Romidepsin combined with pro-apoptotic drugs reduce integrated HIV DNA

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A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Background

One strategy to eliminate T-cells latently infected by HIV in people living with HIV (PLWH) on antiretroviral therapy (ART) is termed "shock and kill"[1].

In recent clinical trials, most single LRAs have induced transient increases in HIV RNA but did not decrease the size of the latent HIV reservoir in all patients[2,3]. This indicates that novel compounds that lead to the death of these reactivated cells are required.

Phosphoinositide 3-kinases (PI3Ks) are secondary messengers that control a wide range of intracellular signalling pathways including cellular apoptosis (programmed cell death)[1]. Activation of the PI3K signalling pathway is believed to promote the maintenance of HIV latency by suppressing apoptosis[1]. Therefore, PI3K inhibitors (PI3Ki) may sensitize cells towards apoptosis. Latently infected T-cells express anti-apoptotic proteins, such as Bcl-2 that block the progression of apoptosis[1]. Latently infected cells can be sensitized towards cells death through the administration of drug compounds that block Bcl-2 such as Venetoclax[1].

LRAs reactivate latently infected cells leading to the expression of pro-apoptotic HIV proteins that results in cell death. The combination of pro-apoptotic drugs such as PI3Ki and VNX with LRAs could drive the reactivated latently infected cell towards apoptosis to clear the latent reservoir[1].

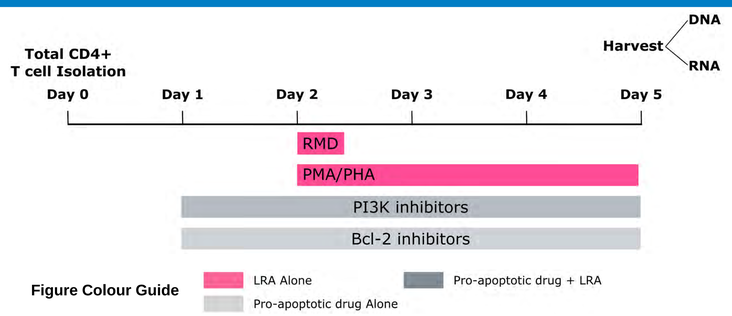
Hypothesis and Aims

Hypothesis: Latently infected cells will undergo apoptosis following administration of pro-apoptotic drugs and reactivation with a latency reversing agent

Aims:

1. Determine non-toxic doses and the timing of administration of pro-apoptotic drugs with LRAs in uninfected resting CD4+ T cells
2. Determine the impact of pro-apoptotic drugs with LRAs in ex vivo CD4+ T cells from PLWH individuals on ART

Experimental Methods



References and Acknowledgments

[1]Kim *et al.*(2018) *Cell Host Microbe,* [2]Rasmussen *et al.*(2014) *Lancet HIV*, [3]Sogaard *et al.*(2015) *PLoS Paths*

We are most grateful to People Living With HIV (PLWH) on ART (Alfred Hospital, Melbourne, Australia) and uninfected donors (Australian Red Cross Blood Service) for generously providing critical samples for this study. We also thank Dr Judy Chang plus the Doherty Institute Flow Cytometry Facility for flow cytometry support (University of Melbourne). This work is supported by a National Health and Medical Research Council of Australia (NHMRC) Program Grant #1052979. SRL is an NHMRC Practitioner Fellow.

Compounds Tested

Pro-apoptotic drug	Name	Mechanism
PI3K inhibitor	IPI-443	Potent dual inhibitor of PI3Kδ/γ isoforms
	IPI-3063	Potent PI3Kδ isoform inhibitor
	Wortmannin	Non-specific PI3K inhibitor
Bcl-2 inhibitor	Venetoclax	Inhibitor of anti-apoptotic molecule Bcl-2

LRA	Name	Mechanism
HDACi	Romidepsin (RMD)	Remodel chromatin structure to reactivate latent HIV
T cell Activation	PMA/PHA	T cell activation during HIV transcription

Results: 1. No significant toxicity in uninfected resting CD4+ T cells pulsed with LRAs

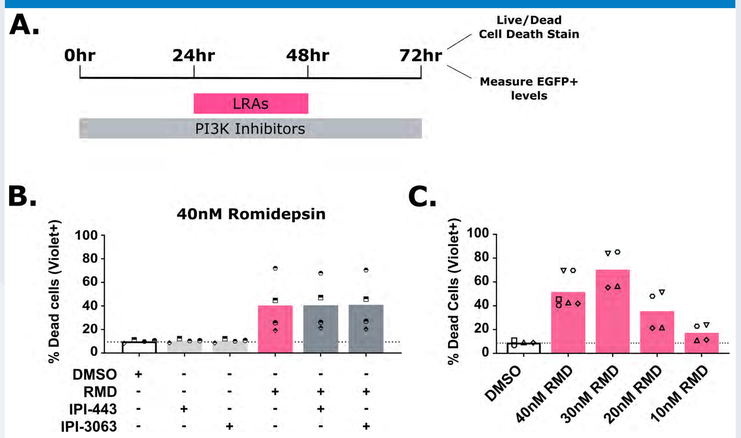


Figure 1. Uninfected resting CD4+ T cells pulsed with romidepsin for 4 hours decreased levels of total dead cells

Pulsing uninfected resting CD4+ T cells with romidepsin and PI3K inhibitors (A, B) for 24 hours resulted in moderate levels of cell death. (C) Pulsing uninfected T cells with lower doses of romidepsin for 4 hours decreased levels of total dead cells compared to 72hr continuous culture in the drug (data not shown). The addition of PI3K inhibitors did not affect cell death. n=4-6, each symbol denotes a separate donor.

Conclusion

- The pro-apoptotic drugs IPI-443, WN and VNX together with the LRA RMD synergistically reduced the levels of integrated HIV DNA in total CD4+ T cells from PLWH on ART
- All pro-apoptotic drugs alone were also able to reactivate latent HIV transcription in total CD4+ T cells from PLWH on ART

Implications

- Demonstrated that combinations of novel pro-apoptotic drugs and LRAs can impact the HIV reservoir
- Discovered a new class of drug compounds that can reactivate latent HIV transcription

2. PI3Ki/Bcl-2i and LRA reduced levels of integrated HIV DNA in ex vivo CD4+ T cells

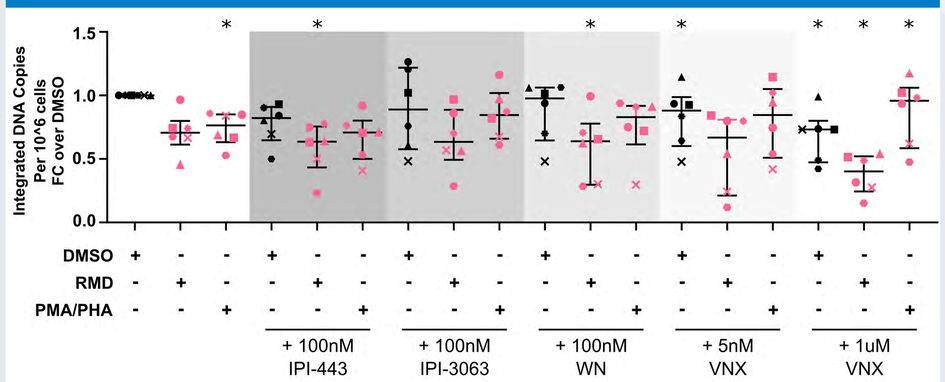


Figure 2. PI3K inhibitor, Bcl-2 inhibitor or LRA alone reduced integrated HIV DNA in total CD4+ T cells from PLWH on ART ex vivo.

*Total CD4+ T cells from PLWH on ART were treated with PI3Ki/Bcl-2i 24hr, pulsed with 20nM romidepsin 4hr and cultured with PI3Ki/Bcl-2i a further 60hr. Cellular DNA was harvested and analysed for HIV integrated DNA by qPCR. Fold change in integrated HIV DNA versus DMSO. WN, wortmannin; VNX, Venetoclax; RMD, romidepsin. *p ≤ 0.05, Wilcoxon matched-paired signed rank test. Each symbol represents a separate donor, n=6, median and interquartile range.*

		Pro-apoptotic drug				
		IPI-443	IPI-3063	WN	5nM VNX	1uM VNX
LRA	RMD	0.19	0.64	0.50	0.32	0.90

Table 1. Bliss Independence Scores of CD4+ T cells treated with pro-apoptotic drugs and RMD

Bliss Independence scores for CD4+ T cells from PLWH on ART treated with pro-apoptotic drugs and RMD were calculated. RMD together with all pro-apoptotic drugs synergistically reduced integrated HIV DNA. Bliss Independence scores above 1 are considered synergistic. RMD, Romidepsin; VNX, Venetoclax; WN, Wortmannin.

3. Romidepsin and pro-apoptotic drugs reactivate latent HIV

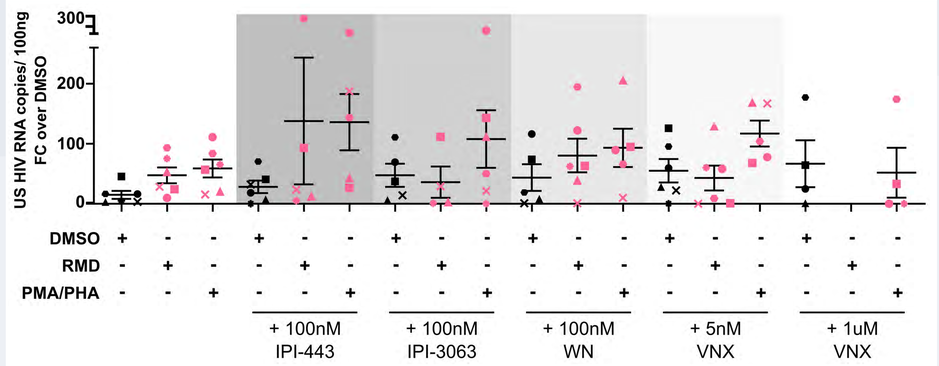


Figure 3. Pro-apoptotic drugs and LRAs reactivate latent HIV transcription in total CD4+ T cells from PLWH on ART ex vivo.

Total CD4+ T cells from PLWH on ART were treated with PI3Ki/Bcl-2i 24hr, pulsed with 20nM romidepsin 4hr and cultured with PI3Ki/Bcl-2i a further 60hr. Unspliced HIV RNA was harvested and analysed by qPCR. Each symbol represents a separate donor, n=4-6, mean and SEM. WN, wortmannin; VNX, Venetoclax; RMD, romidepsin.