Pro-survival Protein Bcl-2 Inhibitor in Combination with a Latency Reversal Agent to Eliminate Latent HIV-Infected Cells

Sharmin Begum[1], Tram NQ Pham[1,2] and Eric A Cohen[1,2]

[1]Human Retrovirology Research Unit, Montréal Clinical Research Institute (IRCM), QC, Canada, [2]Department of Microbiology, Infectiology and Immunology, Université de Montréal, Montréal, QC, Canada

McGill UNIVERSITY

IRCM Montreal Clinical Research Institute

CanCURE Together, we Can CURE HIV

Introduction

- Antiretroviral therapy (ART) suppresses HIV replication, but is not able to eradicate infected cells harboring integrated latent HIV proviruses known as “viral reservoir (VR)”.
- One of the mechanisms to sustain viral latency is by preventing cell death from host immune effectors like CD8+ cytotoxic T lymphocytes (CTLs).
- To enhance immune recognition, latently infected cells are activated using latency reversing agents (LRA) to trigger cytolysis or immune mediated clearance.
- However, recent evidence indicates that VR have an intrinsic resistance to cell death responses modulated by the pro-survival protein Bcl-2.
- Studies in our lab revealed that SMAC mimetics (SM) can be used as a LRA and reactivate HIV in ART-treated mice and induce a small reduction of VR *in vivo.*

Hypothesis

Bcl-2 inhibition alone and/or in combination with SM treatment can achieve optimal reactivation and reductions of HIV harboring reservoirs, a condition that is not optimally achieved with SM or other LRAs alone.

Methods

In Vitro

HIV reactivation and reduction of latently HIV infected cells in CD4+ T cell models of HIV latency and primary CD4+ T cells.

In Vivo

PK/PD of combination treatment in mice. HIV reactivation and VR reduction in ART treated mice.

Figure 1:CD4⁺ T cell models of HIV latency have higher Bcl-2 expression

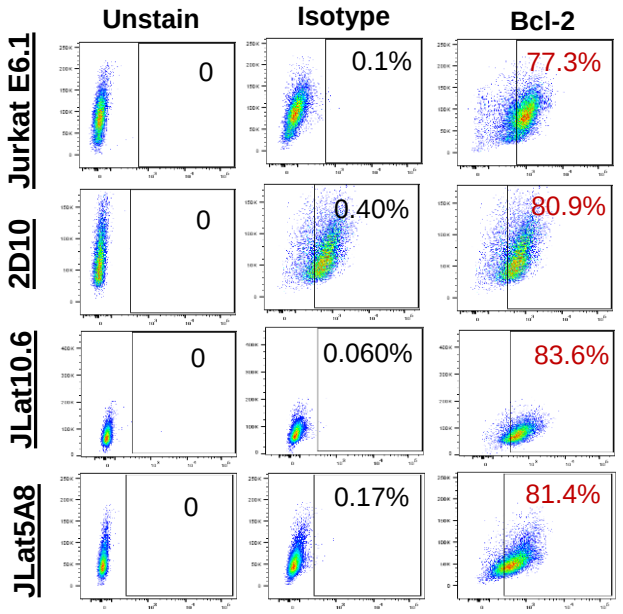


Figure 2: Cell viability of CD4⁺ T cell models of HIV latency treated with Bcl-2 inhibitor

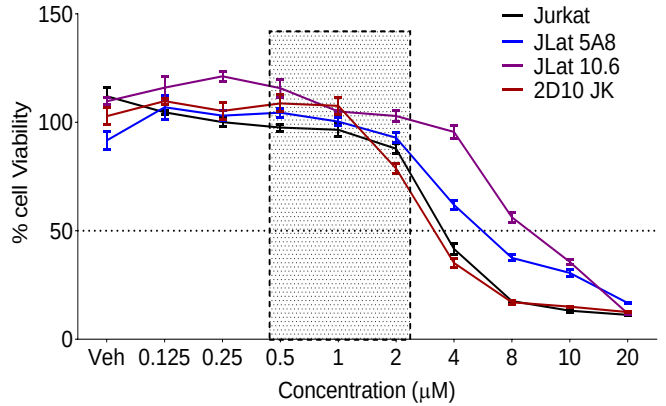
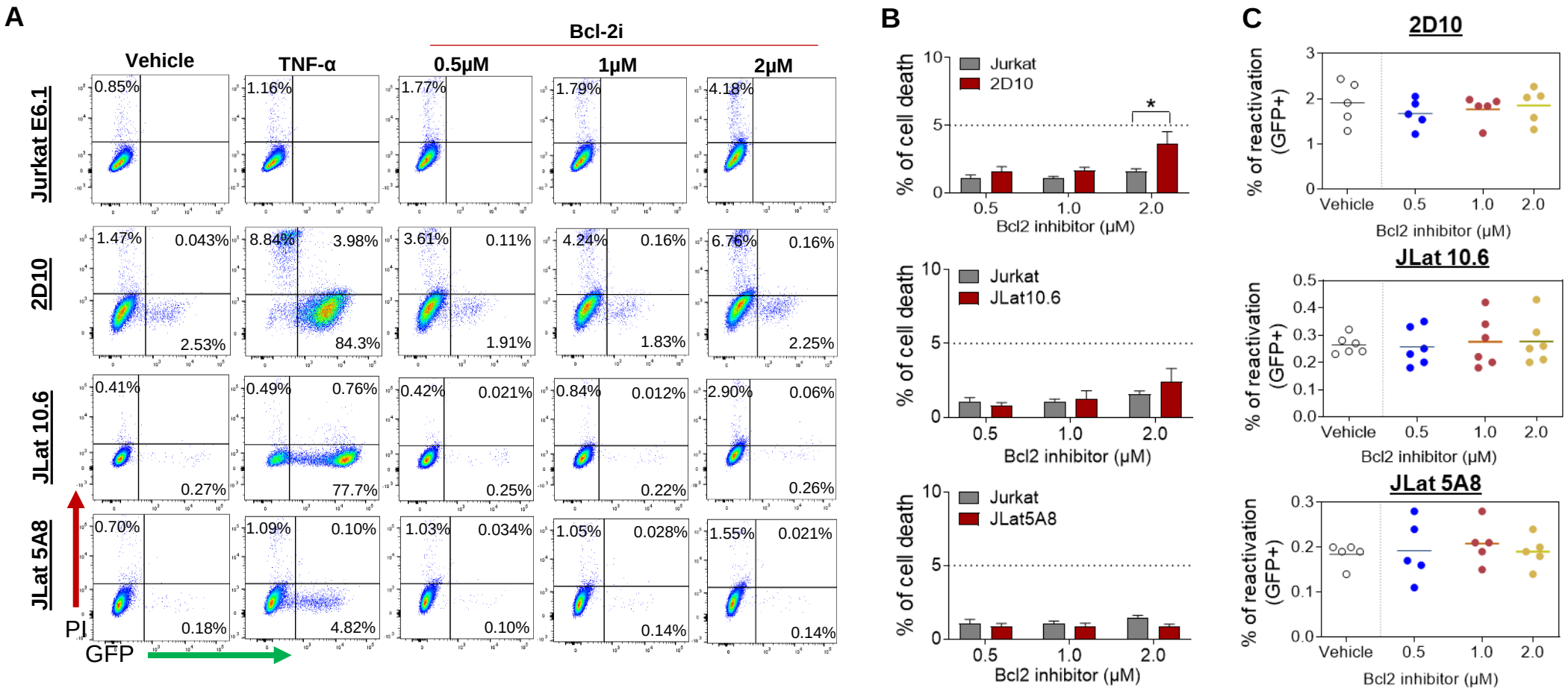
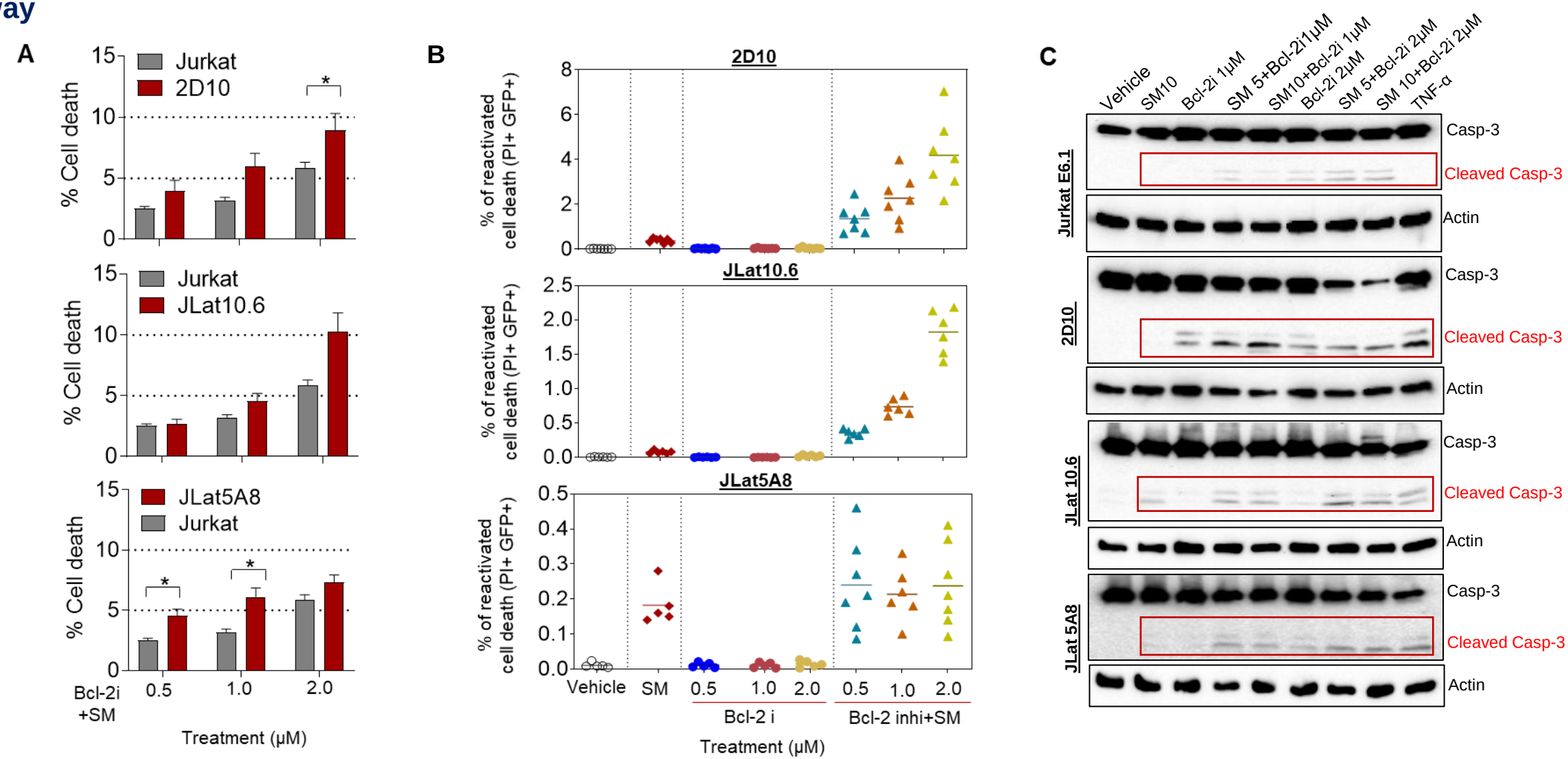


Figure 3: Bcl-2 inhibition reduces frequency of latent HIV-infected cells but does not induce latent HIV reactivation



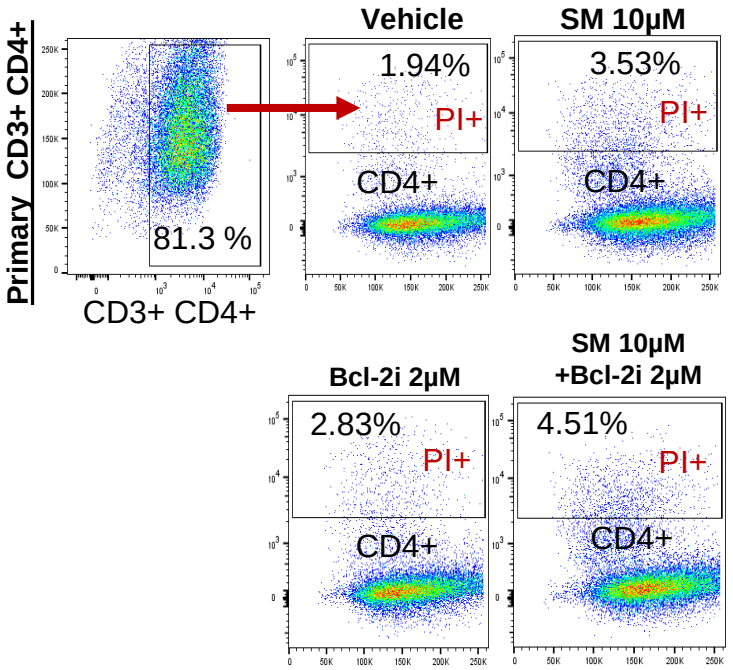
(A) CD4⁺ T cell models of HIV latency or parental Jurkat E6.1 cells were treated with Bcl-2 inhibitor (Bcl-2i) at different concentrations for 24h and assessed as appropriate for cell death and HIV reactivation by flow cytometry. TNF-α (10 ng) treatment was used as a positive control for HIV reactivation.**(B)** Percentage of cell death compared to Jurkat E6.1 cells. **(C)** Percentage of latent HIV-infected cells reactivation following Bcl-2 inhibitor treatment.

Figure 4: Combination of Bcl-2 inhibition and SM treatment reduces latently infected cells via activation of apoptosis pathway



HIV latency model cells were treated with SM (10μM) for 24h followed by different Bcl-2 inhibitor (Bcl-2 inhi 0.5, 1 and 2μM) concentrations and compared with parental Jurkat E6.1 cells for latent HIV-infected cells reactivation and cell death by flow cytometry. **(A)** Percentage of cell death (PI+) of HIV latent model cells compared to Jurkat E6.1 cell following combination treatment. **(B)** Percentage of reactivated cell death (PI+ GFP+) following combination treatment. **(C)** Increased cleaved Caspase-3 observed on Western blot analysis of latent cells compared to parental Jurkat E6.1. SM used as 5 and 10μM concentrations. TNF-α (10ng) treatment used as a positive control for HIV reactivation.

Figure 5: Bcl-2 inhibition alone or in combination with SM induces minimal toxicity to primary CD4+ T cells



Conclusion

- ❑ Bcl-2 expression is higher in CD4⁺ T cell models of HIV latency.
- ❑ Bcl-2 inhibition showed selective toxicity to CD4⁺ T cell models of HIV latency compared to parental Jurkat E6.1.
- ❑ Bcl-2 inhibitor alone is not sufficient to drive reactivation of CD4⁺ T cell models of HIV latency.
- ❑ Combined treatment of SM and a BCL-2 inhibitor additively reduce the frequency of latently infected cells via the activation of apoptotic cell death.
- ❑ Bcl-2 inhibitor alone and/or with SM treatment showed <10 % toxicity in uninfected primary CD4+ T cells cells.

Future directions

- Interrogate the effect of Bcl-2 inhibition in the presence or absence of SM in latently HIV infected primary CD4+ T cells.
- PK/PD analysis of Bcl-2 inhibitor alone and/or with SM in humanized BLT mice.

Significance

This study will expand our knowledge on the survival strategies exploited by latent VR to persist against host immune effectors.