Ex vivo identification of highly effective latency-reversing drug combinations

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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 HIV-1 latency reversal will likely be required for a cure. We set out to identify highly effective combinations using resting CD4+ T cells (rCD4s) from infected individuals and use our ex vivo measurements to predict in vivo efficacy.

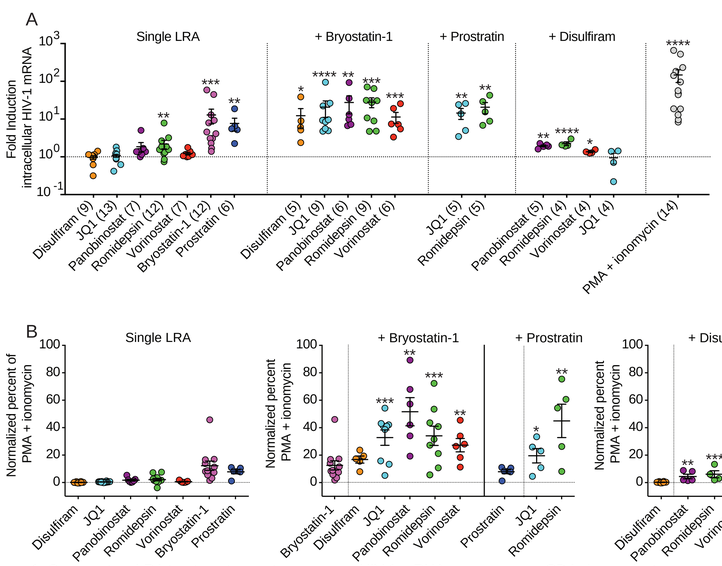


Figure 1. Combination LRA treatment robustly increases HIV-1 mRNA expression in rCD4s from infected individuals on ART. (a) Intracellular HIV-1 mRNA levels in rCD4s, obtained from infected individuals and treated ex vivo with a single LRA or a combination of two LRAs, presented as fold induction relative to DMSO control.(b) Induction of intracellular HIV-1 mRNA by single LRAs, PKC-agonist–containing LRA combinations, and disulfiram–containing LRA combinations presented as a percent of the effect of maximal reactivation with PMA + ionomycin. 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 using a ratio paired T test compared to (a) the DMSO control, (b) bryostatin-1 or prostratin alone, or disulfiram alone.

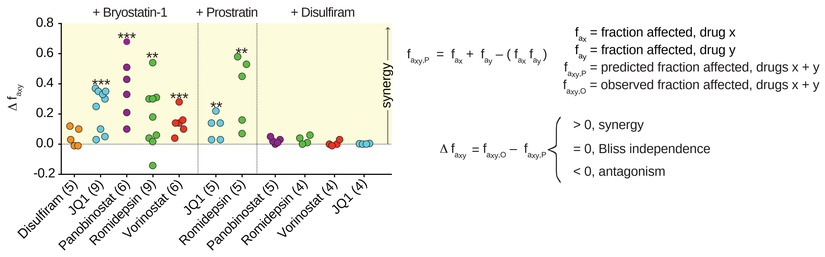


Figure 2. PKC agonists synergize with JQ1 and with HDAC inhibitors to significantly increase HIV-1 mRNA expression in rCD4s from infected individuals on ART. Calculation of synergy for LRA combinations using the Bliss independence model. Numbers in parentheses indicate number of individuals used for each treatment. Data points represent the mean effect of 2 or 3 replicate LRA treatments of 5 million cells for each individual. Statistical significance for the experimental fa was calculated using ratio paired T test compared to the predicted fa for each combination.

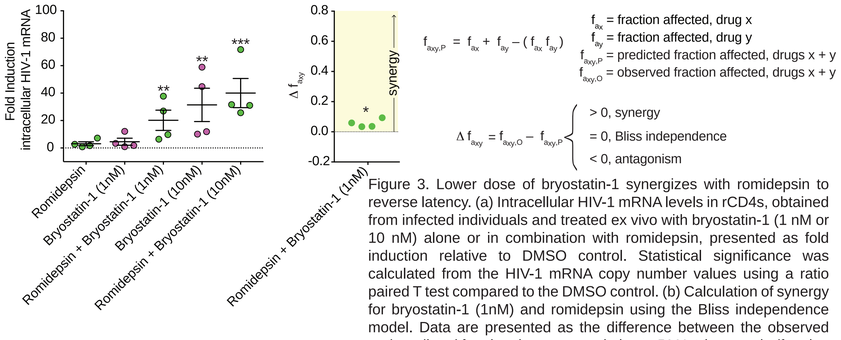


Figure 3. Lower dose of bryostatin-1 synergizes with romidepsin to reverse latency. (a) Intracellular HIV-1 mRNA levels in rCD4s, obtained from infected individuals and treated ex vivo with bryostatin-1 (1 nM or 10 nM) alone or in combination with romidepsin, presented as fold induction relative to DMSO control. Statistical significance was calculated from the HIV-1 mRNA copy number values using a ratio paired T test compared to the DMSO control. (b) Calculation of synergy for bryostatin-1 (1nM) and romidepsin using the Bliss independence model. Data are presented as the difference between the observed and predicted fractional response relative to PMA + ionomycin (fraction affected, fa,). Statistical significance for the experimental fa was calculated using paired T test compared to the predicted fa for each combination.

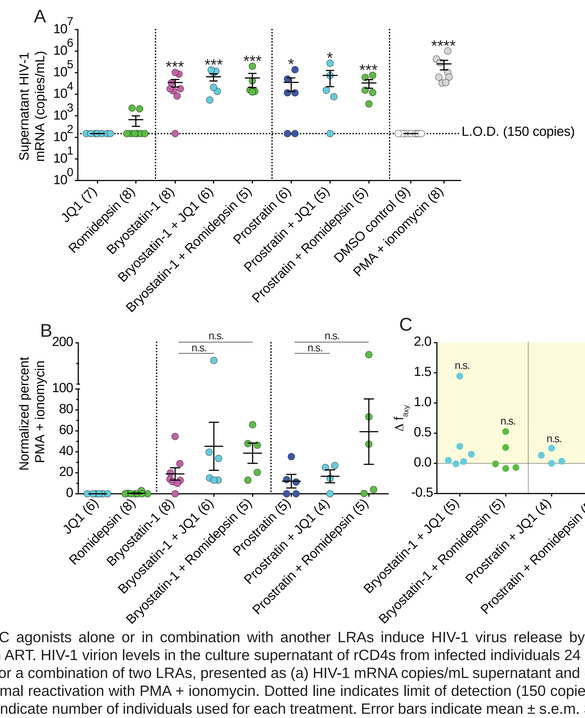


Figure 4. PKC agonists alone or in combination with another LRAs induce HIV-1 virus release by rCD4s from infected individuals on ART. HIV-1 virion levels in the culture supernatant of rCD4s from infected individuals 24 hours after addition of a single LRA or a combination of two LRAs, presented as (a) HIV-1 mRNA copies/mL supernatant and (b) as a percent of the effect of maximal reactivation with PMA + ionomycin. Dotted line indicates limit of detection (150 copies per ml). Numbers in parentheses indicate number of individuals used for each treatment. Error bars indicate mean ± s.e.m. Statistical significance was calculated from the HIV-1 mRNA copy number values using a ratio paired T test compared to (a) DMSO control, or (b) bryostatin-1 or prostratin alone. (c) Calculation of synergy for LRA combinations using the Bliss independence model. Data are presented as the difference between the observed and predicted fraction of supernatant HIV-1 mRNA levels in copies/mL induced by LRA combinations relative to PMA + ionomycin (fraction affected, fa). Statistical significance for the experimental fa was calculated using a ratio paired T test compared to the predicted fa for each combination.

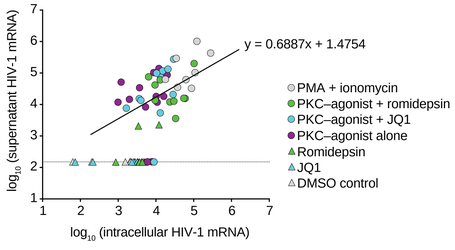


Figure 5. Correlation between intracellular and extracellular HIV-1 mRNA after ex vivo LRA treatment. Plot of intracellular HIV-1 mRNA copy number against supernatant HIV-1 mRNA copy number after exposure of rCD4s from the same infected individual to treatments containing (circles) or lacking (triangles) a PKC agonist. For PKC agonist-containing treatments, a statistically significant correlation was demonstrated by Tobit regression analysis (P = 0.008 for Chi-squared test) .

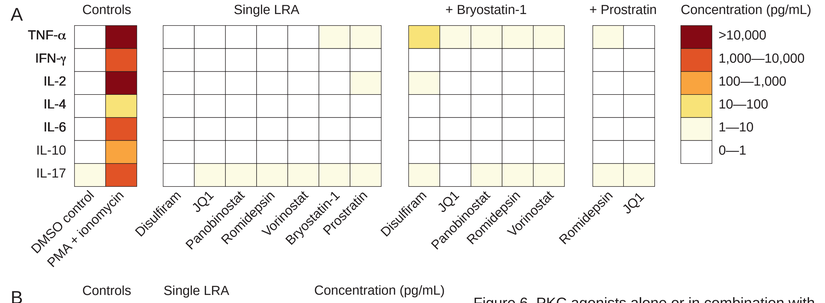


Figure 6. PKC agonists alone or in combination with another LRA do not induce substantial cytokine production. Primary (A) rCD4s or (B) PBMCs were treated with a single LRA or a combination of two LRAs were assayed for supernatant cytokine concentrations (pg/mL). Data are the mean effect of 2 or 3 independent experiments.

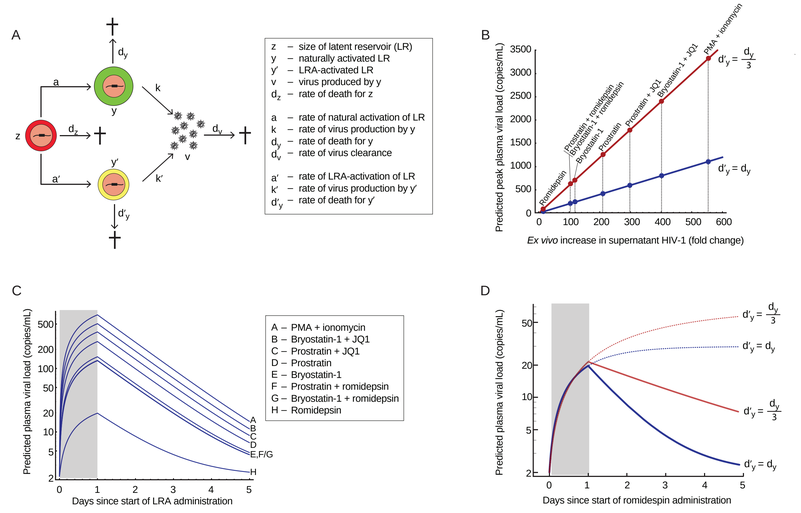


Figure 7. Mathematical model relating ex vivo virus release to predicted increases in plasma HIV-1 RNA levels in vivo. A viral dynamic model (A, detailed in Supplementary Methods) was used to estimate changes in plasma HIV-1 RNA levels in response to the LRA treatments for which ex vivo data on virus release was available. Arrows depict routes from latently infected cells, to productively infected cells after exposure to antigen or LRAs. (B) Predicted peak plasma HIV-1 RNA levels during LRA treatment. For each LRA treatment, median fold change in supernatant HIV-1 versus the DMSO control (x-axis) was used to estimate LRA-driven activation rate a'; this parameter estimate was used to predict peak plasma viral load following continuous administration of the LRA (y-axis). (C) Predicted time-course of viral load (y-axis, log scale) following administration of single-dose LRA treatment that remains active for 1 day. (D) Predicted time-course of viral load (y-axis, log scale) following administration of single-dose romidepsin that remains active for 1 day (solid lines) or that continues indefinitely (dotted lines). Gray shading in C, D indicates duration of LRA activity. Parameters: dy = 1/day; d'y = 1 day-1 (blue curves in B, D, all curves in C) or 1/3 day-1 (red curves in B, D); a + dz = 5.2×10-4 day-1 (reservoir half-life of 44 months), initial viral load = 2 copies/mL.

Methodology: Combinations of latency reversing agents (LRAs) that reverse latency ex vivo to an extent approaching the benchmark of maximal T cell activation (~100 fold induction) have not yet been identified. We therefore measured intracellular HIV-1 mRNA levels and supernatant virion production following individual or combination LRA treatment in rCD4s from infected individuals on suppressive ART. We also determined the effect of LRA treatment on proinflammatory cytokine production. To place our results in broader context and bridge laboratory measures of drug efficacy with clinical trial outcomes, we used a mathematical model to predict the in vivo dynamics of viral load following LRA treatment.

Results: We found that PKC agonists synergize significantly with JQ1 and with HDAC inhibitors to induce intracellular HIV-1 mRNA ex vivo. Combinations of PKC agonists with JQ1 or an HDAC inhibitor also caused significant virus release – in some instances exceeding that 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 LRAs, we then predicted in vivo changes in viral load following LRA treatment. In a realistic clinical scenario, the 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: 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. We demonstrate that this degree of latency reversal can be achieved without inducing functional T cell activation. Finally, we offer a mathematical framework to predict in vivo responses to LRAs using ex vivo measurements that can inform the design of future eradication clinical trials.

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