# Synergistic effects of romidepsin with PI3K inhibitors in reducing the pool of HIV latently infected cells *ex vivo*

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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.

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 with LRAs could drive the reactivated latently infected cell towards apoptosis to clear the latent reservoir[1].

## Hypothesis and Aims

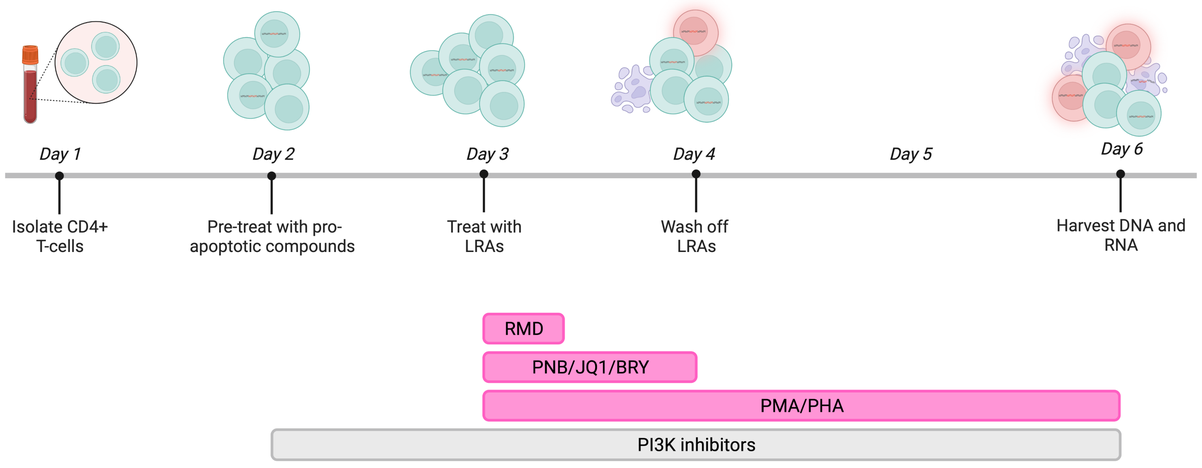
**Hypothesis:** Latently infected cells will undergo apoptosis following administration of pro-apoptotic drugs and reactivation with a latency reversing agent

**Aims:**

**1.** Determine the impact of pro-apoptotic drugs with LRAs on HIV DNA ex vivo CD4+ T cells from PLWH individuals on ART

**2.** Determine the impact of pro-apoptotic drugs with LRAs on HIV RNA ex vivo CD4+ T cells from PLWH individuals on ART

## Methods




## Conclusions

- The pro-apoptotic drugs IPI-443, IPI-3063 and Wortmannin combined with LRA RMD, PNB and JQ1 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 combinations of novel pro-apoptotic drugs and LRAs can impact HIV reservoir

## Acknowledgments

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## Results 1. PI3K inhibitors and LRAs reduce the levels of integrated HIV DNA

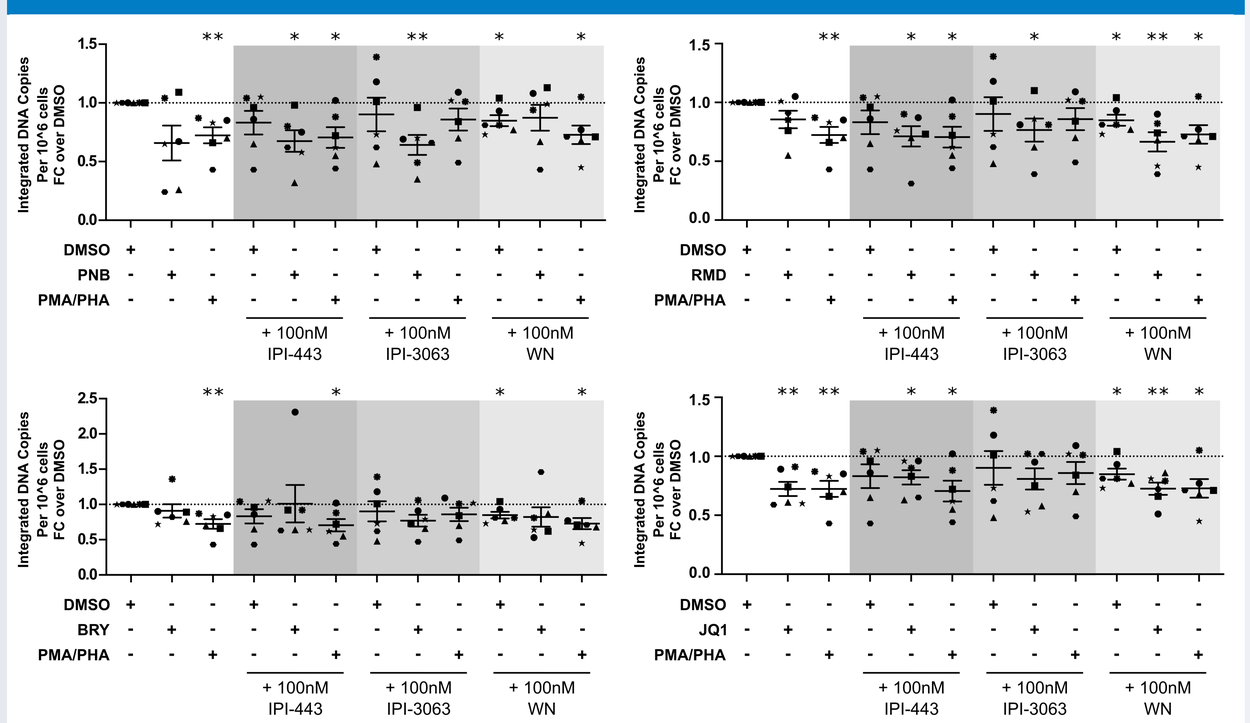


**Fig 1**. *Total CD4+ T cells were isolated from PBMCs collected from PLWH on ART. Cells were then treated with pro-apoptotic compounds, either 100nM IPI-443, 100nM IPI-3063, 100nM wortmannin (WN), or DMSO diluent control for 24 hours with and without an LRA. After the LRA pulse, cells were washed twice in media and cultured for an additional 72 hours in media in the background of the pro-apoptotic drug. All samples were harvested and analysed for HIV integrated DNA per $10^6$ cells using qPCRs for HIV integrated DNA and CCR5. Each symbol represents a different donor, statistical test were done using paired t-test, *p>0.05, **p>0.01*

## 2. PI3Ki and LRAs synergistically reduce integrated HIV DNA

| | | Pro-Apoptotic Drug | | | | | |
|---|---|---|---|---|---|---|---|
| | | IPI-443 | | IPI-3063 | | WN | |
| | | Mean | 95 % CI | Mean | 95 % CI | Mean | 95 % CI |
| LRA | PNB | -0.63 | [-3.39, 2.13] | 0.17 | [-2.40, 2.74] | -1.05 | [-3.58, 1.47] |
| | RMD | 0.28 | [-0.49, 1.06] | 0.42 | [-1.18, 2.01] | 0.51 | [-0.08, 1.10] |
| | BRY | -0.41 | [-5.68, 4.86] | -0.42 | [-5.58, 4.75] | -0.39 | [-3.90, 3.13] |
| | JQ1 | 1.50 | [-2.40, 5.39] | 2.22 | [-2.65, 7.08] | 2.60 | [-2.86, 8.05] |

***Table 1. Bliss Independence Scores of CD4+ T cells treated with pro-apoptotic drugs and LRAs***
*Bliss Independence scores for CD4+ T cells from PLWH on ART treated with pro-apoptotic drugs and LRAs were calculated. LRAs together with all pro-apoptotic drugs synergistically reduced integrated HIV DNA. Bliss Independence scores above 1 are considered synergistic. Values are averages of n=6.*

## 3. PI3Ki can reactivate latent HIV

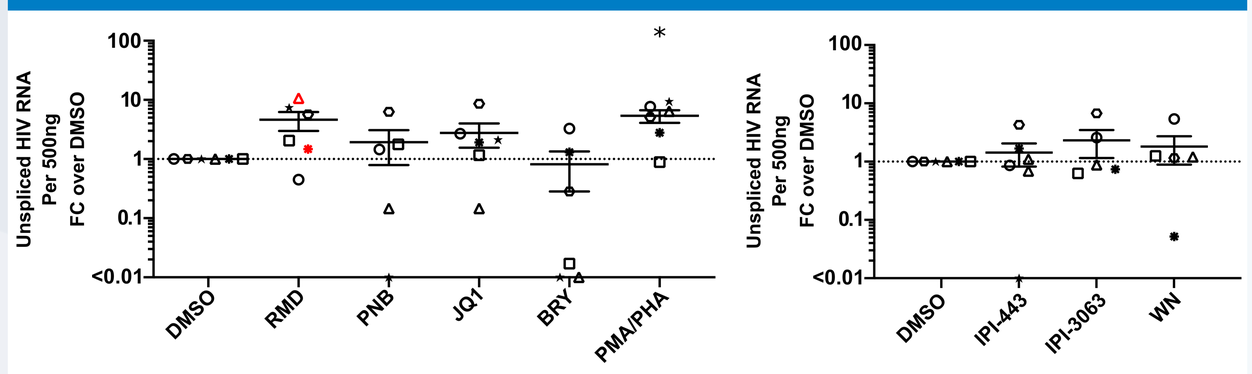


**Fig 2**. *Total CD4+ T cells isolated from PBMCs collected from PLWH on ART were treated with pro-apoptotic compounds, either 100nM IPI-443, 100nM IPI-3063, 100nM wortmannin (WN), or DMSO diluent control for 24 hours. Cells were washed in media and cultured for an additional 72 hours in media in the background of the pro-apoptotic drug. All samples were harvested and fold change in unspliced HIV RNA compared to DMSO are shown for the LRAs and the pro-apoptotic drugs. Each symbol represents a different donor. Statistical test used: paired t-test, *p>0.05*

## References

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