Panobinostat dosing has broad but transient immunomodulatory effects in HIV-patients

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BACKGROUND

Antiretroviral therapy (ART) effectively suppresses viral replication and partially restores immune functions in human immunodeficiency virus type-1 (HIV-1) infected individuals. However, HIV-1 integrates into the host DNA, thus establishing the basis for latent infection. As ART cannot eliminate transcriptionally inactive or latent virus, adjunctive interventions that efficiently activate latent virus are needed to achieve the ultimate goal of a cure for HIV-1 infection. We recently presented data highlighting the latency reversal properties of panobinostat (PANO) (Rasmussen et al Lancet HIV 2014). To evaluate the immunomodulatory effects from HDACi in HIV-infected patients, we investigated a broad range of immune pathways during a latency reversal trial with PANO. Further, as prolonged epigenetic modulation from HDACi treatment has been raised as safety concern, we evaluated gene expression alterations up to 24 weeks after end of PANO dosing.

METHODS

Using flow cytometry, we investigated the impact of PANO on T cell activation (CD69, HLA-DR), T cell exhaustion (PD-1). Further, levels of activated regulatory T cells and their expression of suppressive markers (CD39 and CTLA4) were assessed. To determine broad changes in immune responsiveness to common stimuli, whole blood stimulations with LPS were performed and inflammatory responses by cytokine release were determined using luminex. Lastly, gene expression from purified PBMC's was evaluated using affymetrix HTA 2.0 gene chip. Analysis of probe data was performed by Limma with FC>1.5 and FDR<0.05.

RESULTS

A rapid increase in proportions of both CD4 and CD8 T cells expressing CD69 were observed ($p<0.001$) as early as 24 hrs after first PANO dosing. This was followed by a marked increase in HLA-DR+ CD4 and CD8 T cells ($p<0.001$) observed at day 4. At the same time point, proportions of activated regulatory T cells increased by 40 % four days after treatment initiation ($p<0.01$) and MFI of the suppressive markers CD39 and CTLA4 increased by 12% ($p<0.01$) and 25 % ($p<0.001$), respectively. LPS-induced inflammatory responses as determined by IL-1b, IL-12p40, IL-6 and TNF-a secretion were all significantly down regulated four days after dosing. Evaluating the spontaneous release of cytokines in the whole blood assay revealed a striking observation that IL-18, IFN-g and IP-10 were all significantly secreted from cells on drug compared to baseline.
Importantly, all these PANO-induced immunomodulatory effects were reversible and all markers had returned to pre-treatment levels 4 weeks after end of PANO dosing. Lastly, PANO induced significant changes in the overall gene expression pattern (fold-change >1.5, FDR-corrected $p<0.05$). From baseline to on drug there were 421 differentially regulated transcripts. These alterations in gene expression had regressed considerably by week 4 after end of PANO treatment with only 16 transcripts significantly down-regulated. These changes had normalized entirely by week 24 post-PANO therapy.

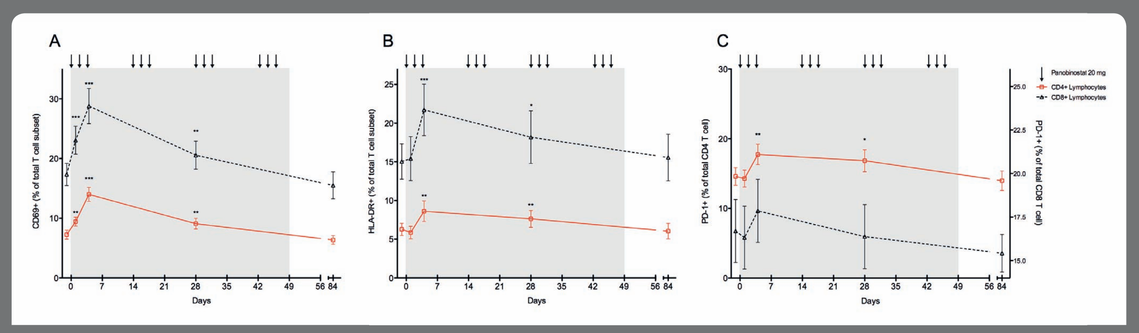

Figure 1.
Lymphocytes (CD3+ and either CD4+ or CD8+) were evaluated for the expression of activation markers A) CD69 and B) HLA-DR as well as the immune exhaustion marker C) PD-1. Levels following dosing were compared to baseline in a paired t-test analysis. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

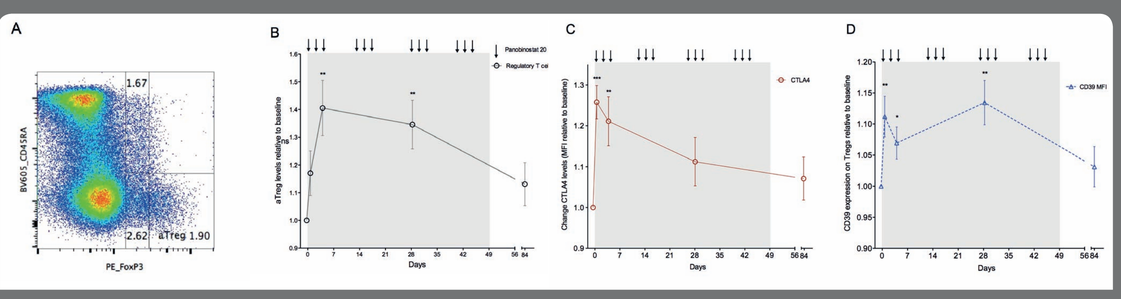

Figure 2.
T regulatory T lymphocytes were defined as CD3+, CD4+, CD45RA- and FoxP3+ as indicated in A). The levels of these activated Treg following PANO dosing is depicted in B). Expression of the suppressive markers C) CTLA-4 and D) CD39 following dosing were compared to baseline in a paired t-test analysis. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

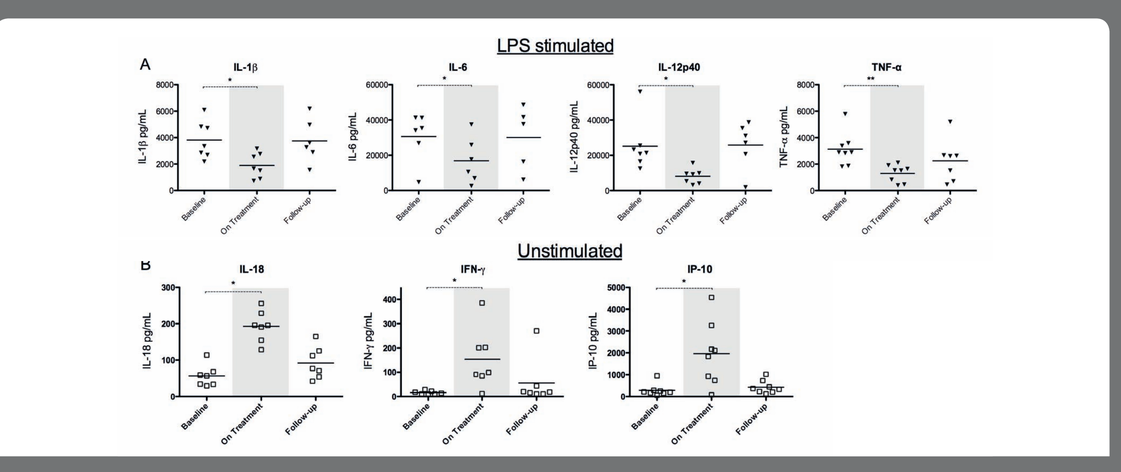

Figure 3.
Whole blood from patients either stimulated with 1 ug/mL LPS (Top panel) or left with media alone (Bottom panel) for 24 hrs. Supernatant harvested and assayed by Luminex. Baseline compared to following dosing with PANO using Wilcoxon matched-pairs signed rank test. *$p<0.05$ **$p<0.01$. Note only 7 evaluable subjects.

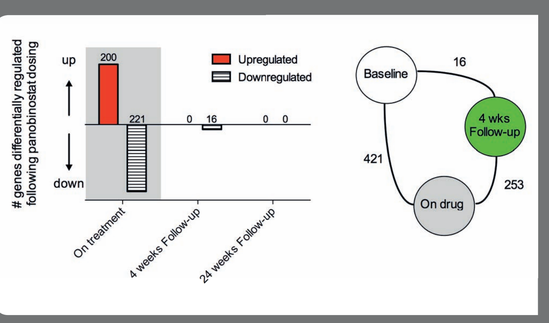

Figure 4.
Graphic representation of gene expression changes from baseline following PANO dosing. On treatment is day 5 (following the first three doses). Analysis of probe data was performed by Limma with FC>1.5 and FDR<0.05.

CONCLUSIONS

PANO significantly but transiently influenced T cells activation status, regulatory T cell phenotype and functional mitogen responsiveness. All measures of immune function had returned to baseline levels 4 weeks after completion of PANO and long-term follow-up revealed no sustained effect on overall gene expression. Collectively, the results suggest that PANO does not cause persistent detrimental epigenetic or immunomodulatory changes in HIV patients.

Layout: Department of Communication, Aarhus University Hospital • MT0215EA

References:
Rasmussen, TA., Tolstrup, M., Brinkmann, CR et al. Panobinostat, a histone deacetylase inhibitor, for latent virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. Lancet HIV 2014.