Targeting HIV Transcriptional Machinery for a Functional Cure of HIV
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Abstract
Antiretroviral therapy (ART) has been used to suppress HIV, but the virus persists in latent CD4+ memory T cells through stable HIV proviral DNA. If ART is interrupted, these latent HIV reservoirs will rebound, typically within a few weeks. A developing strategy for a functional HIV cure, “Block and Lock,” aims to prevent this viral reactivation by suppressing the provirus into a latent state. We examine the efficacy of two compounds to interfere with the transcriptional machinery of HIV provirus to eliminate replication. Compound A inhibits epigenetic modifications of nucleosomes, inducing a silenced structure of local chromatin at the HIV promoter. Compound B inhibits positive transcription elongation factor P-TEFB, which is essential for HIV transcriptional elongation. 2D10 cells were treated with both compounds separately and in combination. Relative proviral expression indicates that both compounds were effective in blocking the transcriptional machinery of HIV. Interruption of treatment resulted in an initial increase in expression, but long-term silencing is still under investigation.

Results
- Suppression of HIV transcription machinery, singly or in combination, caused a significant decrease in proviral expression, indicating that deep latency could be enforced.
- Cellular viability remained consistently around 100%, indicating that the treatments are tolerable to immune cells in vitro.
- Interestingly, cells treated with compound A to suppress epigenetic remodeling indicate a stabilization of HIV expression on Day 63.
- After treatment interruption on Day 49, there was a gradual increase in proviral expression, indicating that the deep silent state may or may not be maintained as the study is still ongoing.

Conclusion
The results show that compound A or B is effective in blocking the transcriptional machinery of HIV. However, whether they can maintain deep silencing for a long-term functional cure is under investigation. In the future, these compounds will be investigated in other cell models.

Background and Introduction
ART is used to suppress the virus in HIV-infected individuals. However, when ART is ceased, the virus rebounds quickly due to latent HIV reservoirs. HIV reservoirs are CD4+ memory T cells that have entered a resting state, but still carry stable HIV proviral DNA inaccessible to ART. A cure for HIV would have to address these latent reservoirs. There are two developing strategies for curing HIV. “Shock and Kill” involves reactivating latent HIV reservoirs using latency-reversal agents followed by immune clearance. “Block and Lock” involves preventing latency reactivation by blocking the transcription of HIV proviruses to enforce HIV provirus into a deep silent state (“deep latency”). We explored the use of two compounds to suppress HIV transcription machinery. Compound A inhibits the acetylation of histones, inducing a silenced structure of local chromatin at the HIV promoter, reducing the stability of RNA. Compound B inhibits P-TEFB, which is a complex that binds to an RNA stem-loop structure formed during transcription of proviral DNA. P-TEFB is essential for HIV transcriptional elongation. Both compounds at low concentration have no effect on cell viability, making them potential candidates for drug development.

Methods
2D10 cells were treated in triplicates for seven weeks with compound A and compound B separately and in combination. The controls were treated with DMSO. Treatment was interrupted on Day 49. The 2D10 cell line contain HIV with green fluorescent protein (GFP) inserted. The cells were cultured in RPMI media with 10% FBS and 1% Penicillin-Streptomycin. Cells were harvested for flow cytometry weekly to detect HIV gene expression by detecting GFP expression. Cellular viability was detected by using a live/dead stain in the harvesting process. Flow cytometry data were used to find the relative proviral expression over control as well as the relative cellular viability over control. Additionally, protein and RNA were harvested for Western Blot and RT-PCR at various points throughout the experiment. Day 35 protein samples were used to detect phosphorylated CDK9.

References

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