# Putting the Kill into Shock and Kill using Pro-apoptotic Drugs with Latency Reversing Agents

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

## HIV/AIDS Research Community Acknowledgement

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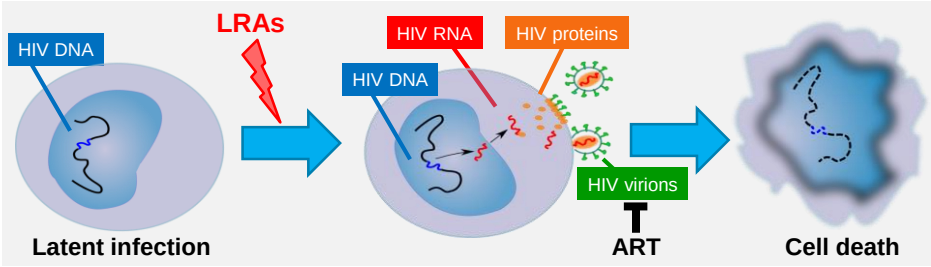
## Disclosure of Interest

SL, JA and YK collaborate with Infinity Pharmaceuticals to test PI3K inhibitors. SL and JA also collaborate with Merck Pharmaceuticals to test novel latency reversing agents.

## Shock & Kill to purge the latent HIV reservoir

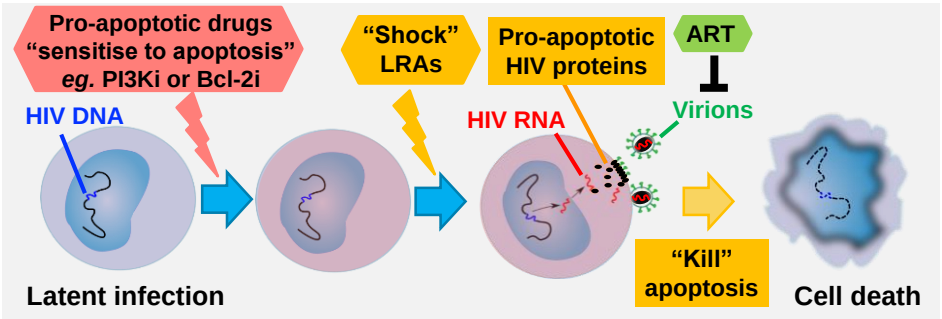
- Latently infected CD4 T cells in individuals on ART key barrier to a cure
  Chun 1997 *Nature*, Finzi 1997 *Science*, Wong 1997 *Science*, Hosmane 2017 *J Exp Med*
- Popular strategy to purge these cells: **shock and kill** Deeks 2012 *Nature*




- Promising LRAs tested alone not deplete HIV infected CD4+ T cells
  Archin 2012 *Nature*, Elliott 2014 *Plos Path,* Archin 2014 *J Infect Dis*, Rasmussen 2014 *Lancet HIV,* Spivak 2014 *Clin Infect Dis,* Sogaard 2015 *Plos Path*, Elliott 2015 *Lancet HIV, Gutierrez* 2016 *AIDS*
- More potent LRAs and **complimentary kill strategies** required

## Pro-apoptotic Drugs with Latency Reversing Agents (LRAs) may deplete the latent reservoir

- Apoptosis (programmed cell death) removes damaged or unwanted cells
  - Regulated by: Bcl-2 family proteins, IAP proteins, PI3K pathway
  - **Drug inhibitors** of phosphoinositide 3-kinases (**PI3Ki**) or Bcl-2 (**Bcl-2i**) **promote apoptosis** Kim 2018 *Cell Host Microbe*
- HIV proteins expressed later in replication have pro-apoptotic functions
  Cummins 2010 *Cell Death Dis*



## HYPOTHESIS:

Combining PI3Ki or Bcl-2i pro-apoptotic drugs to increase cell sensitivity to apoptosis, with LRAs to drive the expression of pro-apoptotic HIV proteins later in viral replication, will induce the selective apoptosis and death of latently infected cells.

## AIM:

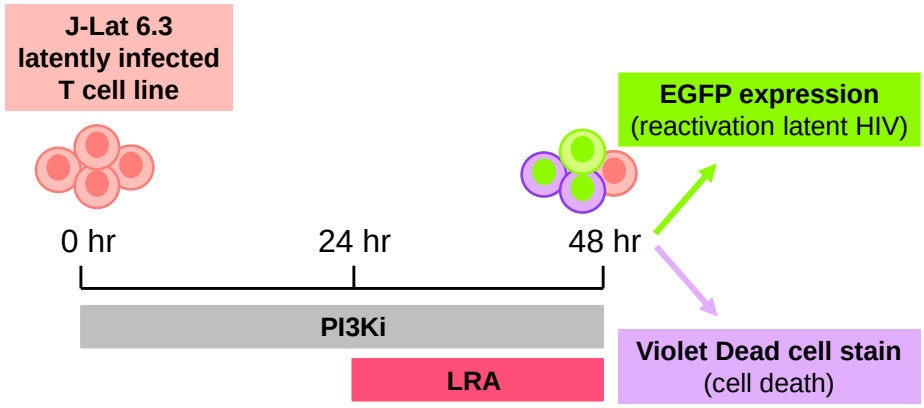
1. Determine the ability of PI3Ki or Bcl-2i pro-apoptotic drug and LRA combinations to reactivate and kill:
    a) J-Lat 6.3 latently infected T cell line
    b) CD4+ T cells from HIV+ individuals on ART *ex vivo*

## Compounds Tested

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

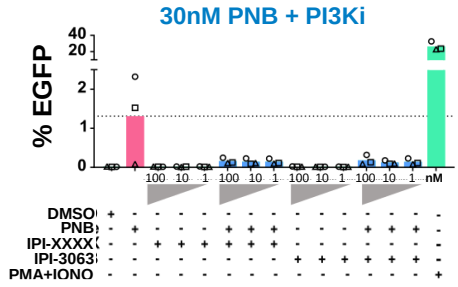
| LRA | Name | Mechanism |
|---|---|---|
| HDACi | Panobinostat (PNB)<br>Romidepsin (RMD) | Remodel chromatin structure to reactivate latent HIV transcription |
| Bromodomain Inhibitor | JQ1 | Inhibit bromodomains and dissociate protein complexes to drive HIV transcription |
| Protein Kinase C (PKC) agonist | Bryostatin (BRY) | Stimulate the PKC pathway leading to active NF-kB to reactivate HIV transcription |
| T cell activation | PMA + Ionomycin<br>PMA + PHA | T cell activation driving HIV transcription |

## PI3Ki and LRA impact on J-Lat 6.3 latent cell line



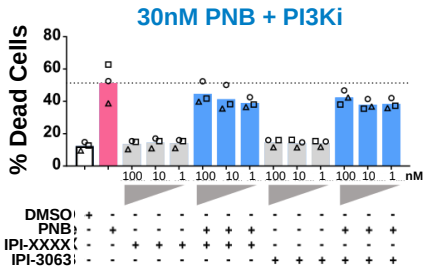

**LRAs:** Panobinostat (PNB), Romidepsin (RMD), Bryostatin (BRY), PMA + Ionomycin (IONO)

## PI3Ki reduce EGFP expression induced by the LRA Panobinostat



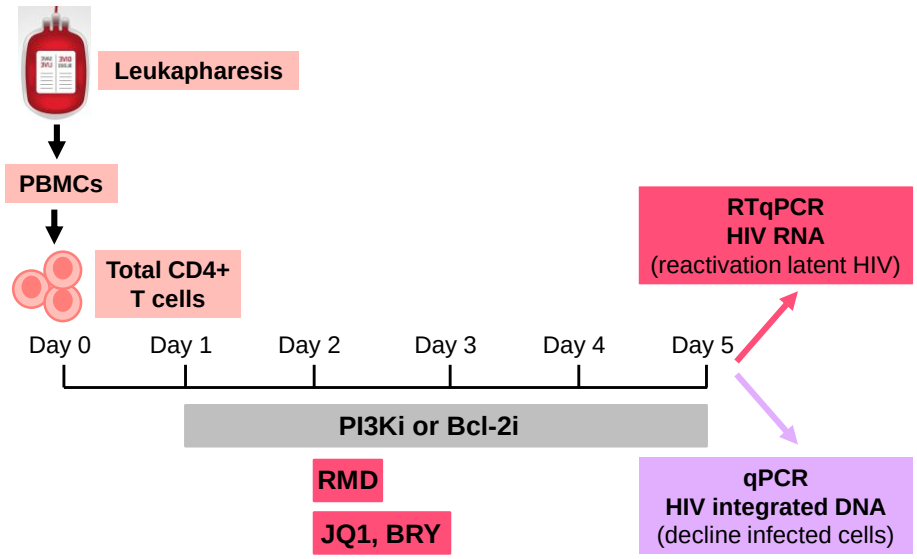

**n = 3**

## No increase in cell death secondary to PI3Ki

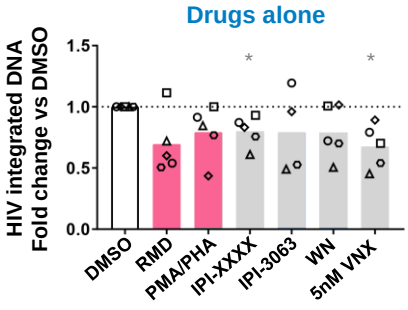



**n = 3**

## Impact of pro-apoptotic drugs & LRAs on total CD4+ T cells from HIV+ individuals on ART

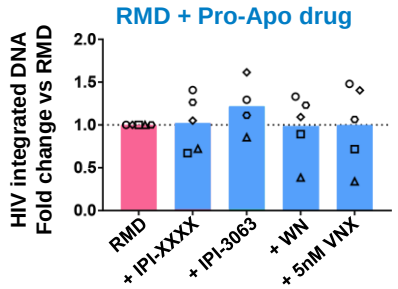


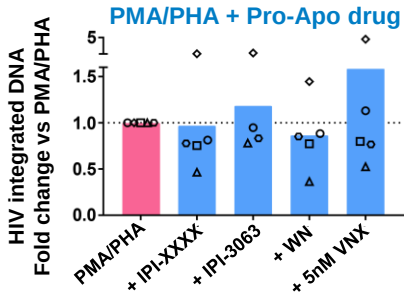
# No synergistic effect of pro-apoptotic drugs with RMD or PMA/PHA on HIV+ patient CD4+ T cells



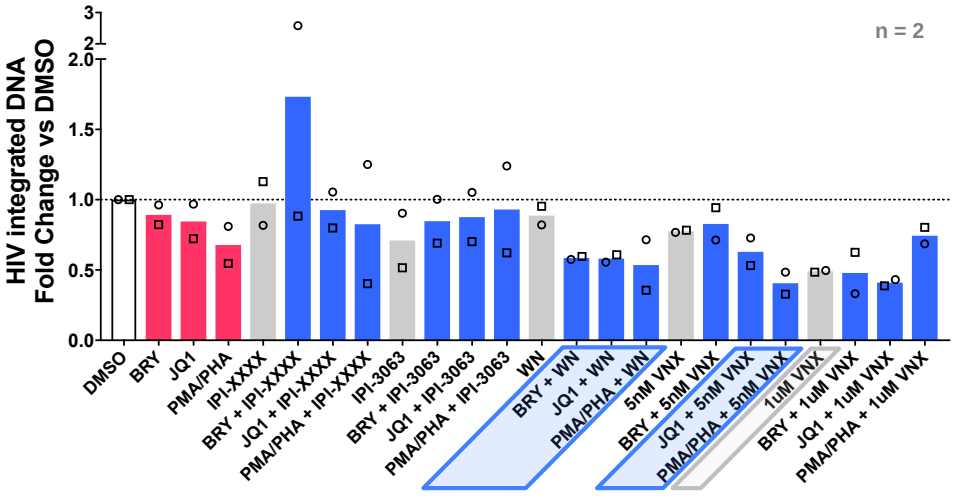

n = 4-5

* P ≤ 0.05




# Greater reduction in HIV DNA from venetoclax or wortmannin together with JQ1, BRY or PMA/PHA




**Drug combination has greater decline in HIV DNA versus pro-apoptotic drug or LRA alone**

## Conclusions and Implications

1. A reduction in integrated HIV DNA ex vivo was observed with:
   - Venetoclax alone (high dose)
   - Venetoclax (low dose) in combination with JQ1
   - Wortmannin in combination with JQ1 or bryostatin

2. Unexpected loss of EGFP+ cells in the J-Lat 6.3 cell line with panobinostat and PI3Ki could potentially be due to death of infected cells or opposing effects on HIV transcription

3. Combining specific LRAs with specific pro-apoptotic drugs may increase selective death of infected cells. Further work needed to understand the mechanism of the interaction.

4. Selective death of HIV infected cells will be confirmed with RNA probes (PrimeFlow), a cell death stain and flow cytometry

## Acknowledgements

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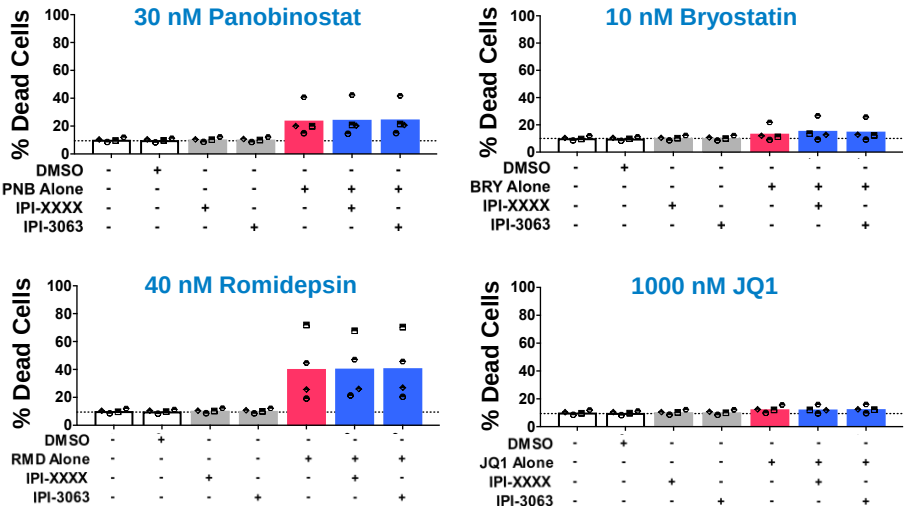
# Uninfected CD4+ T cells: Short LRA pulses limit drug toxicity




**Similar results irrespective of adding PI3Ki before, during or after LRAs**

# Pro-apoptotic drugs & LRA combinations to test

| Pro-apoptotic | Type | Clinical status |
|---|---|---|
| Wortmannin | Pan-PI3K i | Not test: too toxic |
| IPI-3063 | PI3Kdelta i | mouse model |
| Idelalisib | PI3Kdelta i | Licensed: CLL, FL, SLL |
| IPI-594 | PI3Kgamma i | Phase I:advanced solid tumors |
| IPI-4X, IPI-433 | PI3Kdelta+gamma i | mouse model |
| Duvelisib | PI3Kdelta+gamma i | Phase III: CLL, NHL, FL |
| Venetoclax | Bcl-2 i | Licensed: CLL |
| Flurbiprofen | ↑ pro-caspase-8 | Licensed: NSAID |
| Indomethacin | ↑ pro-caspase-8 | Licensed: NSAID |
| Bezafibrate | ↑ pro-caspase-8 | Licensed: ↑ chol, hyperlipidemia |
| Doxycycline | ↑ pro-caspase-8 | Licensed:antibiotic |
| LCL161 | SMAC mimetic | Phase II: MF |
| Birinipant | SMAC mimetic | Phase I/II: HBV |

| LRA | Type | Clinical status |
|---|---|---|
| Romidepsin | HDACi | Licensed: CTCL, PTCL |
| Bryostatin-1 | PKC agonist | Phase I/II: solid tumors, blood tumors, HIV, Alzheimer's |
| PEP005 (PICATO) | PKC agonist | Licensed: gel for actinic keratosis |
| MGN1703 (Lefitolimod) | TLR-9 agonist | Phase I/II: lung, colorectal & advanced tumors |
| JQ-1 | BRDi | Not test: short half life |
| OTX015 | BRDi | Phase I: AML, ALL, DLBCL, MM, advanced solid tumors |
| PMA/PHA | T cell activation | Too toxic |