Pan-Caspase Inhibitors Induce HIV-1 Latency Reversal through Lymphotoxin-α Secretion by Cytokine-Primed NK Cells

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Conclusions

- Cytokine-primed NK cells reactivate latent HIV-1 via LTα secretion after pan-caspase inhibitor treatment.
- Reactivation occurs without reducing NK cell cytotoxicity.
- This supports NK cell-based shock-and-kill strategies in HIV-1 cure research.

Introduction

HIV-1 latency in CD4⁺ T cells hinders a cure. The HIV-1 shock-and-kill strategy aims to reactivate latent HIV with LRAs and eliminate infected cells. Shock-and-kill strategies face challenges due to weak latency reversal agents (LRAs). NK cells, with antigen-independent cytotoxicity, show promise. This study aims to identify an LRA secreted by NK cells after pan-caspase inhibitor treatment.

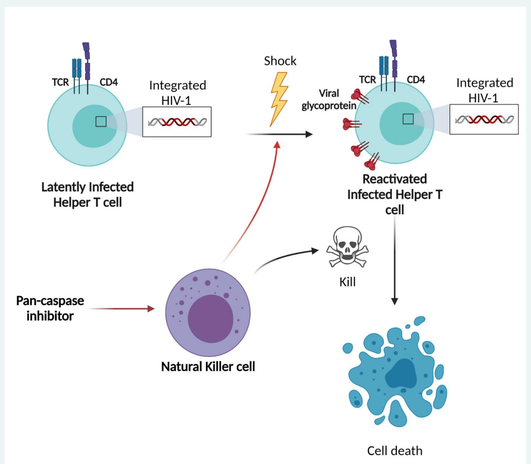

Figure 1. Schematic overview of our previous findings and proposed HIV-1 shock-and-kill strategy. Created with BioRender.com.

We aim to identify the **latency reversal agent (LRA)** secreted by **NK cells** after **pan-caspase inhibitor** treatment and assess its role in **reactivating latent HIV-1** without compromising NK cell cytotoxicity.

Cytokine priming is required for Z-VAD-FMK-induced LTα secretion and LRA activity in human NK cells

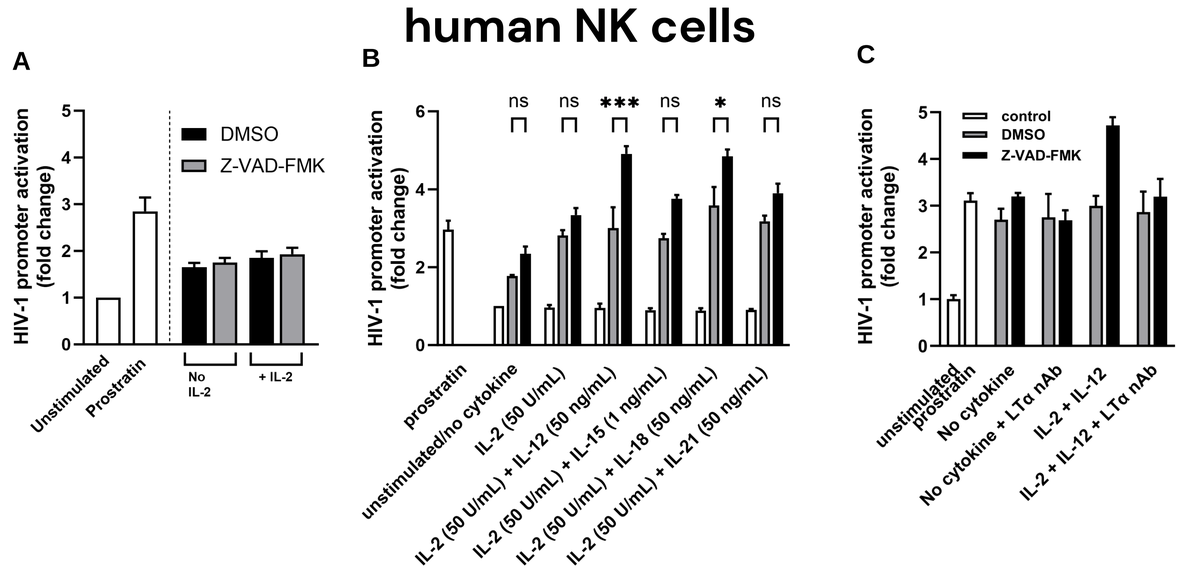

Figure 3. Human primary NK cells were enriched from PBMCs. After culturing the enriched NK cells with cytokines for 24h, they were incubated with Z-VAD-FMK (or only DMSO as control) for another 24h. Then supernatants were collected and added on TZM-bl reporter cells. TZM-bl cells have an integrated copy of the luciferase gene under the HIV-1 LTR promoter. Promoter activity is measured through a luciferase assay. For neutralization, the supernatants are first pre-incubated with neutralizing antibodies (nAb) before adding to the TZM-bl reporter cells. Data points are plotted as mean ±SD from three individual experiments each with technical triplicates.

(A) Z-VAD-FMK failed to induce LRA activity in resting NK cells or those treated with low-dose IL-2 alone.
(B) LRA activity is particularly increased when NK cells were primed with IL-2 in combination with IL-12 or IL-18.
(C) LTα neutralization blocked LRA activity in IL-2/IL-12-primed NK cells, showing that LTα secretion is necessary for Z-VAD-FMK-induced effect.

Pan-caspase inhibitor-treated NK cells display a different secretome profile

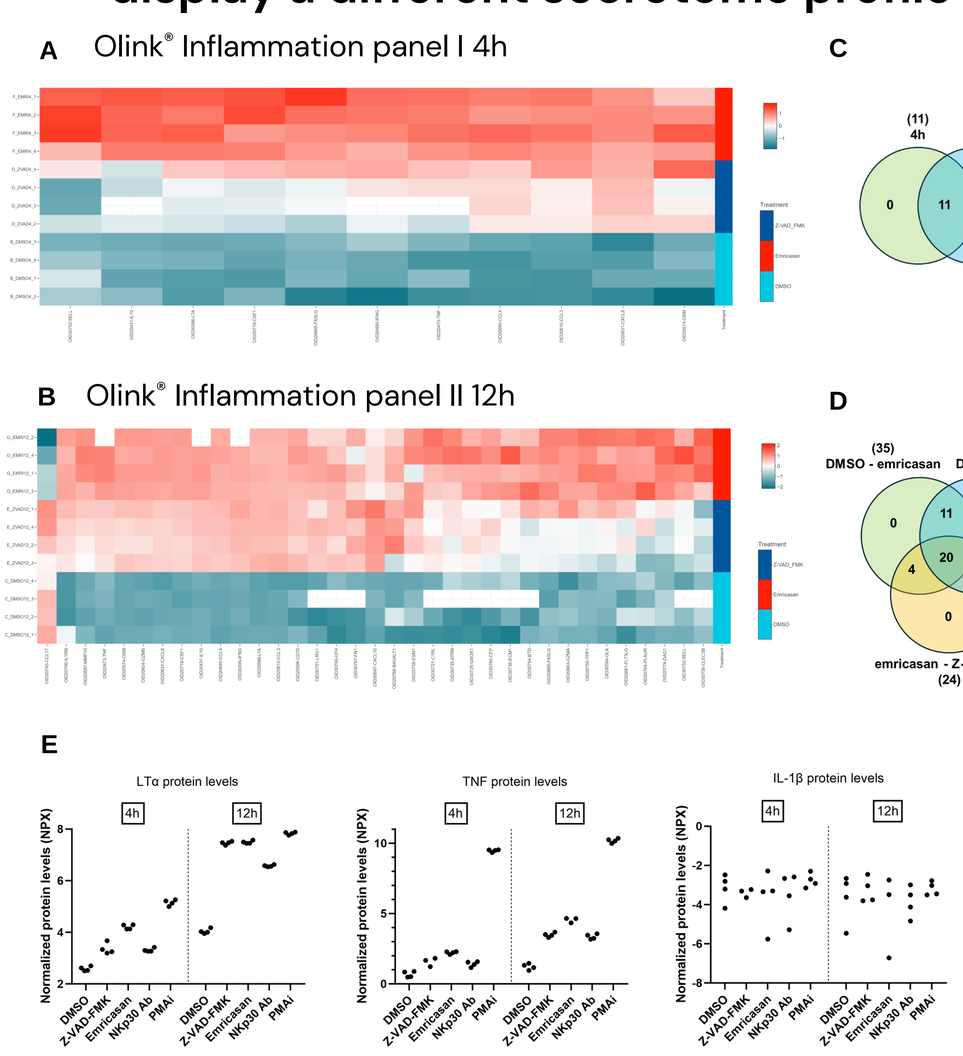

Figure 2. NK lymphoma cell line KHYG-1, was incubated with DMSO (0.5 %), Z-VAD-FMK (50 μM), emricasan (50 μM) for 4h or 12h (each condition n=4) after which supernatants were collected and relative protein levels were analyzed by PEA Olink panels. (A-B) Heat maps for panels for 4h (A) and 12h (B). Venn diagrams depicting the number of secreted proteins with significantly different protein levels between the 4h and 12h time points (C), and between the three groups at 12h (D). Normalized protein levels for TNF, lymphotoxin-α (LTα) and IL-1β (E) are depicted.

Z-VAD-FMK does not compromise the ability of NK Cells to kill target cells in a shock-and-kill strategy

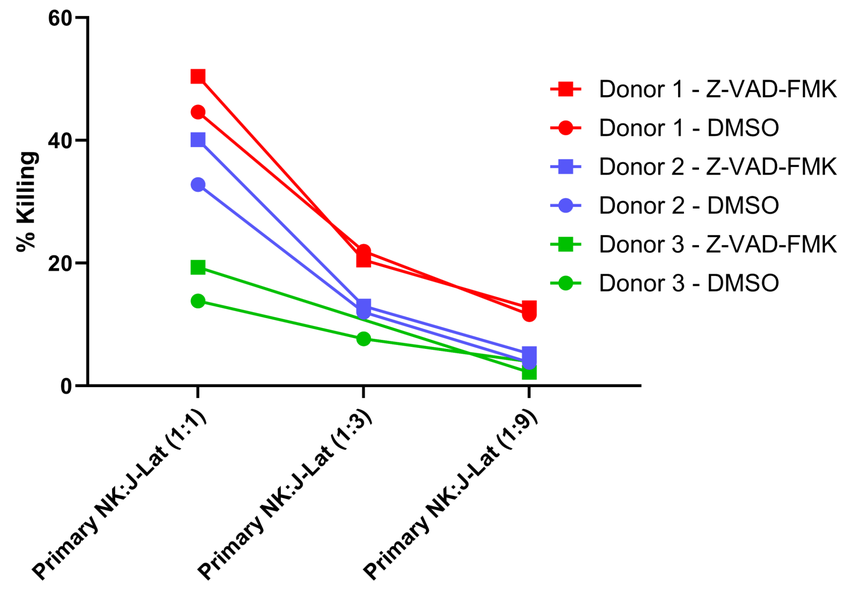

Figure 4. Human primary NK cells from 3 donors were primed with IL-2/IL-12 followed by Z-VAD-FMK or DMSO incubation for 1h and co-cultured with J-Lat target cells at various effector-to-target (E:T) ratios (1:1, 3:1, and 9:1) for 4h. NK cell cytotoxicity was assessed using flow cytometry to determine the percentage of target cell death. Results from all donors showed a minor increase in target cell killing by Z-VAD-FMK-treated NK cells compared to DMSO-treated controls. Data points are plotted as mean for each condition. The colors represent individual donors: red (donor 1), blue (donor 2), and green (donor 3). The shapes represent treatments: circles (Z-VAD-FMK) and squares (DMSO).

Future perspectives

- Map the downstream pathways by which primary NK cells become activated after pan-caspase inhibitor treatment.
- Analyze the extent of primary NK cell activation by pan-caspase inhibitors in the context of HIV-1 infections.
- Further assess the cytotoxic potential of pan-caspase inhibitor-treated NK cells and its exploitation in an HIV-1 shock-and-kill strategy

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