HIV-1 Reactivation Increases Mitochondrial Priming of the Latent Reservoir

Jeremy A. Ryan[1], Allison L. Schure[2], Zelda Euler[3], Anthony Letai[1], Athe M.N. Tsibris[4,5]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA; [2]Massachusetts General Hospital, Boston, MA, USA; [3]Ragon Institute of MGH, MIT, and Harvard, Boston, MA, USA; [4]Division of Infectious Diseases, Brigham and Women's Hospital, Boston, MA, USA; [5]Harvard Medical School, Boston, MA, USA

Abstract

Background: Histone deacetylase inhibitors (HDACi) induce cancer cell death through mitochondrial apoptosis, a pathway regulated by the Bcl-2 family of proteins. The extent to which HDACi alter apoptosis priming in non-neoplastic cells is unknown.

Methods: We used BH3 profiling, a flow cytometry-based mitochondrial phenotyping assay, to investigate the mitochondrial priming effects of activation stimuli on peripheral blood mononuclear cell (PBMC) subpopulations. PBMC isolated from HIV-uninfected and HIV-infected, treated, virologically suppressed participants - defined as HIV-1 plasma RNA levels <50 copies/mL for ≥1 year - were studied. Cells were incubated in the presence or absence of anti(α)-CD3/anti(α)-CD28 beads, vorinostat (SAHA), panobinostat (PNB), or romidepsin (RMD). Dose-ranging studies were performed and, after treatment with a standardized panel of death peptides, BH3 profiles were generated. To determine the selectivity of priming for latently HIV-infected cells, we used the J89 cell line in a series of dual HDACi and small-molecule Bcl-2 inhibitor experiments.

Results: Marked changes in the magnitude and phenotype of CD4⁺ T cell mitochondrial priming were observed after immune activation or exposure to PNB and RMD, compared to untreated HIV-infected PBMC. BH3 profiles varied as a function of drug type, dose, and duration of exposure. Increased priming in response to the peptide BAD and decreased permeabilization to the peptide NOXAA was observed with both transcriptional and immune activating stimuli, signifying an increased dependence on the anti-apoptotic Bcl-2 protein. The rank order of increased mitochondrial priming was RMD > PNB > α-CD3/α-CD28 beads >> SAHA. Similar magnitudes of priming occurred in central and transitional memory CD4⁺ T cell subsets. Increasing concentrations of the Bcl-2 inhibitors ABT-263 or -199 in the presence of RMD 20nM led to progressive losses of HIV-expressing J89 cells.

Conclusions: Histone deacetylase inhibition increased primary CD4⁺ T cell mitochondrial priming and cellular reliance on the anti-apoptotic protein Bcl-2. The mitochondrial phenotypes that we observed were specific to the immune activation or transcriptional re-activation stimulus being used. Combination HDACi and Bcl-2-inhibition selectively eliminated HIV-expressing cells in a latency cell line. Targeted small molecule Bcl-2 inhibition merits further investigation as a targeted HIV eradication strategy.

Background

A complex network of Bcl-2 family proteins tightly regulates intrinsic apoptosis and comprises pro-apoptotic, anti-apoptotic, and sensitizer proteins (**Figure 1**). HDACi such as vorinostat, romidepsin, and panobinostat, have been reported in cancer cell lines and primary cancer cells to modulate the activity of many proapoptotic Bcl-2 family members. The extent to which histone deacetylase inhibition modulates the activity of Bcl-2 family proteins in normal cells is unknown.

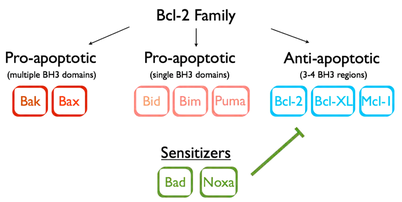


Figure 1. Schematic diagram of the Bcl-2 family of proteins involved in apoptosis via the intrinsic pathway.

Methods

Subject Selection

We used BH3 profiling to explore the mitochondrial priming effects of immune and transcriptional activation stimuli on blood-derived CD4⁺ T cell subsets, including central and transitional memory T cells that comprise the circulating HIV-1 reservoir (2). We studied PBMC isolated from a total of 8 HIV-uninfected donors and 14 HIV-infected, treated, virologically suppressed subjects (plasma HIV-1 RNA levels <50 copies/mL for ≥1 year.

Sample Collection and BH3 Profiling

Five million PBMC per condition were incubated in the presence or absence of activation stimulus for 24-48 hours. Treated cells were stained with CD3, CD4, CD45RA, CD45RO, CCR7, CD27 and cytochrome c mAbs and BH3 profiles in T cell subsets determined.

Follow-on Bcl-2 Inhibition

After a 24-hour incubation in the presence or absence of HDACi, cells were washed. A live/dead stain excluded all cells that died prior to ABT-263/199 exposure. PBMC were incubated with ABT compound for 2 hours, followed by annexin V staining and fixation.

BH3 Profiling

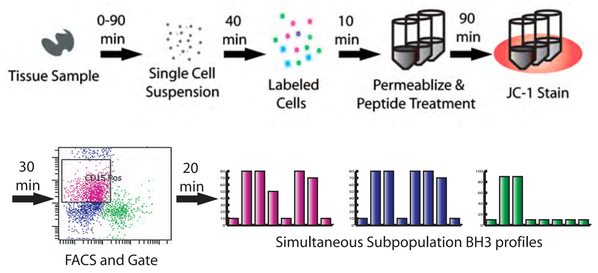


Figure 2. BH3 profiling, a flow cytometry-based mitochondrial phenotyping assay, measures the proximity of cell mitochondria to their apoptotic threshold, a property referred to as priming. Primed mitochondria respond more robustly to both the activator and sensitizer peptides, are more predisposed toward apoptosis, and are more sensitive than unprimed cells to cell stressors.

Priming after HDACi exposure

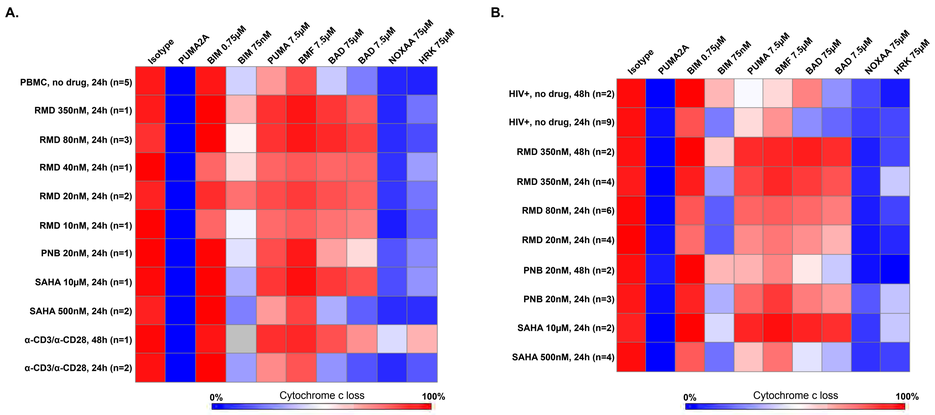


Figure 3. Summary heat maps for BH3 profiling of CD4⁺ T lymphocytes. (**A**) HIV-uninfected CD4⁺ T cells, (**B**) Latently HIV-infected CD4⁺ T cells. The magnitude of cytochrome c loss is displayed colorimetrically on a sliding scale from 0% (bright blue) to 100% (bright red). The number of replicates for each condition is denoted by n. PBMC, freshly isolated HIV-uninfected PBMC comparator; HIV+, freshly isolated HIV-infected PBMC without HDAC; RMD, romidepsin; PNB, panobinostat; SAHA, vorinostat.

T cell subset priming, HDACi

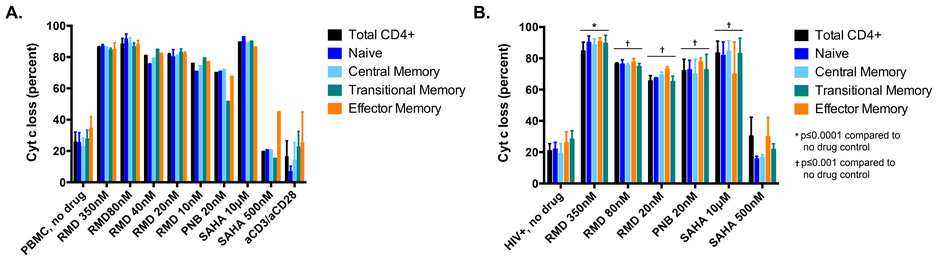


Figure 4. BAD peptide-induced cytochrome c losses in T cell subsets after 24 hours. (**A**) HIV-uninfected CD4⁺ T cells, (**B**) Latently HIV-infected CD4⁺ T cells. Significant differences in cyt c loss were determined by one-way ANOVA with corrections for multiple comparisons. PBMC, freshly isolated HIV-uninfected PBMC comparator; HIV+, freshly isolated HIV-infected PBMC without HDAC; RMD, romidepsin; PNB, panobinostat; SAHA, vorinostat.

Summary Heat Map, Bcl-2 inhibitors

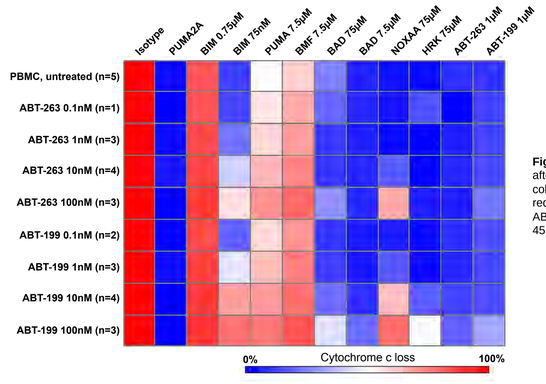


Figure 5. BH3 profiling heat map for HIV-uninfected CD4⁺ T lymphocytes after Bcl-2 inhibition. The magnitude of cytochrome c loss is displayed colorimetrically on a sliding scale from 0% (bright blue) to 100% (bright red). The number of replicates for each condition is denoted by n. ABT-263 and -199 were added to these BH3 profile panels to simulate a 45 minute ABT compound exposure at a 1μM dose.

T cell subset priming, Bcl-2i

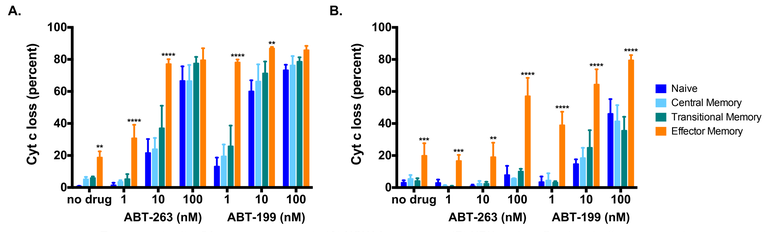


Figure 6. Cytochrome c losses in T cell subsets after 24 hours in response to (**A**) NOXAA peptide and (**B**) HRK peptide. Significant differences in cyt c loss were determined by two-way ANOVA with corrections for multiple comparisons. **, p<0.01, ***, p<0.001, ****, p<0.0001, each for effector memory cell-associated cyt c loss when compared to naïve, central memory, and transitional memory (all 3) for a given drug and concentration.

Bcl-2 inhibition after HDACi priming

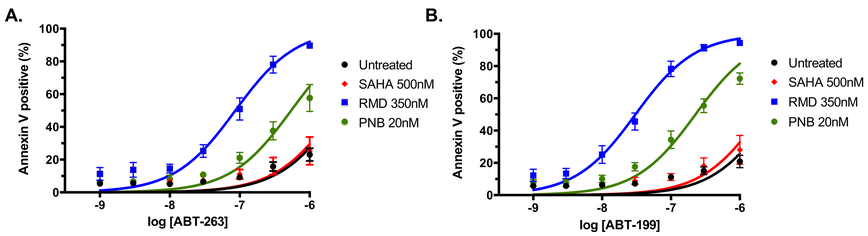


Figure 7. The effect of ABT-263 and ABT-199 on the induction of T cell apoptosis after HDACi treatment. (**A**) ABT-263, (**B**) ABT-199. PBMC were isolated from treated, virologically suppressed, HIV-infected participants. Untreated, no HDACi exposure; RMD, romidepsin; PNB, panobinostat; SAHA, vorinostat.

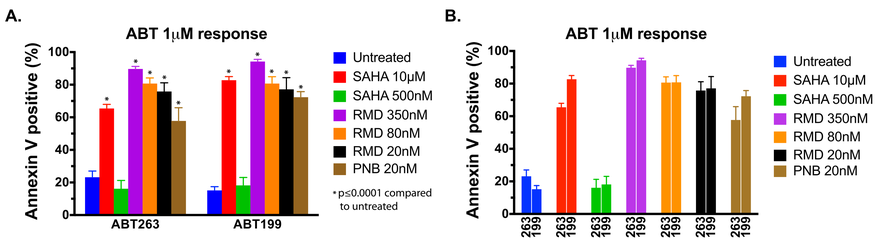


Figure 8. Maximum observed CD4⁺ T cell annexin V induction with HDACi exposure and follow-on 1μM ABT-263 and ABT-199. (**A**) Annexin response by ABT group, (**B**) Annexin V response by HDACi group. The exact same data is displayed in panels A and B. T cells were isolated from treated, virologically suppressed, HIV-infected participants. Significant differences in annexin V expression were determined by one-way ANOVA with corrections for multiple comparisons. Untreated, no HDACi exposure; RMD, romidepsin; PNB, panobinostat; SAHA, vorinostat.

HIV-1 selectivity

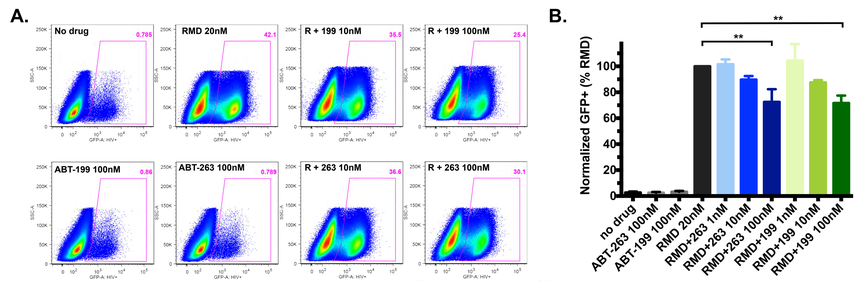


Figure 9. Loss of GFP expression in J89 cells exposed to combination RMD and Bcl-2 inhibitor. (**A**) Flow panels from a representative experiment showing absolute GFP expression losses on the x-axis, (**B**) Summary data showing GFP loss relative to the percent GFP expression induced by RMD 20nM. A minimum of 3 experiments were conducted for each condition and error bars represent the standard error of the mean. Significant differences in normalized GFP expression were determined by one-way ANOVA with corrections for multiple comparisons, ** p<0.01. RMD (R), romidepsin; 263, ABT-263; 199, ABT-199.

Conclusions

- **HDAC inhibition primes non-neoplastic CD4⁺ T cells for apoptosis**
- **HDAC-induced priming increases cell reliance on Bcl-2 for continued survival**
- **Follow-on small molecule Bcl-2 inhibition induces near-complete HIV-1 reservoir apoptosis**
- **Combination HDAC/Bcl-2 inhibition is selective for but not exclusive to latently HIV-1 infected, reactivated cells**

Acknowledgments

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