The Latency reversing agent 3-hydroxy-1,2,3-benzotriazin-4(3H)-one (HODHBt) synergizes with IL-15 to enhance cytotoxic capacities of HIV-specific CTL

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Background

To date, 'shock and kill' approaches aimed at reducing HIV reservoirs have not yet yielded this outcome in clinical trials, pointing to the need for novel and more potent therapeutic agents. 3-hydroxy-1,2,3-benzotriazin-4(3H)-one (HODHBt), an enhancer of STAT activation, increases occupancy of the transcription factor STAT5 on the HIV LTR, thereby reactivating latent HIV in CD4+ T-cells (Bosque et al, *Cell Reports,* 2017). In particular, we have previously shown that HODHBt enhances IL-15 mediated reactivation in cells isolated from ARV-suppressed PLWH (Sorensen et al, *AAC*, 2020). STAT5 can also enhance CD8+ T-cell effector functions by transducing IL-15 signaling. Since HODHBt enhances STAT5 activation, we hypothesized that HODHBt would act synergistically with IL-15 to enhance cytotoxic function (granzyme B release) of *ex vivo* HIV-specific CD8+ T-cells from antiretroviral therapy (ART) treated individuals.

Methods

Granzyme B (GZMB) ELISpots were performed on PBMCs from 14 people living with HIV who had been on suppressive ART for an average of 10.9 years (range, 7.4-18). Cells were stimulated with peptide pools spanning Gag, Pol, Nef, or Env, CMVpp65, with or without 1 ng/ml IL-15 and/or 50 ug/ml HODHBt. Supernatants were collected and cytokine secretion was analyzed using the CorPlex Human Cytokine Panel 1 10-Plex array (IFN-γ, IL-1b, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-22 and TNF-α).

Conclusions

In addition to its LRA potential, we show that HODHBt synergizes with IL-15 to markedly enhance HIV-specific cytotoxic T-cell responses in *ex vivo* PBMCs from ART-treated PLWH. Our results highlight that pharmacologic enhancement of IL-15 mediated STAT activation can be a therapeutic strategy with the potential to enhance both the 'shock' and the 'kill' components of strategies aimed at depleting HIV reservoirs.

Results

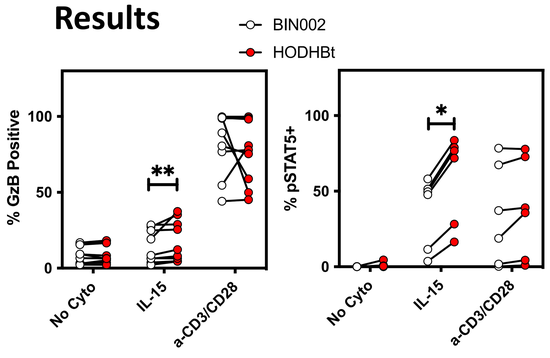


Figure 1. CD8+ T cells treated with IL-15 and HODHBt synergize and express increased pSTAT5 ($p<0.05$), and GZMB ($p<0.001$), relative to IL-15 alone. HODHBt alone does not increase pSTAT5 or GZMB in CD8+ T cells assessed by flow cytometry.

Results

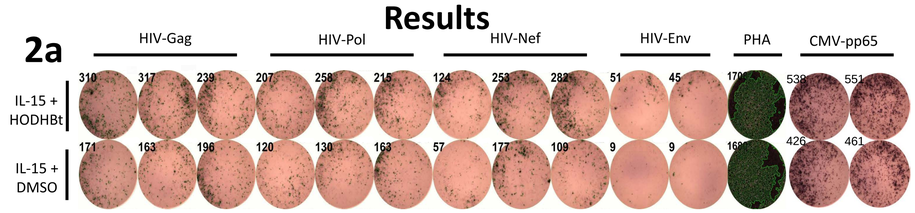


Figure 2a. Representative plot of GZMB responses to HIV and CMV peptides pools and PHA. Treatment with IL-15 + HODHBt significantly increases HIV and CMV responses.

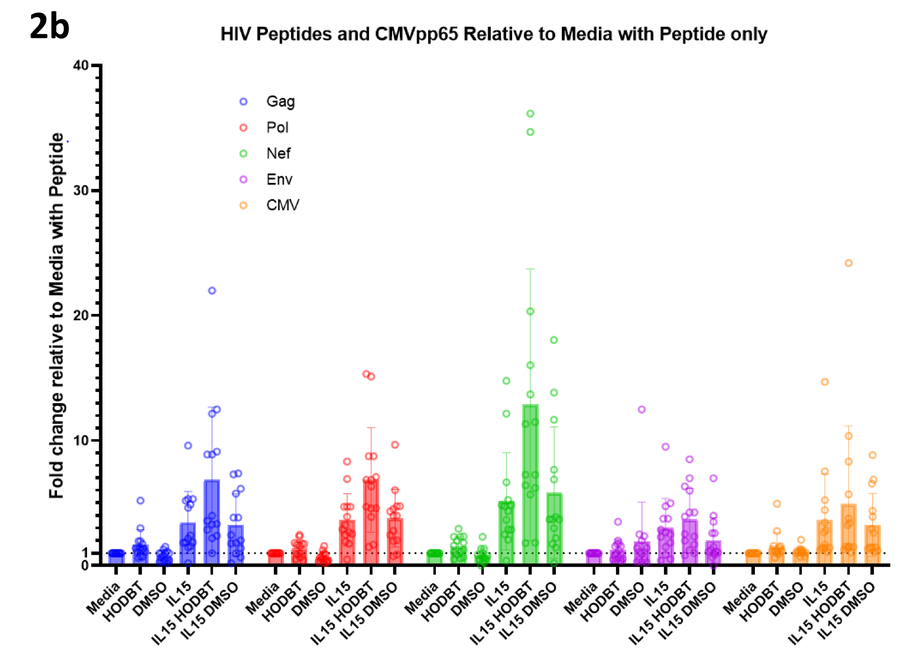


Figure 2b. HIV and CMV responses were substantially increased relative to enhancements with IL-15+DMSO alone. HODHBt enhances the GZMB release in response to multiple HIV peptides, typically enhancing GZMB release by more than 2-fold, for Gag, Pol, and Nef. HIV-specific GZMB-releasing responses were enhanced by treatment with HODHBt in combination with IL-15, relative to medium only. This points out the relative fold change increase in HIV specific response are consistently enhanced by the addition of HODHBt in combination with IL-15.

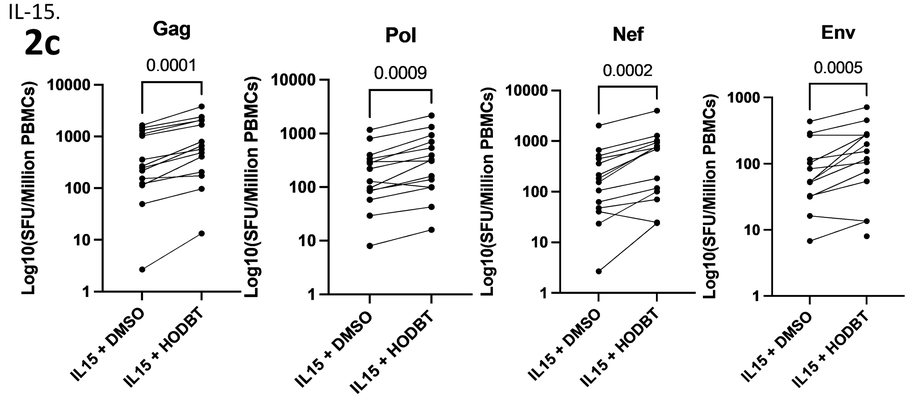


Figure 2c. Spot forming units (SFUs) between matched samples treated with IL-15 + DMSO and IL-15 + HODHBt. An increase in HIV specific responses by GZMB release were detected in almost every case.

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Proposed Mechanism of Action

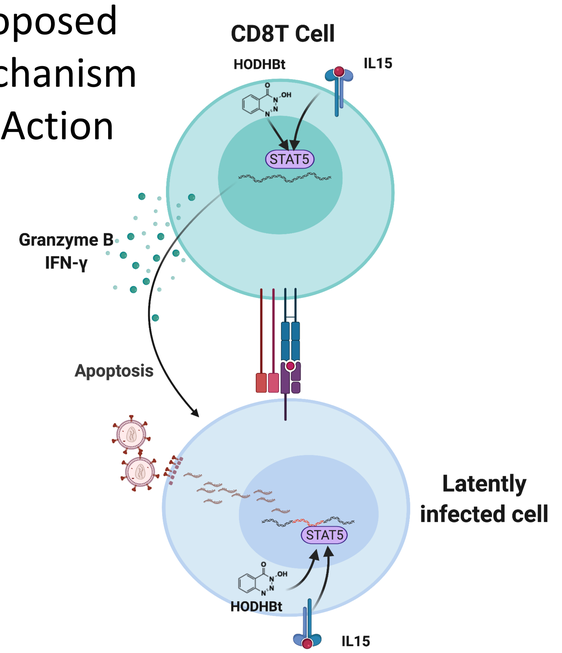


Figure 4. GZMB release was significantly positively correlated with cytokines IFNγ ($r= 0.89$, $p<0.0001$) and IL22 ($r=0.73$, $p<0.001$) across all conditions and peptides, except CMV. Showing that that increase in GZMB release is likely an effective agent at inducing cytolytic responses upon exposure to HIV antigen.

