**Background:** HIV-1 persists in a latent reservoir despite ART, and reactivation of this reservoir has been proposed as a cure strategy. Effective drug combinations that achieve high levels of viral suppression in ex vivo tests can delay reactivation of this reservoir. Here, we report the identification of drug combinations using sorting CD4+ T cells (cCD4s) from infected individuals and use ex vivo measurements to predict in vivo efficacy.

**Results:** We performed an ex vivo latency reversal assay using two strategies: (1) combination of single LAAs or a combination of two LAAs, presented as fold induction relative to DMSO control; and (2) induction of intracellular HIV-1 mRNA by single LAAs. PKC–agonist–containing LAAs combinations presented a synergistic effect of maximal reactivation with PMA + JQ1. Data points represent the mean effect of 2 or 3 replicate LRA treatments of 5 million cells for each individual. For panels A and B, statistical significance was calculated from the HIV-1 mRNA copy number values by using a paired T test compared to (a) the DMSO control, (b) bradykinin-1 or prostratin alone, or (c) combination LAAs.

**Conclusion:** Using multiple assays for latency reversal, we have completed the first ex vivo comparative study to identify highly effective LRA combinations. We demonstrated that select PKC–agonist-containing combinations reverse latency at levels approaching those seen with maximal T cell activation. These combinations did not induce the release of proinflammatory cytokines. Using our ex vivo measurements of virus production in response to LAAs, we then predicted in vivo changes in viral load following LRA treatment. In a realistic clinical scenario, viral load would be expected to decay immediately after LRA activity ceases. In the most conservative scenario considered by our model, plasma viral loads of over 100 copies/mL are predicted for all treatments we investigated that contain a PKC agonist.

**Methodology:**

**Correlation between intracellular and extracellular HIV-1 mRNA levels after ex vivo treatment**

Using multiple assays for latency reversal, we have completed the first ex vivo comparative study to identify highly effective LRA combinations. We demonstrated that select PKC–agonist-containing combinations reverse latency at levels approaching those seen with maximal T cell activation. These combinations did not induce the release of proinflammatory cytokines. Using our ex vivo measurements of virus production in response to LAAs, we then predicted in vivo changes in viral load following LRA treatment. In a realistic clinical scenario, viral load would be expected to decay immediately after LRA activity ceases. In the most conservative scenario considered by our model, plasma viral loads of over 100 copies/mL are predicted for all treatments we investigated that contain a PKC agonist.

**Conclusions:**

**Acknowledgements and Funding:** We thank the study participants without whom this research would not be possible. We also thank Linda Aston, Adam Longworth, Holly McHugh, and Anitha Devasseri for assistance with some experiments. Funding: Martin Delaney CARE and AidsLIFE Collaboratives (NIH AI096113, 1U1A096109), amfAR 108165-50-RQRL, Johns Hopkins CFAR, NIH grant RO143222, Howard Hughes Medical Institute, NIH grant F31AI116316 (in support of G.M.L.

**Study approval:** The Johns Hopkins Institutional Review Board granted approval for this study. All research participants enrolled in this study provided written informed consent prior to inclusion in this study.