Role of RIPK1 in SMAC mimetics-induced apoptosis in primary human HIV-infected macrophages

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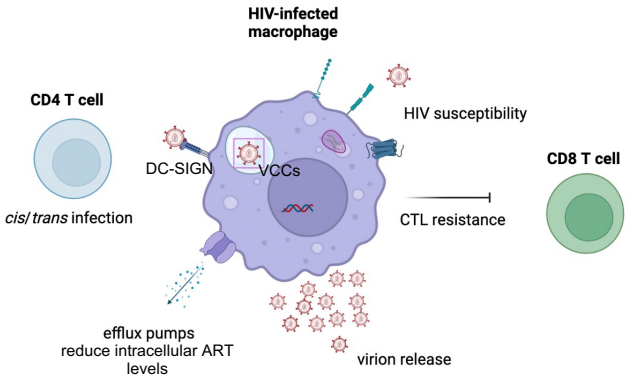
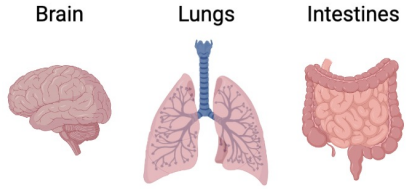
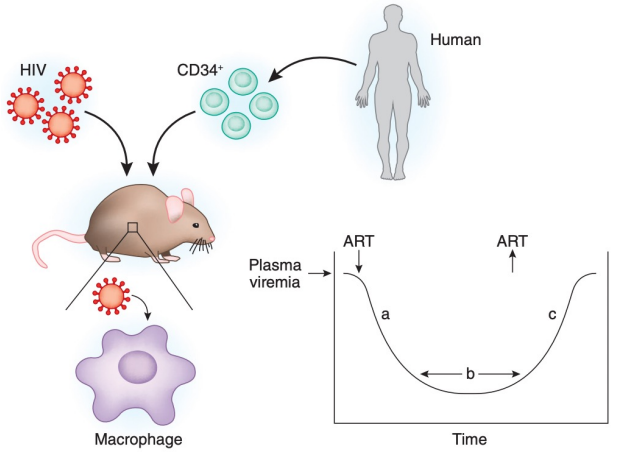
Macrophages play an important role in the pathogenesis of HIV-1



tissue-resident macrophages harbor HIV-DNA and have the potential to serve as viral reservoirs



Macrophages support viral rebound upon ART-interruption



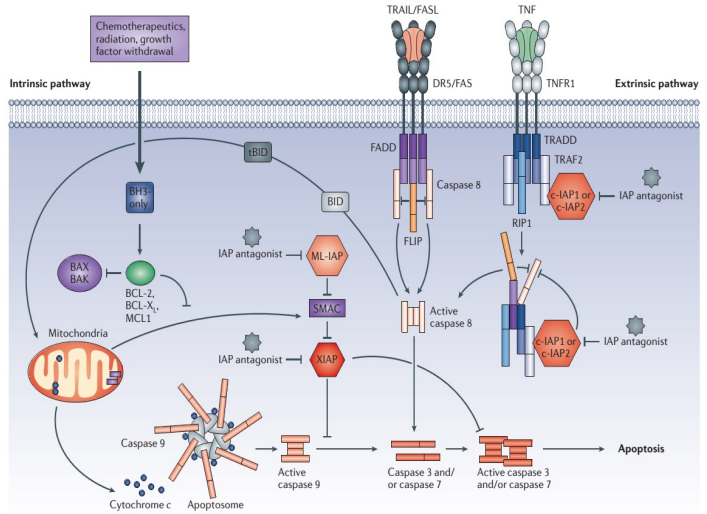
Mario Stevenson 2017, *Nature Medicine*

Humanized BLT mice reconstituted with only human macrophages harbor replication-competent HIV and a source of viremia upon treatment interruption

Macrophages are resistant to HIV-induced cell death. Eradication of HIV-infected macrophages may require manipulation of programmed cell death pathways

Second mitochondrial activator of caspases (SMAC)-mimetics (SM) are antagonists of Inhibitor of apoptosis proteins (IAPs) and promote apoptosis

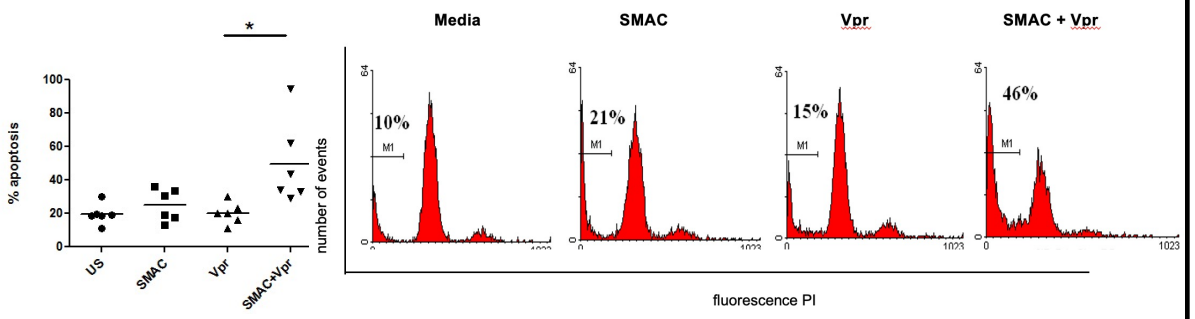
SM are well-tolerated and are currently in clinical trials as anti-cancer drugs



Cellular IAP1/2, survivin, and other members of the IAP family are involved in survival of HIV-infected CD4+ T cells and macrophages from HIV-infected individuals. (Campbell *et* al 2018 *Cell Host* Microbe, Kuo *et al* 2018 Immunity, Campbell *et al* 2020 *Cell Death Dis)*

Fulda, S., & Vucic, D. 2012, *Nature reviews Drug discovery*.

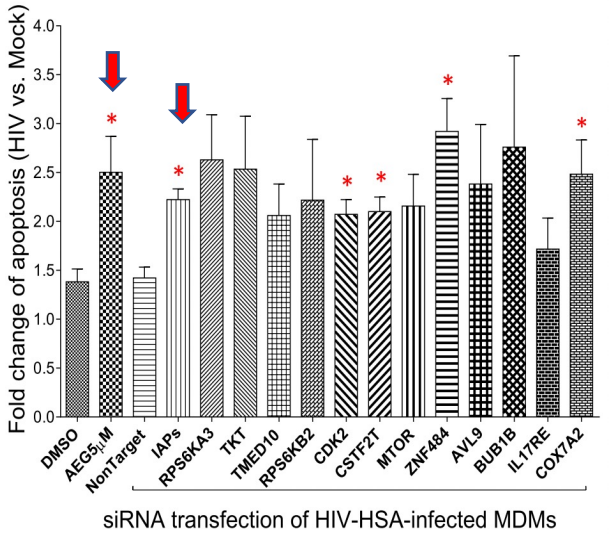
Rationale and Research Question



Previously in the Kumar lab, we showed that monocytes are susceptible to HIV-Vpr induced cell death, but acquires resistance during differentiation to macrophages due to upregulation of cellular inhibitor of apoptosis proteins (IAPS)

SMAC mimetics target IAPs for proteosomal degradation and sensitizes MDMs to HIV-Vpr induced cell death

Busca *et al.* 2012, *Journal Biological Chemistry*



HIV infection dysregulates the expression of many host genes essential for the survival of infected cells.

We used pooled-shRNA-based genome wide screening to identify novel gene targets whose inhibition selectively induced apoptosis in HIV-infected macrophages.

Dong *et al* 2021, *BMC Infectious Diseases*

Overarching goal: Identification of apoptosis-related genes and signalling proteins involved in resistance of HIV-infected macrophages to apoptosis is crucial to the development of therapeutic intervention

Research Question: Can modulation of the IAP-signalling pathway be exploited to eradicate HIV-infected macrophages?

Hypothesis:
SMAC-mimetics induce cell death of HIV-infected human monocyte-derived macrophages through apoptosis

Aim 1: Assess the effect of SM on the viability of HIV-infected myeloid cells

Aim 2: Elucidate the underlying mechanism of selective SMAC-induced killing of HIV-infected macrophages

Research Methods

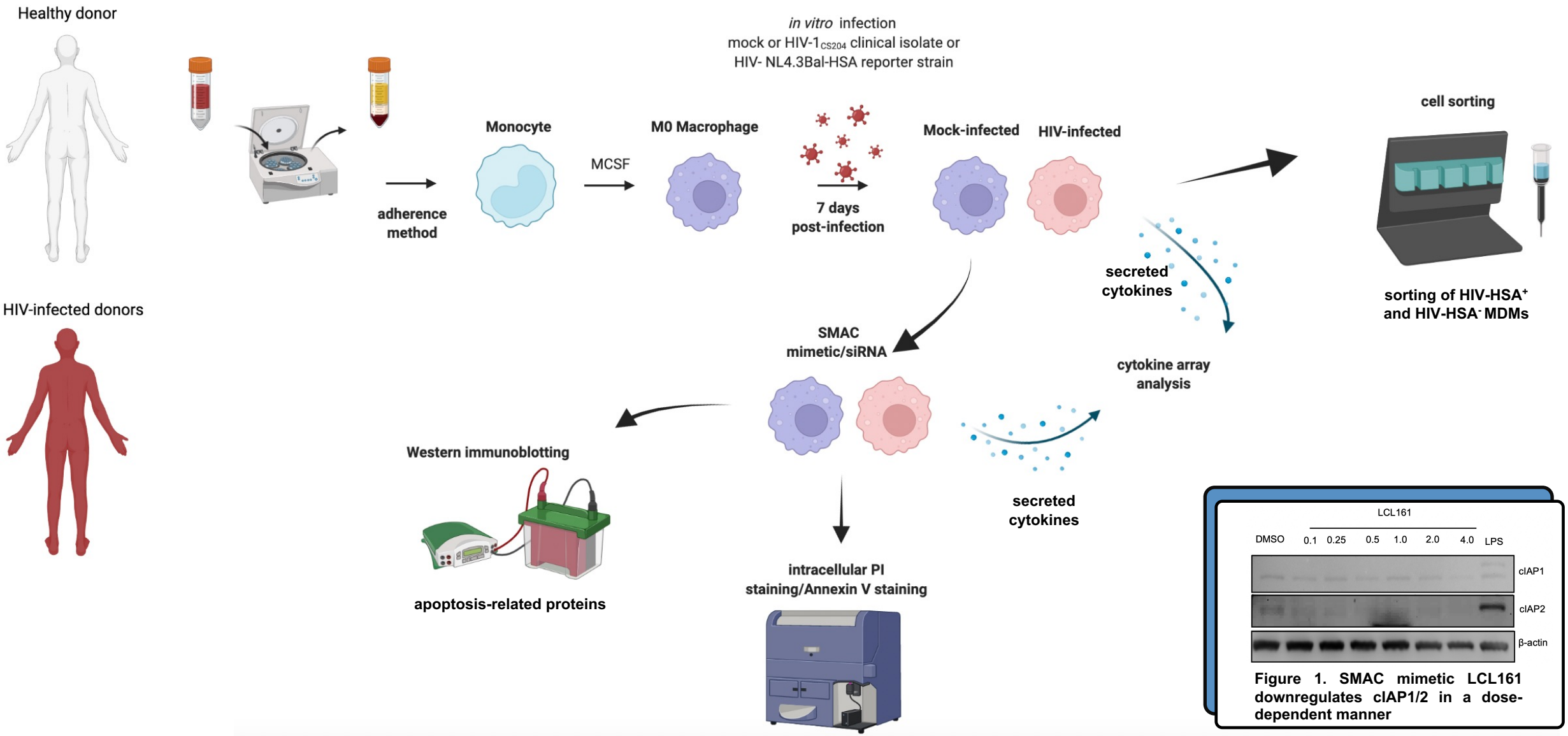


Figure 1. SMAC mimetic LCL161 downregulates cIAP1/2 in a dose-dependent manner

Results: SMAC mimetics induce apoptosis of HIV-infected macrophages

LCL161 induces cell death in undifferentiated and differentiated U1 cells, but not in the uninfected U937 cells

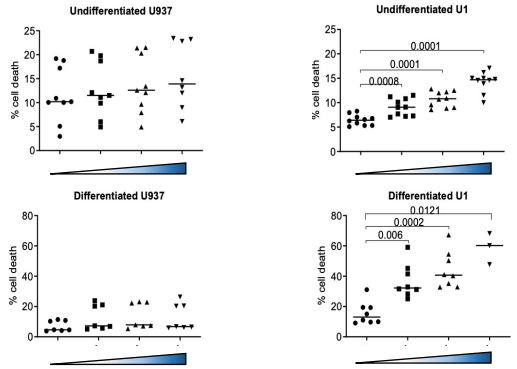


Figure 2. SM induces cell death of chronically HIV-infected myeloid cells. Treatment: DMSO or increasing conc. of LCL161

Primary macrophages infected with HIV-1 undergo cell death upon LCL161 treatment

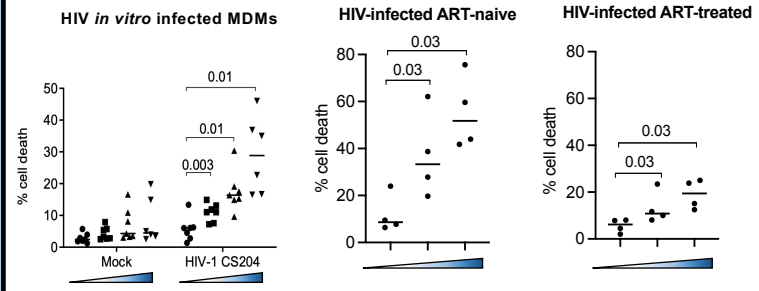


Figure 3. SM induces cell death of *in vitro* HIV-infected MDMs and MDMs generated from ART-naïve and ART-treated donors. DMSO or LCL161 treatment

SM-induced cell death is mediated by caspase activation

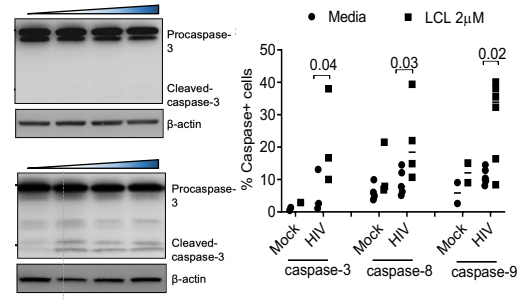
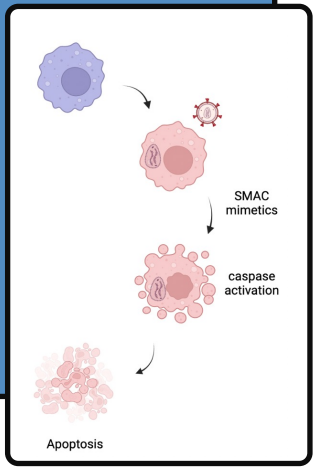


Figure 4. SM treatment leads to activation of apoptosis in chronically infected U1 cells and HIV-infected MDMs. DMSO or LCL treatment

Induction of apoptosis by SMAC mimetics



HIV-HSA⁺ MDMs are specifically targeted by monovalent LCL161 and divalent AEG40730 and leads to a reduction in frequency of HIV-HSA⁺ MDMs in culture

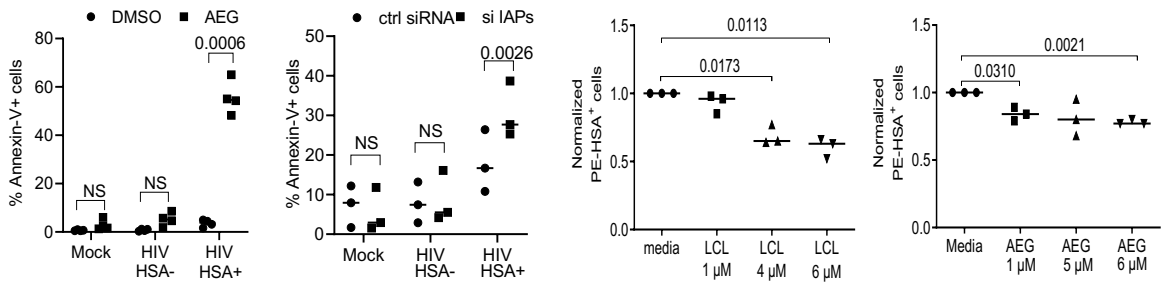
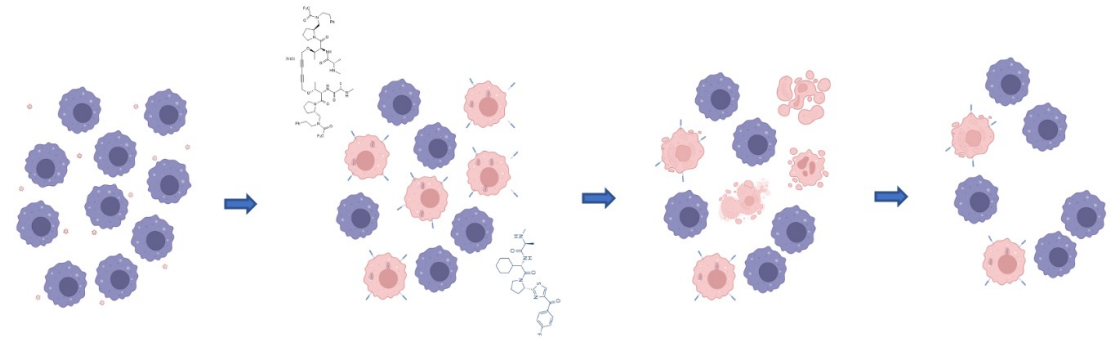


Figure 5. SM selectively induces cell death of HIV-HSA⁺ MDMs. Treatment: DMSO or AEG and control sIRNA and cIAP1/2 siRNA.

SMAC mimetics as potential therapeutic strategy to eradicate HIV-infected macrophages



Results: SM induced cell death is not mediated by TNFα, but with concomitant downregulation of RIPK in HIV-infected MDMs

HIV-infection upregulates TNFα production, but SMAC mimetics and TNFα do not synergize to induced apoptosis in MDMs, unlike in cancer cells

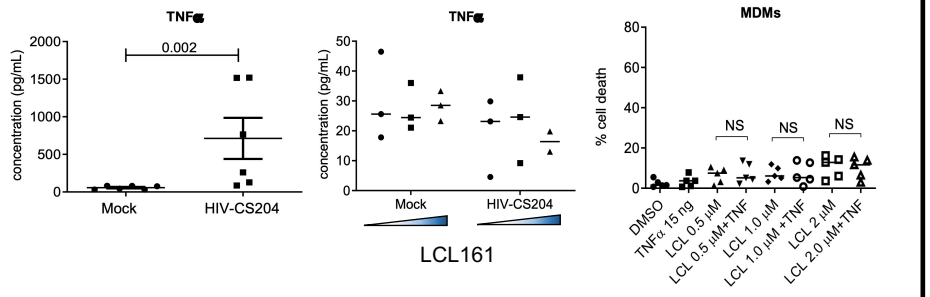


Figure 6. TNFα does not synergize with SM to induce cell death of MDMs. rTNFa and LCL161 treatment

HIV-infection downregulates RIPK1 in MDMs and treatment with SMAC mimetics results in greater downregulation of RIPK1 in

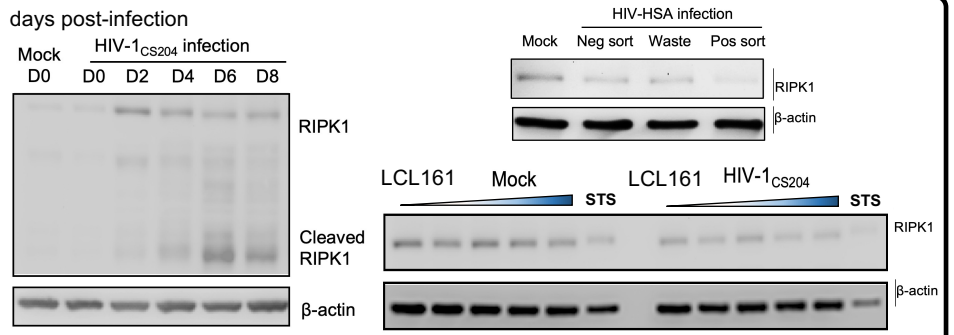


Figure 7. RIPK1 levels are reduced in HIV-infected macrophages

In MDMs derived from healthy donors, concomitant downregulation of cIAP1/2 by SMAC mimetics and RIPK1 inactivation by necrostatin-1 results in activation of cell death by apoptosis

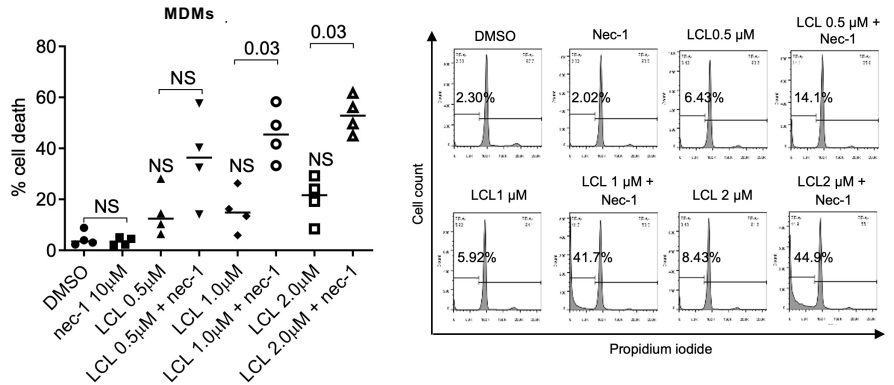


Figure 8. Simultaneous cIAP1/2 downregulation and RIPK1 inactivation induces cell death in healthy MDMs. Necrostatin-1 inhibits RIPK1

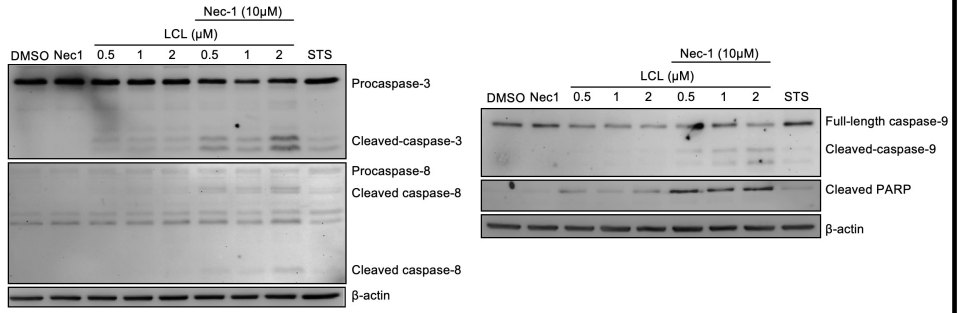
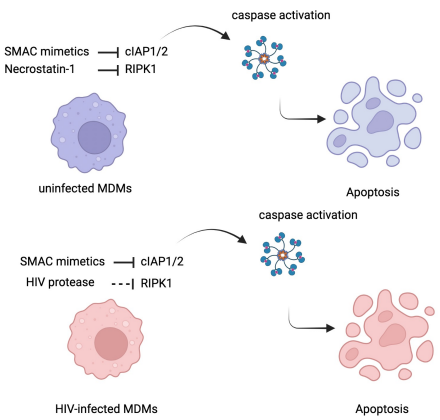


Figure 9. Concomitant downregulation of cIAP1/2 and RIPK1 inactivation in MDMs derived from healthy donors results in activation of apoptosis

Conclusions



1. SMAC mimetics (LCL161 and AEG43730 induce cell death in HIV infected macrophages but not in healthy control. RIPK1 downregulation may mediate the apoptotic effect of SMAC mimetics on infected cells.

2. Modulation of the IAP-signalling pathway may be a therapeutic target to clear HIV-infected macrophages

Acknowledgments

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