Mechanisms of HIV-Infected Cell Persistence Following Exposure to HIV Protease Activators

584

Ishak Irwan, Bally Randhawa, Alessia Corrado, Cameron M Soulette, Ricardo Ramirez, Mark Bartlett, Jeffrey Murry

Gilead Sciences, Inc., Foster City, CA, USA

Conclusions

- Cells that survived exposure to HIV protease activator treatment showed reduced GagPol expression, and HIV protease activators were less potent in cells with low GagPol levels. Nevertheless, at the concentrations tested, HIV protease activators still efficiently killed infected cells expressing sub-virion quantities of GagPol
- RNA sequence analysis of survivor cells revealed an altered pro-survival transcriptional profile characterized by increased proliferation, enhanced reactive oxygen species control and detoxification, and reduced inflammatory signaling
- Whole-genome CRISPR screens demonstrated that knockout of genes involved in pyroptosis (CARD8 and caspase-1), mitochondrial import, and reactive oxygen species generation enhanced the survival of HIV protease activator–treated cells
- Inhibition of mitochondrial reactive oxygen species generation with S3QEL2 weakened HIV protease activator potency and led to a modest increase in the number of HIV protease activator survivor cells
- Knockout of top CRISPR screen hits involved in reactive oxygen species generation, *AHCY* and *DNAJC11*, increased HIV protease activator survival
- HIV protease activator survivor cells were more proliferative and exhibited increased mitochondrial damage and oxidative stress

Plain Language Summary

- A new class of drugs, called HIV protease activators, are designed to kill HIV-infected cells and may help support strategies aimed at eventually curing HIV
- This study looked at why some infected cells survive even when treated with these drugs. We discovered that infected cells were more likely to survive if they:
  - Had lower amounts of a viral protein
  - Were dividing more rapidly
  - Had lower activity in their mitochondria, the part of the cell that produces energy and also creates harmful molecules called reactive oxygen species
- We also used a genetic screening approach to:
  - Reveal the role of mitochondria-generated reactive oxygen species in triggering cell death
  - Confirm a known cell death pathway involving a protein called CARD8
- Together, these findings help researchers better understand how HIV protease activators work and may guide the development of more effective HIV cure strategies

References: **1.** Chun TW, et al. *Nat Immunol.* 2015;16:584-9. **2.** Wang K, et al. *Science.* 2021;371:1240-5. **3.** Clark KM, et al. *Nat Chem Biol.* 2023;19:431-9. **4.** Converso A, et al, inventors. US patent 20220242847A1. August 4, 2022.

Acknowledgments: This study was funded by Gilead Sciences, Inc. Editing and production assistance were provided by Parexel and were funded by Gilead Sciences, Inc. Editorial support was provided by Jane Abolafia, PhD, of Lumanity Communications Inc., and was funded by Gilead Sciences, Inc.

Correspondence: Ishak Irwan, ishak.irwan@gilead.com

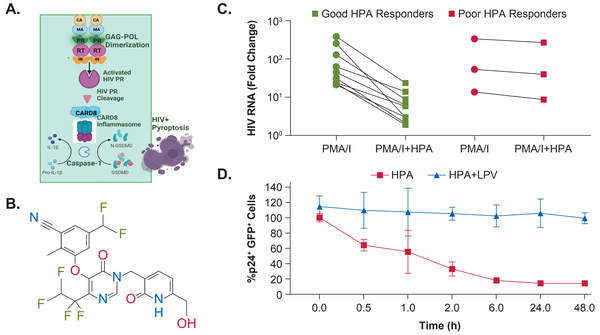
Disclosures: All authors are employees of and may own stocks or stock options in Gilead Sciences, Inc.

These data, in part, have been previously presented at the EU2Cure HIV Symposium (September 14-17, 2025; Ghent, Belgium).

Introduction

- Persistent HIV reservoirs that evade antiretroviral therapy remain the primary obstacle to achieving an HIV cure[1]
- HIV protease activators (HPAs) are a class of non-nucleoside reverse transcriptase inhibitor that can rapidly and selectively eliminate HIV-infected cells in the latent reservoir by activating HIV protease and triggering pyroptotic cell death via a caspase activation and recruitment domain 8 (CARD8)–mediated pathway[2,3] (**Figure 1**)
- However, a subset of infected cells survives HPA treatment both in vitro and ex vivo
- Elucidating the mechanism of resistance is critical for optimizing the use of HPAs in HIV cure strategies

Figure 1. HPA-Triggered Pyroptotic Cell Death and Survivor Cells



(A) HPA dimerizes the viral GagPol polyprotein, causing premature activation of the HIV protease. The activated protease induces cleavage of host proteins, including CARD8, triggering the pyroptotic signaling cascade and activating CASP1, GSDMD, and IL-1β, which kills the infected cell. **(B)** Structure of HPA tool compound used.[4] **(C)** Heterogeneity in HPA killing is seen in ex vivo reactivated CD4 T-cell reservoirs from people with HIV, with some showing minimal reduction in supernatant viral RNA. Poor responders were defined as having a <0.5 log reduction in viral RNA after treatment. **(D)** Infected healthy primary CD4 T cells are rapidly killed within 6 hours, but a subset of cells survives and persists beyond 72 hours. In contrast, inhibiting HIV protease activity with LPV prevents cell killing by HPA.
CA, capsid; CARD8, caspase activation and recruitment domain 8; CASP1, caspase-1; GFP, green fluorescent protein; GSDMD, gasdermin D; HPA, HIV protease activator; IL-1β, interleukin 1β; IN, integrase; LPV, lopinavir; MA, matrix; N-GSDMD, N-terminal of gasdermin D; PMA/I, phorbol 12-myristate 13-acetate/ionomycin; PR, protease; RT, reverse transcriptase.

Methods

- Doxycycline-inducible GagPol cell lines were used to assess the changes in HPA potency and cell killing at various intracellular amounts of GagPol
- Bulk RNA sequencing was performed on infected THP-1 and primary CD4 T cells that survived HPA-mediated killing to identify transcriptional changes associated with survival
- Whole-genome CRISPR negative selection screens were used to identify host factors contributing to HPA resistance

Results

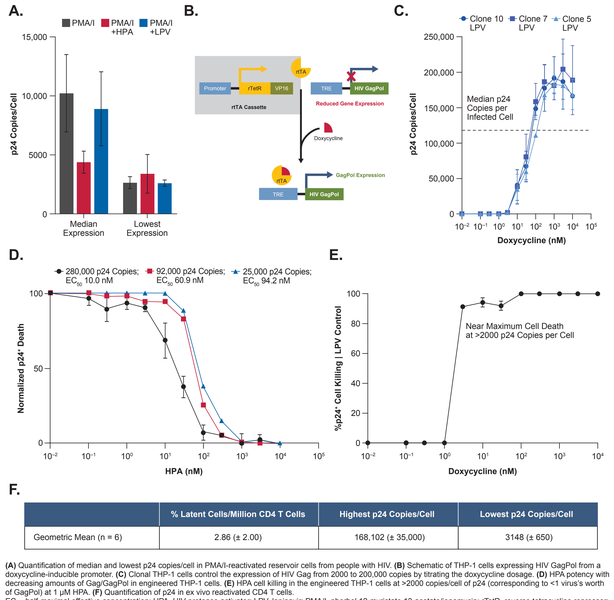
Survivor Cells Express Sufficient GagPol to Be Eliminated by HPA (Figure 2)

- Engineered THP-1 cells demonstrated reduced HPA potency with decreasing amounts of Gag/GagPol
- Quantification of the HIV p24 capsid protein in ex vivo reactivated CD4 T cells revealed sufficient p24 expression for HPA-mediated killing at 1 μM

HPA Survivor Cells Showed a Distinct Transcriptional Profile Marked by Increased Cellular Proliferation and Control of ROS Production That Conferred Resistance to Subsequent HPA-Mediated Cell Death (Figure 3)

- CD4 T cells that survived initial HPA exposure were more resistant to subsequent rounds of treatment
- Transcriptomic profiling of survivor cells revealed upregulation of pathways related to cell proliferation, survival, and reactive oxygen species (ROS) detoxification
- Downregulated pathways were associated with mitochondrial function, hypoxia, and inflammatory signaling

Figure 2. Expression of GagPol in Survivor Cells Versus HPA-Induced Cell Death

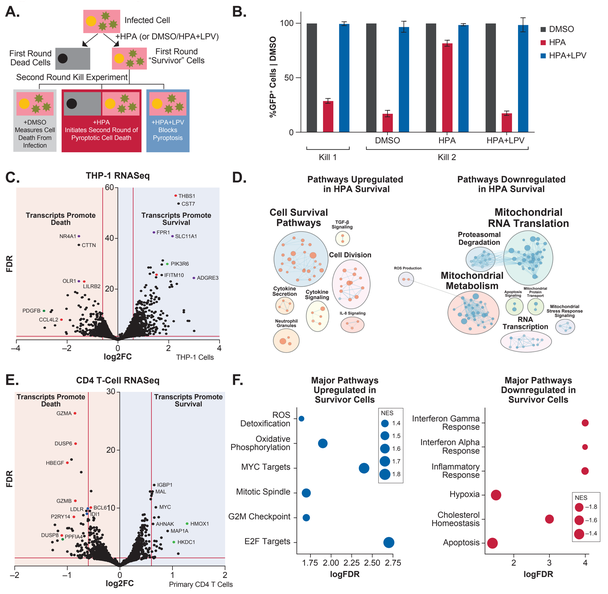


F.

	% Latent Cells/Million CD4 T Cells	Highest p24 Copies/Cell	Lowest p24 Copies/Cell
Geometric Mean (n = 6)	2.86 (± 2.00)	168,102 (± 35,000)	3148 (± 650)

(A) Quantification of median and lowest p24 copies/cell in PMA/I-reactivated reservoir cells from people with HIV. **(B)** Schematic of THP-1 cells expressing HIV GagPol from a doxycycline-inducible promoter. **(C)** Clonal THP-1 cells control the expression of HIV Gag from 2000 to 200,000 copies by titrating the doxycycline dosage. **(D)** HPA potency with decreasing amounts of Gag/GagPol in engineered THP-1 cells. **(E)** HPA cell killing in the engineered THP-1 cells at >2000 copies/cell of p24 (corresponding to <1 virus's worth of GagPol) at 1 μM HPA. **(F)** Quantification of p24 in ex vivo reactivated CD4 T cells.
EC_{50}, half-maximal effective concentration; HPA, HIV protease activator; LPV, lopinavir; PMA/I, phorbol 12-myristate 13-acetate/ionomycin; rTetR, reverse tetracycline repressor; rtTA, reverse tetracycline transactivator; TRE, tetracycline response element; VP16, herpes simplex virus protein vmw65.

Figure 3. Transcriptional Profile of HPA Survivor Cells

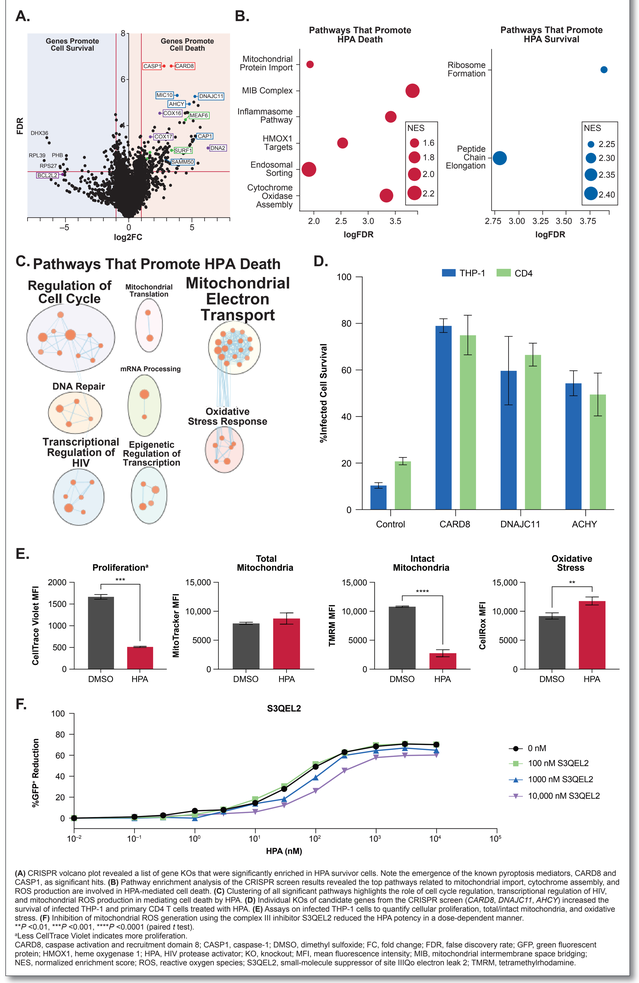


(A) Experimental design of 2 rounds of HPA killing. **(B)** Isolated survivors from infected CD4 T cells treated with HPA were allowed to recover and subjected to a second round of HPA killing. **(C)** RNASeq volcano plot of DEGs in HPA survivor cells in THP-1 cells. **(D)** Pathway analysis of DEGs from THP-1 HPA survivor cells. **(E)** Volcano plot of DEGs in HPA survivor cells in primary CD4 T cells. **(F)** Major pathway analysis of DEGs from primary CD4 T cells that survived HPA treatment.
DEG, differentially expressed gene; DMSO, dimethyl sulfoxide; FC, fold change; FDR, false discovery rate; GFP, green fluorescent protein; HPA, HIV protease activator; IL-8, interleukin 8; LPV, lopinavir; NES, normalized enrichment score; ROS, reactive oxygen species; TGF-β, transforming growth factor β.

CRISPR Screen Confirmed the Role of CARD8-CASP1 and the Importance of Mitochondrial ROS Generation to HPA Survival (Figure 4)

- A whole-genome CRISPR screening identified a list of putative gene knockouts that were significantly enriched in HPA survivor cells, including the known pyroptosis mediators CARD8 and caspase-1 (CASP1)
- Pathway enrichment revealed that higher cell proliferation and lower mitochondrial function and ROS production were key contributors in HPA-mediated cell survival
- Knockouts of the top hits involved in mitochondrial function and ROS generation (*DNAJC11* and *AHCY*) showed increased HPA survival
- HPA survivor cells were confirmed to be more proliferative and exhibited increased mitochondrial damage and oxidative stress
- Inhibition of mitochondrial ROS generation using the complex 3 inhibitor, small-molecule suppressor of site IIIQo electron leak 2 (S3QEL2), reduced HPA potency in a dose-dependent manner and modestly increased (~10%) the number of infected cells that survived HPA treatment

Figure 4. Whole-Genome CRISPR Knockout Screen Confirmed the Importance of Mitochondrial ROS Generation to HPA-Mediated Cell Death



(A) CRISPR volcano plot revealed a list of gene KOs that were significantly enriched in HPA survivor cells. Note the emergence of the known pyroptosis mediators, CARD8 and CASP1, as significant hits. **(B)** Pathway enrichment analysis of the CRISPR screen results revealed the top pathways related to mitochondrial import, cytochrome assembly, and ROS production are involved in HPA-mediated cell death. **(C)** Clustering of all significant pathways highlights the role of cell cycle regulation, transcriptional regulation of HIV, and mitochondrial ROS production in mediating cell death by HPA. **(D)** Individual KOs of candidate genes from the CRISPR screen (*CARD8*, *DNAJC11*, *AHCY*) increased the survival of infected THP-1 and primary CD4 T cells treated with HPA. **(E)** Assays on infected THP-1 cells to quantify cellular proliferation, total/intact mitochondria, and oxidative stress. **(F)** Inhibition of mitochondrial ROS generation using the complex III inhibitor S3QEL2 reduced the HPA potency in a dose-dependent manner.
***P* <0.01, ****P* <0.001, *****P* <0.0001 (paired *t* test).
*Less CellTrace Violet indicates more proliferation.
CARD8, caspase activation and recruitment domain 8; CASP1, caspase-1; DMSO, dimethyl sulfoxide; FC, fold change; FDR, false discovery rate; GFP, green fluorescent protein; HMOX1, heme oxygenase 1; HPA, HIV protease activator; KO, knockout; MFI, mean fluorescence intensity; MIB, mitochondrial intermembrane space bridging; NES, normalized enrichment score; ROS, reactive oxygen species; S3QEL2, small-molecule suppressor of site IIIQo electron leak 2; TMRM, tetramethylrhodamine.