Viral reservoir disruption with Panobinostat and IFN-α: First results

Ciputra Adijaya Hartana[1], Theresa Flynn[2], Amy Sbrolla[2], Carina Bannach[1], Jane E. Blackmer[1], Joshua M. Chevalier[1], Pilar Garcia-Broncano[1], Xu G. Yu[1,3], Rajesh T. Gandhi[2], Daniel Kuritzkes[2,3], Mathias Lichterfeld[1,2,3]

Affiliations: [1]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA; [2]Massachusetts General Hospital, Boston, MA, USA; [3]Brigham and Women's Hospital, Boston, MA, USA

Poster # 0341

Supported by NIH/NIAID under U01 AI117841

BACKGROUND

- Upon reactivation of viral transcription, viral reservoir cells are sensitized to immune-mediated killing resulting in the reduction of long-term persistence of virally-infected CD4⁺ T cells in ART-treated individuals.
- The ACTIVATE study is an ongoing, prospective, randomized, dose-escalation clinical trial, which administered histone deacetylase inhibitor (HDACi) Panobinostat as a latency-reversing agent in combination with pegylated IFNα2a as an innate immune modulator.

METHODS

Figure 1. Study outline

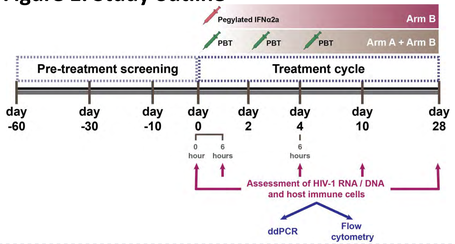

Figure 1: The framework of the study. ART-treated participants were randomized to receive three consecutive doses of 5mg (phase I) or 10mg (phase II) of panobinostat alone (Arm A, n=2 participants in stages I and II each), or in combination with one dose of pegylated IFNα2a (Arm B, n=6 participants in stages I and II each). At multiple timepoints during study drug administration, cell-associated HIV-1 RNA from the CD4⁺ T cells were quantified using droplet digital PCR (ddPCR)[1], meanwhile HIV-1 DNA was evaluated using the intact proviral DNA assay (IPDA)[2]. Moreover, innate and adaptive immune responses and acetylated H3 expression were analyzed by flow cytometry.

Figure 2. The expression of acetylated histone H3 in different treatment timepoints

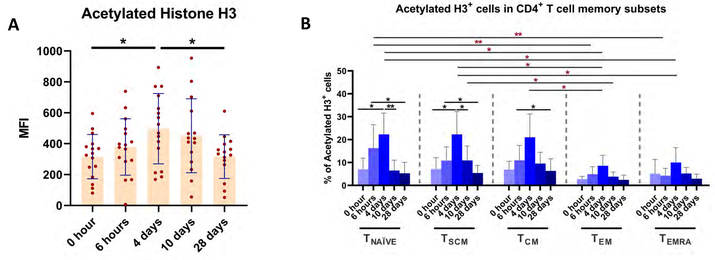

Figure 2: Increased expression of acetylated histone H3, notably in T_{naive}, T_{SCM} and T_{CM} CD4⁺ T cells after 3 doses of Panobinostat. (A) The expression of acetylated histone H3 in total lymphocytes is shown as mean fluorescence intensity (MFI) as measured by flow cytometry. The five treatment timepoints from both Arm A and Arm B (n=16) are compared. The values are mean ± SD. *P* values were calculated by repeated measures ANOVA. **(B)** Comparison of acetylated H3⁺ cell frequencies among various memory CD4⁺ T cell subsets in different treatment timepoints from both Arm A and Arm B (n=16). The values are mean ± SD. *P* values among timepoints were calculated by repeated measures ANOVA (black asterisk). *P* values among memory subsets were calculated by one-way ANOVA (red asterisk). *p<0.05; **p<0.01

RESULTS

Figure 3. HIV-1 RNA expression following panobinostat administration

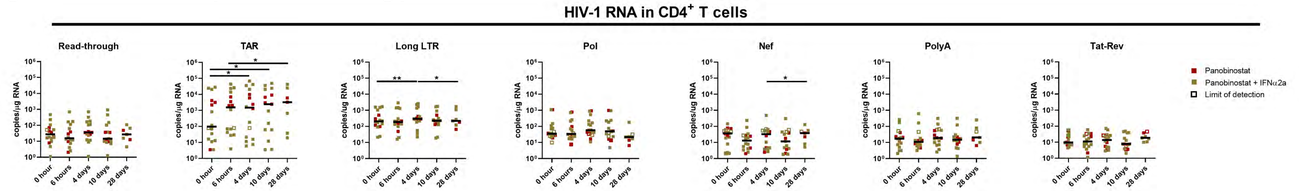

Figure 3: HIV-1 gene transcription increased after the administration of panobinostat. The seven HIV-1 RNA transcripts (Read-through, TAR, Long LTR, Pol, Nef, PolyA and Tat-Rev)[1] were measured by droplet digital PCR (ddPCR). These HIV-1 transcripts were compared among treatment timepoints in Arm A (n=4; red squares) and Arm B (n=12; brown squares). Median values are represented as black line. *P* values were calculated by Wilcoxon signed-rank test. *p<0.05; **p<0.01

Figure 4. The frequency of NK cell activation markers CD38 and NKp30

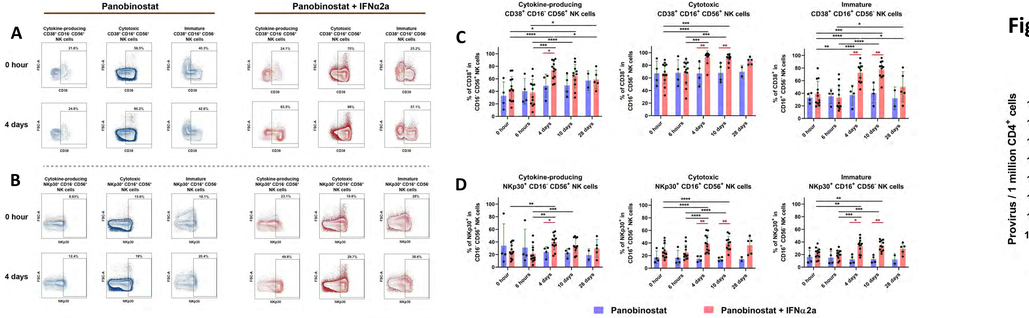

Figure 4: The frequency of cells expressing activation markers CD38 and NKp30 increased in cytokine-producing, cytotoxic and immature NK cells. (A) The contour plot showing the expression and frequency of CD38 in cytokine producing (CD16⁻ CD56⁺), cytotoxic (CD16⁺ CD56⁺) and immature (CD16⁺ CD56⁻) NK cells from a representative patient as measured by flow cytometry. The gating was based on fluorescence minus one (FMO) control. **(B)** The contour plot of NK cell subsets expressing NKp30 as in (A). **(C)** The frequency of CD38⁺ NK cells in cytokine producing (CD16⁻ CD56⁺), cytotoxic (CD16⁺ CD56⁺) and immature (CD16⁺ CD56⁻) subsets. **(D)** The frequency of cells expressing NKp30 in NK cell subsets as in (C). The frequency of CD38⁺ and NKp30⁺ NK cells were compared among the five treatment timepoints from Arm A (n=4; blue bars) and Arm B (n=12; red bars). The values are mean ± SD. *P* values among timepoints were calculated by repeated measures ANOVA (black asterisk). *P* values between treatment arms were calculated by Mann-Whitney test (red asterisk). *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Figure 5. Polyfunctionality of HIV-1 specific CD4⁺ T cells

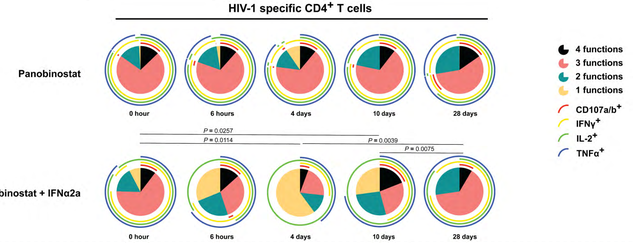

Figure 5: The proportion of IL-2-producing HIV-1-specific CD4⁺ T cells increased during treatment with IFNα2a, while IFNγ secreting CD4⁺ T cells were reduced. The frequency of HIV-1 specific CD4⁺ T cells co-expressing CD107a/b, IFNγ, IL-2 and TNFα was measured by flow cytometry. The net value was calculated by subtracting the frequency of cytokine⁺ cells measured in DMSO control (negative control), analyzed using Boolean gating and run on the SPICE program. The cytokine expression was compared among the five treatment timepoints from Arm A (n=4; top rows) and Arm B (n=12; bottom rows). *P* values among timepoints were calculated by permutation test.

Figure 6. HIV-1 DNA levels during treatment

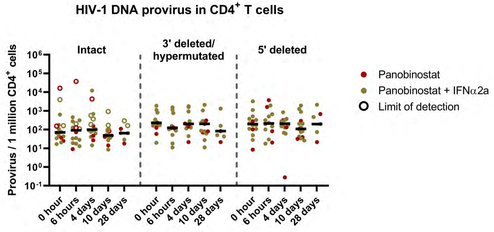

Figure 6: There were no visible changes in HIV-1 DNA levels among timepoints and between treatment arms. The HIV-1 DNA profile was measured by intact proviral DNA assay (IPDA)[2]. The HIV-1 DNA proviral status was determined as intact, 3' deleted/hypermutated or 5' deleted and was compared among treatment timepoints in Arm A (n=4; red dots) and Arm B (n=12; brown dots). Median values are represented as black line. Repeated measures ANOVA was use as the statistical test.

CONCLUSIONS

First results from the ACTIVATE study indicate that:

- ✓ **The study medication induces HIV-1 transcription and augments innate and adaptive immune effector cells.**
- ✓ **No visible effect on HIV-1 DNA levels is seen in our current analysis.**

Phase III in which panobinostat is administered at a dose of 15mg is currently ongoing.

REFERENCES

[1]Yukl, S.A., et al. 2018. HIV latency in isolated patient CD4⁺T cells may be due to blocks in HIV transcriptional elongation, completion, and splicing. Sci. Transl. Med. 10(430)

[2]Bruner, K.M., et al. 2019. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature* 566, 120–125.