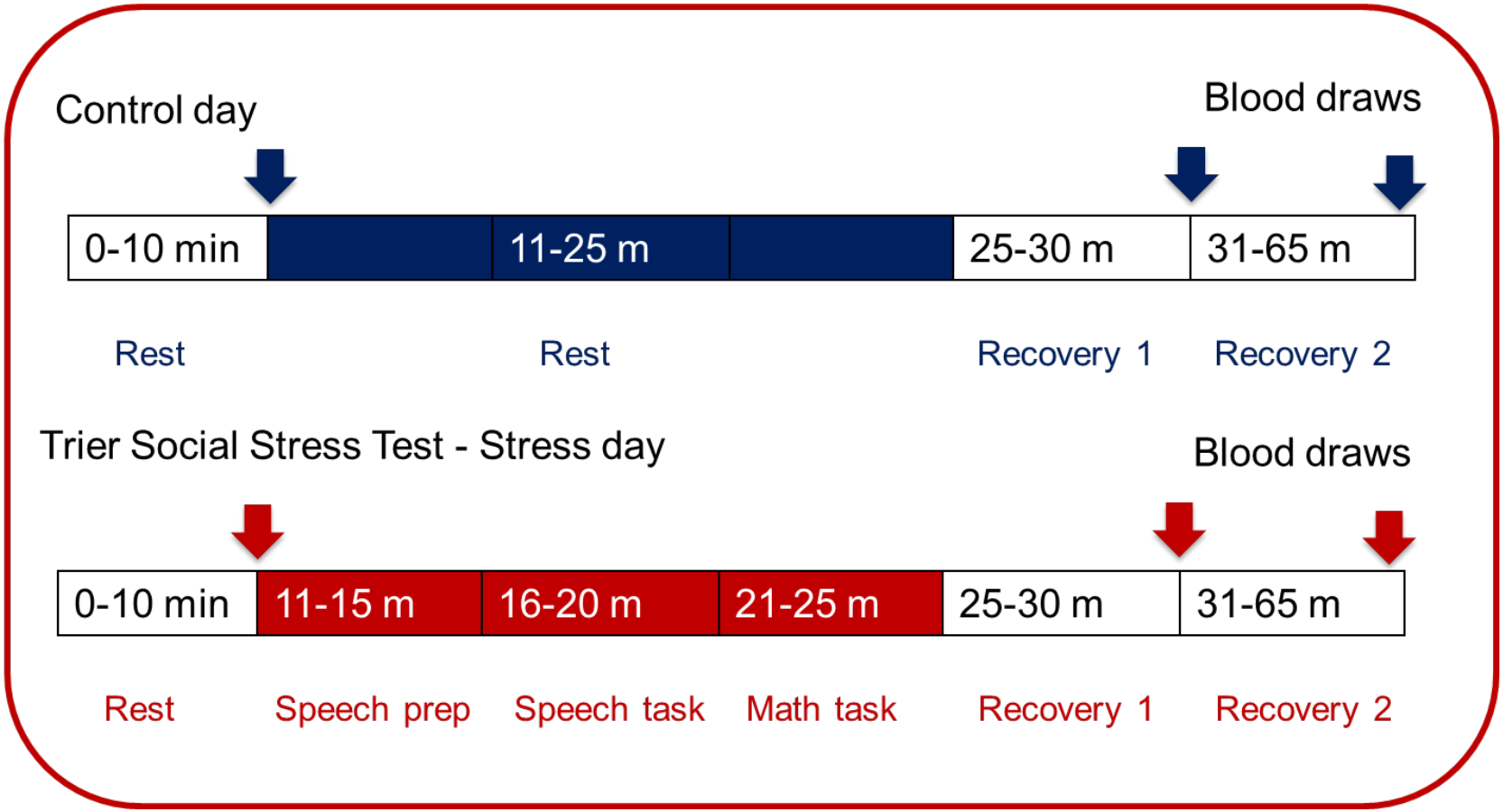
Chang et al., AIDS 2018

Summary

- Natural variation in CA-US HIV RNA in individuals on ART with a clear relationship to time over a 24 hour period
- BMAL-1 was the only circadian gene that was associated with both CA-US HIV RNA and time.
- BMAL-1/CLOCK forms a heterodimer and activated HIV transcription through binding to the second E-box
- The CLOCK/BMAL-1 pathways could be exploited to identify new latency reversing agents (LRA) or potentially enhance activity of LRAs based on time of administration

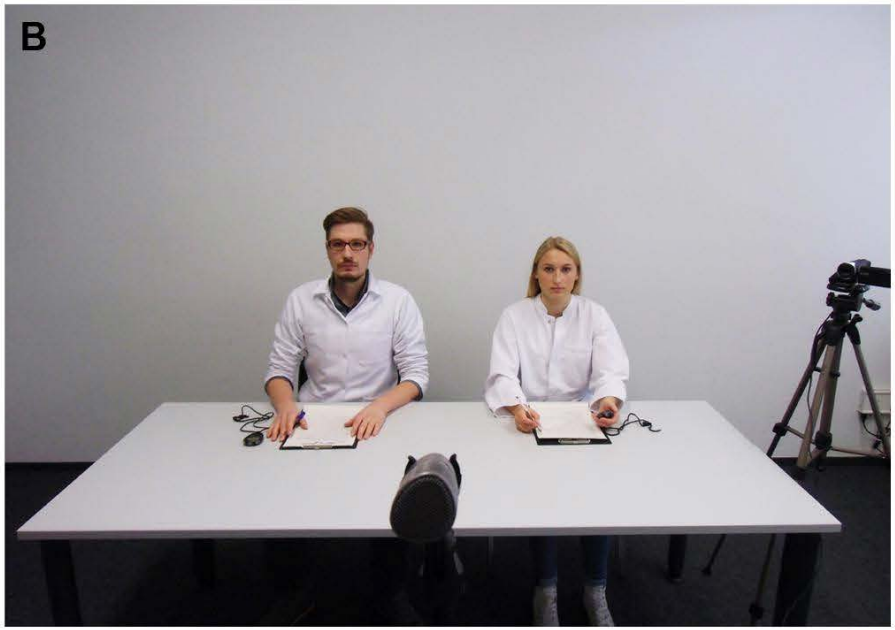
Effect of stress on HIV transcription

Evaluation of stress: control and stress day



N=25 HIV-infected individuals on ART, CD4 current = 637 cells/ul; nadir = 240 cells/ul

Evaluation of stress: Trier Social Stress Test



Physiological evaluation:

Heart rate variability= parasympathetic

- Respiratory Sinus Arrhythmia (RSA) = vagal tone

Impedance cardiography = sympathetic

- Pre-ejection period (PEP)
- Cardiac output

HIV evaluation

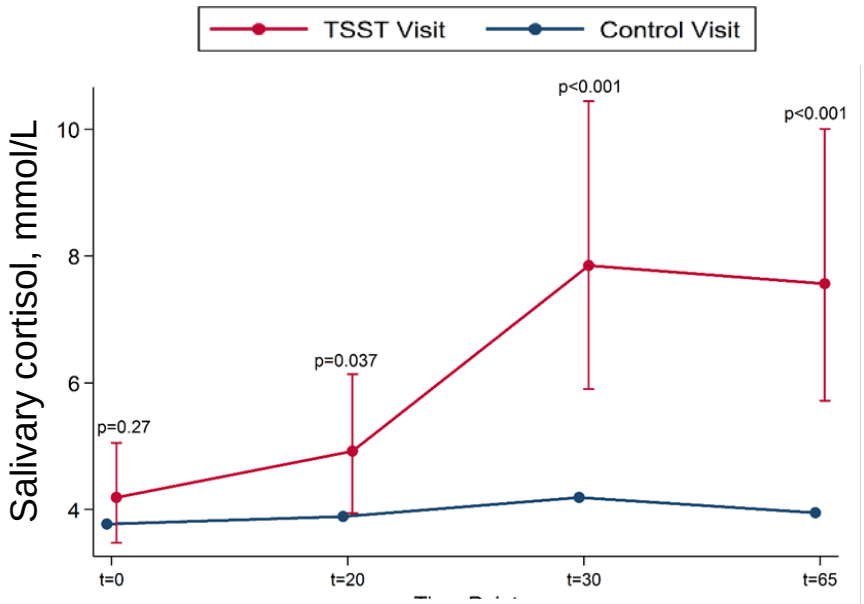
Virology:

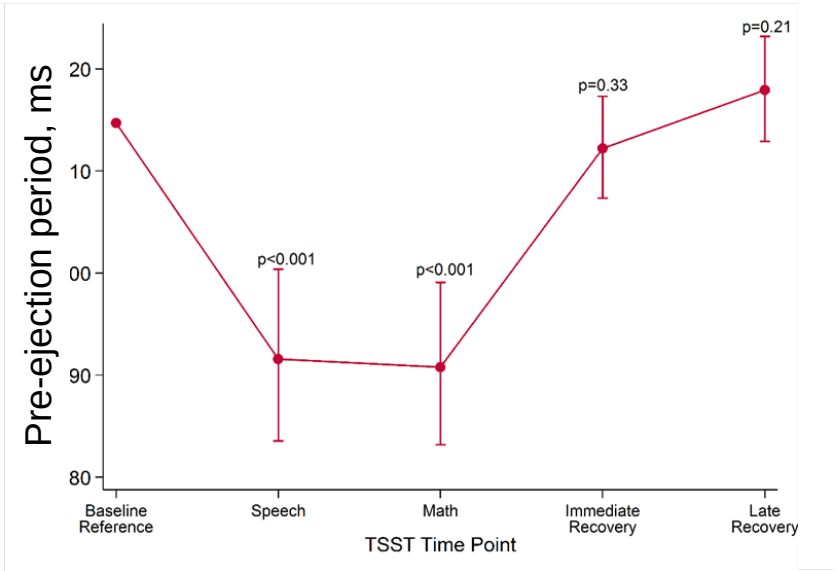
- HIV DNA and US RNA

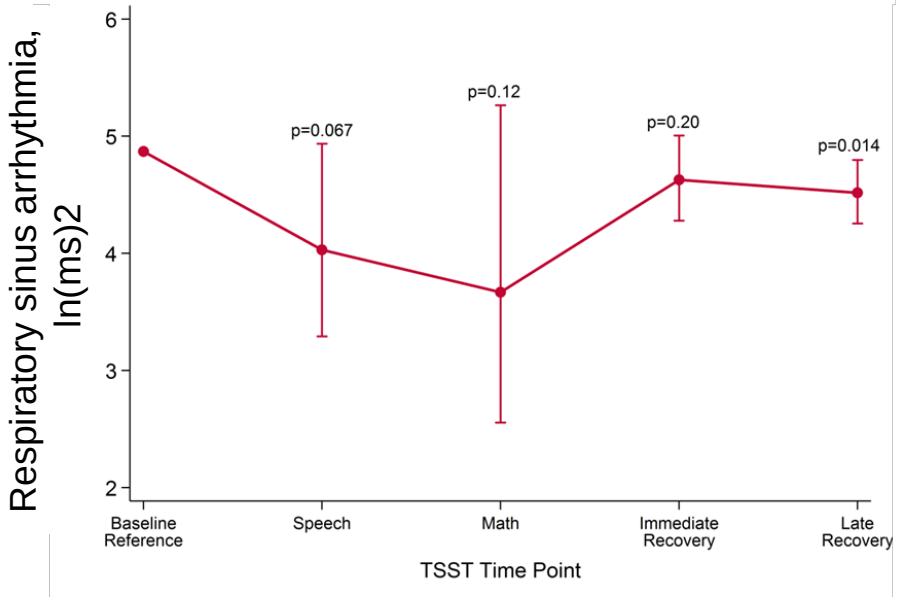
Immunology

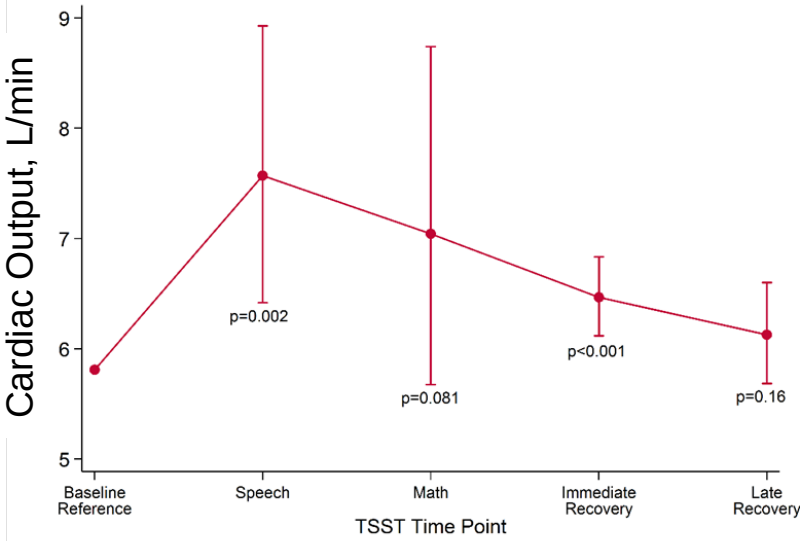
- T-cell subsets
- Activation markers

TSST induces physiological stress

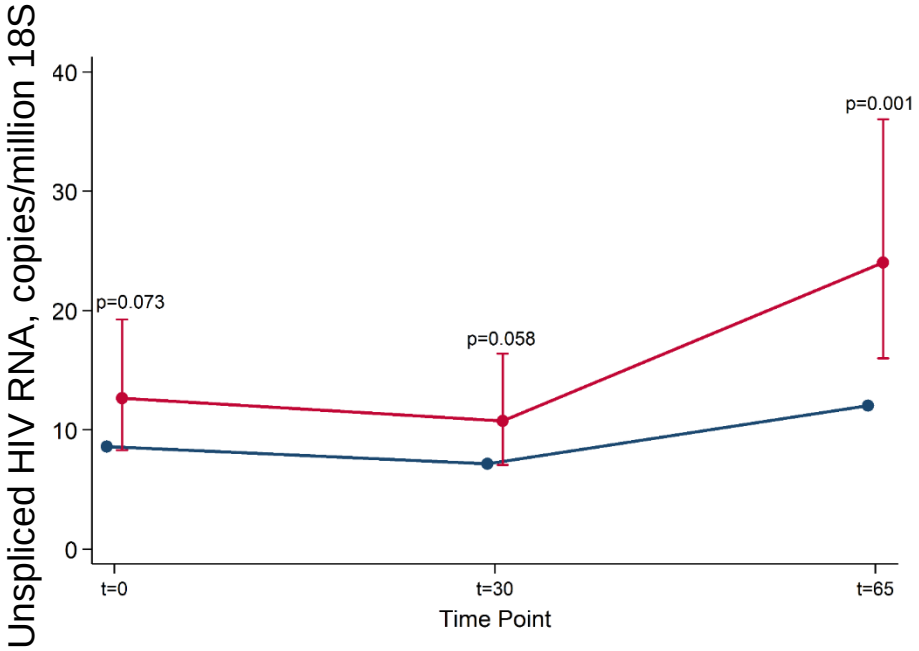


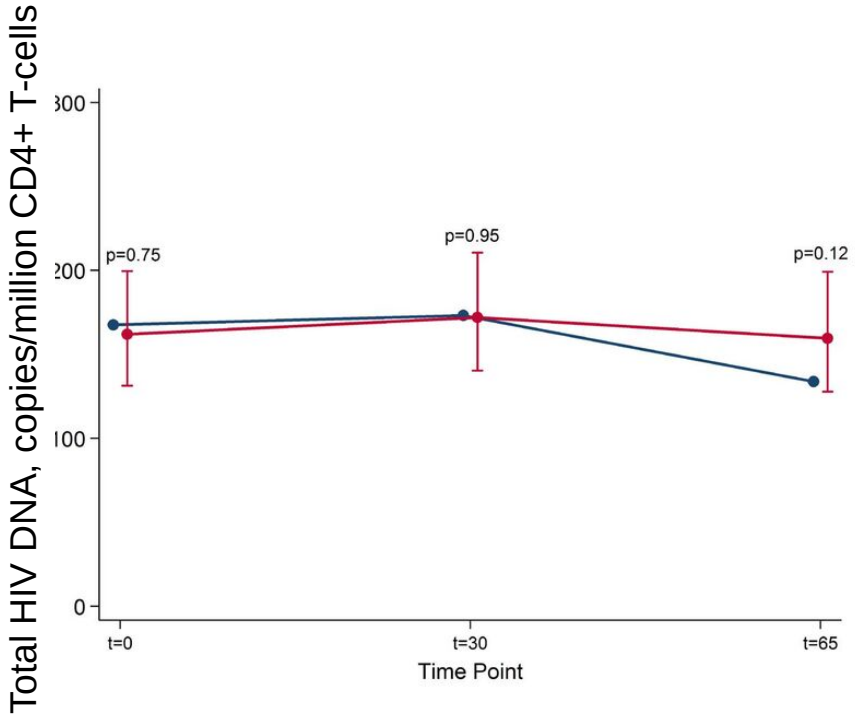






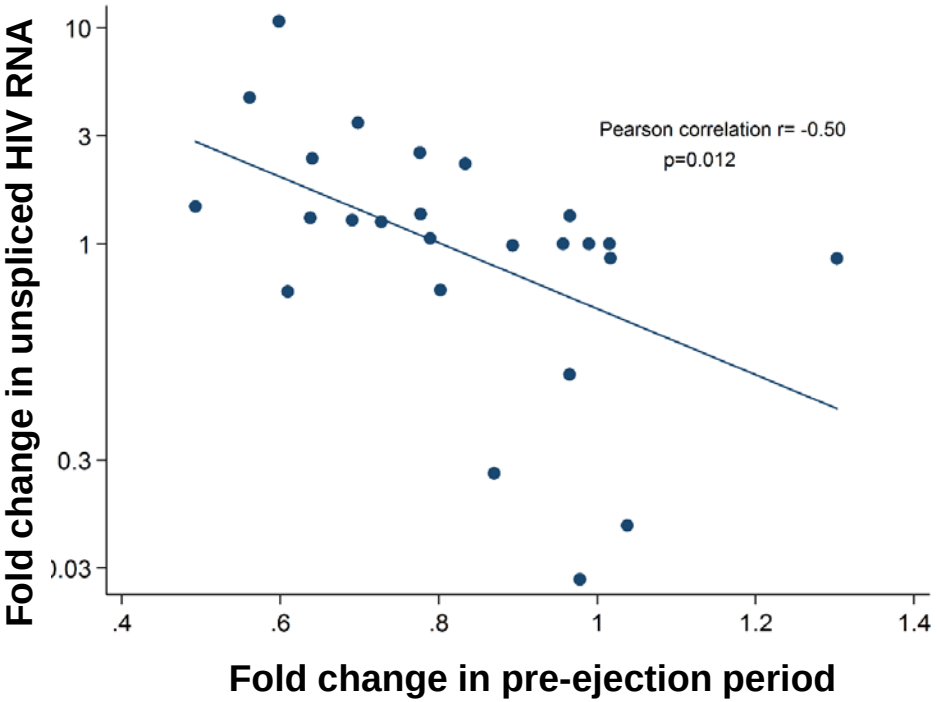
Significant increase in unspliced HIV RNA with stress





No significant changes in T-cell subsets or activation following stress

Autonomic nervous system (ANS) changes associated with changes in unspliced HIV RNA but not HIV DNA



ANS Measure	US RNA		DNA	
	Spearman rho	P-value	Spearman rho	P-value
Pre-ejection Period (PEP)	-0.59	0.002	-0.09	0.68
Respiratory Sinus Arrhythmia (RSA)	-0..05	0.81	-0.01	0.96
Cardiac Output (CO)	0.60	0.003	-0.02	0.94

Summary and implications

- Significant natural variation of cell associated unspliced HIV RNA in individuals living with HIV on ART with a clear effect of time andstress. This needs to be considered in the design of clinical trials of LRAs
- The circadian transcription factors, CLOCK and BMAL1, upregulate HIV LTR-mediated transcription initiation and this upregulation requires an intact E-box 2 motif
- Circadian proteins and or stress represent pathways that could potentially be exploited for latency reversal through development of novel drugs or optimising the timing of administration of LRAs
- Other cell associated markers of RNA transcription such as multiply spliced RNAs may be better biomarkers of latency reversal in clinical trials

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Delaney AIDS Research Enterprise
DARE
to find a cure

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