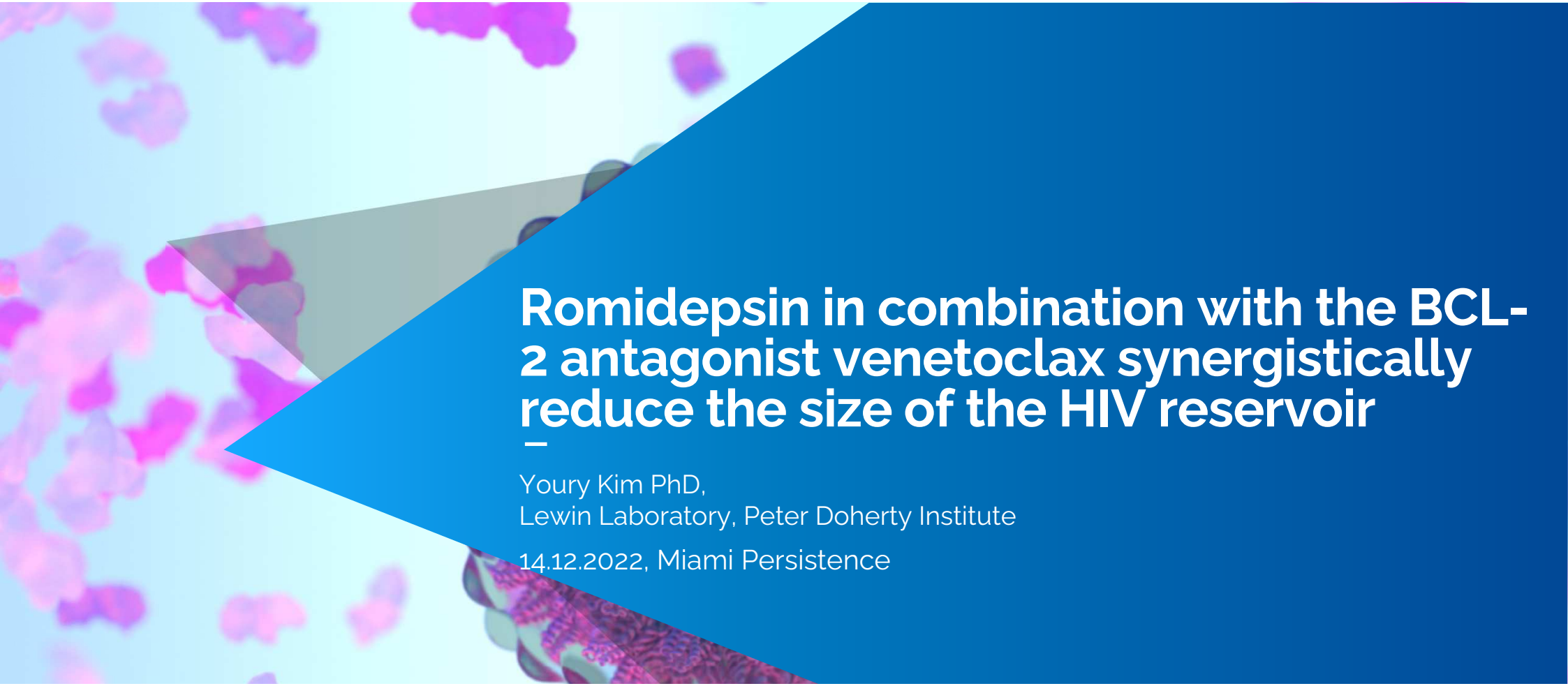


Romidepsin in combination with the BCL-2 antagonist venetoclax synergistically reduce the size of the HIV reservoir

Youry Kim PhD,
Lewin Laboratory, Peter Doherty Institute

14.12.2022, Miami Persistence

Doherty Institute

THE UNIVERSITY OF MELBOURNE

The Royal Melbourne Hospital

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

BCL-2 proteins, BCL-2 antagonists and effects on HIV

- The BCL-2 inhibitor, venetoclax increases the sensitivity of malignant cells to death and is now FDA approved and **licensed for the treatment of chronic lymphocytic leukemia** (Abbvie)[1,2]

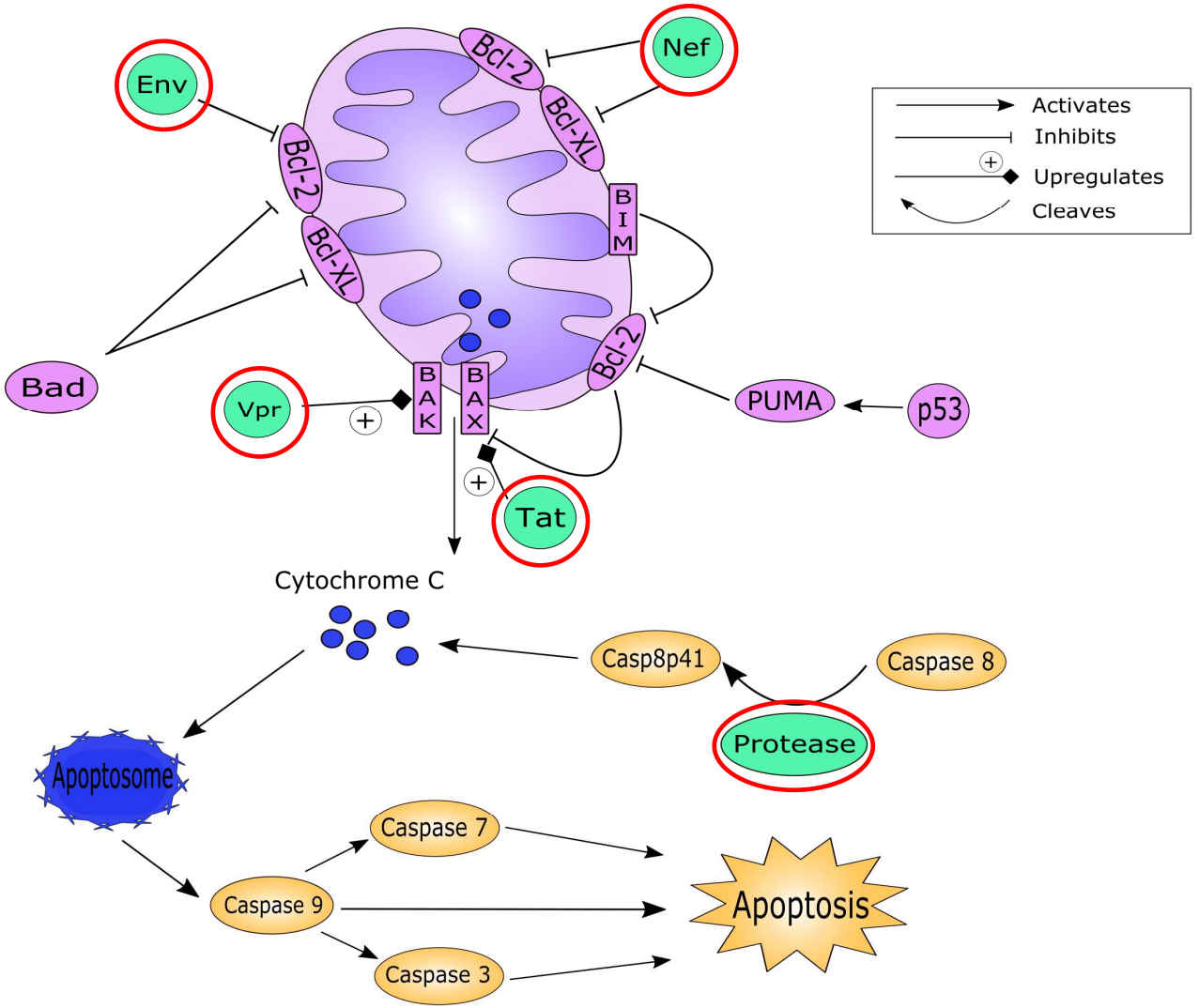
- **Venetoclax combined with anti CD3/CD28** induced a decline in total HIV DNA in CD4+ T cells collected from people living with HIV on ART with no observed cellular toxicity[3,4]

- Bcl-2 antagonist alone or in combination with **LRA Bryostatin** was able to reduce the levels of HIV DNA in an *in vitro* model of HIV latency and enhanced cell death in *ex vivo* CD4+ T cells, but only in the presence of cytotoxic T lymphocytes[5]

- The triple combination of **anti-CD3/CD28 antibodies, HIV-specific effector CD8+ T cells and venetoclax** led to consistent, significant reductions in both HIV DNA and IUPM using CD4+ T cells from people living with HIV on ART[6]

[1] Souers et al. Nat Med 2013; 2 Roberts A, N Engl J Med 2015; 3 Chandrasekar et al Clin Micro Rev 2019; 4 Cummins et al., J Virol 2018; 5 Ren et al., *JCI* 2020, 6 Ren et al., *J Virol* 2021

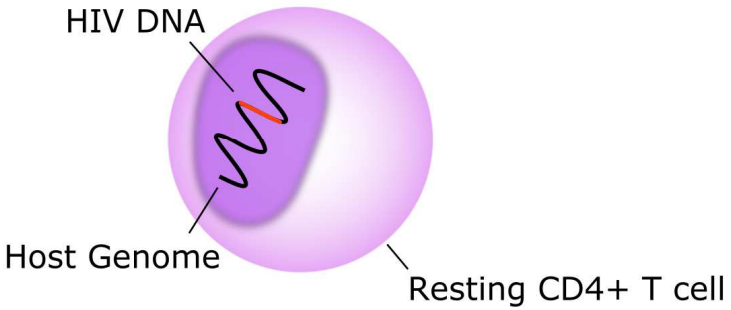
Multiple HIV proteins have pro-apoptotic effects



Kim, Anderson and Lewin, Cell Host Microbe 2018

Hypothesis

We **hypothesise** that the combination of pro-apoptotic drugs to increase cell sensitivity to apoptosis together with latency reversing agents (LRAs) can lead to the death of HIV latently infected T-cells

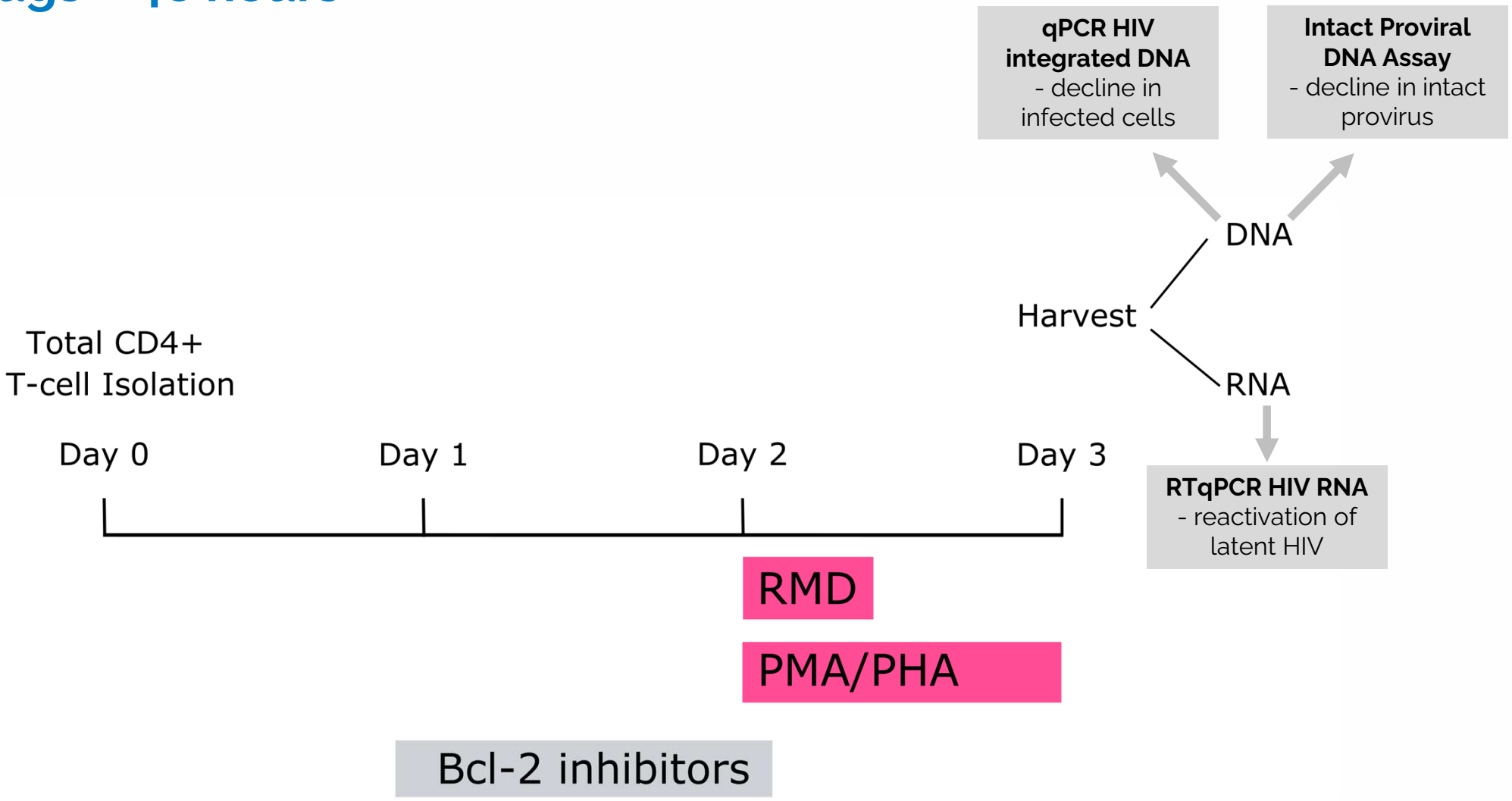


Aims

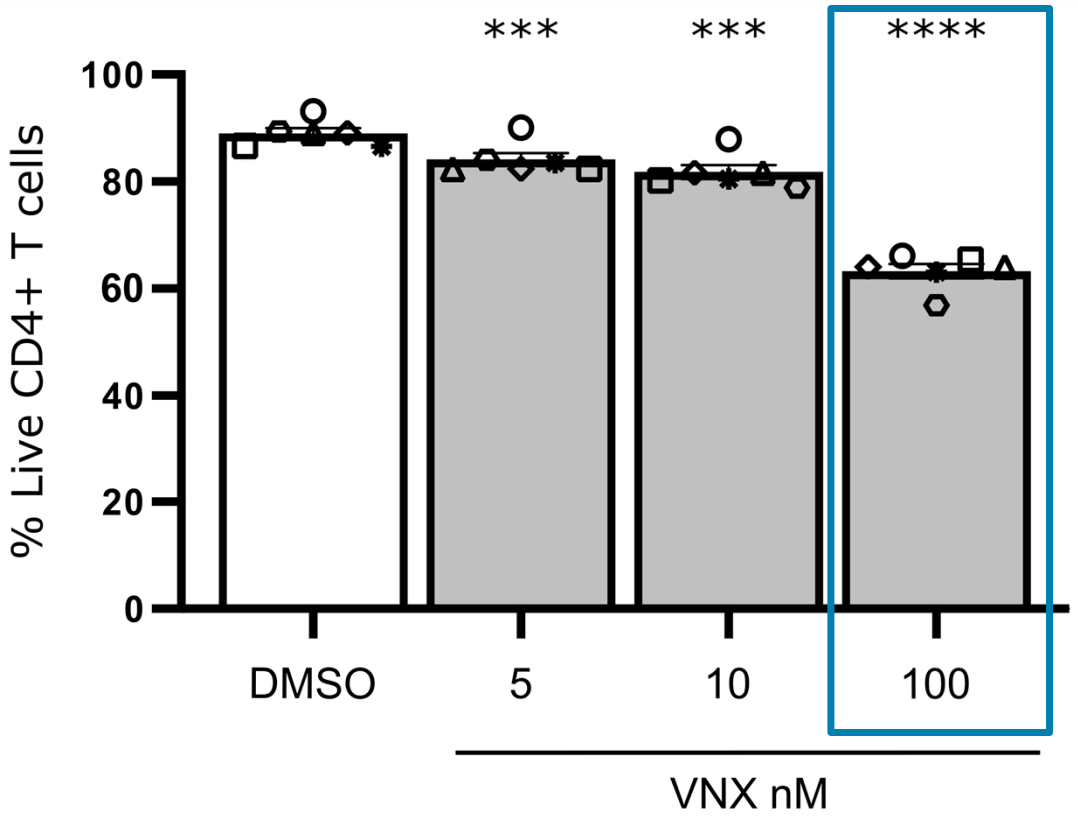
1. To determine the effect of the BCL2 antagonist, venetoclax on the number of total and latently infected CD4+ T-cells

2. To determine whether the combination of an LRA (to increase HIV proteins) and pro-apoptotic drugs lead to a synergistic reduction in latently infected CD4+ T-cells

Method: combination treatment with LRAs and pro-apoptotic drugs – 48 hours



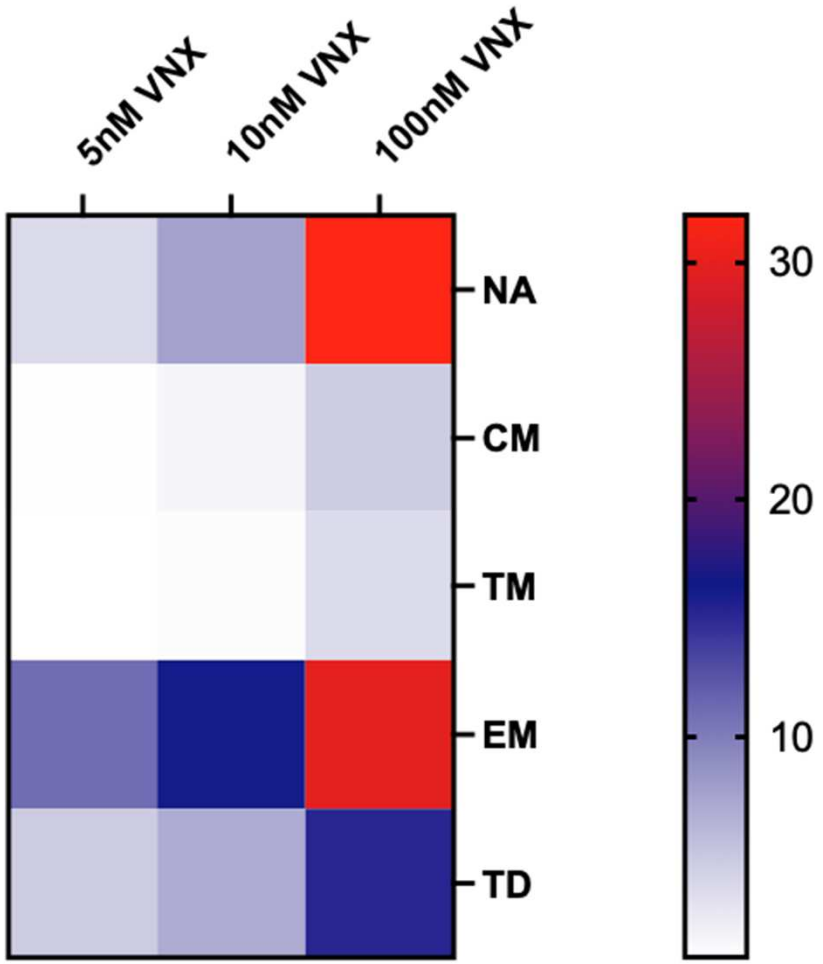
Dose Dependent effects of VNX on cytotoxicity



Mean+SEM; paired t test, ***p<0.001, ****p<0.0001

People living with HIV on ART with an undetectable viral load for >3 years underwent leukapheresis. Total CD4+ T cell were isolated and stimulated ex vivo for 48 hours

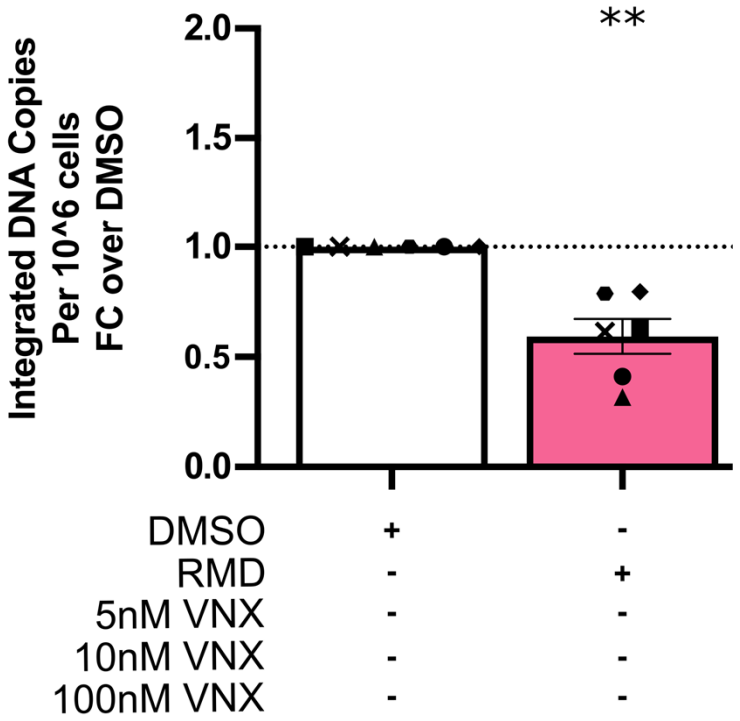
Venetoclax induces high levels of cell death in naïve and effector memory subset of T cells



- Venetoclax alone induces high levels of cell death within subsets of T cells
- Toxicity was significantly higher in naïve and effector memory T cells following treatment with venetoclax compared to the DMSO control
- The varying effects of VNX may be due to the different basal levels of BCL-2 in T cell subsets[1]

[1]Akbar *et al.* (1993) *J Exp. Med.*

Romidepsin together with Venetoclax reduces the levels of integrated HIV DNA in CD4+ T cells *ex vivo*



Mean+SEM;
* p<0.05, **<0.005,
***<0.0005, paired t test, n=6

Synergistic effects on decline in integrated DNA with romidepsin and venetoclax

		Latency Reversing Agent RMD
Pro-apoptotic Drug	5nM VNX	0.36
	10nM VNX	0.59
	100nM VNX	0.20

Bliss independence model

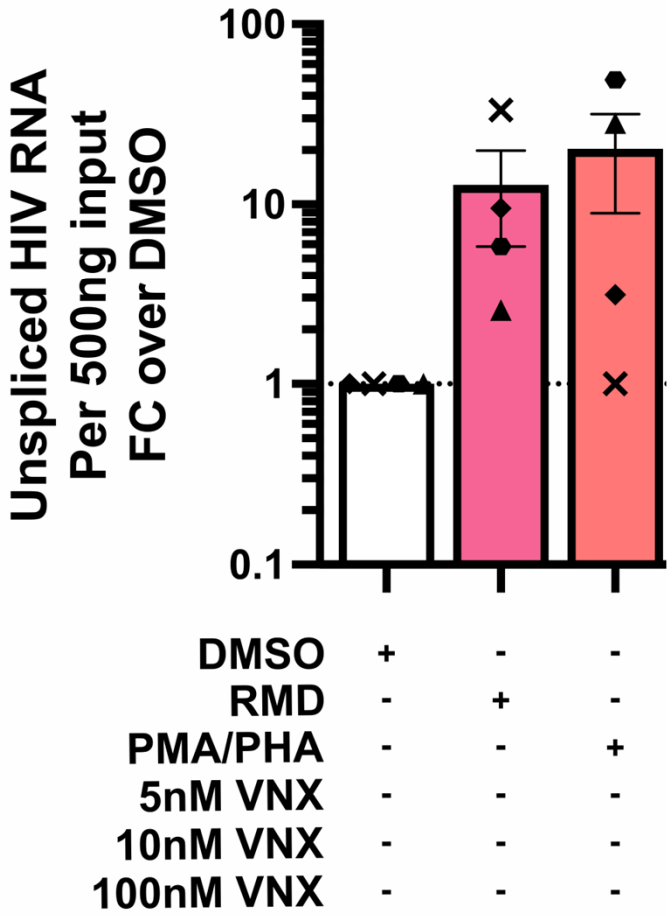
$$f_{axy,P} = f_{ax} + f_{ay} - (f_{ax}\, f_{ay})$$

f_{ax} = fraction affected, drug x
f_{ay} = fraction affected, drug y
$f_{axy,P}$ = predicted fraction affected, drugs $x + y$
$f_{axy,O}$ = observed fraction affected, drugs $x + y$

$$\Delta f_{axy} = f_{axy,O} - f_{axy,P} \begin{cases} > 0, \text{ synergy} \\ = 0, \text{ Bliss independence} \\ < 0, \text{ antagonism} \end{cases}$$

Laird et al., J Clin Inv 2015

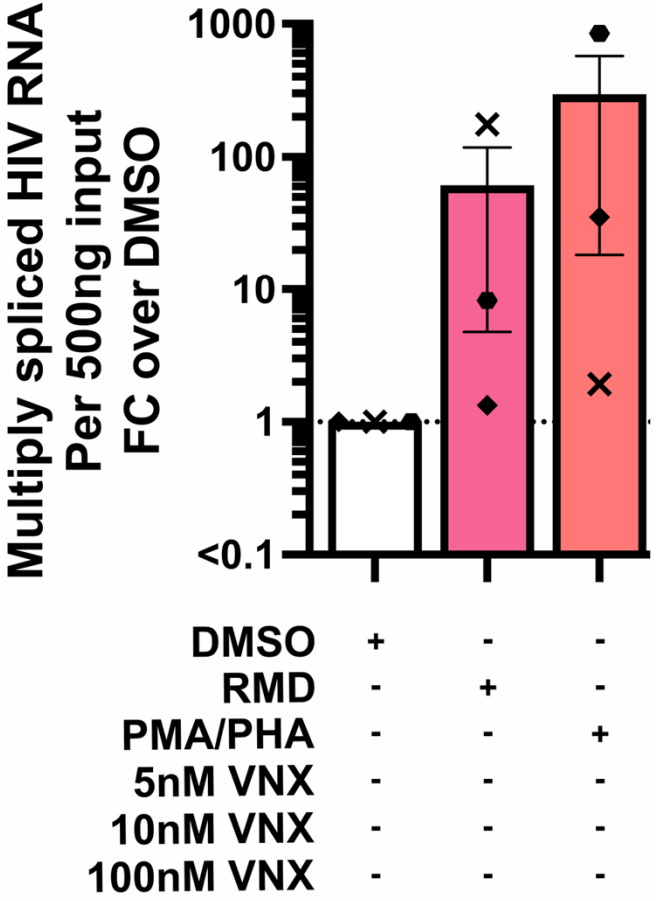
Romidepsin and PMA/PHA treatment results in increases in cell associated Unspliced RNA



Courtesy of Ajantha Rhodes

n=3-4

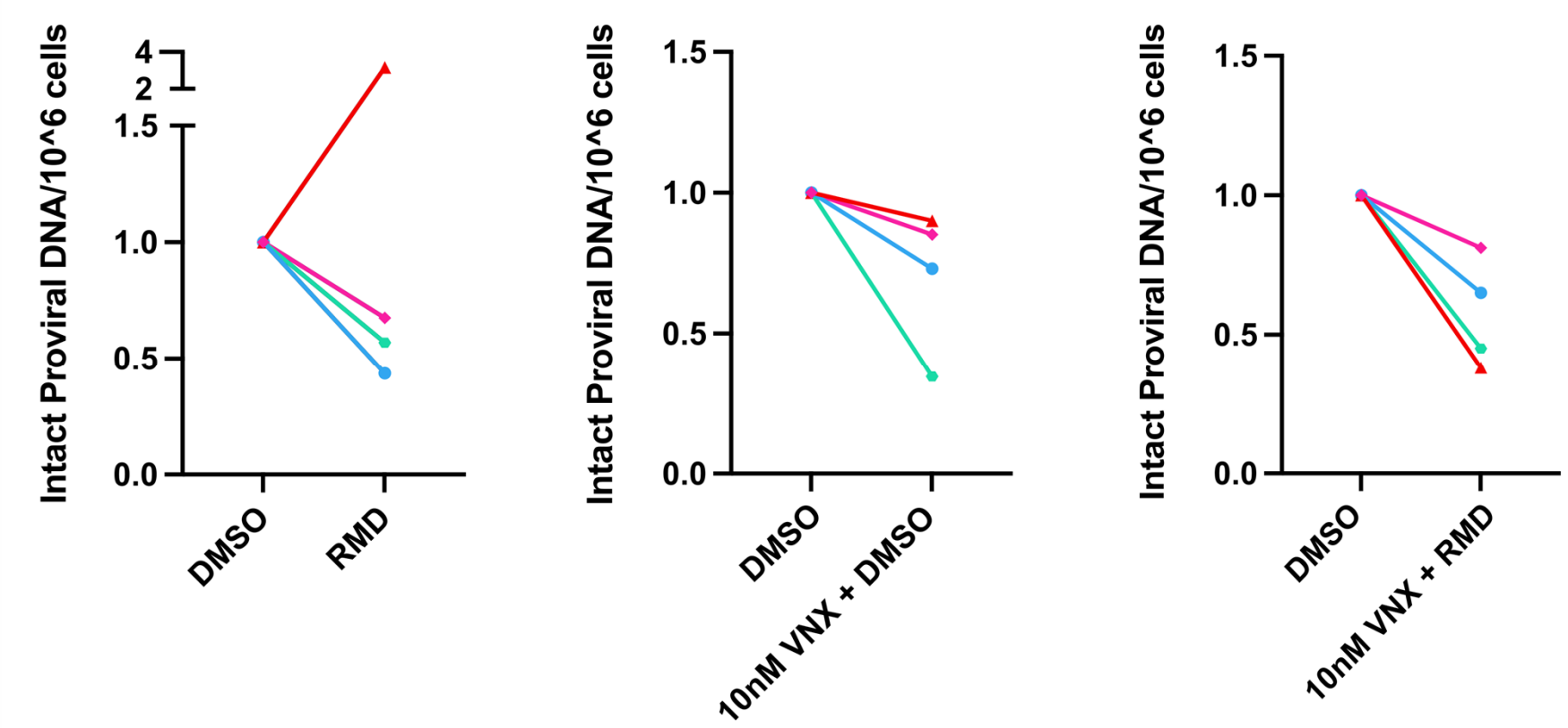
Romidepsin and PMA/PHA treatment results in increases in cell associated Multiply Spliced RNA



Courtesy of Ajantha Rhodes

n=3

Treatment of CD4+ T cells with 10nM VNX + RMD decreased levels of intact HIV DNA



- Similar findings with 100nM VNX + RMD

n=4, symbols present an individual donor

Summary

- Venetoclax alone leads to a **reduction in integrated HIV DNA** *ex vivo* at a concentration of 100nM. Potentially explanations include
  - Latently infected cells are primed to die or
  - Over expression of BCL-2 in latently infected cells drives survival
- Naïve T cells and effector memory T cells compared to other T-cell subsets were more susceptible to VNX treatment
- When **combined with romidepsin**, venetoclax significantly reduced integrated HIV DNA, and this decline was synergistic. Similar patterns of decline in intact virus using IPDA on 4 donors
- Modest increase in US RNA with venetoclax (far less that that observed with RMD or PMA/PHA which also increased MS RNA.

Implications

- Synergism between romidepsin and venetoclax potentially explained by
  - Impact on cell death of pro-apoptotic viral proteins increased following **latency reversal**
  - Direct effects of **romidepsin on multiple pro-apoptotic pathways** including increasing reactive oxygen species (ROS), caspase 3 and BAK proteins or reduction in inhibitors of apoptosis proteins (XIAP)

- Further evaluation of **venetoclax alone or in combination with romidepsin** as a strategy for elimination of latently infected cells should be explored in pre-clinical animal models or potentially clinical trials

[1] Valdez et al., Blood Cancer J 2015; Circu, M.L. and T.Y. Aw, Free Radic Bio Mol 2010; Ramakrishnan Hematologica 2019; Makena Mol Cancer Ther 2017

What was the **key question** asked?

- Can the combination of a latency reversing agent and pro-apoptotic drug lead to the death of latently infected cells?

What was the **key finding** and **take home message?**

- Combination of romidepsin and venetoclax was able to reduce the levels of integrated HIV DNA and intact provirus.
- Achieved a ”Shock and Kill” strategy

What are the **next steps?**

- Determine which death pathway led to the decline in latently infected cells
- Potentially trial the combination of romidepsin and venetoclax in animal models

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DARE
to find a cure

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