**In vivo effects of panobinostat and romidepsin on HIV-1-specific CD8+ T cell immunity**

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**BACKGROUND**

Panobinostat (the CLEAR trial; Rasmussen et al. Lancet HIV 2014) or romidepsin (the REDUC trial; IAS 2014) were administered to virologically suppressed HIV-1 infected adults on ART (Table 1). In two clinical trials, treatment of HIV-1 infected patients with panobinostat or romidepsin did not decrease the levels of HIV-1 specific EM and TD CD8+ T cells. Further, we observed no decrease in the levels of IFNγ and TNFα produced by HIV-specific CD8+ T cells in response to HLA-A2 restricted epitope peptides.

**METHODS**

Vital study eligibility criteria and enrolment (CLEAR trial). Panelists, as well as indicators of viral suppression (AIDS-Defining conditions), were administered to endocrine syndromes. Patients with diabetes were subjected to the below-described analyses (Figure 1A).

**RESULTS**

The effect of romidepsin treatment on the levels of cytokines produced by HIV-specific CD8+ T cells was assessed in two clinical trials, the CLEAR trial and the REDUC trial (Fig. 2). In the CLEAR trial, patients were divided into two groups: the control group (n=5) was administered no treatment, while the treatment group (n=6) was administered romidepsin. The levels of cytokines produced by HIV-specific CD8+ T cells were measured using a cytokine array. The data were analyzed using a statistical software package (SPSS). The results were expressed as a mean ± standard deviation. The significance of differences between the two groups was assessed using an unpaired t-test. The significance level was set at p < 0.05.

**ACKNOWLEDGMENTS**

The CLEAR and REDUC trials were funded by a grant from the Research Council of Norway (GLOBVAC) program (Nr: 208317/V10). The CLEAR trial was sponsored by the SSAC Foundation and Aarhus University, Aarhus University Hospital. The REDUC trial was sponsored by the MAIA Foundation, the GLOBVAC program (NR: 208317/V10), and Aarhus University Hospital. Informed consent was obtained from all participants before the start of the study. The study was conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. The study was approved by the local ethics committee.

**CONCLUSIONS**

In two clinical trials, treatment of HIV-1 infected patients with panobinostat or romidepsin did not decrease the levels of HIV-1 specific CD8+ T cells. Further, we observed no decrease in the levels of IFNγ and TNFα produced by HIV-specific CD8+ T cells in response to HLA-A2 restricted epitope peptides. These results are consistent with earlier observations that neither panobinostat nor romidepsin blunts the HIV-specific CD8+ T cell response in vivo, supporting their use in combination with therapeutic vaccination in future clinical trials.
**In vivo effects of panobinostat and romidepsin on HIV-1-specific CD8+ T cell immunity**

**BACKGROUND**

In two clinical trials, treatment of HIV-1 infected patients with panobinostat or romidepsin did not result in the reactivation of HIV-1 latent infection. Here, we evaluated HIV-1-specific CD8+ T cells and memory subset composition before and after treatment with panobinostat or romidepsin.

**METHODS**

We performed a retrospective analysis of two phase II trials: the CLEAR trial and the REDUC trial. Total CD8+ T cell counts and memory subset distribution were measured in 23 HIV-1-infected patients before, during, and after treatments with panobinostat or romidepsin. The effect on the frequencies of intracellular cytokine (IFN-γ and TNF-α) producing CD8+ T cells was evaluated by intracellular cytokine staining (IFN-γ and TNF-α) was evaluated by intracellular cytokine staining (IFN-γ and TNF-α).

**RESULTS**

- During panobinostat treatment we saw no statistically significant change in the levels of staphylococcal enterotoxin B (SEB)-responsive CD8+ T cells (Figure 4A-B). This was true for both EM and TD subsets. The effect on the frequencies of intracellular cytokine (IFN-γ and TNF-α) producing CD8+ T cells was evaluated by intracellular cytokine staining (IFN-γ and TNF-α).
- We observed no statistical significant changes in total CD8+ T cell counts and memory subset distribution during panobinostat treatment (Figure 3A-B).
- CDs4 T cell counts and memory subset distribution before, during and after panobinostat treatment (Figure 3A-B) or romidepsin treatment (Figure 3C-D). Red represents limit for detection of positive response.

**ACKNOWLEDGMENTS**

The CLEAR trial was partly funded by the American Foundation for AIDS Research (AmfAR), the Danish Council for Strategic Research, but also received contributions from the Danish AIDS Foundation, The Novo Nordisk Foundation, and Aarhus University Hospital. The REDUC trial was partly funded by the Research Council of Norway (231474/200) and the SSAC Foundation and Aarhus University Hospital. The REDUC trial (amfAR), The Danish Council For Strategic Research, but also received contributions from the SSAC Foundation and Aarhus University Hospital. The REDUC trial (amfAR), The Danish Council For Strategic Research, but also received contributions from the SSAC Foundation and Aarhus University Hospital.

**CONCLUSIONS**

In two clinical trials, treatment of HIV-1 infected patients with panobinostat or romidepsin did not alter the levels of IFN-γ and TNF-α production by virus-specific CD8+ T cells in response to HIV gag peptide. These results are in contrast to recent reports showing that panobinostat and romidepsin activated HIV-1 latency. Further investigation is needed to determine the impact of these HDAC inhibitors on the levels of IFN-γ and TNF-α produced by CD8+ T cells in response to HIV gag peptide.
In vivo effects of panobinostat and romidepsin on HIV-1-specific CD8+ T cell immunity

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BACKGROUND

In clinical trials, HDAC inhibitors including panobinostat and romidepsin, are currently being evaluated in experimental clinical trials for their ability to induce reverse transcriptase in infected cells. In vitro studies have demonstrated that HDAC inhibitors are able to induce HIV expression in latently infected cells with the overall aim of decreasing the latent HIV reservoir. The rationale for targeting the latent HIV reservoir through disrupting HDAC complexes is supported by evidence from both experimental and clinical studies showing that HDAC inhibitors can reactivate the virus-encoding cells and induce the expression of viral antigens in an HIV-specific immune response. However, recent studies have suggested that HDAC inhibitors may also have an effect on the in vivo CD8+ T cell response, which is necessary for productive vaccine development.

METHODS

We performed a double-blind, placebo-controlled, randomized trial (the CLEAR trial) or romidepsin (the REDUC trial) to induce a significant increase in HIV transcription in CD8+ T cells, as well as increase in HIV-specific CD8+ T cell responses. We included patients with CD8+ T cell immunity and excluded patients with CD8+ T cell immunity above 30%. We evaluated CD8+ T cell responses in patients with CD8+ T cell immunity above 30%.

RESULTS

In two clinical trials, treatment of HIV-1 infected patients with panobinostat or romidepsin did not significantly affect the levels of HIV-1 specific CD8+ T cells.

ACKNOWLEDGMENTS

The CLEAR and the REDUC trial were supported by grants from the American Foundation for AIDS Research (AFAR) and the Danish Medical Research Council (DAN130114).

CONCLUSIONS

In two clinical trials, treatment of HIV-1 infected patients with panobinostat or romidepsin did not significantly affect the levels of HIV-1 specific CD8+ T cells. Further studies are needed to determine the levels of IFN-γ and TNF-α induced by the HIV-1 specific CD8+ T cells in response to HIV gag peptides. These results are consistent with earlier studies that show paradoxical CD8+ T cell responses in vivo, supporting their use in combination with therapeutic vaccination in future clinical trials.
In vivo effects of panobinostat and romidepsin on HIV-1-specific CD8+ T cell immunity

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BACKGROUND

HDAC inhibitors (HDACi) including panobinostat and romidepsin, are currently being evaluated in experimental viral models for their ability to induce transcriptional reprogramming of viral elements in latently infected cells, leading to the reactivation of latent HIV infection. In humanized mice, HDACi have been shown to activate latent HIV reservoirs, providing evidence that HDACi may diminish effector functions of CD8+ T cells, thus impairing the elimination of virus-expressing cells (Jones et al. 2014 PLoS. Pathogens).

In vivo γ-δ T cells stained with viability dye followed by surface staining (CD8, CCR7 and CD45RA) and intracellular staining with IFN-γ and TNF-α memory and terminally differentiated cells and primarily produced only IFN-γ (naïve phenotype producing only IFN-γ and TNF-α) and terminally differentiated cells and primarily produced only IFN-γ (naïve phenotype producing only IFN-γ and TNF-α). The effect of panobinostat or romidepsin treatment on the frequencies of HIV-specific EM and TD CD8+ T cells.

METHODS

Total of 14 patients participated and were enrolled in the REDUC trial (NTC02092116). Samples were analyzed on a BD Fortessa and 6 out of 6 patients showed significant increase in HIV transcription in CD4+ T cells, as well as increase in HIV viral load, providing evidence that panobinostat and romidepsin activate latent HIV from latency. We evaluated panobinostat CD8+ T cell responses during clinical administration of panobinostat and romidepsin.

RESULTS

The effect of panobinostat or romidepsin on the frequencies of HIV-specific CD8+ T cell responses during clinical administration of panobinostat and romidepsin. The REDUC trial: at baseline (base, n=14), on treatment: early (PANO-1, n=6) and late (PANO-2, n=13) and at follow-up (post, n=13). (C-D) The CLEAR trial: at baseline (base, n=14), on treatment: early (PANO-1, n=6) and late (PANO-2, n=13) and at follow-up (post, n=13).

In both trials the majority of the HIV-specific CD8+ T cells were effector memory and terminally differentiated cells and primarily produced only IFN-γ (naïve phenotype producing only IFN-γ and TNF-α) and terminally differentiated cells and primarily produced only IFN-γ (naïve phenotype producing only IFN-γ and TNF-α). The effect of panobinostat or romidepsin treatment on the frequencies of HIV-specific EM and TD CD8+ T cells.

ACKNOWLEDGMENTS

The CLEAR and the REDUC trial were funded by the American Foundation for AIDS Research (AFAR), the Doris Duke Charitable Foundation, and the National Institutes of Health (NIH). The researchers would like to thank all the patients and healthy volunteers who participated in the studies. The researchers would also like to thank all the technicians who contributed to the successful execution of the studies.

CONCLUSIONS

In two clinical trials, treatment of HIV-infected patients with panobinostat or romidepsin did not change the levels of IFN-γ and TNF-α, as measured by ELISA. Further, we observed no increase in the levels of IFN-γ and TNF-α cytokines produced by the HIV-1 specific CD8+ T cells in response to HIV gag peptide. These results were consistent in that neither panobinostat nor romidepsin blunted the HIV-1 specific CD8+ T cell response in vivo, supporting their use in combination with therapeutic vaccination in future clinical trials.