Development of Cytotoxic Enhancing Agents (CEAs) to Improve Shock-and-Kill Strategies.

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Abstract

BACKGROUND: Elimination of the latent reservoir is crucial towards efforts to eradicate HIV. Therapeutic interventions against latent HIV have been mainly focused on 'shock and kill' strategies. These strategies are based on the transcriptional activation of latent HIV with a Latency-Reversing Agent (LRA) with the consequent killing of the reactivated cell by either the cytopathic effect of HIV or an arm of the immune system. Several clinical trials targeting the latent reservoir with LRAs have resulted in limited to no clinical effect on the size of the latent reservoir. Some potential explanations to the failing of these strategies are: a reduced effector function of immune cells; reactivation of defective proviruses that do not produce necessary viral antigens; or a survival advantage of latently infected cells. To that end, strategies that can reactivate latent HIV and also enhance immune responses against HIV may overcome some of the disadvantages of current cure efforts.

METHODS: We have developed a primary cell model to identify cytotoxic enhancing agents (CEAs) that will enhance the killing capacity of CD8T cells. This method relies on a mix-lymphocyte reaction (MLR) in which polyclonally activated CD8T cells are co-cultured with allogeneic polyclonally activated CD4T cells from a different donor. CD4T cells are activated in conditions to generate central memory CD4T cells (TCM) cells, as this cell subset is the major contributor to the latent reservoir and is more resistant to apoptosis than other subsets of CD4T cells. Cell death in both CD4 and CD8 subsets is measured by flow cytometry.

RESULTS: We have characterized two potential CEAs. The first one is the LRA HODHBt, a STAT SUMOylation inhibitor. HODHBt enhances the cytotoxic capacity of CD8T cells by increasing their cytolytic potential. The second one, ABT-199, is a Bcl-2 inhibitor that increases the sensitivity of CD4T cells to the killing machinery of CD8T cells.

CONCLUSIONS: We have developed a reliable primary cell paradigm to study and screen strategies that will enhance the cytotoxic capacity of CD8T cells. This new primary cell model will help in the development of effective 'shock and kill' strategies to reduce or eliminate the latent reservoir.

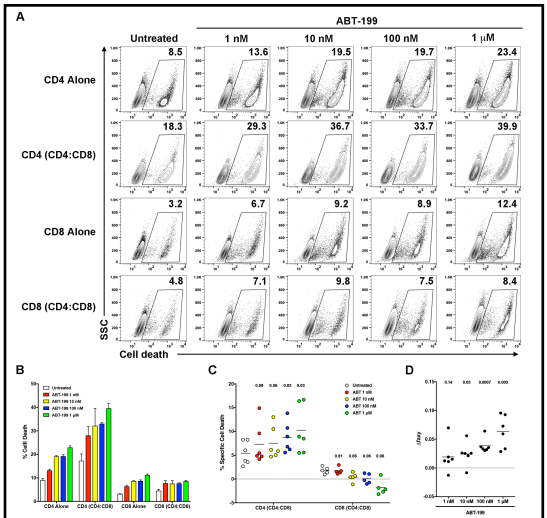

Figure 3. The BCL-2 Antagonist ABT-199 Enhances CD8⁺ T-cell Mediated Elimination of Allogeneic CD4⁺ T-cells. **(A)** Representative data from one MLR showing cell viability as measured by flow cytometry. **(B)** Mean ± SD of 3 replicates. The data show a dose-dependent increase in death of CD4⁺ T-cells with ABT-199 treatment. **(C)** Summary data of specific cell death in MLRs from 6 different pairs of donors indicate a significant and dose-dependent enhancement in CD4⁺, but not CD8⁺ T-cell death by the addition of ABT-199. P values are shown above each plot, representing Wilcoxon matched-pairs signed rank statistical test over untreated control (**D**) The Bliss independence model was utilized to calculate synergy in the ability to induce CD4 T cell death between ABT-199 and CD8T cells (n=6). Synergy is defined as Δfaxy>0. P values for Wilcoxon signed rank statistical tests are shown above each plot.

References

(1) Bosque and Planelles, 2009, *Blood.*
(2) Chomont et al., 2009, *Nat Med.*
(3) Riou et al., 2007, *J Exp Med.*
(4) Bosque et al, 2017, *Cell Reports.*

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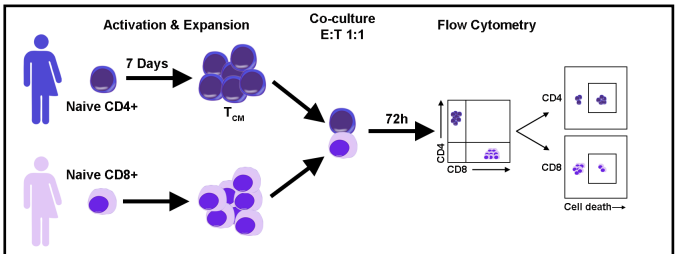

Figure 1. Primary Cell Model to Identify CEAs. Experimental diagram of the MLR killing assay of TCM cells. In this assay, polyclonally activated CD8 T cells are co-cultured with polyclonally activated CD4 T cells from a different donor to resemble a mixed-lymphocyte reaction (MLR)at an effector:target ratio (E:T) 1:1. CD4 T cells are activated in conditions to generate central memory CD4 T cells (TCM) cells, as this cell subset is the major contributor to the latent reservoir (1,2). Furthermore, CD4 TCM are more resistant to apoptosis than other subsets of CD4 T cells (3). Cell death in both CD4 and CD8 subsets is measured at 72h using a fixable viability dye.

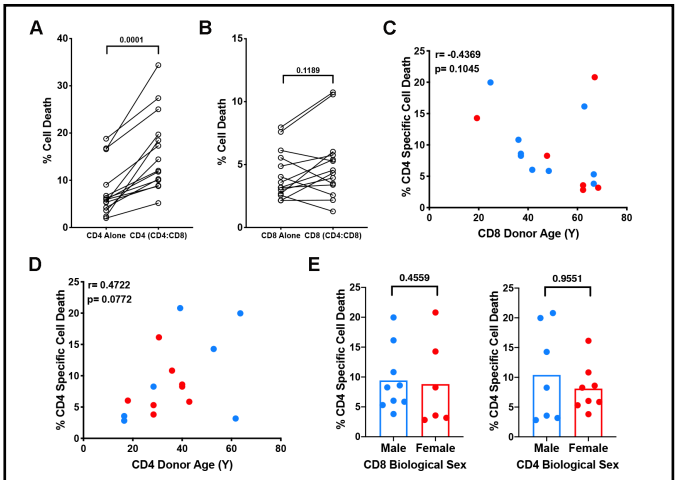

Figure 2. The MLR Assay identified Age as a Potential Biological Variable in the Effector Function of CD8 T Cells. **(A)** Percentage of CD4 cell death either alone or in co-culture with CD8 cells, E:T ratio 1:1, from 14 HIV-negative donors. Nonparametric Wilcoxon matched-pairs signed rank test was used to calculate p values. **(B)** Percentage of CD8 cell death either alone or in co-culture with CD4 cells, E:T ratio 1:1, from 14 HIV-negative donors. Nonparametric Wilcoxon matched-pairs signed rank test was used to calculate p values. **(C)** Spearman correlation between CD4 specific cell dead (%CD4 cell death in the co-culture with CD8 - %CD4 cell death in the monoculture) and the age of the donor CD8 T cells. **(D)** Spearman correlation between CD4 specific cell dead and the age of the donor CD4 T cells. **(E)** Comparison of CD4 specific cell dead and the biological sex of the donor CD8 T cells (left panel) or the donor CD4 T cells (right panel). Mann-Whitney test was used to calculate p values.

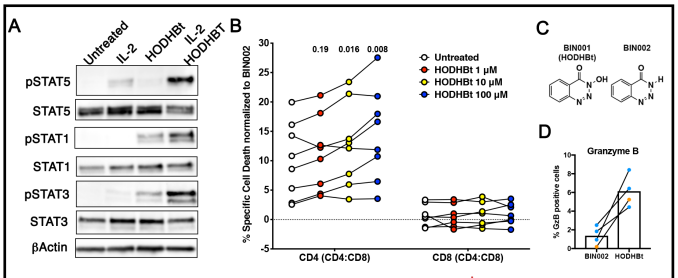

Figure 4. The STAT SUMOylation inhibitor HODHBt Enhances CD8⁺ T-cell Mediated Elimination of Allogeneic CD4⁺ T-cells. **(A)** CD8 T cells were cultured with IL-2 in the presence or absence of HODHBt and levels of phosphorylated STAT5, STAT1 and STAT3 were measured by western blot. As previously shown for CD4 T cells (4); HODHBt enhances the phosphorylation levels of STAT5 in CD8 T cells. Furthermore, HODHBt alone increased the phosphorylation levels of both STAT1and STAT3, and enhanced those induced by IL-2. **(B)** Summary data of specific cell death in MLRs from 8 different pairs of donors indicate a significant and dose-dependent enhancement in CD4⁺, but not CD8⁺ T-cell death by the addition of HODHBt. P values are shown above each plot, representing Wilcoxon matched-pairs signed rank statistical test over untreated control. **(C)** Structure of 3-Hydroxy-1,2,3-benzotriazin-4(3H)-one (HODHBt or BIN001) or the inactive compound 1,2,3-Benzotriazin-4(3H)-one (BIN002). **(D)** Levels of intracellular expression of Granzyme B in CD8 T cells treated with HODHBt or BIN002. Blue dots correspond to male donors and orange dots to female donors.