A novel assay to evaluate the response of patient-derived virus to latency reversing agents *ex vivo*

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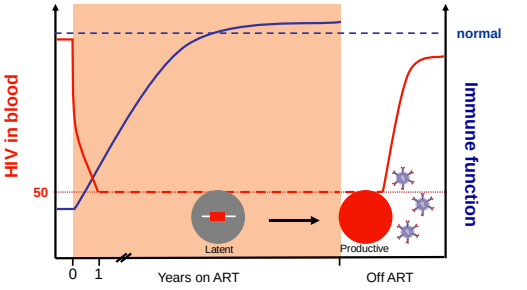
Disclosure statement

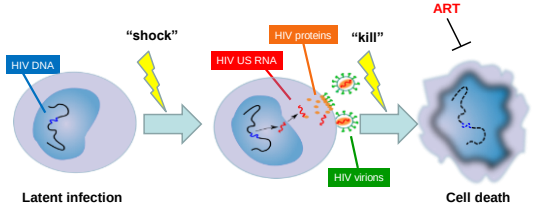
- No conflicts of interest to disclose

Rapid rebound viraemia after ART cessation



Gulick *et al.*, N Engl J Med, 1997; Hammer *et al.*, N Engl J Med, 1997; Davey *et al.*, Proc Natl Acad Sci USA, 1999; Chun *et al.*, Nat Med 2000

Shock and kill strategy



Latency reversing agents in clinical development

Epigenetic modifiers
HDACi
Methylation inhibitors
Methyltransferase inhibitor
Bromodomain inh

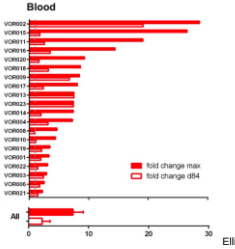
TLR agonists
TLR7 (GS9620)
TLR3 (polyICLC)
TLR 9
TLR4

PKC agonists
Prostratin
Bryostatin
Ingenol B / PEP 005

Other
Disulfiram
Quinolines
IL-15

Variability in response to latency reversing agents (LRA) stimulation

- Although *in vitro* studies using LRAs (e.g. vorinostat) have shown consistent reactivation of latent HIV, *ex vivo* and *in vivo* studies have shown variability in response



Elliott *et al.*, PloS Pathog, 2014

Hypothesis and Aims

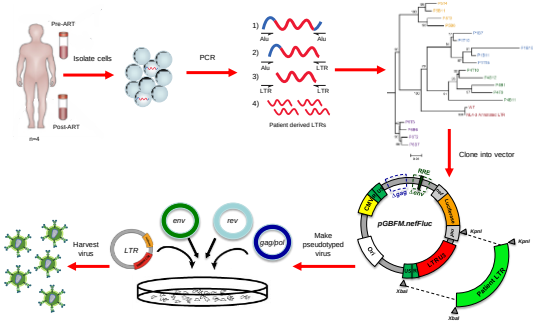
Hypothesis

- Since HIV transcription is dependent on the activity of the HIV promoter – the **long terminal repeat (LTR)** – variability in reactivation to LRAs could be attributed to changes in the **sequence and/or function** of the HIV LTR

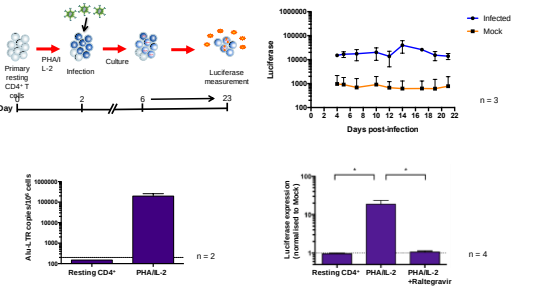
Aims

- 1) Establish a model of HIV latency using patient-derived HIV LTRs
- 2) Determine the potency of various LRAs on HIV reactivation using patient-derived LTRs

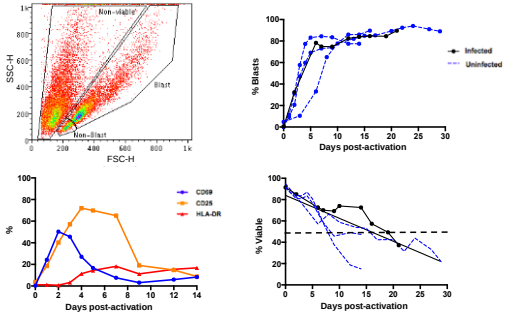
Isolating patient-derived LTRs and creating pseudotyped virus



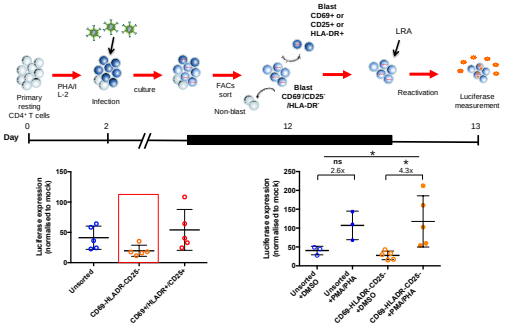
Infection led to integration and luciferase expression



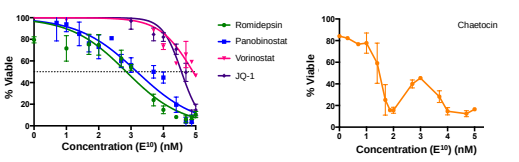
Phenotypic analysis of CD4+ T-cells post PHA/IL-2 activation



Enrichment of latently infected cells

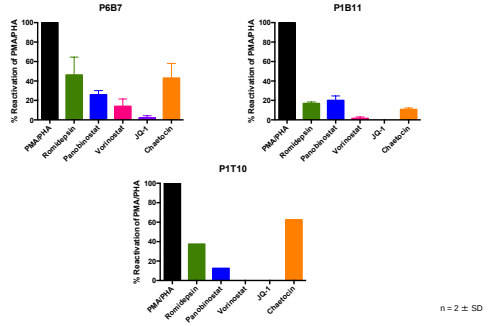


Cytotoxicity data



	Romidepsin	Panobinostat	Vorinostat	JQ-1	Chaetocin
Functions	Class I selective HDACi	Pan-HDACi	Pan-HDACi	BET Bromodomain Inhibitor	Histone Methyltransferase Inhibitor
CC_{50} (nM)	715	1519	82906	36330	NA
Used (nM)	40	50	500	1000	10

Romidepsin and chaetocin induce high levels of HIV transcription



Summary

- We have developed a novel primary cell model of HIV latency that allows the assessment of patient-derived HIV LTRs and their response to LRAs
- Inducible expression of luciferase from integrated virus was detected in blast cells that didn't express activation markers, potentially consistent with post-activation latency
- Romidepsin and chaetocin induced high levels of HIV transcriptional activity
- Further experiments are required using a wider panel of HIV LTRs to fully assess variability in response to LRAs

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