Assessment of Monovalent and Bivalent SMAC mimetics to Both Shock and Kill the HIV Reservoir

Kiho Tanaka[1], Youry Kim[1], Rachel Pascoe[1], Carolin Tumpach[1], Jesslyn Ong[1], Michael Roche[1], Sharon R Lewin[1, 2, 3]

[1]Department of Infectious Diseases, The University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria 3000, Australia.

[2] Victorian infectious Diseases Service, Royal Melbourne Hospital at The Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria 3000, Australia.

[3]Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne, Victoria, Australia.

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

Introduction

"**Shock and Kill**' is one of the approaches being investigated to cure HIV. The main goal is to reverse latency by using latency reversal agents (LRAs) and induce viral protein expression so that the infected cells can be eradicated by immune or viral mediated killing[1]. However, to date LRAs have been shown to reverse latency but do not necessarily die[1]. Therefore, a better LRA that can induce cell death in latently infected cells is desired[1].

A new class of LRAs, second mitochondria-derived activator of caspase mimetics (**SMACm**) inhibit inhibitors of apoptosis proteins (IAPs)[1] and have been shown by others to reverse HIV latency reversal via the non-canonical nuclear factor kappa B (ncNFkB) pathway[1]. Bivalent SMACm with two protein binding motifs have demonstrated higher potency for latency reversal but in clinical trials for cancer, have been shown to induce greater toxicity[1].

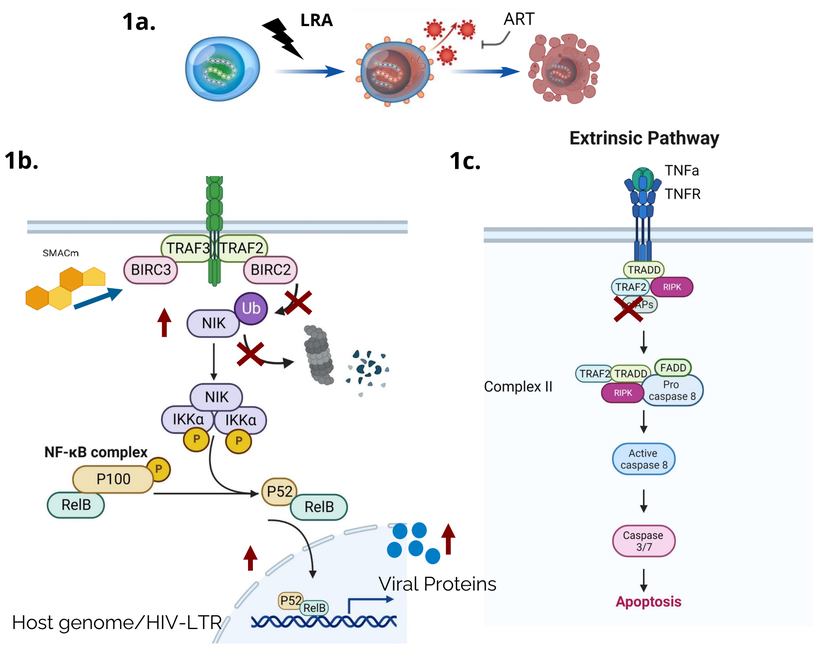


Fig 1a. Shock and Kill. HIV latency is reversed using LRA in the presence of antiretroviral therapy (ART) and the infected cell is eradicated.
Fig 1b. SMACm utilises nc-NFkB pathway to induce latency reversal. SMACm induces IAP (BIRC2/3) degradation which leads to the accumulation of NIK and cleaving of p100 into p52. The p52/RelB complex translocates into the nucleus and binds to the NFkB binding site of HIV-LTR to induce viral transcription and translation – latency reversal.
Fig 1c. SMACm induced depletion of IAP enhances the TNF dependent apoptosis. TNF ligation with TNFR leads to the formation of Complex II in the absence of IAPs and as a result, induces caspase activation and apoptosis.

Hypothesis

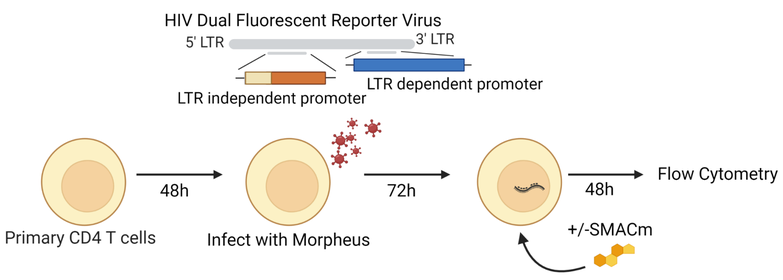
Given that **IAPs** are **upregulated** in **latently infected cells**, we hypothesised that SMACm will induce **death** of latently infected cells and that this would be **enhanced** in the presence of Tumour Necrosis Factor (TNF). Furthermore, the effects of SMACm on **death** of infected cells will be **enhanced** in the presence of **cytotoxic T-cells** which produce TNF.

Methods

1. SMAC mimetics

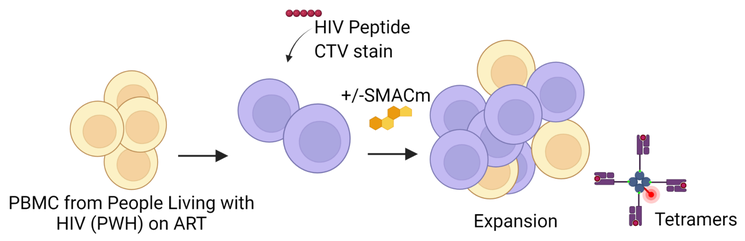
Monovalent	Bivalent
GDC0152	AZD5582
GDC0197	BV6
LCL161	Birinapant
Xevinapant	

2. Dual Reporter Virus –Morpheus[2]



The Morpheus reporter virus consists of a **productive marker** that is regulated by the LTR-dependent promoter and a **latent marker** downstream of the PGK promoter which is regulated by a host protein that is constitutively expressed in the cell.

3. Proliferation of HIV specific Cytotoxic CD8+ T-cells



1. SMACm Reactivate Latent HIV in a Latently Infected Cell Line

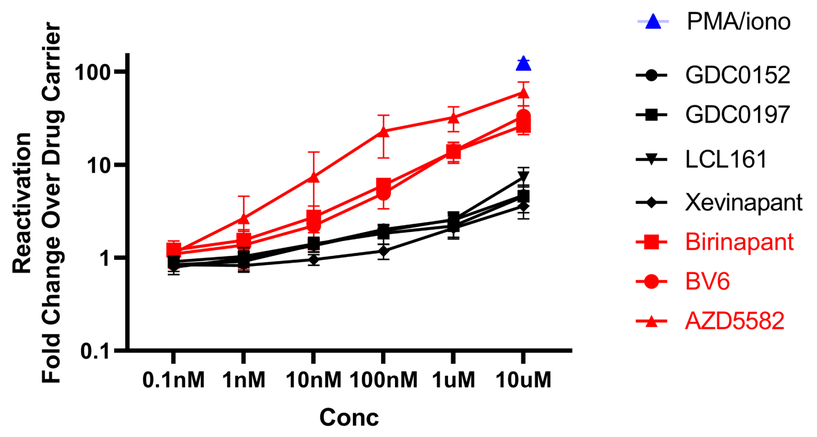


Fig 2. Both monovalent and bivalent SMACm induce HIV reactivation in J-Lat 10.6. J-Lat10.6 cells were treated with varying concentrations of SMACm for 48h and expression of green fluorescent protein (GFP) and cell viability were measured using flow cytometry. Bivalent SMACm – Red; monovalent – Black. n=3±SEM.

2. SMACm Induce cIAP Degradation

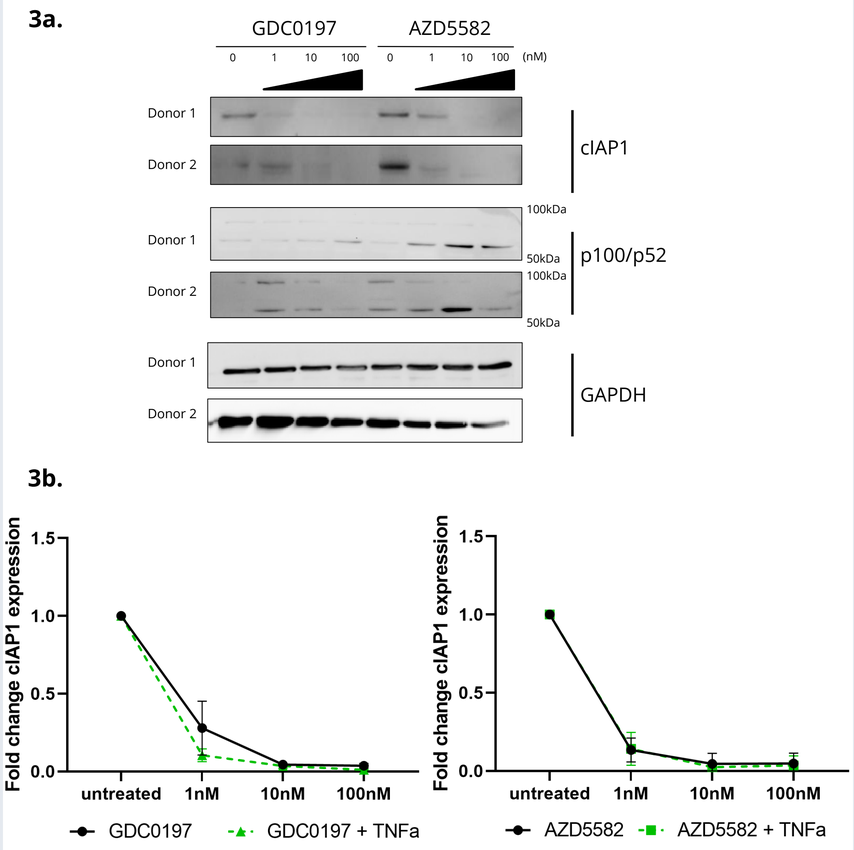


Figure 3. SMACm induce degradation of cIAP1 in HIV negative primary CD4+ T-cells as well as in cells from PWH on ART, and the addition of TNF may enhance this effect. 3a) HIV negative primary CD4+ T-cells were treated with increasing concentrations of monovalent (GDC0197) and bivalent (AZD5582) SMACm for 48h and the level of cIAP1 protein and p100/p52 protein were analysed by western blotting. 3b) Cells from PWH on ART were analysed for cIAP1 degradation in the presence and absence of 20ng/ml TNF, and the lysates were analysed by western blotting.

3. TNF Does Not Alter SMACm Toxicity

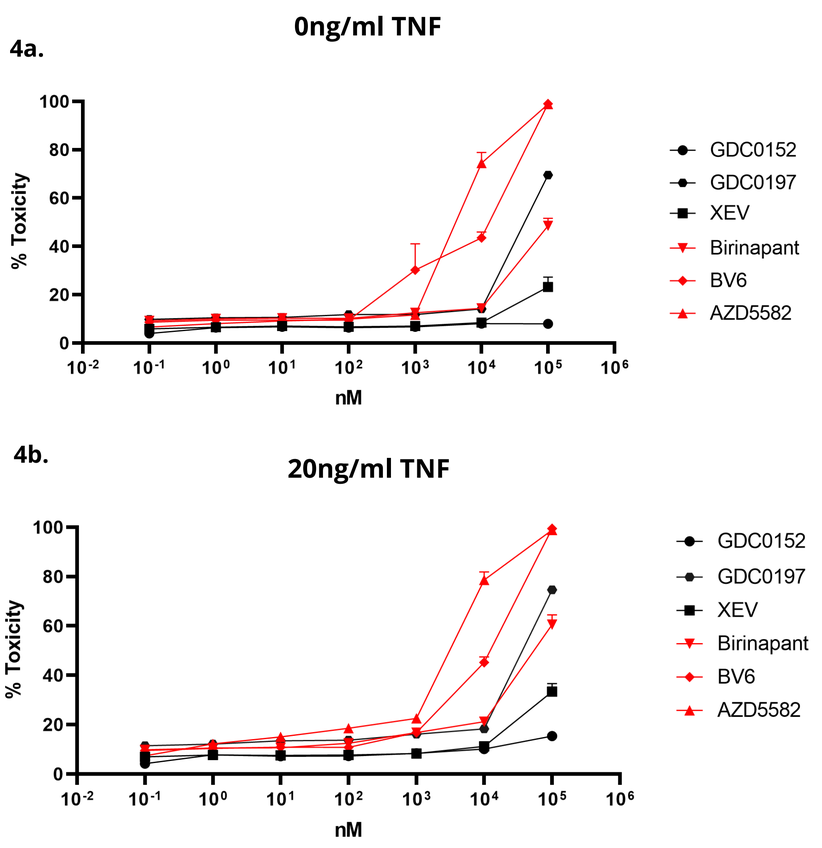


Figure 4. Bivalent compared to monovalent SMACm have greater toxicity and this isn't changed by TNF. 4a) HIV negative primary CD4+ T-cells were treated with varying concentrations of SMACm for 48h without and 4b) with 20ng/ml TNF and their cell viability was measured using flow cytometry . Bivalent SMACm – Red; monovalent – Black. n=4-8±SEM

4. SMACm Reactivate Latent HIV in Primary Cell Model

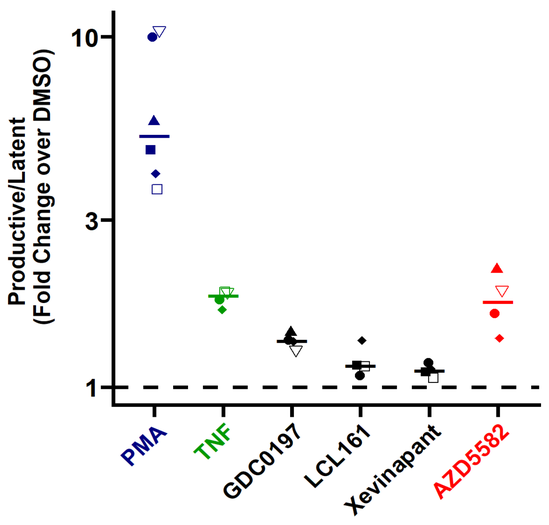


Fig 5. SMACm induce latency reversal in a primary cell model. HIV negative primary CD4+ T-cells were infected with the Morpheus reporter virus. At 72h post infection, 100nM of each SMACm were added and cultured for another 48h.

The expression levels of the productive marker (mCherry) and latent marker (NGFR) were analysed using flow cytometry. The cells were cultured with 10U/ml IL-2 throughout. Symbols represent independent donors. n=4-6, median shown.

5. Monovalent SMACm Increases Expansion and Reduce PD-1 Expression in HIV Specific CD8+ T-cells

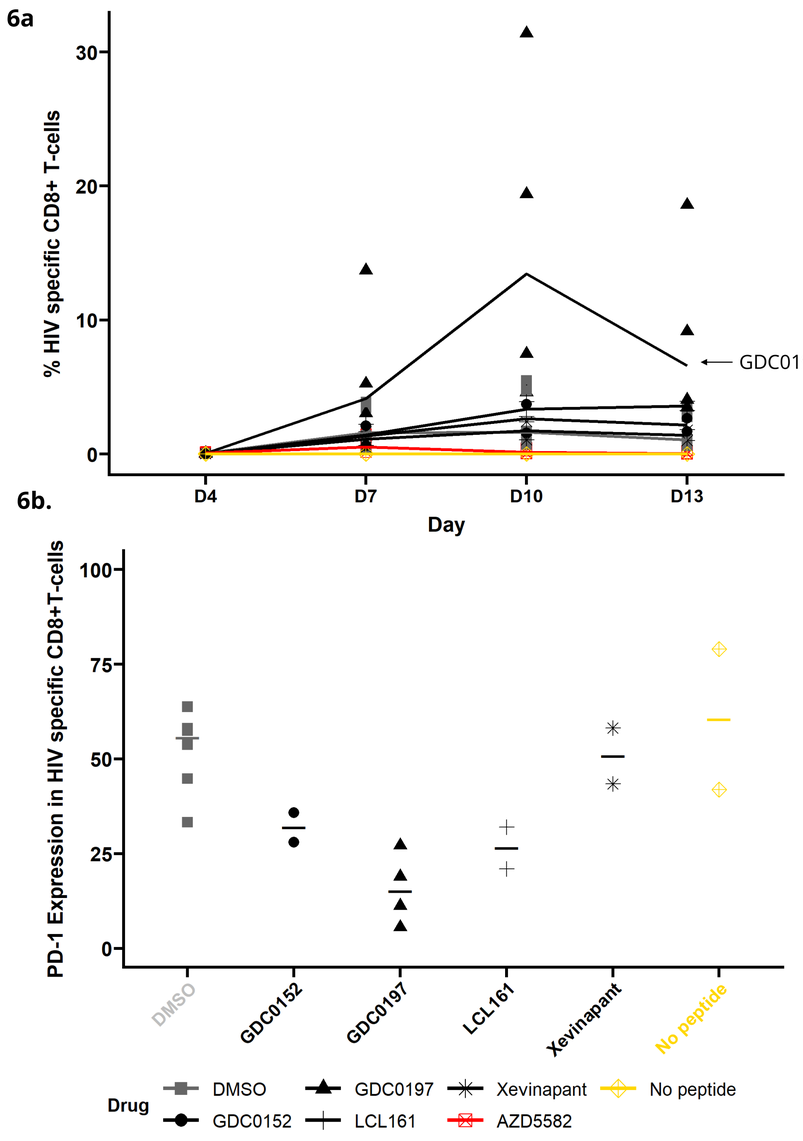


Fig 6. A novel SMACm, GDC0197, increases the recall of HIV specific CD8+T-cells and reduces the expression of the exhaustion marker, PD-1. 6a) PBMC from PWH on ART were stained with CellTrace Violet and treated with monovalent and bivalent SMACm. The cells were cultured for 13 days. The HIV specific CD8+T-cells were identified using tetramers and the cells were analysed using flow cytometry. 6b) PD-1 Expression on HIV specific CD8+ T-cells on Day 10. n=2-4, independent donors, median shown.

Conclusion

We have shown:

- SMACm induce **latency reversal** in two models of HIV latency
  - Bivalent SMACm have a higher potency
- SMACm can have a direct effect on HIV-specific T-cells
  - **Expansion of total numbers and reduction in PD1 expression**

Next Steps:

- Determine whether SMACm selectively induce apoptosis in infected cells using the primary cell model (to distinguish latently and productively infected cells) and cells from PWH on ART with and without TNF
- Determine if SMACm treated HIV specific CD8+ T-cells can enhance their ability to kill HIV-infected target cells

Acknowledgement

This work was funded by **NIH DARE** and mHIVE (Melbourne HIV Exchange Gilead Research award). We'd like to thank Prof. Viviana Simon from Icahn School of Medicine at Mount Sinai, Prof. Marc Pellegrini from WEHI and the Flow core team at the University of Melbourne.

Reference. 1) Tanaka, K, Kim, Y, Roche, M, Lewin, SR. The role of latency reversal in HIV cure strategies. J Med Primatol. 2022; 51: 278- 283. doi: 10.1111/jmp.12613
2) Kim EH, Manganaro L, Schotsaert M, Brown BD, Mulder LCF, Simon V. Development of an HIV reporter virus that identifies latently infected CD4+ T cells. Cell Rep Methods. 2022 Jun 13;2(6):100238. doi: 10.1016/j.crmeth.2022.100238. PMID: 35784650; PMCID: PMC9243624.