Oral Abstract Session 2: Activating latent HIV infection in vitro and in vivo

OA2-1

Histone deacetylase inhibitors alter the accumulation of spliced HIV mRNA - implications for virus production

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Background: Clinical trials in HIV-infected patients on antiretroviral therapy with histone deacetylase inhibitors (HDACi) have demonstrated an increase in cell-associated unspliced (CA-US) HIV RNA, variable changes in plasma HIV RNA and no change in the number of latently infected cells. We aimed to define the effects of latency reversing agents (LRAs) on HIV mRNA splicing.

Methods: Resting CD4+ T cells isolated from the blood of HIV-negative individuals were treated with the chemokine CCL19 and infected with wild type HIVNL4.3 to establish latency (n=5). Latently infected CCL19-stimulated cells were then cultured with vorinostat, romidepsin, JQ1, romidepsin+JQ1 or PMA/PHA, all in the presence of an integrase inhibitor (L8). Cells and supernatant were harvested at 6, 24, 48, and 72 hours. Reverse transcriptase (RT) was quantified in supernatant and CA-US and multiply spliced (MS) HIV RNA were quantified by real time qPCR.

Results: In latently infected CCL19-treated CD4+ T-cells, stimulation with PMA/PHA led to a significant exponential increase in both US-RNA and MS-RNA, and by 48 hours reached a mean fold increase above baseline of 80-fold for US-RNA and 56-fold for MS-RNA ($p=0.03$ for both, relative to DMSO). There was a significant increase in RT in supernatant following stimulation with PMA/PHA but no change following any LRA (n=2). In contrast, following stimulation with each LRA, there was only a modest increase in CA-US RNA that was not statistically significantly different from DMSO ($p=0.56$). MS-RNA increased transiently (mean 2.7-fold change at 6hr with romidepsin) and then significantly declined over time following treatment with romidepsin and romidepsin+JQ1 ($p=0.02$ and 0.002 respectively), with a mean fold reduction by 72 hours compared to baseline of 0.15-fold and 0.17-fold respectively ($p=0.02$ for both compared to DMSO) in the absence of any cellular cytotoxicity.

Conclusions: In this in vitro model of latency, PMA/PHA and the potent HDACi romidepsin had strikingly different effects on the accumulation of US-RNA, MS-RNA and virus production. While successful HDACi agents yield small increases in US-RNA, synergistic strategies that achieve a larger accumulation of MS RNA may result in enhanced release of latent HIV.