This study explores the potential of a BRD4-selective small molecule modulator (ZL0580) to “block and lock” HIV transcription in brain myeloid cells (BrMCs), a potential HIV reservoir, within the central nervous system (CNS). Primary microglia isolated from fresh autopsy brain tissue was used to test the effects of ZL0580 to block and lock HIV expression. HIV transcription was measured through digital droplet PCR. After a week of treatment, we observed that variable doses of ZL0580 resulted in increased levels of HIV RNA in the supernatants of BrMCs. After prolonged post-treatment of BrMCs with ZL0580 for 16 days, we discovered that HIV latency reversal induced by a combination of LRAs (JQ-1, SAHA, and CM272) was reduced, compared with the untreated control. The data suggest that BrMCs isolated from human brain tissues can be successfully infected with HIV in vitro. Additionally, ZL0580 exhibited a potential to reactivate HIV infection. Thus, our data do not entirely suggest the observations that ZL0580 may enforce HIV into deep latency in the CNS reservoir cells. Further studies are needed to optimize the treatment conditions and test the impact of ZL0580 on HIV transcription and latency. We may also study some new approaches to reduce HIV reservoirs by different killing compounds, including Dexamethasone (DEXA), Polycytidylic acid (Poly I:C), and Nurr1/Nor1 agonist 6-MP. These studies may help us better understand the molecular mechanisms of HIV latency in long-lived BrMCs in people living with HIV on antiretroviral therapy.