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Ingenol efficiently reactivates latent HIV in cells from aviremic patients

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Background: The HIV-1 latent reservoir represents a major barrier to viral eradication in aviremic HIV-1+ patients taking antiretroviral therapy (ART). Here, we describe a careful characterization of the promising LRA properties of Ingenol dibenzoate, panobinostat, and bromosporin, in cells from aviremic patients, including the effects on cellular activation. To accomplish this, we utilized a new-generation, rapid assay performed ex vivo with patient cells.

Methods: We formulated a rapid ex vivo assay using cells from healthy, aviremic HIV-1+ volunteers on stable ART. After phlebotomy, resting CD4+ T cells were isolated via negative magnetic bead purification and cultured in aliquots of 5x10⁶ cells / 1mL RPMI-based culture media. Cell aliquots were exposed to media alone (negative control), individual LRAs at concentrations previously demonstrated to induce viral reactivation, combinations of LRAs with unique mechanisms of action or antibodies against CD3 and CD28 to induce T cell receptor stimulation and cellular activation (positive control). After 48 hours in culture, quantitative rtPCR was performed using both culture supernatant and cell-associated RNA to detect HIV-1 viral mRNA. Cryopreserved cell aliquots from each condition were evaluated by flow cytometry for biomarkers of drug activity, cellular activation and toxicity.

Results: Ingenol 3,20-dibenzoate, a PKC α agonist, demonstrated viral reactivation comparable to CD3/28 antibody stimulation (median reactivation = 49% of positive control). CD69, an early marker of T cell activation known to be up-regulated by ingenol, increased in all cell aliquots exposed to ingenol (MFI = 81% of positive control). Bromosporin, a BRD inhibitor, and Panobinostat, an HDAC inhibitor, demonstrated modest activity in a subset of patients.

Conclusions: Because of the diversity of cell culture models of latency and the lack of uniformity in the responses across models, we made an effort to develop a rapid ex vivo assay using cells from aviremic HIV-1+ patients to confirm bioactivity and characterize latency-reversing potential of candidate LRAs individually and in combination. This assay was used to characterize the activity of Ingenol as an exciting LRA candidate, as it combines a potent reactivation ability with a very low toxicity profile, which sets it apart from other PKC agonists that are, unfortunately highly toxic.