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HIV-specific latency reversing therapies that exploit novel pathways for suboptimal Tat protein expression

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Background: We have identified a footprint of viral Tat expression in latent HIV infected cells. Suboptimal levels of Tat arise from an IRES-mediated translation of chimeric cell-HIV mRNAs that arise from alternative splicing of read-through mRNA transcripts from cellular promoters adjacent to latent integrated provirus. To simulate the role of RNA-processing pathways in HIV latency we recapitulated the low level Tat-expression from cellular-provirus read-through transcripts present in HIV latency reporter cells that express low-level Tat using the native IRES that underlies the first coding tat exon and a second, different Click-Beetle-Luciferase, expressed from a CMV-IE promoter to test specificity. Novel compounds and drug combinations were screened to identify HIV-specific drugs that synergize with this latent-viral signature. HIV-specific activation was further examined in T-cell models.

Methods: We screened [5,6]00 compounds in a known drug library and a library comprising of 114,000 drug-like compounds using a 293.IRES HIV-specific reporter cell line that contained CMV-CBG/LTR-CBR luciferase reporter system. Hits were identified that activated the LTR-CBR while having a minimal effect on the CMV-CBG reporter. A rigorous selection verification included 11-point titration in the normal and counter-screen assay cell lines, in dsRED-expressing J-Lat cells, and activity in primary cell models of latent HIV.

Results: From this screening cascade two known BET bromodomain and four HDAC inhibitors were found to significantly and specifically activate LTR promoter whereas compounds such as Vorinostat exhibited non-specific activity and increased global transcription. Several drug combinations that target different mechanisms implicated in HIV-1 latency were found to synergistically reactivate the virus with high potency. Importantly, seven novel compound classes were identified in the 114,000 compound library screen. Analogues of these seven classes were obtained and examined in 11-point assay with CMV-CBG/LTR-CBR reporter cell lines and 106 compounds gave a clear indication of early structure-activity relationships.

Conclusions: Seven novel classes of HIV-specific latency purging drugs were found that activate HIV provirus in synergy with a low intrinsic expression of HIV RNA and Tat. These novel small molecule leads warrant further development to iteratively enhance their HIV-1 specificity and potency. We also identified new drug combinations that synergistically activate expression from the latent HIV-1 LTR.