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Identification and targeting of metabolic profiles in CTL-resistant, HIV-infected CD4+ T-cells

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INTRODUCTION

- While viral latency is a main contributor to HIV persistence, a degree of HIV transcription and surveillance by HIV-specific CTL persists even under long-term ART.
- Previous work from our lab and others has proposed that a subset of HIV infected, transcriptionally active CD4+ T-cells is resistant to CTL targeting and may contribute to long-term HIV persistence, via mechanisms including the overexpression of BLC2, SERPINB9, and PVR among other factors.
- In this study we characterize the transcriptional landscape and surface protein profile of HIV infected, CTL-resistant CD4+ T-cells at the single cell level and describe a quiescent profile with low oxidative stress as a mode of CTL resistance.

METHODS

- Central memory CD4+ T-cells were differentiated from HLA-B57^{+} donors, and then infected with either HIV$_{JRSCF}$ or an HIV$_{JRSCF_TW10esc}$ variant containing a escape mutation in the Gag-TW10 epitope (Non-polarized infection model; NPIM).
- CD4+ T-cells infected with these strains were dyed with CTFR (WT) or CFSE/CTV (TW10esc), mixed and killing assays were performed with a TW10 epitope-specific CTL clone.
- **Surviving WT** and **bystander TW10esc** cells were sorted based on HIV envelope surface expression and profiled by CITE-seq.
- Cellular reactive oxygen species (ROS) levels were measured via flow cytometry using CellROX and DCFH-DA.
- HIV-infected CD4+ T-cells we pre-treated with FDA-approved ROS inducer atovaquone (ATQ) and/or a hypoxia inducing compound in additional killing assays
- EX-vivo CD4+ T-cells from PLWH were sorted based on levels of ROS detection.

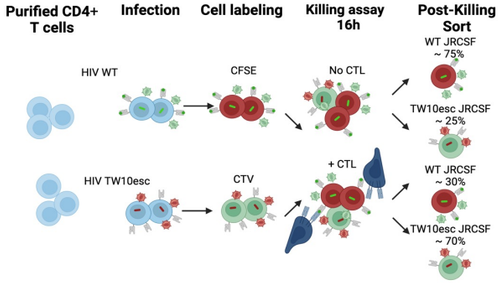


Figure 1. Sorting schematic for surviving and bystander infected CD4+ T-cell after CTL targeting .

RESULTS

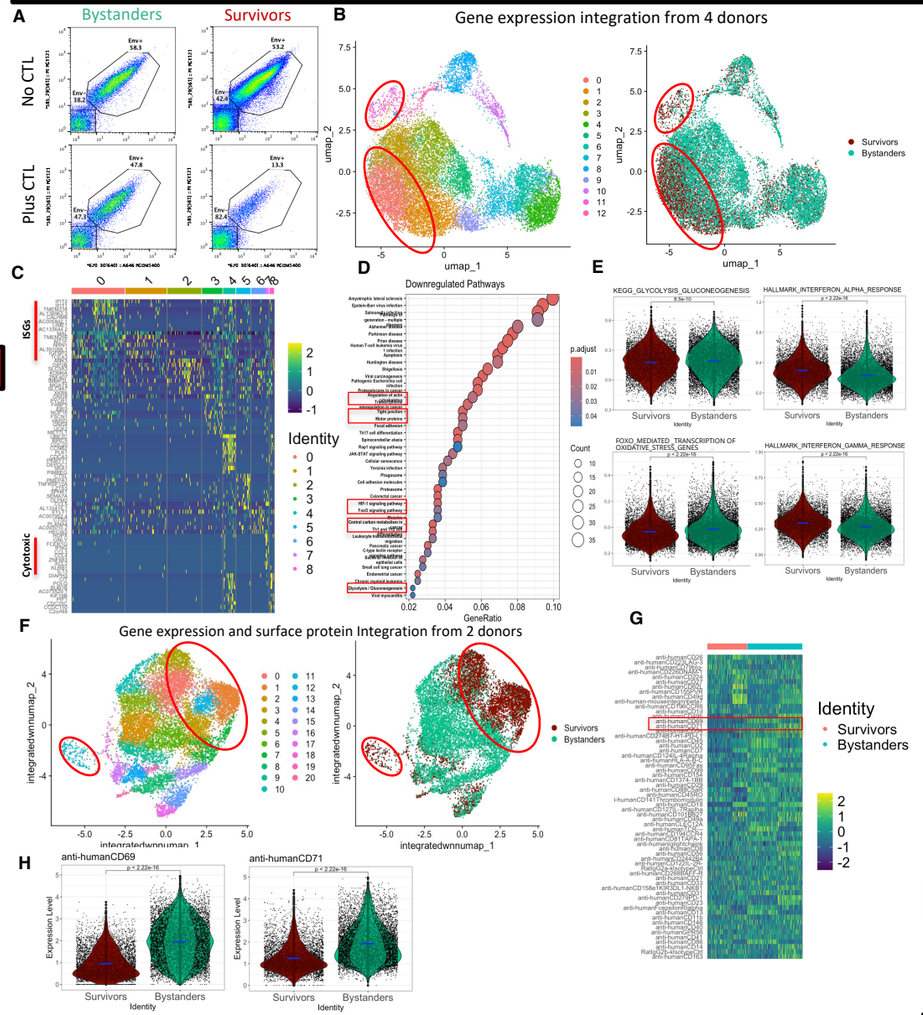


Figure 2. HIV-infected, CTL-resistant CD4+ T-cells targets have a unique transcriptional and surface protein profiles, denoting low levels of activation and oxidative stress. A, Representative flow cytometry plots showing gating of surviving and bystander HIV-infected cells sorted from killing assays. **B**, Unbiased gene expression clustering of surviving and bystander cells from 4 donors (left) colored by identity (right). **C,** Heatmap of differentially expressed genes by cluster. **D,** Pathway enrichment results showing downregulated pathways in survivors. **E,** Violin plots showing scoring of affected gene modules. **F,** Clustering of survivors and bystanders from 2 donors using integrated gene expression and surface protein modalities. **G,H,** Heatmap of differentially expressed proteins and violin plots of selected markers under the control of activation and metabolic signals.

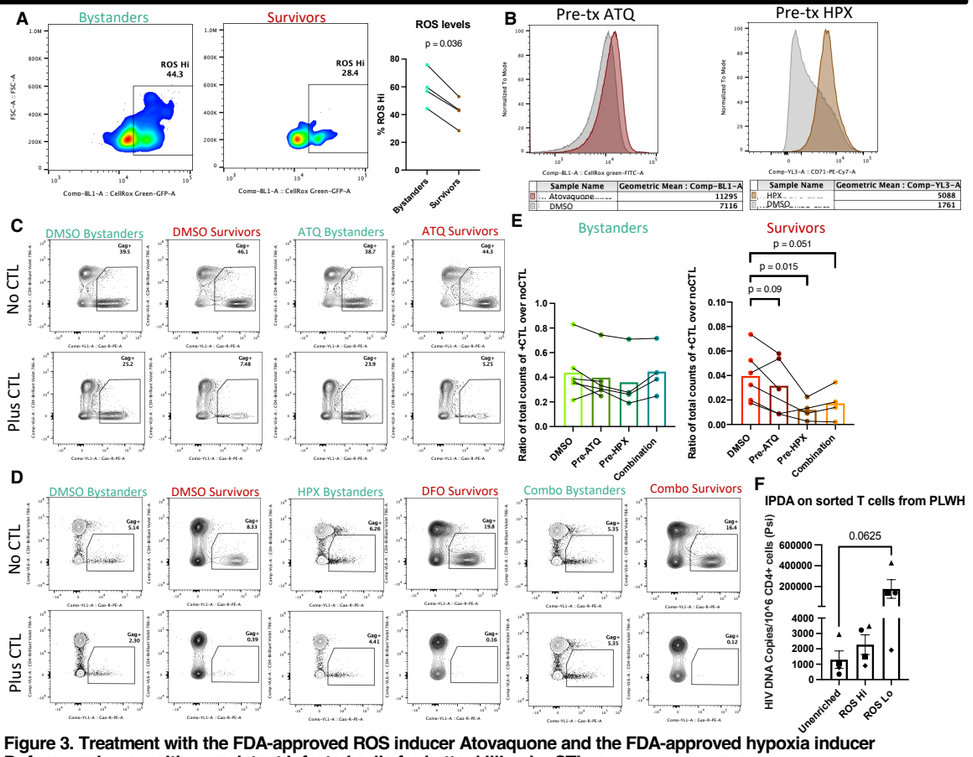


Figure 3. Treatment with the FDA-approved ROS inducer Atovaquone and the FDA-approved hypoxia inducer Deferoxamine sensitizes resistant infected cells for better killing by CTL. A, Representative FACS plots and quantification of baseline ROS accumulation in survivors and bystanders after a killing assay. **B**, Representative FACS histograms showing the ability of Atovaquone (ATQ) and an FDA-approved hypoxia inducing compound (HPX) to induce sub-lethal oxidative stress (left) and upregulation of the transferrin receptor (right) respectively in infected cells. **C, D,** Representative FACS plots showing Gag-p24 expression of survivors and bystanders in no CTL vs plus CTL conditions with the indicated treatments. **E,** Quantification of flow cytometry results showing ratios of total counts from no CTL over plus CTL conditions in cultures of surviving targets (right) and bystanders (left) plotted by treatment condition. **F,** IPDA results from unenriched, and FACS sorted ROS Hi and ROS Lo CD4+ T-cells from PLWH showing number of HIV DNA copies per million CD4+ T-cells.

CONCLUSIONS AND FUTURE DIRECTIONS

- Surviving CTL-resistant CD4+ T-cell targets have an enriched transcriptional profile and a unique surface protein expression pattern compared to non-targeted bystanders, denoting low levels of activation, metabolism processes and oxidative stress.
- Infected cells can be sensitized to upregulate ROS and the transferrin receptor (CD71), key components of ROS mediated cell death and ferroptosis, and become vulnerable to elimination by CTLs. Cells harboring provirus are enriched in CD4+ T-cells with low levels of oxidative stress from PLWH.

ACKNOWLEDGEMENTS

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