Aminobisphosphonates reverse latency in HIV-seropositive individuals

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BACKGROUND

- Aminobisphosphonates (N-BPs), such as Pamidronate (PAM), zoledronate (Zol) and Alendronate (ALN) are approved for the treatment of bone related diseases. They inhibit the formation of farnesyl pyrophosphate groups, used for protein prenylation.
- This post-translational modification is critical for the proper function of certain proteins involved in cell signaling and HIV replication.
- We hypothesized that N-BPs may disrupt HIV latency, and thus here we explore their potential as a novel latency reversing agent.

METHODS

***Ex vivo*:**

- PBMCs from HIV-seropositive donors on suppressive ART
- Unspliced HIV caRNA transcripts were measured in isolated rCD4 cells from nine participants by qPCR.
- Quantitative viral outgrowth assay (QVOA) was performed using isolated rCD4 cells from seven donors.
- Mechanism of action was investigated in rCD4 cells from seven participants by RNA-seq.

***In vivo*:**

- We obtained frozen PBMC and plasma samples (basal / week 2 / week 24 / week 48) from the clinical trial ACTG A5163 (2004-2007) that analyzed the effect of weekly ALN on bone mineral density in individuals on ART:
  - Arm A: ALN + 500mg calcium carbonate + vitamin D
  - Arm B: Placebo + 500mg calcium carbonate + vitamin D
- We measured caRNA and total HIV DNA levels in PBMCs.

RESULTS

N-BPs induce perturbations of the latent HIV reservoir *in vivo*

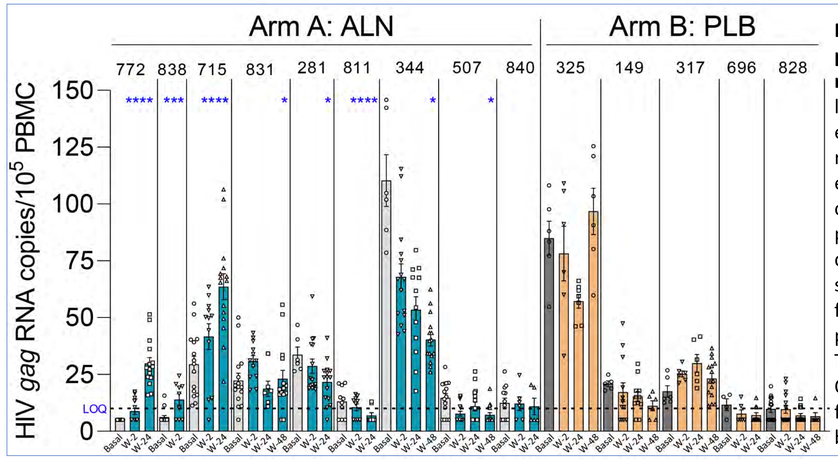


Figure 2. N-BPs induce perturbations on the latent HIV reservoir *in vivo*. HIV caRNA levels were altered overtime in eight of nine individuals who received ALN. Four participants experienced an increase compared to basal and four participants experienced a decrease. One individual did not show alterations. Participants from the PLB arm did not show perturbations in caRNA levels. Two-sided Mann-Kendall test. Overtime levels in participants from ALN arm represented in blue and PLB overtime in orange.

Reduction in total HIV DNA levels in three participants treated with ALN *in vivo*

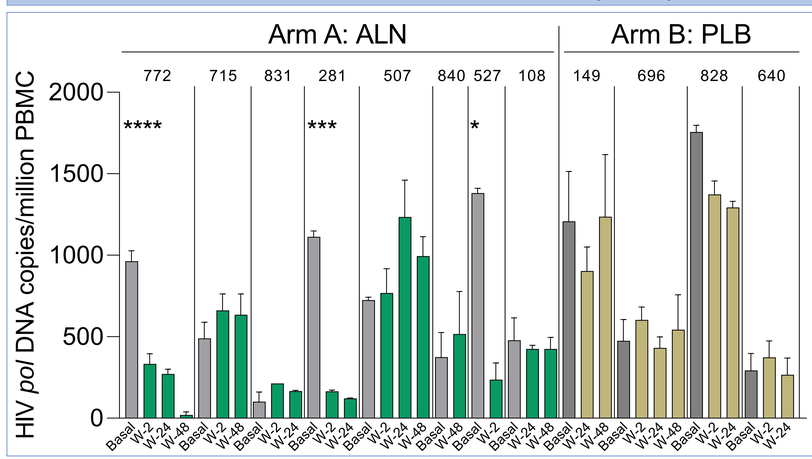


Figure 3. Quantification of total HIV DNA levels *in vivo*. ALN treatment resulted in a mean of 2.9 to 49.1-fold decrease in HIV DNA levels in three of eight participants treated with ALN. Total HIV DNA levels remained unmodified in four individuals tested from Arm B. Two-sided Mann-Kendall test.
Limited sample availability prevented us to measure caRNA and HIV DNA levels in the same participants.

N-BPs reactivate latent HIV *ex vivo*

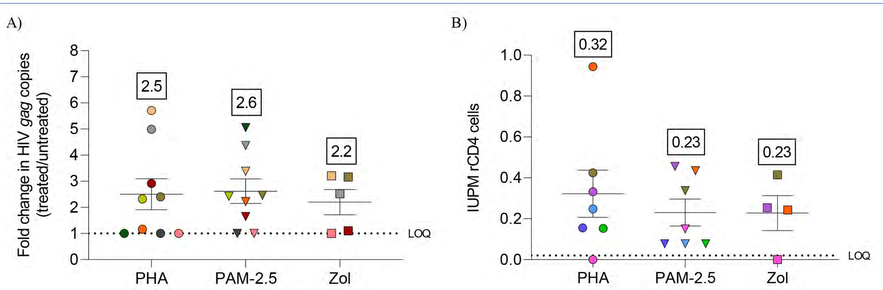


Figure 1. N-BPs induce reactivation of latent HIV in rCD4 cells. A) Comparable HIV caRNA levels between the positive control and both PAM and Zol. **B)** Recovery of replication-competent HIV from QVOA in samples treated with N-BPs and PHA were similar. Wilcoxon-paired signed rank tests.

Aminobisphosphonates are a novel class of latency reversing agents that led to a decrease in the latent reservoir of some ART-suppressed HIV-seropositive participants *in vivo*

Mechanism of action of N-BPs

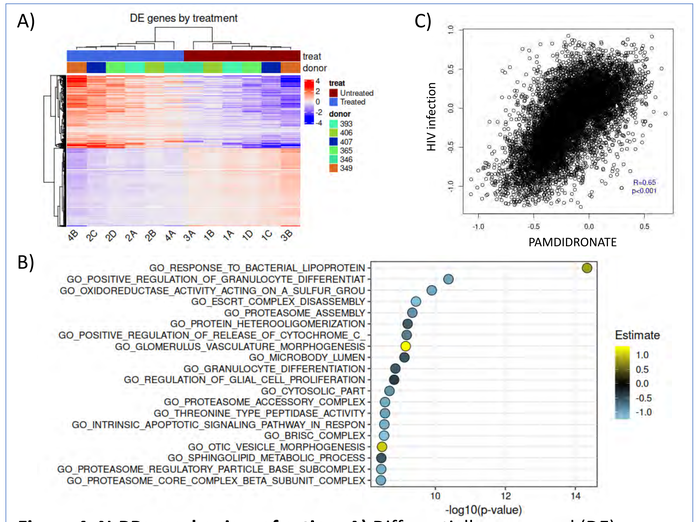


Figure 4. N-BPs mechanism of action. A) Differentially expressed (DE) genes in paired untreated and PAM-treated samples from seven individuals. Wald's statistic conditioned on donor, q-value<0.05, FDR correction and **B)** Top differentially expressed pathways. GO gene sets, q-value<0.05, FDR correction. **C)** Strong correlation between pathways altered by HIV infection and pathways targeted by PAM treatment. Wald's statistic.

CONCLUSIONS

- We demonstrate the novel use of N-BPs as potent latency reversing agents that induce reactivation of persistent HIV both *ex vivo* and *in vivo*. Ongoing work is focused on finding immune correlates to explain positive and negative trends in caRNA levels.
- We present the first evidence in humans that treatment with a single agent is able to reduce the frequency of persistent HIV infection *in vivo*.
- Correlation between pathway coefficients for HIV infection and PAM demonstrates that pathways are altered similarly.

Our study shows that the "shock and kill" strategy may be a feasible approach that could lead to sustained ART- free HIV remission or cure.

ADDITIONAL KEY INFORMATION

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