#317 HIV infection of target cells in pediatric tonsils is inhibited by an Hsp90 inhibitor

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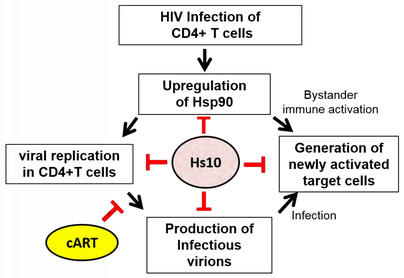
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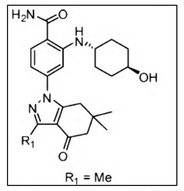
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Introduction

- Transmission of HIV via breastfeeding accounts for approximately 50% of the 150,000 pediatric infections occurring annually, most of which cannot be readily diagnosed.
- These postnatally infected infants require long time to achieve virologic control upon initiation of combined anti-retroviral therapy (cART).
- HIV-induced bystander immune activation generates activated target cells resulting in cell-to-cell viral spread and increased HIV reservoir size.
- **Hypothesis: Targeting HIV-induced bystander immune activation in combination with cART, can be an alternative antiviral strategy to attain viral control and limit reservoir size in established infections.**





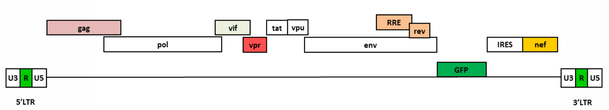
Hs10: Analogue of SNX2112, currently in clinical trial for cancer therapy

Objective

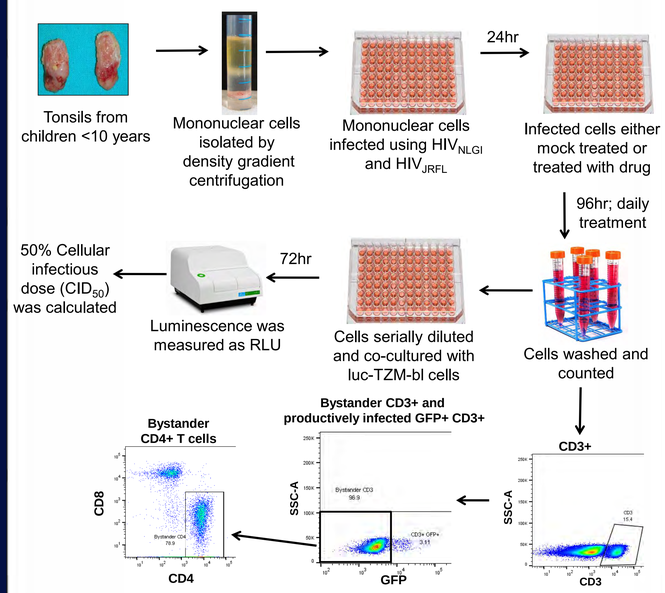
To investigate the synergistic role of cART and a small molecular inhibitor of Hsp90 (Hs10) on HIV replication and spread, in a physiologically relevant pediatric tonsillar tissue model of HIV replication.

Methods

Viruses used for infection: CXCR4-tropic HIV-1_{NLGI} and CCR5-tropic HIV-1_{JRFL}



***In vitro* HIV infection and transfer model:**



Statistical Analysis: Wilcoxon matched-pair signed rank test was performed to test the significance between mock treated and drug treated groups.

Results

Hs10 inhibits HIV replication in pediatric tonsillar cells *in vitro*

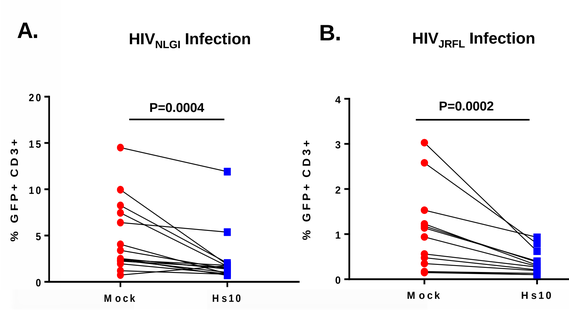


Figure 1. Effect of Hs10 on established tonsillar cell infection: Mononuclear cells isolated from tonsils of 13-15 donors were infected with **A)** HIV_{NLGI} and **B)** HIV_{JRFL}. The infections were either mock or 100nM Hs10 treated . Productive HIV infection (CD3+GFP+) was quantified by flow cytometry. Hs10 inhibited HIV_{NLGI} infection by 49.2% and HIV_{JRFL} infection by 63.6%.

Hs10 inhibits infectious tonsillar cell viral transfer

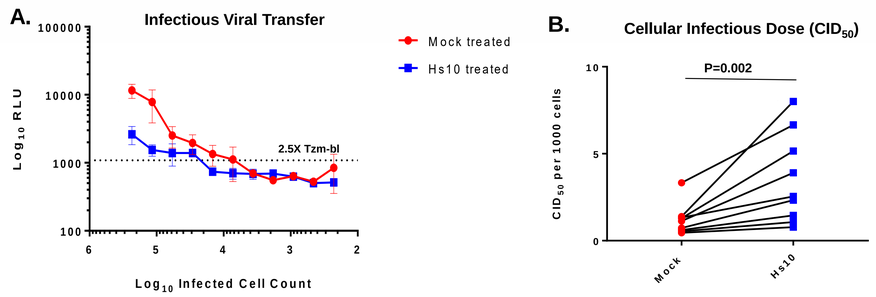


Figure 2. Infectious viral transfer by infected tonsillar mononuclear cells was inhibited in the presence of Hs10: Mononuclear cells isolated from pediatric tonsils (n=10) were infected with HIV_{JRFL}. The infections were either mock or Hs10-treated, and cocultured with luciferase-expressing Tzm-bl reporter cells. **(A)** Luminescence of the mock treated vs. Hs10 treated group was measured. The detection limit of infection, represented by the dotted line, was defined as 2.5X average RLU for the Tzm-bl only wells from 10 independent experiments. **(B)** The number of infected mononuclear cells required to initiate 50% infection of the Tzm-bls (CID_{50}) was determined using the Reed-Muench method. Our data demonstrate that Hs10 treatment resulted in 437% increase in the CID_{50} per 1000 cells.

Hs10 inhibits bystander activation and proliferation of CD4+ T cells, reducing target cells

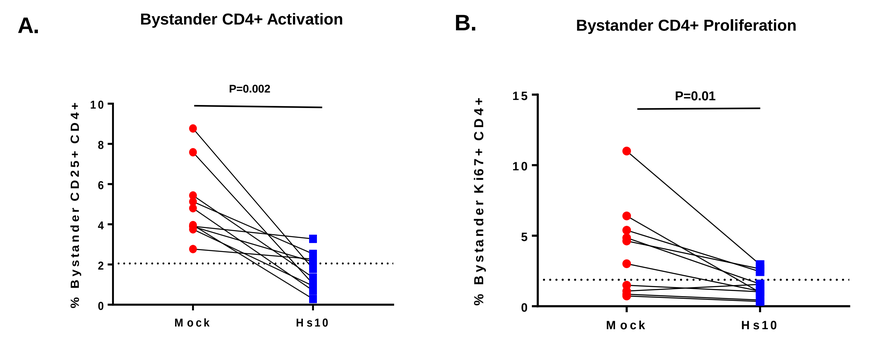


Figure 4. Effect of Hs10 treatment on bystander T cell activation and proliferation: Mononuclear cells isolated from tonsils (n=10) were infected with HIV_{JRFL} and treated with Hs10. Bystander CD4+ T (CD3+ GFP- CD4+) cells were stained for **A)** T cell activation marker CD25 and **B)** T cell proliferation marker ki67. Hs10 reduced the proportion of bystander CD4+CD25+ cells by 67.3%, and the proportion of bystander CD4+ki67+ cells by 61.8%. The dotted line represents the mean proportion of cells in the uninfected-treated group.

Results

Hs10 increases the proportion of CD4-CD8- T cells by downregulating CD4+ expression, altering target cell phenotype

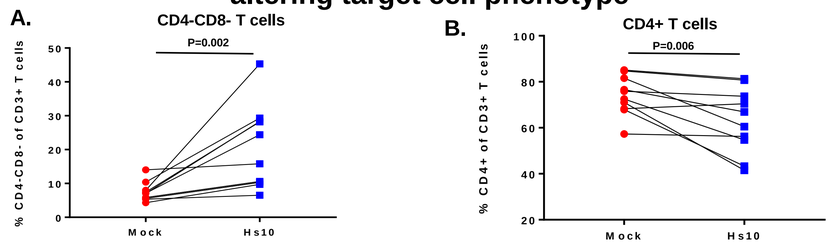


Figure 5. Proportion of CD4-CD8- T cells and CD4+ T cells in infected tonsillar mononuclear cells treated with Hs10: Mononuclear cells isolated from tonsils (n=10) were infected with HIV_{JRFL}. and treated with Hs10. Proportion of CD4+ and CD4-CD8- cells were measured by flow cytometry. Hs10 increased the proportion of CD4-CD8- population by 177%, by downregulating CD4 expression by 99%. There was no significant change in the proportion of CD3+ and CD8+ T cells in the Hs10-treated and mock-treated group (data not shown).

Hs10 synergizes with combined anti-retroviral therapy (cART) to inhibit HIV replication

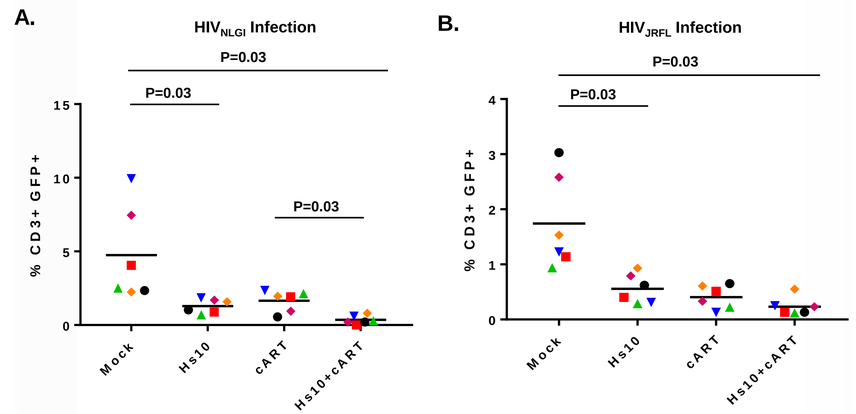


Figure 5. Synergistic effect of Hs10 and cART on HIV replication: Mononuclear cells isolated from pediatric tonsils (n=6) were infected with **A)** HIV_{NLGI} and **B)** HIV_{JRFL}. The infections were either mock treated, or treated with Hs10, or a triple drug ARV consisting of optimized doses of Lamivudine, Zidovudine and Nevirapine, or a combination of Hs10 and ARVs. Productive infection of CD3+ T (CD3+GFP+) cells were quantified by flow cytometry. Incorporation of Hs10 in the ARV panel resulted in a greater inhibition of HIV replication (HIV_{NLGI}: Mean inhibition 65.4% and HIV_{JRFL} mean inhibition: 76.5%), compared to ARV alone (HIV_{NLGI} mean inhibition: 92.6% and HIV_{JRFL} mean inhibition: 86.5%).

Summary

Incorporation of an Hsp90 inhibitor in the cART regime synergize for effective HIV replication and spread via reducing bystander T cell activation and downregulating CD4+ expression.

Future Directions and Implications

In future, we will evaluate the safety and efficacy of incorporating an Hsp90 inhibitor in the current ARV regime in SHIV-infected neonatal rhesus macaques, for reduction of SHIV reservoir size. Rapid control of viral replication, and reduced reservoir size might result in functional cure by lengthening the time to viral rebound upon discontinuation of therapy

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