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Effective and targeted latency reversal in CD4$^+$ T cells from individuals on long term combined antiretroviral therapy initiated during chronic HIV-1 infection

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ABSTRACT

To date, an affordable, effective treatment for an HIV-1 cure remains only a concept with most "latency reversal" agents (LRAs) lacking specificity for the latent HIV-1 reservoir and failing in early clinical trials. We assessed HIV-1 latency reversal using a multivalent HIV-1-derived virus-like particle (HLP) to treat samples from 32 people living with HIV-1 (PLWH) in Uganda, US and Canada who initiated combined antiretroviral therapy (cART) during chronic infection. Even after 5–20 years on stable cART, HLP could target CD4$^+$ T cells harbouring latent HIV-1 reservoir resulting in 100-fold more HIV-1 release into culture supernatant than by common recall antigens, and 1000-fold more than by chemotherapeutic LRAs. HLP induced release of a divergent and replication-competent HIV-1 population from PLWH on cART. These findings suggest HLP provides a targeted approach to reactivate the majority of latent HIV-1 proviruses among individuals infected with HIV-1.

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Introduction

While combination antiretroviral therapy (cART) provides durable, long-term control of viremia, HIV-1 remains non-curative for people living with HIV-1 (PLWH) due to the establishment of a latent reservoir of integrated provirus within susceptible host cells [1,2]. At all times during infection, HIV-1 infection/propagation is supported in activated CD4$^+$ T cells, an activation mediated by antigen presentation through MHC-II on Antigen Presenting Cells (APCs) to a cognate T cell receptor (TCR) on T cells. The process leading to memory T cell generation from activated T cells can occasionally carry along an integrated HIV-1 proviral DNA into latency. Recent studies describe proviral HIV-1 populations in some myeloid cell types [3,4] but memory CD4$^+$ T cell populations still appear to be the major cell reservoir for replication-competent, recrudescing virus [5].

Therapeutics leading to cART-free remission, or a cure, needs to target those cells carrying the latent reservoir to drive out and kill the remaining replication-competent HIV-1 ("shock-and-kill" approach) [6,7]. In individuals receiving stable cART, latent, integrated HIV-1 was primarily found in transcriptionally silent regions of human chromatin [8]. Diminutive but significant HIV-1 latency reversal was observed with drugs that induce cellular mRNA transcription including global T cell activators [9–13], heterochromatin de-condensers (e.g. HDAC inhibitors) [14–18], Toll-Like Receptors [19], epigenetic reader inhibitors [20,21], and cell signalling cascade modulators (e.g. PKC agonists) [22–25]. However, minimal changes in the latent HIV-1 pool

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were observed in clinical trials involving repeated sub-toxic dosing by these chemotherapeutic drugs and as such, testing has been largely discontinued [14,17,26].

With limited success of broad spectrum, transcriptional activators, attention turned to identifying biomarkers labelling the small percentage of T cells harbouring latent HIV-1 in hopes of developing a targeted strategy for a cure [27,28]. A recent study described an enrichment of the latent HIV-1 reservoir in memory $CD4^+$ T cells with upregulated expression of CD44, CD28, CD127, and releasing IL-21 [29] but relating these findings to a targeted cure therapeutic will be challenging. Earlier studies showed that prior to cART, replicating HIV-1 was mostly housed in HIV-1-specific as opposed to other antigen-specific $CD4^+$ T cells [30–32]. Our therapeutic approach bore out of (1) the assumption that upon cART, the latent HIV-1 would remain in the HIV-1-specific $CD4^+$ T cells during its transition to the memory cell pool, and (2) of the knowledge that the highest level of HIV-1 latency was observed upon activation of TCR-based cell signalling. Thus, we developed an HIV-1/virus-like particle (HLP) as a "shock-and-kill" biotherapeutic which targets and activates $CD4^+$ T cells through their HIV-1-specific TCR. Considering that different HLA class II restricted $CD4^+$ T cells recognize and respond to different HIV-1 peptides among individual infections, we have constructed, produced and validated a safe, multivalent HLP representing approximately 95–98% of all HLA class II restricted HIV-1 peptides [33,34].

Our hypothesis was first tested by using HLP in samples from individuals treated with combined antiretroviral *t*herapy during *a*cute/early stages of *H*IV-1 *i*nfection (tAHI) [34]. HLP induced nearly 100-fold greater HIV-1 latency reversal than mitogens (e.g. PMA/Ionomycin and anti-CD3/CD28 antibodies). Thus, latent HIV-1 was found primarily in HIV-1-specific $CD4^+$ T cells after 2–3 years of stable cART, initiated during acute/early infection [34]. However, less than 5% of people living with HIV-1 received cART during acute/early infection. As such, HLP requires testing in samples from those individuals who have been on cART for years during chronic disease, sometimes with this treatment initiated years following primary infection (tCHI cohort). Targeted latency reversal by HLP in samples from the tCHI cohort presupposed that HIV-1 was primarily replicating in HIV-1-specific over other antigen-specific, activated $CD4^+$ T cells during years of chronic infection and upon cART, mostly memory HIV-1-specific $CD4^+$ T cells would harbour the latent HIV-1 pool.

The study herein demonstrated that HLP can effectively reverse latency in samples from 32 participants from the US, Uganda, and Canada, all of whom initiated treatment during chronic infection and were on stable cART for a median of 13.2 years. Furthermore, HLP targets the $CD4^+$ T cells harbouring the latent reservoir, providing 100- to 1000-fold higher specificity and HIV-1 activation than previously tested latency reversal agents (LRAs) and even T cell mitogens. HLP represents a cost-effective antigen-driven biotherapeutic, administered by intramuscular injections, that eliminates the HIV-1 latent reservoir and provides a potential cure (or remission) for the 25 + million living with HIV-1 and receiving stable cART.

Methods

M.1 Ethics Statement

PLWH ($n = 32$) were recruited from the Toronto Maple Leaf clinic in Ontario, Canada, from the Special Immunology Unit in Cleveland, USA, and from the Rakai Health Sciences Program in Rakai, Uganda. Ethical approval was obtained from the Institutional Review Boards at Johns Hopkins, the Uganda Virus Research Institute, the Uganda National Council for Science and Technology, and the National Institute of Allergy and Infectious Diseases. These chronic cohorts of individuals were enrolled with their HIV-1 subtype determined: either subtype B or subtype D (with 2 participants subtype A) (Supplementary Table 1). Peripheral blood was obtained from PLWH volunteers who had suppression of viremia to <40 copies HIV-1 RNA/mL for >6 months on cART. All study subjects used in this manuscript were adults and gave prior written informed consent. All volunteers were Hepatitis B and C negative at enrolment.

M.2 Production of HIV-1/virus-like particle (HLP) Formulation

The new subtype A and D HLP was produced as previously described for subtype B HLP [33,34]. These resultant HLP formulations contain three major modifications: (1) it is devoid of a 5' LTR (thus cannot serve as template for reverse transcription), (2) cannot encapsidate viral gRNA into budding particles (due to a disrupted Ψ region, by a 9-point nucleotide substitution), and (3) does not contain a functional integrase gene (due to an AAH > RRK amino acid substitution). The HLPs are thus lacking an HIV-1 genome and non-infectious.

M.3 Latency Reversal Assay

Isolation and culture of monocyte-derived dendritic cells (MDDCs) and donor $CD4^+$ T lymphocytes

Monocytes were purified from HIV-1-infected peripheral blood mononuclear cells (PBMCs) through plastic

adherence for 2 h and differentiated into Monocyte-derived dendritic cells (MDDCs) over 6 days [34]. The non-adherent cells from PBMCs were collected and CD magnetically-labeled microbeads were used to negatively select for $CD4^+$ T lymphocytes ($CD4^+$ T cell isolation kit, Miltenyi Biotec, USA) as per manufacturer instructions [34].

Latency reversing co-culture assay

As previously described, MDDCs were pulsed overnight on day 6 following plastic adherence with 5 μg/mL (based on p24) of RT-equivalent heterologous HLP (subtype B, D, or combined A + D) or CMV–tetanus–flu (CTF) recall antigen cocktail [34]. Fresh, autologous CD4-purified T cells were co-cultured with antigen-pulsed MDDCs on day 7 following plastic adherence. Cells were co-cultured at a ratio of 4 T cells to 1 MDDC at a concentration of 1×10^6 $CD4^+$ T cells/mL (i.e. 2.5×10^5 MDDC/mL) in 96 well ELISpot plates. For conditions that did not require MDDC-pulsing [i.e. 1/500 (v/v) media-diluted phorbol 12-myristate 13-acetate (PMA) + Ionomycin (Iono) (PMA/Iono) (Invitrogen, CA), 40,000 beads/well Dynabeads® Human T-Activator CD3/CD28 agonist antibodies (Gibco, CA), 335 nM of HDAC inhibitor (HDACi) Vorinostat/SAHA (Caymen Chemicals, USA), 40 nM of HDACi Romidepsin (Cedarlane, CA), 30 nM of HDACi Panobinostat (Selleckchem, USA), and 10 nM of protein kinase C (PKC) agonist Bryostatin-1 (Sigma-Aldrich, CA)], stimuli were added on day 7 directly to T cell culture [34]. Fusion inhibitor Enfuvirtide (T20; 10 μM) was added to purified $CD4^+$ T cells prior to any culturing to prevent *de novo* HIV-1 infection of bystander T cells by activation of the HIV-1 from the latent pool.

IFN-γ ELISpot assay

As previously described, human IFN-γ enzyme-linked immunosorbent spot (ELISpot) assays (Mabtech, USA) were carried out with MDDC:$CD4^+$ T cell co-cultures as per the manufacturer's instructions [34]. After 16 h of ELISpot incubation, culture supernatants were gently aspirated and removed for additional experiments. Spots were developed using 1 μg/mL of biotinylated anti-IFN-γ antibody for 2 h, followed by 1 μg/mL of streptavidin-ALP for 1 h, a reaction that was stopped with 100 μl/well of "BCIP/NBT-plus substrate for ELISpot" (Mabtech, USA), then incubated for 15 min in the dark to develop the plate, with D-PBS washes in between. Spot forming units (SFU) were counted using an ImmunoSpot S5 UV Analyzer (Cellular Technology Ltd, USA) and ImmunoSpot 5.0.9 software. The raw SFU counts were corrected to 1×10^6 $CD4^+$ T cells/mL and analyzed using GraphPad Prism version 8.0.1 (GraphPad Software, USA).

Quantification of cell-free supernatant HIV-1 genomic RNA and intracellular msRNA

A reverse transcription quantitative real-time PCR (qRT-PCR) workflow was performed to assess each latency reversal agent's (LRA's) reactivation ability of the proviral reservoir in HIV-1-infected $CD4^+$ T cells as described [34]. Briefly, 96-well cell co-culture plates were centrifuged (500 g, 5 min) after 72 h at 37°C, 5% CO_2 to pellet the cell-fraction and remove the cell-free supernatant. HIV-1 RNA was extracted using a QIAamp Viral RNA Mini Kit following manufacturer instructions (Qiagen, USA) from 0.2 mL of cell-free supernatant. cDNA was produced using Superscript IV reverse transcriptase (Invitrogen, USA) according to manufacturer's protocol using the f-as primer, specific for the 5' end of the HIV-1 Gag gene (Supplementary Table 2). To detect and quantify only the activated HIV-1 genomic RNA and not any contaminating RNA from HLP, the "B.A.D. 5' LTR PA" and "B.A.D. GagR" primer pair were used for real-time PCR (qPCR) with QuantStudio5 (Applied Biosystems, CA) using PowerTrack SYBR Green Master Mix (Applied Biosystems, CA). Details of cycling conditions and standard curve preparation are detailed in the Supplementary Materials.

The cell-fraction from the co-culture was lysed using TriZol Reagent (Invitrogen™, USA) to isolate intracellular HIV-1 RNA for detection via qRT-PCR using a tat-rev primer set: Tat 1.4, Tat 2.2, and Rev 2.0 (Supplementary Table 2). A cDNA synthesis step was performed using SuperScript™ III Reverse Transcriptase (Invitrogen™, USA) and random hexamers. Next, a pre-amplification step was performed using Platinum Taq DNA polymerase as per manufacturer's instructions (Thermofisher, USA) using primers Tat 1.4 and Rev 2.0. qPCR was subsequently performed as described above with the primers Tat 2.2 and Rev 2.0.

M.4 Analyzes of LRA-induced HIV-1 RNA by Deep Gene Sequencing

The library preparation and data analysis for next generation Illumina sequencing using MiSeq was well described previously [34]. Briefly, nested PCR was used to amplify C2-V3 amplicons from LRA-induced HIV-1 RNA. Then, the Nextera XT Index kit system (NexteraXT Index Kit v2 Set A, Illumina) was used to attach the Illumina dual-index sequence adapters to the amplified and purified PCR products. The pooled samples were processed at our in-house ImPaKT facility using the Illumina MiSeq (Illumina Inc., San Diego, USA).

The library was run on an Agilent High Sensitivity DNA Bioanalyzer chip (Agilent Technologies Inc., CA, USA) to assess size distribution and qRT-PCR

(Kapa Biosystems, Inc., Wilmington, MA, USA) was used to assess the quantity. The library was diluted to 4 nM and sequenced on an Illumina MiSeq using a 2 × 300 paired end run with 10% PhiX (according to the Illumina protocol "Preparing Libraries for Sequencing on the MiSeq, Rev. C").

With the processing of the FASTQ data files, *env* sequences (HXB2 positions nt 6858-7338) of the induced virus were compared to a collapsed consensus sequence to identify the unique sequence reads using a customized version of the MiCall pipeline (https://github.com/PoonLab/MiCall-Lite; see Supplementary Materials). Finally, highlighter type plots were generated using the R package "highlineR" (https://github.com/PoonLab/highlineR), to demonstrate the frequency and diversity of unique reads in each sample. A maximum-likelihood tree of the unique reads was also reconstructed for each sample using IQ-TREE (version 2.2.2.6) [35].

For the Gag gene, cDNA products produced by RT with primer HIV-Gag-Rev6 were amplified a first round with B.A.D 5' LTR PA and HIV-Gag-Rev8 primers and in a second round with the HIV-U5-sense and HIV-Gag-Rev4 primers (Supplementary Table 2) to produce a 800 nt 5' UTR/Gag gene product (HXB2 nt 610-1429) using a hot start Phusion Taq polymerase and protocol (Invitrogen, USA). PCR products were purified and submitted to Plasmidsaurus for Nanopore sequencing (OR, USA), and London Regional Genomics Centre at Robarts Research Institute (London, Ontario, CA) for Sanger sequencing.

M.5 Statistical Analyzes

Sample size calculations were based on the effects of HLP (termed ACT-VEC) in pilot study using samples obtained from individuals diagnosed and treated for HIV-1 during acute/early infection. Power calculations considered the 90% effect of HLP treatment to the 10% CTF recall antigen treatment (from the pilot study; > 4-fold activation in the patient sample) resulting in N = 5 for a $p < 0.05$ and power of 80%. Our cohort size of 32 had sufficient power to measure a 60% effect in the HLP treatment versus 25% in the CTF treatment. Other analyzes were secondary outcomes in this study. Statistical analyzes were shown in Supplementary Table 3, and were performed using GraphPad Prism version 8.0.1 (GraphPad software, USA). A p value less than 0.05 served as a threshold for statistical significance. Kruskal–Wallis followed by Dunn's post-hoc multiple comparisons tests with Bonferroni correction method were applied to address type 1 errors. Two-tailed Mann–Whitney non-parametric (U) T tests, and two-tailed Wilcoxon matched-pairs signed rank tests were used to test for inter- and intra- sample significance, respectively. Correlative data was interpreted using a two-tailed Pearson Correlation Coefficient (r) test where indicated.

Results

HIV⁺ volunteer characteristics

For this study, peripheral blood mononuclear cell (PBMC) samples were obtained from participants (designated P1-P32) who received cART late into their (*c*hronic) *H*IV-1 *i*nfection, i.e. tCHI participants. Study participant blood samples were collected from the Maple Leaf Clinic in Toronto, Canada ($n = 11$), from the Special Immunology Unit in Cleveland, USA ($n = 4$), and from the Rakai Health Sciences Program in Rakai, Uganda ($n = 17$). All tCHI donors in Toronto and Cleveland harboured HIV-1 subtype B infection while the Ugandan cohort had HIV-1 subtype D ($n = 15$) and A ($n = 2$) infections. Of the 32 tCHI participants enrolled in this study, 30 had durable viral suppression and undetectable viremia during cART. This was maintained for a median of 16 years in Canada/US cohorts and 11 years in Uganda prior to blood donation (ranges 13–20 and 5–15 years, respectively) (Figure 1A). At the time of PBMC sample collection, the subtype B and D $CD4^+$ nadir averaged 1.6×10^6 cells/mL (range = 0.1–84.6 × 10^6 cells/mL). A relationship between viral load and time of treatment was plotted using the best-fit curve model (Figure 1B). Only P3 and P17 had viral load blips greater than 1000 copies/mL that persisted over more than one time point. P3 was responsive to cART (Bictegravir/Emtricitabine/tenofovir alafenamide) from 2005 to 2021 where a treatment interruption appeared related to substantial rebound (days 5165–5186) in 2019; but resumption of the same cART brought viral loads back to undetectable for another two years, i.e. the time of sample collection. P17 had detectable viral loads in the range of 8000–50,000 copies/mL after three years, again related to stoppage of Zidovudine + Lamivudine/Efavirenz treatment. When the same cART was resumed in 2009, viral load remained undetectable for 11 years. As described below, several analyzes were run with original treatment start date of P3 and P17 and then repeated analyzes with the treatment interruption at years 2.2 and 9.9 (Supplementary Figure 7) now considered the "treatment start date".

HLP treatment reverses HIV-1 latency in $CD4^+$ T cells from HIV^+ individuals, diagnosed and cART treated at chronic stage of infection

We measured the *ex vivo* efficacy of HLP to induce HIV-1 latency reversal in tCHI PBMC samples after a median of 13.2 years (range 5–20) of cART treatment. From these blood samples, monocyte-derived dendritic cells (MDDCs) pulsed with HLP (MDDC

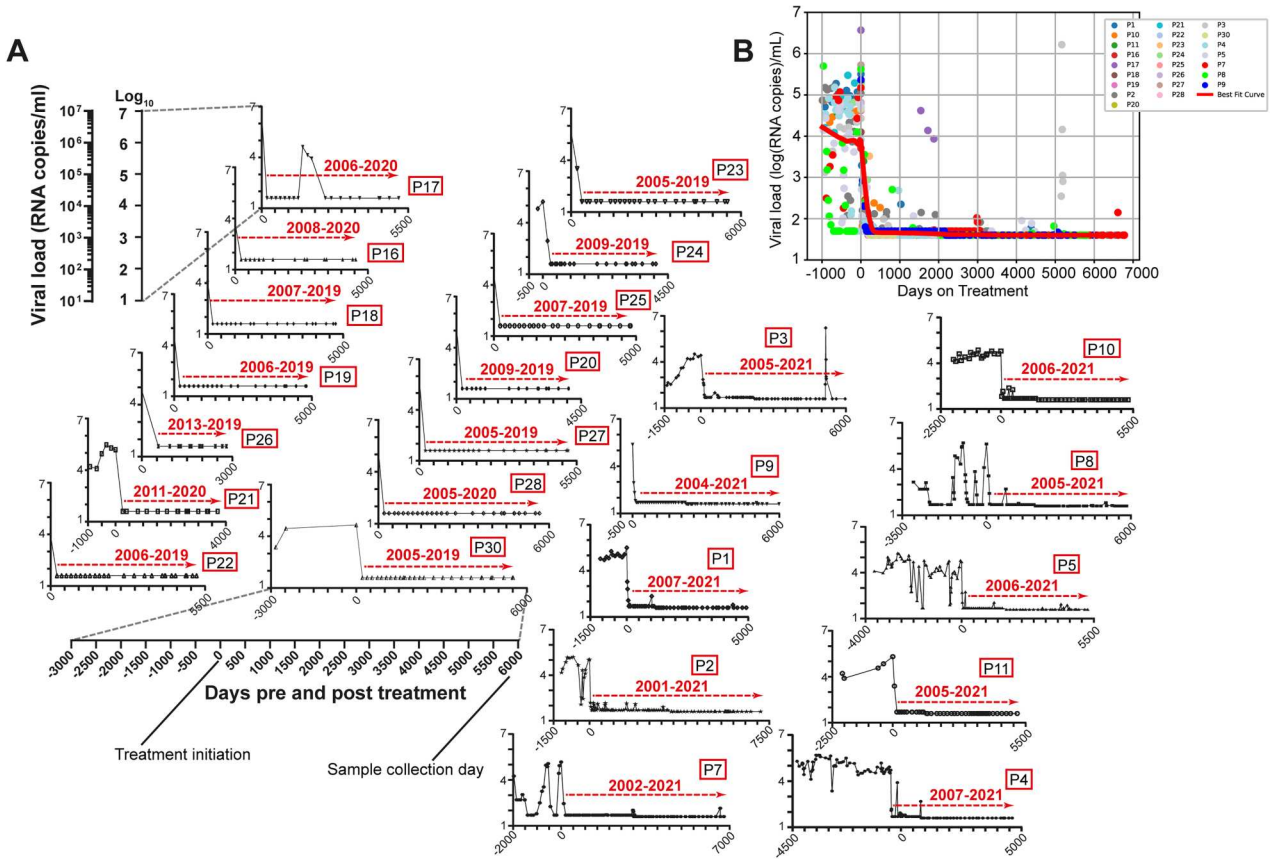

Figure 1. Viral load graphs of some of the tCHI participants in this study. (A) Individual participant tracking shown. P12, P13, P14, and P15 received stable cART for less than two years at the time of sample collection. P6, P29, P31, and P32 had intermittent tracking of viral load during the course of their 225, 4221, 2889, and 2581 days on treatment, respectively. Graphs for these participants are not shown. (B) One cumulative overlay of the participants graphed in Panel A, best fit curve was constructed using LOWESS smoother by statsmodels v.0.15.0.

+ HLP) and then co-incubated with autologous, latently-infected $CD4^+$ T cells resulted in high levels of intracellular HIV-1 multiply-spliced RNA (msRNA) (mean: 8.7×10^6 copies/mL, ranges: 8.3×10^4–6.3×10^7; Supplementary Figure 1) and release of HIV-1 RNA (vRNA) into culture supernatants, measured as total virus release from 1×10^6 $CD4^+$ T cells herein (mean: 1.0×10^5, ranges: 496–3.7×10^8) (Figure 2A).

The measured levels of HLP-mediated reactivation and release of HIV-1 RNA into culture supernatants were 75-fold ($p < 0.0002$) higher than treatment with recall antigens – CMV/Tetanus Toxoid/Flu co-incubated with MDDC (MDDC + CTF), 97-fold ($p < 0.0001$) higher than treatment with the common T cell mitogen (PMA/Iono) (Figure 2A; **paired analyzes,** Supplementary Figure 2), and 60-fold ($p = 0.013$) higher than direct treatment with anti-CD3/anti-CD28 antibodies using selected samples (Supplementary Figure 3). The 72 h co-culture of $CD4^+$ T cells with MDDC + HLP (and other stimuli) was done in the presence of 10 μM Enfuvirtide to prevent *de novo* HIV-1 propagation and indicates that the viral RNA detected in supernatant reflects the release of HIV-1 from only the activated $CD4^+$ T cells that harboured latent HIV-1. It is important to note that the released HIV-1 in supernatant was capable of viral transcription, translation, assembly, budding and release. Whereas HLP treatment resulted in HIV-1 release from 100% (32/32) of the tCHI participants' $CD4^+$ T cells, only 71% (17/24) of the participant $CD4^+$ T cells treated with MDDC + CTF and 61% (19/31) treated with PMA/Iono resulted in detectable latency reversal, as measured by viral RNA in the culture supernatant (Figure 2A).

Considerably more PBMC were available from the donors P18, P23, P26, P30, and P31 from Uganda than from the North American cohorts, providing more vRNA released upon MDDC + HLP treatments. We performed next generation sequencing as described in the Materials and Methods for the amplification of HIV-1 *gag* and *env* C2-V3 regions. All HIV-1 *gag* sequences derived from HLP stimulation were unique to each Ugandan participant sample and clustered with subtype D HIV-1 reference sequences (Figure 3A). With P30, the *gag* vRNA released by HLP matched that released by PMA/Iono treatment but there was insufficient vRNA for *env* vRNA sequencing. Except for this P30 sample, PMA/Iono and CTF treatments resulted in nearly

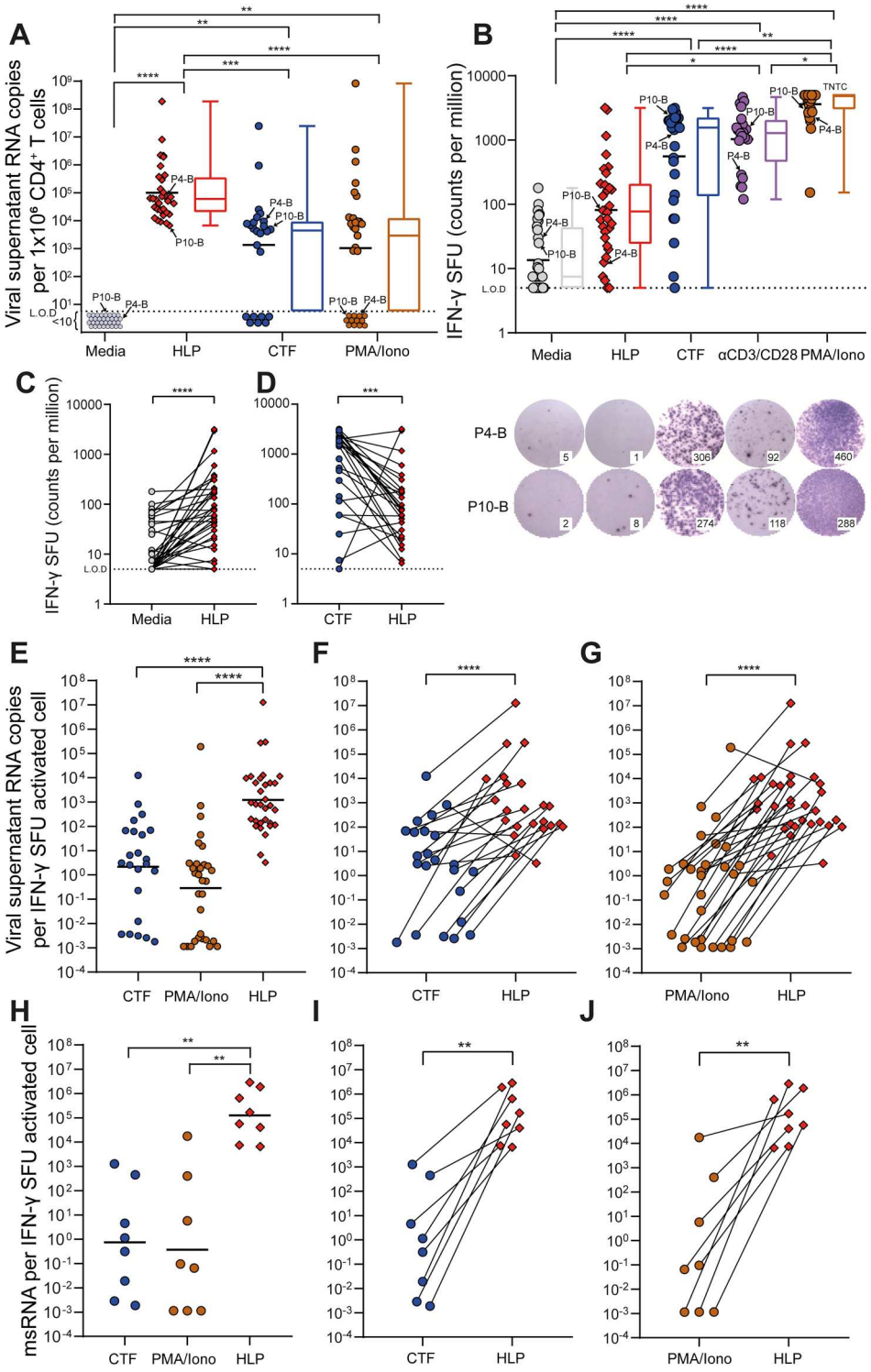

Figure 2. HLP_B induces potent latency reversal in $CD4^+$ T cells from tCHI. Latency reversal assay was conducted using HLP, CTF, αCD3/CD28, and PMA/Iono. (A) Viral copy number was determined via qRT-PCR from culture supernatants (geometric mean shown for scatter, median is shown for box–whisker plots). (B) IFN-γ was measured via ELISpot (geometric mean shown for scatter, median for box–whisker). ELISpot wells were shown and purple dots are spot forming units (SFU), from participant 4 and 10 with subtype B infection. Raw SFU counts used 1 or 2×10^5 cells/well, and were presented in the graph adjusted to 1×10^6 cells/mL for consistency to assay for viral release analysis in Panel A. Next, intra-donor comparison for T cell activation by IFN-γ ELISpot was analyzed between HLP and (C) Media or (D) CTF. (E) The ratio of HIV vRNA copies per IFN-γ activated cell from culture supernatants was calculated (geometric mean), with intra-donor pairwise alignment comparison between HLP and either (F) CTF or (G) PMA/Iono. (H) The ratio of HIV msRNA copies per IFN-γ activated cell from infected cells was calculated, with intra-donor pairwise alignment comparison between HLP and either (I) CTF or (J) PMA/Iono. Each pair of datapoints connected by a line is from one donor. Kruskal–Wallis with Dunn's post-hoc tests were performed to account for inter-sample multiple comparisons, and two-tailed Wilcoxon matched-pairs signed rank tests were performed for intra-sample significance. Paired comparisons between every condition were performed, only the statistically significant comparisons are denoted. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; L.O.D, limit of detection; SFU, spot forming units; msRNA, multiply-spliced RNA; TNTC, too numerous to count.

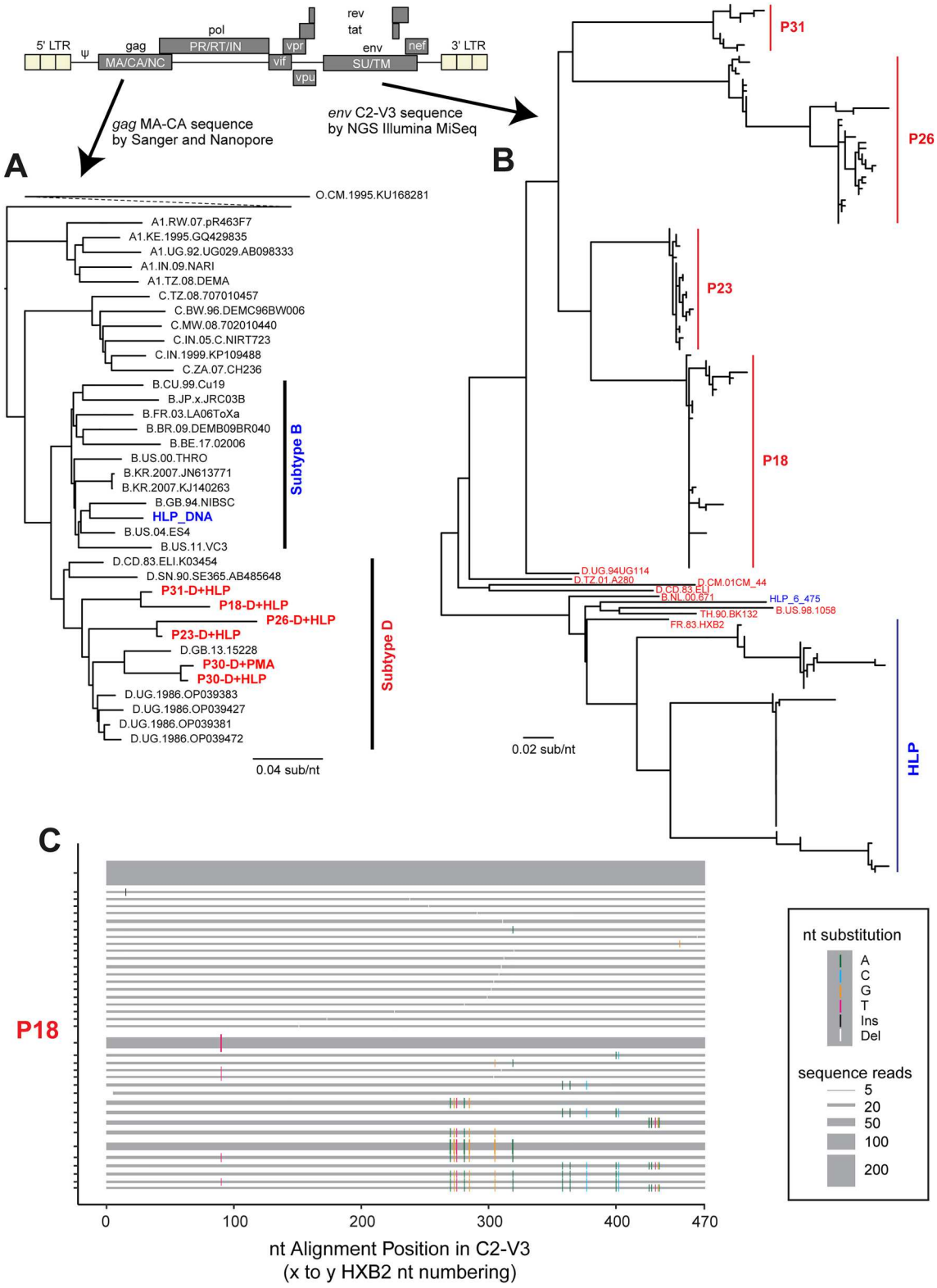

Figure 3. HLP-induced HIV-1 has high genetic diversity when reactivated from tCHI samples. The genetic diversity of induced HIV-1 subtype D sequences after latency reversal is shown. HIV-1 sequences spanning the LTR-*gag* and *env* (C2-V3) regions were analyzed using Sanger and Oxford Nanopore (A) and Illumina MiSeq (B), respectively. Reference sequences for subtypes A, B, C, and D were included for comparison, while HLP_DNA sequence was also included. (C) A HighlineR plot showing HLP-induced HIV-1 C2-V3 diversity from participant 18. The horizontal grey bands correspond to each unique sequence read, while the band sizes represent the frequency of each read. The highlineR package designates the most common read as the master sequence and places it at the top of the plot. The diversity of other reads is then computed relative to this master sequence. Differences relative to the master sequence are shown as coloured tick marks on each grey band, indicating the nucleotide positions with change. For the brevity and easy visualization, only reads with frequencies >3 were shown in the plot. HighlineR plots for remaining donors are found in Supplementary Figure 4.

100-fold less viral RNA (when compared to HLP treatment), i.e. levels insufficient for longer RT–PCR products and next generation sequencing. Moreover, from an input of approximately 1000–10,000 vRNA copies, high genetic diversity in the *env* C2-V3 regions was observed in the HIV-1 released from the latent pool upon MDDC + HLP treatments; the released vRNA from donors P18, P23, P26, and P31 were consistent with years of viremia prior to the initiation of cART (Figure 3B,C and Supplementary Figure 4). In contrast, our previous study [34] described a very homogenous HIV-1 population induced by HLP in samples from participants who received cAR*T* early into their (*a*cute) *H*IV-1 *i*nfection (i.e. tAHI samples), consistent with HIV-1 evolution being limited to only a few weeks or months and not years before the initiation of cART.

HLP targets the CD4$^+$ T cells harbouring latent HIV-1 for latency reversal

Using an IFN-γ ELISpot assay, we next measured the number of tCHI CD4$^+$ T cells activated by MDDC + HLP, MDDC + CTF (recall antigen mix), and global T cell activators (Figure 2B). As expected, PMA/Iono, followed by anti-CD3/anti-CD28 antibodies were the most potent T cell activators with 100% (32/32 and 20/20 respectively) of participants having CD4$^+$ T cells responding with mean spot forming units (SFU) of 3609 and 1031 SFU/10^6 cells respectively. With MDDC + CTF, i.e. presentation of common recall antigens, 100% (26/26) of participants had CD4$^+$ T cells that responded and with a mean of 557 SFU/10^6 cells/participant. Finally, the lowest level of T cell activation as measured by IFN-γ ELISpot assay was with MDDC + HLP treatment. All participants (32/32) had CD4$^+$ T cells responding to MDDC + HLP with a mean of 81 SFU/10^6 cells; but significantly lower response compared to the mitogen exposures in the grouped analyzes (Figure 2B). For a more accurate comparison of response to the different treatments in terms of CD4$^+$ T cell activation and virus release, each treatment was compared to HLP in paired analyzes (Supplementary Figure 2). All of the statistical significance observed in the grouped analyzes (Kruskal–Wallis with Dunn's test) were also significant in the paired analyzes except for HLP vs Media and HLP vs CTF when analyzing T cell activation (Figure 2B). In Figure 2C,D, paired analyzes of HLP vs Media and HLP vs CTF are shown to be significant (two-tailed Wilcoxon matched-pairs signed-rank test). It is important to note that the participant population used in this study differed based on dates of treatment initiation, viral load at treatment, and length of stable treatment prior to sample collection; variables that would impact T cell activation response and viral release in a mixed population analysis.

Following the various treatments, virus released from CD4$^+$ T cells as measured by vRNA detected in culture supernatants was compared relative to the number of activated CD4$^+$ T cells (Figure 2E–G). As shown, MDDC + HLP resulted in a 571-fold and 4290-fold higher release of vRNA per activated CD4$^+$ T cell compared to MDDC + CTF ($p < 0.0001$) and PMA/Iono ($p < 0.0001$), respectively (Figure 2E), providing the first evidence of HLP targeting of the latent HIV-1 pool for reactivation. We next evaluated the intra-donor relationship between induced vRNA in cell-free supernatant and the number of activated CD4$^+$ T cells. In all but two donors (P15 and P22), the vRNA:SFU/10^6 cell ratios were significantly higher with MDDC + HLP compared to MDDC + CTF and PMA/Iono treatments ($p < 0.0001$; Figure 2F,G). In a previous study, chemotherapeutic LRAs such as HDACi were shown to induce low levels of HIV-1 mRNA transcription from latent tAHI HIV-1-specific CD4$^+$ T cells, but minimal cell release of vRNA in cell-free supernatant, indicative of virus particle production [34]. MDDC + HLP induced exceptionally high levels of HIV-1 multiply-spliced (ms) mRNA in tCHI CD4$^+$ T cell lysates compared to other LRAs and to treatments with MDDC + CTF and PMA/Iono, herein. When comparing the levels of HIV-1 msRNA per IFN-γ positive CD4$^+$ T cell from a subset of tCHI ($n = 8$), MDDC + HLP induced an average five Log increase in the quantities of HIV-1 msRNA in cell lysates compared to MDDC + CTF ($p = 0.0056$), and PMA/Iono stimulation ($p = 0.0016$) (Figure 2H). Evaluating the intra-donor relationship, the msRNA:SFU/10^6 cell ratios were significantly higher with MDDC + HLP compared to MDDC + CTF and PMA/Iono treatments ($p = 0.0078$; Figure 2I,J). It is important to note that HLP is a virus-like particle formulation with reduced genomic vRNA packaging (mutation in Psi), which is incapable of producing proviral DNA (5'LTR deletion), integrating proviral DNA (mutation in integrase active site), or transcribing HIV-1 msRNA (no transcription initiation or splice donor site) [33].

HLP triggers greater latency reversal in tCHI samples compared to tAHI samples

In this tCHI cohort, participants were infected for years prior to the initiation of treatment. In contrast, past studies on a tAHI cohort involved participants who were treated within six months of infection. HLP mediated 164-fold greater HIV-1 release from the CD4$^+$ T cells in the tCHI samples than with the same number of CD4$^+$ T cells in the tAHI samples ($p < 0.0001$) (Figure 4A). These findings suggest that prolonged infection prior to initiation of cART seeds a larger HIV-1 reservoir in memory CD4$^+$ T cells. Increased latency reversal in the tCHI over the tAHI cohort was quite striking considering that the samples

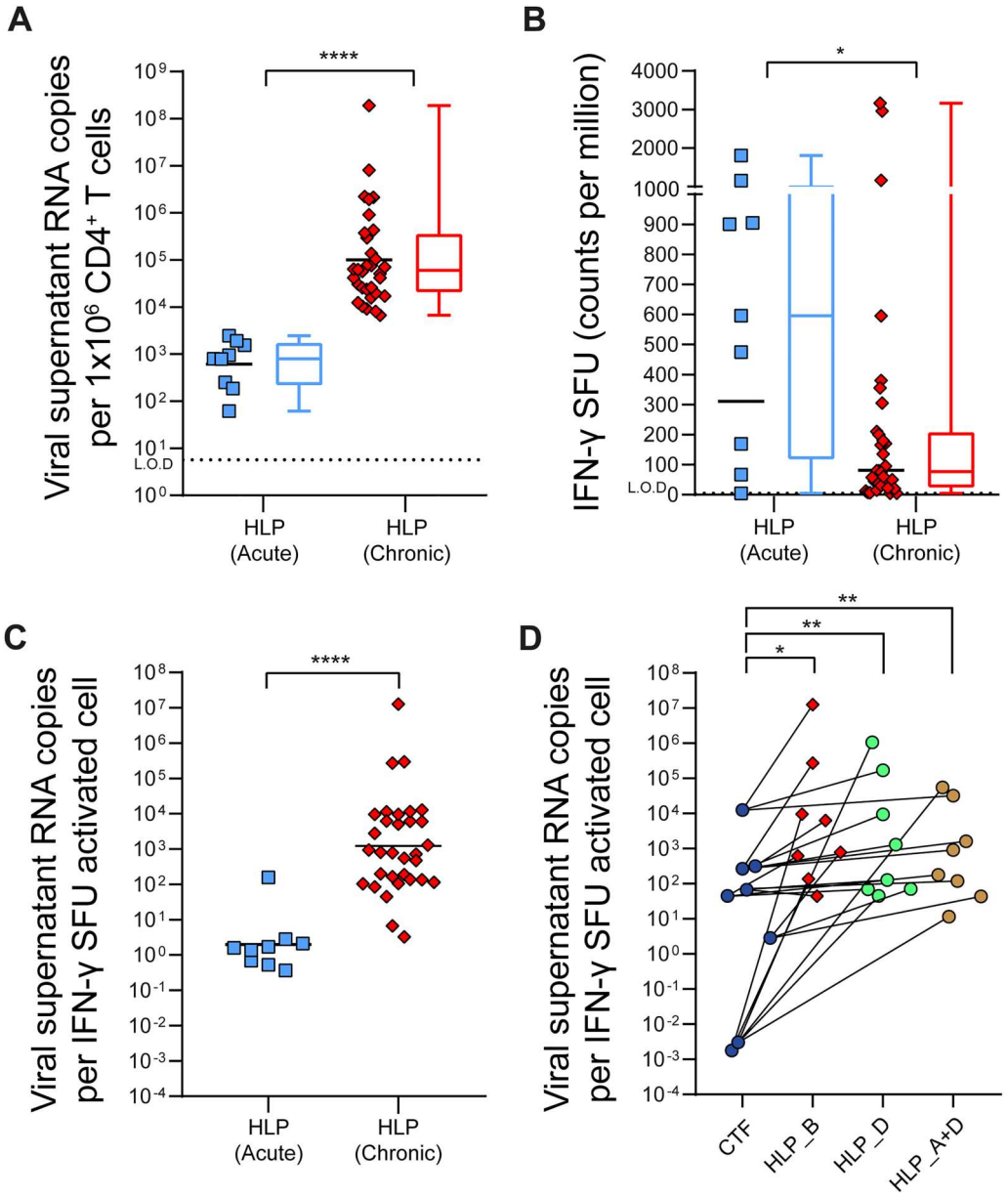

Figure 4. HLP_B induces significantly greater latency reversal in tCHI samples than in tAHI samples. (A) Viral copies in culture supernatant from latency reversal assay were measured by qRT-PCR. (B) Activation of $CD4^+$ T cells was determined by SFU count via IFN-γ ELISpot assay. (C) The ratio of HIV vRNA copies per IFN-γ activated cell was calculated. Each datapoint represents one donor from the respective cohort. (D) Comparison of three HLP formulations (HLP_B, _D, and _A + D) on latency reversal efficiency in the tCHI cohort and intra-donor pairwise alignment comparison among three HLP formulations and CTF was shown. Each pair of datapoints connected by a line is from one donor. Two-tailed Mann–Whitney tests were performed to test inter-sample significance, and two-tailed Wilcoxon matched-pairs signed rank tests were performed to test intra-sample significance. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. HLP, HIV-1/virus-like particle; SFU, spot forming units; L.O.D, limit of detection. Geometric mean shown for scatter, median is shown for box–whisker plots.

used in these analyzes were collected in tCHI participants after a median of 13.2 years as compared to just 2–3 years of stable cART in the tAHI participants. As recently reported [36] and described below, the infectious HIV-1 reservoir in memory $CD4^+$ T cells decays at a very slow rate. Interestingly, the number of $CD4^+$ T cells activated to release IFN-γ as measured by ELISpot, was significantly reduced in tCHI samples compared to the tAHI samples when stimulating with MDDC + HLP ($p = 0.0340$; Figure 4B). Thus, we observed a 627-fold increase in latent HIV-1 released per activated $CD4^+$ T cell in the tCHI versus the tAHI samples ($p < 0.0001$; Figure 4C).

HLP reverses latency in $CD4^+$ T cells irrespective of subtype of HIV-1 infection

HIV-1 is a highly genetically diverse human pathogen with different HIV-1 subtypes predominating in different regions of the world, e.g. subtype B in the US and Canada and subtypes D and A in Uganda. Using the same methods for construction of the

heterologous HLP_B, formulations of HLP were prepared from the proviral genomes of HIV-1 subtype D and A infected individuals [34]. The $CD4^+$ T cells from the Ugandan tCHI donors infected with subtype D were treated with HLP_B, HLP_D, or a mixture of HLP_A + HLP_D, presented by MDDCs. The levels of latency reversal relative to $CD4^+$ T cells were similar among all the tCHI donor samples when activated by the HLP_B, HLP_D, and HLP_A + D formulations. All the HLP formulations stimulated greater latency reversal than the common recall antigens, CTF, also presented by MDDCs ($p < 0.05$–0.01; Figure 4D).

HLP induces significantly greater latency reversal than HDACi and PKC agonists

Several LRAs that function as HDAC inhibitors have been evaluated or are currently being investigated as an HIV-1 cure in clinical trials. We therefore compared the latency reversal properties of MDDC + HLP to clinically relevant LRAs such as Vorinostat [16–18], Romidepsin [15], Panobinostat [14,37], and Bryostatin [21,22] using $CD4^+$ T cells from tCHI samples ($n = 17$) and in the presence of Enfuvirtide over three days of stimulation. Significantly higher quantities of HIV-1 vRNA in cell-free supernatant were observed with MDDC + HLP compared to each of the three HDACi in nearly every intra-sample pair (Figure 5A; Supplementary Figure 5). Specifically, MDDC + HLP induced two Log-higher quantities of HIV-1 vRNA release into culture supernatants compared to Vorinostat ($p = 0.0113$, Kruskal–Wallis with Dunn's test, Figure 5A; $p = 0.0081$, Wilcoxon MP-SR test, Supplementary Figure 5A), three Log-higher than Romidepsin ($p = 0.0063$, Figure 5A), and three Log more vRNA than Panobinostat ($p = 0.0195$, Figure 5A). More notable was the failure of HDACi and Bryostatin to reverse latency in half of the donor samples (6/13, 5/10, 5/10, 3/8, respectively), whereas HLP induced latency reversal in all samples. It is important to note that there was an average of 118-fold increased virus release (ranges: $85–1.43 \times 10^6$ fold) with HLP versus with Bryostatin in 7 of 8 tCHI participant samples but did not reach significance ($p = 0.5469$, Wilcoxon MP-SR test) due to one sample with high virus release with both HLP and Bryostatin. Aside from Bryostatin and HLP, the HDACi did not appreciably activate the $CD4^+$ T cells to release IFN-γ (see Figure 5B and Supplementary Figure 5F–I).

Comparing latency reversal by stimuli and dynamics of the latent HIV-1 pool during the time on cART

As expected, proviral DNA load at the time of cART initiation directly correlated with the size of the proviral DNA reservoir in the samples collected after years of stable cART ($p = 0.015$; Figure 6A). Also, length of time on stable cART correlated with lower proviral DNA loads at the time of sample collection ($p = 0.0062$; Figure 6B). The decay rate of the proviral pool from the start of cART to sample collection was a median loss of 0.5% (IQR = 0.14) per $CD4^+$ T cell per year (Supplementary Figure 6A).

During stable cART, the HIV-1 reservoir contains mostly defective and dormant HIV-1 proviral DNA, and only a small proportion is infectious, replication-competent latent HIV-1 DNA. The quantitative viral outgrowth assay (qVOA) is a common method

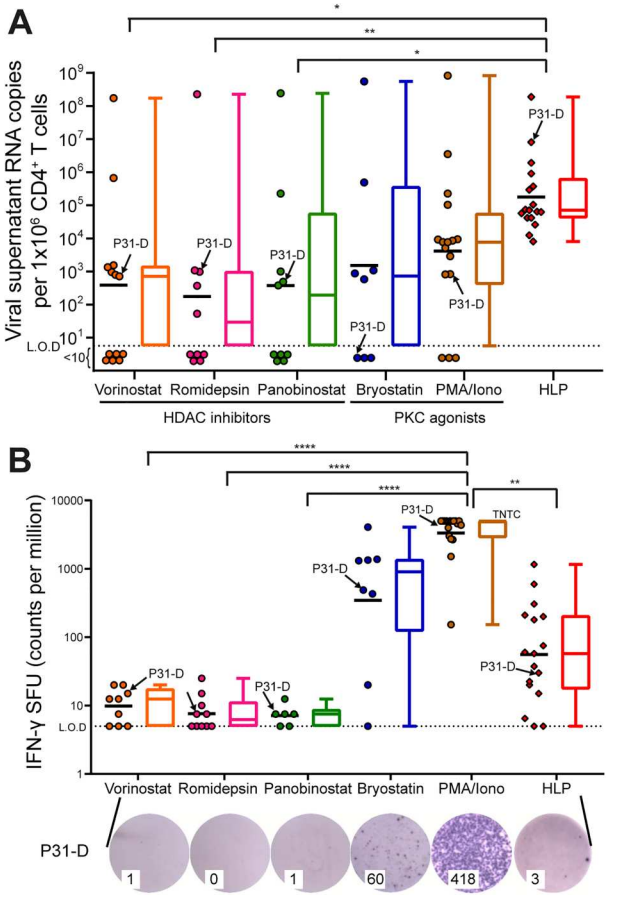

Figure 5. HLP outperforms clinically relevant LRAs at induction of viral transcripts. (A) After LRA stimulations, we measured induced HIV-1 reactivation by qRT-PCR of viral RNA detected in supernatant. (B) Activation of $CD4^+$ T cells with LRAs was determined by IFN-γ ELISpot. Purple dots are spot forming units (SFU) from ELISpot wells, from participant 31 with subtype D infection. Raw SFU counts used 1 or 2×10^5 cells/well, and were presented in the graph adjusted to 1×10^6 cells/mL for consistency to assay for viral release analysis in Panel A. Each datapoint represents one donor. Kruskal–Wallis with Dunn's post-hoc tests were performed to account for inter-sample multiple comparisons. Paired comparisons between every condition were performed, only the statistically significant comparisons are denoted. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$. HLP, HIV-1/virus-like particle; LRA, latency reversal agent; L.O.D, limit of detection; TNTC, too numerous to count. Geometric mean shown for scatter, median is shown for box–whisker plots.

for measuring the frequency of latently infected cells, i.e. capable of producing replication-competent virus upon activation. For the Ugandan cohort, sufficient cells were available to measure infectious viral units per million cells (IUPM) using qVOA [38,39]. We compared the IUPM by qVOA to the amount of virus released into supernatant from the $CD4^+$ T cells treated with HLP or PMA/Iono. The amount of HIV-1 RNA released from CTF was too low for this analysis. With HLP, the amount of virus released from 10^6 tCHI $CD4^+$ T cells directly correlated with replicating HIV-1 (i.e. IUPM using qVOA) on the same samples collected from each participant ($p = 0.040$; Figure 6C). Viral release from tCHI $CD4^+$ T cells by PMA/Iono treatments did not correlate with the same IUPM values measured by qVOA (Supplementary Figure 6B). It is important to stress that the qVOA induces the latent virus pool per cell based on a specific activation protocol (e.g. PMA/Iono or αCD3/αCD28 antibody treatment). When compared to HLP in our bulk memory $CD4^+$ T cell activations, PMA/Iono stimulations induced less HIV-1 released from the latent pool from the same tCHI samples. Finally, it should be noted that propagating, replication-competent HIV-1 was released from the tAHI samples treated with HLP using a qVOA assay (with HLP instead of PMA/Iono as the activator) [34]. Finally, the latent HIV-1 released by HLP treatment correlated both with the duration of treatment and proviral DNA load at the time of sample collection (Figure 6D). This three-way 3D-plot reveals that HLP continues to specifically activate the infectious, latent HIV-1 pool, even as it slowly decays during years of cART. Even PMA/Iono, capable of stimulating most $CD4^+$ T cells (and thus highly toxic for therapy), does not have the same specificity and efficiency of inducing the latent HIV-1 reservoir (Figure 6E). Finally, we repeated the analyzes in Figure 6B,D. By first removing the datapoints for P3 and P17, we observed a retention of statistical significance $p = 0.0089$ (Supplementary Figure 7B). A second analysis assumed that treatment interruption (followed by resumption of the same regimen) observed with P3 and P17 was the "treatment start date", i.e. 2.2 and 9.9 years after the actual treatment start date respectively. With treatment interruption/resumption as the treatment start date, we no longer observed a significant correlation between Days on Treatment and Relative proviral DNA copy numbers at time of sample collection (Supplementary Figure 7C). The correlations of Days on Treatment vs Relative proviral DNA copy numbers at time of sample collection vs HLP stimulation (Viral RNA copies/10^6 $CD4^+$ T cells) modelled in the landscape plot (Figure 6D) maintained a smooth plane regardless if the P3 and P17 datapoints were included (Supplementary Figure 7A,D) or not (Supplementary Figure 7B,E). With the revised P3 and P17 treatment start dates, the landscape plot now resulted in "jagged peaks" (Supplementary Figure 7C,F). These findings could suggest that a new latent HIV-1 reservoir was not fully re-established with treatment interruption, an observation that requires further study.

Discussion

An HIV-1 treatment for a cure or remission is a global strategy necessary to reduce the dependency of life long combined antiretroviral therapy (cART) provided to 25 + million, with a total 40 + million predicted by 2030 according to UNAIDS targets [2]. Other pandemics, geopolitical issues, and wars/conflict result in competing challenges to maintain a global supply and access to anti-HIV treatments. As such, cART failure and emergence of drug resistance is at least five times more common in low-to-middle income countries (LMICs) bearing 95% of HIV-1 cases than in high income countries (HICs). Dealing with HIV-1 drug resistance with new treatments will continually drives costs well over $100 billion per year for global HIV-1 treatment strategy [2]. An effective HIV-1 cure therapeutic is paramount to maintain control and reverse the HIV-1 pandemic. To date, the only demonstrated Cure requires a stem cell transplant from CCR5Δ donor, which is unaffordable even for HICs. As described herein, the low cost HLP is (1) 100-fold more effective than any other HIV-1 Cure therapeutic tested, (2) can drive out/eliminate the latent HIV-1 pool from individuals anytime for at least 20 years of stable cART, and (3) would be administered by simple intramuscular "prime" and "boost" injections much like a seasonal flu or SARS-CoV-2 vaccination. The following will provide the leading hypotheses on why the HLP can target the latent HIV-1 pool, why it is so effective at HIV latency reversal, and how the HLP induces an anti-HIV-1 immune response to augment cART to eradicate this HLP-induced, remnant HIV-1 for a cure or remission.

Since the identification of HIV-1 reservoirs during cART in the mid-90s, many approaches have been employed to "drive out" the latent provirus within $CD4^+$ T with limited success [17,18,22,40,41]. Because the integrated proviral DNA is often found in transcriptionally silent sites (e.g. heterochromatin) [8,42] within quiescent, memory $CD4^+$ T cells, chemotherapeutic agents that activate cells/transcription factors (e.g. PKC agonists like Bryostatin) or involve in chromatin de-condensation [8] (e.g. HDAC inhibitors like Vorinostat or Panobinostat) have no cell targeting, but could induce low level HIV-1 mRNA transcription in latently infected cells. To avoid toxicity, repeated low dose treatments were expected to randomly activate

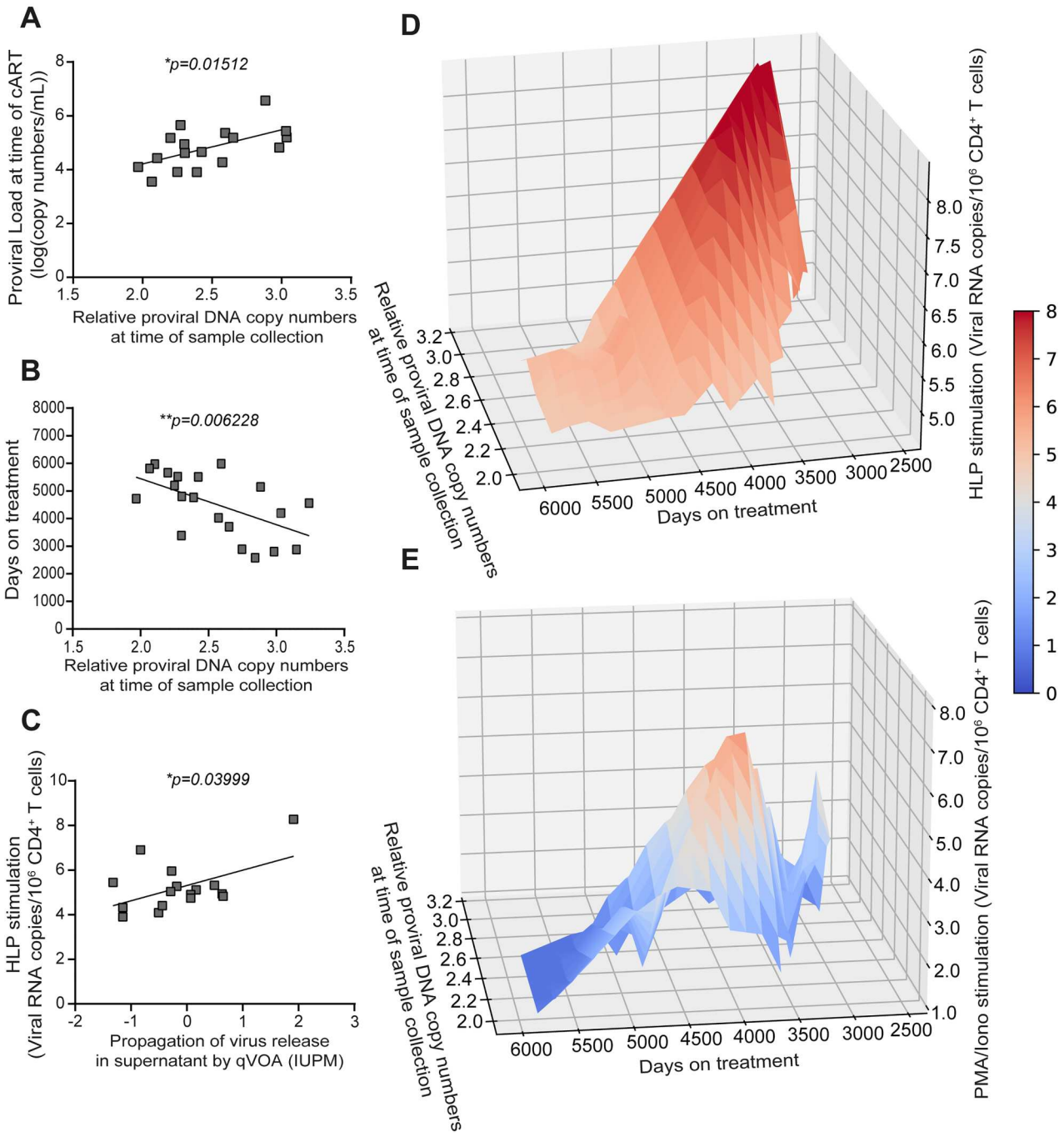

Figure 6. Correlation analysis between proviral load, propagation of virus release in supernatant by qVOA (IUPM), and viral copies in supernatant stimulated by HLP measured by qRT-PCR. (A) Correlation between proviral load at the time point starting combination anti-retroviral treatment (cART) and proviral load at sample collection. (B) Correlation between proviral load at sample collection and time of cART treatment (days). (C) Correlation between viral RNA copies in culture supernatant from latency reversal assay by HLP and measured by qRT-PCR, and a quantitative viral outgrowth assay (qVOA) used a limiting dilution of primary T cells co-cultured with MOLT-4/CCR5 cells to stimulate those cells harbouring latent HIV to propagate further infection, an amount that was expressed as IUPM, infectious units per million cells. Each datapoint represents one donor. (D) A 3D correlation was mapped comparing time of treatment (days) and proviral DNA copy numbers at time of sample collection and viral RNA copies in culture supernatant from latency reversal assay by HLP and measured by qRT-PCR. (E) A 3D correlation was mapped comparing time of treatment (days) and proviral DNA copy numbers at time of sample collection and viral RNA copies in culture supernatant from latency reversal assay by PMA/Iono and measured by qRT-PCR. Colour bar corresponds to the z-axis. A two-tailed Pearson Correlation Coefficient test was used to assess significance (*$p < 0.05$; **$p < 0.01$). HLP, HIV-1/virus-like particle.

and hopefully hit the one in a million CD4$^+$ T cells harbouring latent HIV-1 provirus leading to HIV-1 mRNA transcription and protein production for immune recognition. However, the level of latency reversal was insufficient for continued development beyond phase I/II human trials [14,17,26]. The HLP mediates high level HIV-1 mRNA transcription, protein production, and release of replication-competent virus particles from tCHI CD4$^+$ T cells, a response 2- to 3-logs greater than that observed in treatments

with Bryostatin, Vorinostat, and Panobinostat [8]. Remarkably, this high level of latency reversal by HLP was derived from HIV-1 antigen-specific $CD4^+$ T cells, representing less than 1% of all memory $CD4^+$ T cells. Thus, HLP treatment resulted in both high specificity and high efficiency to drive out the remnant HIV-1 in samples from tCHI. HLP treatment released HIV-1 of high genetic diversity, suggesting the deposition of HIV-1 in the years of chronic infection prior to initiation of stable cART. It is important to note that HLP induced a relatively homogenous HIV-1 population from tAHI samples, indicative of minimal HIV-1 evolution in the weeks-months preceding cART [34].

Why latent HIV-1 might be primarily housed within the HIV-1-specific $CD4^+$ T cells from individuals receiving cART late into their infection is not fully understood from this study alone. Previously, studies provided evidence that before cART initiation, HIV-1 might be primarily infecting HIV-1-specific T cells as compared to other antigen-specific T cells [30–32]. Various studies examining lymph nodes of infected individuals show activation of the $CD4^+$ T cells in the T cell zones surrounding the HIV-1-specific germinal centres [43–45]. Immunohistochemistry-based studies helped to establish the idea that $CD4^+$ T cell activation and exhaustion was mostly related to the ongoing infection surrounding germinal centres [46–48]. Thus, a latent HIV-1 infection during the transition of activated T cells to memory T cells could lead to establishment of latently infected $CD4^+$ T cell populations, mostly within the T cell zones surrounding the HIV-1-specific germinal centres [49]. Density of these proliferating T cells surrounding the HIV-1-specific germinal centres may focus replication to these T cell zones and reduce HIV-1 migration/diffusion into other antigen-specific germinal centres [31]. With the advent of *in situ* single cell sequencing technologies, testing for the mRNA of HIV-1 and of HIV-1-specific TCRs in the T cell zones surrounding specific germinal centres is feasible during acute and chronic infections. However, in the era of test-and-treat, future studies would have a short window of lymph node collection prior to initiation of cART. Studies like this may help to determine why latent HIV-1 is more prevalent in HIV-1-specific T cells than in other antigen-specific T cells. Recently, intact HIV-1 provirus was observed in $CD4^+$ T cells (likely HIV-1-specific based on studies herein) that had upregulation of CD44, CD28, CD127, and the IL-21 receptor [29], providing the possibility for very targeted "shock and kill" approaches with a combination of HLP and modulating antibodies to these markers. Effectiveness of HIV-1 control by these targeted approaches could be monitored by CD33/Sig expression on $CD4^+$ T cells [50] and sensitive detection of the remaining proviral DNA [51,52].

It was hypothesized that the optimal induction of latency reversal would require a heterogenous HLP formulation. As such, we cloned the HIV-1 quasispecies from the blood of five treatment-naïve individuals infected with highly divergent HIV-1 subtype B isolates [33]. This process was repeated to produce HLP formulations based on subtype A and subtype D. As described herein, a subtype D HLP was not superior to a subtype B HLP at latency reversal in samples of HIV-1 subtype D infected individuals. A comprehensive bioinformatic study is now examining the putative HIV-1 epitopes (from the entire proteome) in the HIV-1 sequence database that could be presented by MHC II, i.e. encoded by the common HLA class II alleles. In this study, based on the HIV-1 sequences of each subtype D infected participant and of the HLP construct, over 95% putative HIV-1 epitopes presented by common HLA class II alleles would be presented with the current subtype B-derived HLP formulation. Studies are now underway to determine if an autologous HLP formulation may be more effective than the current multivalent HLP formulations. There is trade-off. Determining the HLA class II alleles and HIV-1 sequence in each HIV-1-infected individual for the production of personalized, autologous HLP for a cure would be of high cost with minimal increased benefit. By comparison, a universal, multivalent HLP that has high epitope coverage for all HIV-1 subtypes and HLA alleles, could be highly effective for a cure, produced and administered at a lower cost for all.

Our previous study revealed that HLP induced transcriptional reactivation and release of replication-competent HIV-1 from latently infected $CD4^+$ T cells from nine participants who initiated antiretroviral treatment during acute/early infection (tAHI) [34]. Latency reversal in this tAHI study represented a minimal bar for preclinical HLP development because (1) less than 5% of the HIV-1 infected population receive cART during acute/early infection and (2) residence of latent HIV-1 in activated $CD4^+$ T cells with TCRs specific for HIV-1 was predicted to be greatest during acute/early infection. Multiple infections by, and vaccination against different pathogens will occur during months to years of chronic HIV-1 infection. Thus, we expected a higher proportion of the latent HIV-1 pool in T cells with TCRs specific to other antigens rather than just in HIV-1-specific $CD4^+$ T cells. In contrast to expectations, HLP induced even higher latency reversal in $CD4^+$ T cells from those receiving stable cART for years, initiated during chronic infection (tCHI), when compared to those who initiated treatment during acute/early infection (tAHI) [34]. In the tCHI samples, HLP induced latency reversal at two to

three logs higher than T cell activators/mitogens, recall antigens/peptides and clinically relevant chemotherapeutic agents.

In this report, we have demonstrated the effectiveness of antigen-driven latency reversal *ex vivo* through HIV-mimetics to specifically target the dominant latent HIV-1 reservoir. In the millions of people living with HIV-1 for years before initiating cART, our HIV-1 virus-like particle formulation (HLP) appears to reactivate the replication-competent portion of the HIV-1 reservoir, but again, this based on limitations of the qVOA assay as a comparator. Just as the HLP can target the HIV-1-specific $CD4^+$ T cells for latency reversal, the same mechanism of HIV-1 antigen presentation will also induce a secondary HIV-1-specific immune response that in association with ongoing cART, could prevent any rebound HIV-1 replication. With completion of ongoing animal toxicity testing, phase I clinical trials could involve HLP administered to individuals on cART through intramuscular injections at specific anatomical sites to ensure uptake and delivery by antigen presenting cells to the draining lymph nodes in that region. We are proposing multiple administrations separated by 6-week intervals while cART is maintained and with active monitoring for reduction in the proviral DNA levels and of the latent HIV-1 pool, i.e. replication-competent provirus. It is important to stress that HLP are dead HIV-1 particles with complete HIV-1 proteomes, including an inactive, mutated integrase encoded by HIV-1 mRNA that lacks the genetic elements for encapsidation and required for reverse transcription [33]. Finally, HLP serves as both a latency reversal agent as well as treatment that will boost HIV-1-specific immune responses, assisting cART in killing of reactivated HIV-1 [33]. The potency of HLP in this study on tCHI samples from Canada, the US, and Uganda suggests the possibility of a global, cost-effective HIV-1 cure strategy for the future.

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Disclosure statement

E.J.A. and J.F.S.M. are the inventors of patent US11596682B2, and E.J.A. of patent AU2007217275A1, both associated with the vectors used in this study. The remaining authors declare no competing interests.

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Authors contributions

M.H.N, J.P., and R.C.Y.H. performed all the experimentation with the assistance of R.P., K.K., and Y.L for the primary cell isolations and analyzes, T.B. and E.N. for the HLP cloning and preparations, R.C. for qRT-PCR, T.B. and A.S.O. for bioinformatic analyzes. Participant screening, sample collection, and viral RNA analyzes involved the team at Rakai Health Sciences Program/John Hopkins University/NIAID (S.T., S.J., A.A., T.K., P.B., R.M.G., S.J.R., T.C.Q., A.D.R, J.L.P.), at CWRU (R.A., J.M.J., D.H.C.), and University of Toronto (C.K.). The RHSP/JHU/NIAID team also measured the virus infectious units and proviral DNA content in the participant samples from Uganda. E.J.A., J.F.S.M., M.H.N., J.P., and R.C.Y.H. have directly accessed and verified the underlying data reported in the manuscript. J.F.S.M. and E.J.A. conceptualized the project, interpreted data and together with M.H.N, J.P., and R.C.Y.H., wrote the manuscript. The entire team provided edits and comments to earlier drafts of the manuscript. M.H.N, J.P., and R.C.Y.H. contributed equally to this manuscript as co-first authors.

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