“Block and Lock” HIV Persistence in Human Brain Myeloid Cells

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Background and Introduction

Globally, there are more than 38 million people living with HIV (PLWH). The HIV provirus integrates itself into the host genome. Antiretroviral therapy (ART), a combination of antiviral drugs that target various stages of the replication cycle, is currently the standard treatment for controlling human immunodeficiency virus type-1 (HIV-1) replication. ART is effective at reducing viremia in the periphery and controlling active viral replication, but it cannot entirely eliminate HIV infection due to latent HIV reservoirs, causing the virus to rebound after therapy interruption. Although many studies have shown that CD4+ T cells serve as a reservoir for HIV-1, recent studies from our group and others have revealed latent HIV infection in brain myeloid cells (BrMCs), which have the potential to serve as a true HIV reservoir within the central nervous system (CNS).

To eliminate such reservoirs, two strategies have been proposed. The first strategy, called “shock and kill,” involves the use of latency reversing agents (LRAs) to reactivate latent HIV reservoirs, followed by immune clearance to eliminate the cells with reactivated HIV-1. It is not clear whether this approach is ideal to eliminate brain myeloid reservoirs since the induction of HIV RNA or protein may be toxic to the neuronal cells. The second strategy, called “block and lock,” aims to permanently block and lock HIV transcription. In this study, we explore the use of a BRD4-selective small molecule modulator (ZL0580), which has been shown to silence HIV transcription in latent myeloid cell line model systems. Here, we use primary microglia directly isolated from fresh autopsy brain tissue as a novel, physiologically relevant platform to test the effects of ZL0580 in blocking and locking HIV expression.

Methods

- To evaluate the efficacy of deep silencing of HIV proviruses in the brain microglia, primary microglia infected with patient brain-derived HIV were subjected to the treatment of BRD4 modulator, ZL0580, known to silence HIV in T cells.
- HIV transcription was measured by digital droplet PCR (ddPCR), which includes three steps: viral RNA purification, RT IV reverse transcription, and PCR.

Results

- The HIV-specific ddPCR accurately detected the presence of HIV RNA in the culture supernatants of the infected BrMCs **(Figure 1)**.
- One week-post treatment, we observed that variable doses of ZL0580 resulted in enhanced levels of HIV RNA **(Figure 2)**.
- In the cells treated with ZL0580 for 16 days, we discovered that the prolonged treatment with ZL0580 prevented HIV reversal, compared with the untreated control **(Figure 3).**

Figure 1. The representative interface of digital droplet PCR assay to measure HIV RNA released in the supernatants of HIV infected BrMC cultures.

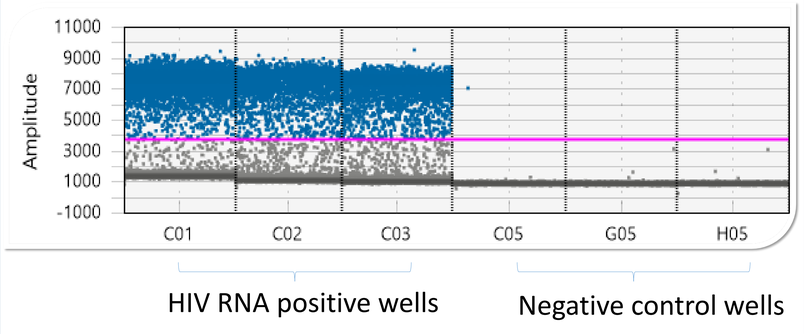


Figure 2. HIV RNA released in the supernatants from BrMC culture 7-days post-ZL0580 treatment.

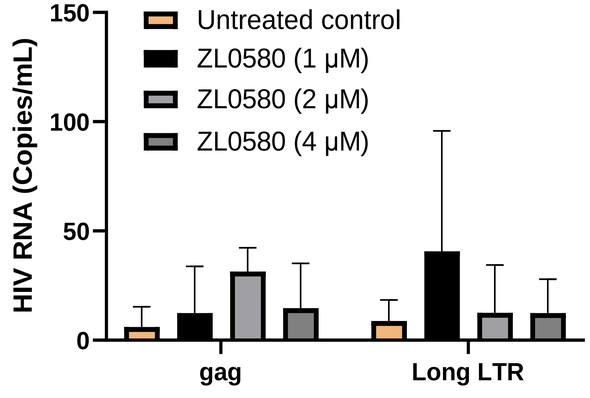
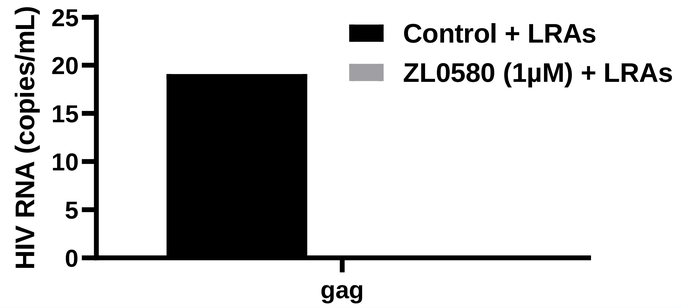


Figure 3. ZL0580 may prevent HIV from latency reversal in BrMCs. Sixteen days post-treatment, a combination of LRAs (JQ-1, SAHA, and CM272) reactivated latent HIV.



Discussion

- We successfully infected BrMCs isolated from human brain tissues with HIV in vitro.
- Our results indicate that a short-term treatment (7 days) with ZL0580 has the potential to reactivate latent HIV in BrMCs. Our findings further suggest that prolonged treatment (16 days) with ZL0580 can block HIV reactivation from latency reversal. These results are different from the previous studies conducted on microglia cell line models, which may be attributed to the different features of primary microglial cells.

Future Steps

- To optimize conditions of our study and generate replicable results to solidify our understanding of the impact of BRD4 modulation by ZL0580 in BrMCs.
- To conduct new studies with LRAs and/or killing compounds:
  - Dexamethasone (DEXA)
  - Polycytidylic acid (Poly I:C)
  - Nurr1/Nor1 agonist 6-MP

Acknowledgements

I would like to thank Dr. Yuyang Tang, Dr. Guochun Jiang, and the members of the Jiang Lab for their unconditional mentorship and support throughout this project.