Efficient HIV-1 *Trans* Infection of CD4⁺ T Cells Occurs in the Presence of Antiretroviral Therapy

Giovanna Rappocciolo,¹,² Nicolas Sluis-Cremer,¹ and Charles R. Rinaldo¹,³

¹Department of Infectious Diseases and Microbiology, Graduate School of Public Health, and Departments of ²Medicine and ³Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

**Background.** Antiretroviral therapy (ART) has dramatically improved the quality of life of people with HIV-1 infection (PWH). However, it is not curative, and interruption of ART results in rapid viral rebound. Cell-to-cell transfer of HIV-1, or *trans* infection, is a highly efficient mechanism of virus infection of CD4⁺ T cells by professional antigen-presenting cells (APCs), that is, dendritic cells (DCs), macrophages, and B lymphocytes.

**Methods.** APC from HIV seronegative donors treated with ART *in vitro* (CCR5 agonist, NRTI, PI and NNRTI, alone or in combination), were loaded with HIV R5-tropic HIV_Bal and mixed with autologous or heterologous CD4⁺ T lymphocytes to assess *trans* infection. Ex vivo APC from chronic HIV-infected MACS participants before and after initiation of ART, were also loaded with HIV R5-tropic HIV_Bal and tested for trans infection against autologous or heterologous CD4⁺ T lymphocytes. Virus replication was measured by p24 ELISA.

**Results.** Here we show in vitro that antiretroviral drugs did not block the ability of DCs and B cells to *trans*-infect CD4⁺ T cells, although they were effective in blocking direct *cis* infection of CD4⁺ T cells. Moreover, ex vivo DCs and B cells from ART-suppressed PWH mediated efficient HIV-1 *trans* infection of CD4⁺ T cells, which were resistant to direct *cis* infection.

**Conclusions.** Our study supports a role for HIV-1 *trans* infection in maintenance of the HIV-1 reservoir during ART.

**Keywords:** antigen-presenting cells; ART; B lymphocytes; dendritic cells; HIV; *trans* infection.
METHODS

Ethics Statement
Biological samples were acquired and studied from consented individuals according to University of Pittsburgh International Review Board–approved protocols. All recruited participants were over the age of 18 and provided informed consent before sample collection or use.

Participants
We studied 10 HIV-1 chronically infected participants of the Pittsburgh portion of the MACS who were receiving ART who had an undetectable viral load and CD4+ T-cell counts >500 cells/mm^3 at the time of the study. Two HIV-1 nonprogressors (NPs) who chose to initiate ART were also studied. HIV-1-seronegative blood bank donors were used to test the effect of ART on trans infection in vitro. A standard HIV-1-seronegative donor was always tested in parallel with MACS participants as a control for assay performance.

Cell Isolation and Culture
CD4+ T lymphocytes, B lymphocytes, and monocytes were positively enriched from freshly isolated or frozen peripheral blood mononuclear cells (PBMCs) from consented Pittsburgh MACS participants or anonymous blood bank donors using anti-CD4, CD19, or CD14 monoclonal antibody (mAb)–coated magnetic bead separation (Miltenyi Biotech), according to the manufacturer’s instructions. DCs were derived from monocytes by culture with 1000 U/mL of granulocyte-macrophage colony-stimulating factor (GM-CSF; Miltenyi Biotech) and 1000 U/mL of recombinant human interleukin 4 (rhIL-4; R&D Systems) for 5 days in AIM-V medium (Gibco). CD4+ T cells and B cells were activated for 48 hours with 10 U/mL of delectedinated interleukin 2 (IL-2; Roche) and 2 ug/mL of phytohemagglutinin (PHA; Sigma) or 1000 U/mL of rhIL-4 (R&D Systems) and 0.1 ug/mL of CD40L (Enzo Life Sciences), respectively.

R5-tropic HIV-1^rav^ purified from PM1 cells (obtained through the National Institutes of Health [NIH] AIDS Reagent Program, Division of AIDS, NIAID, NIH. Lusso et al [18]) was used for the cis and trans infection experiments. Virus stock titration and experimental HIV-1 Gag p24 measurements were acquired by ELISA using the HIV-1 p24 Antigen Capture Immunoassay kit (SAIC-Frederick), per the manufacturer’s instructions.

ART Drugs
The following ART drugs were tested at the indicated final concentrations: maraviroc (1 μM), tenofovir (100 μM), rilpivirine (500 nM), and darunavir (500 nM).

Trans and Cis Infection of CD4+ T Cells

Trans Infection
To measure trans infection, 1 × 10^6 APCs were loaded with HIV-1^rav^ at 10^-3 multiplicity of infection (m.o.i.) in minimal volume for 2 hours at 37°C, washed 3 times with cold medium, and co-cultured with autologous PHA/IL-2-activated CD4+ T cells at a 1:10 ratio in complete medium [11]. APCs were also exposed to ART drugs alone or in combination before use in the trans infection experiments.

Cis Infection
CD4+ T cells untreated or exposed to ART drugs were infected directly in cis with HIV-1^rav^ at an m.o.i. of 10^-3 to determine susceptibility to infection. Supernatants from the trans and cis cultures were sampled every 4 days and tested for HIV-1 Gag p24 by ELISA.

Statistics
Data were analyzed by 1-way analysis of variance, followed by the Student t test. GraphPad prism 7.0 Software was used for statistical analysis.

RESULTS

APC-to-CD4+ T-Cell Trans Infection by HIV-1 Is Not Inhibited by ART In Vitro
We previously reported [10, 11] that HIV-1 trans infection of CD4+ T cells by DC and B cells results in highly efficient virus replication in CD4+ T cells, as measured by levels of HIV-1 Gag p24 over 12 days of co-culture. Here we set out to determine the effect of ART drugs on APC-mediated cell-to-cell HIV-1 trans infection. We first exposed B cells, DCs, and CD4+ T cells obtained from healthy, de-identified, HIV-1-negative blood donors to the protease inhibitor (PI) darunavir, the nucleoside reverse transcriptase inhibitor (NRTI) tenofovir, the nonnucleoside reverse transcriptase inhibitor (NNRTI) rilpivirine, or the CCR5 co-receptor-blocking agent maraviroc (Figure 1A, B, and C, respectively). All drugs were used at concentrations that blocked direct HIV-1 cis infection of CD4+ T cells (Figure 1C). We used HIV-1 Gag p24 levels in culture supernatants over time as a measure of productive infection in CD4+ T cells. Therefore, only levels of infectious, replicating HIV-1 were measured. CD4+ T cells from the same donors were also tested for their susceptibility to HIV-1 cis infection, either in the presence of ART drugs or when left untreated. Finally, the amount of input virus loaded into DCs and B cells for assessing trans infection was chosen to be highly inefficient for productive infection of CD4+ T cells in cis [8, 10, 11]. This allowed us to focus on the levels of p24 measured in the APC-T-cell co-cultures resulting from trans infection of CD4+ T cells.

As shown in Figure 1A, B-cell-mediated trans infection of CD4+ T cells was not significantly affected over 12 days by exposure to the drugs tested. Among the drugs tested, treatment with darunavir and maraviroc did not affect the ability of both B cells and DCs to efficiently transfer infectious, replicating virus to the target CD4+ T cells. Only rilpivirine and tenofovir treatment significantly reduced the efficiency of trans infection at the earlier time points, although we were still able to detect HIV-1 Gag p24 production by day 12 in culture comparable to that in untreated cultures.

2 • OFID • Rappocciolo et al.
Figure 1. Effect of antiretroviral drugs on B cell and DC mediated\textit{trans} infection in \textit{vivo}. Panel A and B. B cells or DC were treated with the indicated drugs, loaded with HIV\textsubscript{Al} at 10\textsuperscript{-3} m.o.i. and mixed with activated, purified autologous CD4\textsuperscript{+} T cells as described in Methods. Panel C. Cell-free \textit{cis} infection of CD4\textsuperscript{+} T cells with HIV\textsubscript{Al} at 10\textsuperscript{-1} m.o.i. was conducted in parallel to determine CD4\textsuperscript{+} T cells susceptibility to infection. Cell culture supernatants were collected at the indicated time points and HIV-1 Gag p24 was measured by ELISA. Data are mean values ±SE; n=4 experiments. GraphPad prism 7.0 Software was used for statistical analysis (one-way ANOVA followed by Students t test. *p<0.05) ‡= below limit of detection. Abbreviation: ns, nonsignificant.
Notably, treatment with maraviroc, a CCR5-blocking agent, significantly enhanced trans infection of target CD4+ T cells by 8 days of co-culture when B lymphocytes were used, but this effect was not detected at day 12 (Figure 1A). Maraviroc has been reported to increase CCR5 expression on CD4+ T cells in both humans and macaques [19, 20], suggesting that it could actually increase the susceptibility of T cells to HIV-1 infection. Interestingly, we did not observe a maraviroc-enhancing effect on trans infection in the DC-CD4+ T-cell co-cultures, which was present when B cells were used as APCs.

Considering that most ART regimens are now comprised of >1 drug, we tested the efficiency of trans infection in the presence of a 3-drug combination, that is, darunavir/rilpivirine/tenofovir (DRV/RPV/TVF). As shown in Figure 2, efficient trans infection occurred even in the presence of the DRV/RPV/TVF combination using either DCs or B cells as APCs (Figure 2A and B, respectively). As a comparison, the effect of the TVF/RPV/DRV combination on effectively reducing cell-free cis infection of CD4+ T cells is shown in Figure 2C.

**Figure 2.** Effect of combination antiretroviral drugs on B cells and DC mediated trans infection in vitro. B cells (panel A) or DC (panel B) were treated with the indicated drugs in combination, loaded with HIV at 10^-3 m.o.i. and mixed with activated, purified autologous CD4+ T cells. Purified CD4+ T cells were also infected in cis in the presence of the drug combination (panel C). Drug concentrations are described in Methods. Data are mean values ±SE; n=4 experiments. Panel D and E: B cells and DC were treated with up to 9 times the concentration of maraviroc or of the three drug combination, respectively and used in trans infection experiments as described above. Panel F: purified CD4+ T cells were also infected with cell free virus in the presence of maraviroc or of the three drug combination in decreasing concentrations. Cell cultures supernatants were collected at the indicated time points and HIV-1 Gag p24 was measured by ELISA. Data are representative of 2 independent experiments and are mean values ±SE of triplicate wells. GraphPad prism 7.0 Software was used for statistical analysis (one-way ANOVA followed by Students t test. *p<0.05) ‡= below limit of detection. Abbreviations: DC, dendritic cell; DRV/RPV/TVF, darunavir/rilpivirine/tenofovir.
It has been postulated that the lack of effect of ART on trans infection is a consequence of high virus particle delivery at the cell-to-cell juncture [13, 21]. Therefore, we tested whether HIV-1 trans infection could be blocked by increasing the drug concentrations to overcome the trans infection effect. As shown in Figure 2D and E, increasing drug concentrations up to 9 times the level needed to block cis infection resulted in suppression of HIV-1 trans infection by B cells and DCs, respectively. By the same token, reducing the drug concentration up to 9 times allowed for cis infection of CD4+ T cells (Figure 2F).

Taken together, these data show that APC-mediated, efficient trans infection of T cells with HIV-1 can take place in the presence of ART drugs and can be overcome only by high ART concentrations.

**Ex Vivo APCs From Participants on ART Can Trans-Infect CD4+ T Cells**

We next addressed whether APCs derived from HIV-1-infected individuals on virus-suppressive ART can trans-infect autologous CD4+ T cells ex vivo. We tested 10 participants under suppressive ART and found that B cells and DCs derived from these individuals mediated efficient trans infection of autologous CD4+ T cells compared with trans infection mediated by APCs archived before therapy initiation (Figure 3A and B, respectively). We also determined if potential ex vivo carryover of antiviral drug activity could affect direct cis infection of CD4+ T cells, comparing PBMCs obtained from the same MACS participants before and after initiation of ART. As shown in Figure 3C, CD4+ T cells post-ART were not able to support HIV-1 replication as well as T cells from pre-ART blood, indicating that the in vivo antiviral effect of their ART carried over ex vivo. Even in the face of this antiviral effect on the CD4+ T cells ex vivo, we found that both DCs and B cells of HIV-1-infected participants on ART were able to trans-infect autologous CD4+ T cells derived from the same blood samples (Figure 2A and B, respectively) with levels of HIV-1 Gag p24 at least 1 log10 higher than by cis infection (Figure 2C). As we observed in seronegative participants, B-lymphocyte-mediated trans infection resulted in higher levels of virus replication compared with DCs. Thus, APCs from participants on virus-suppressive ART and tested ex vivo can efficiently mediate HIV-1 trans infection of CD4+ T cells that are resistant to direct cis infection.
We have previously shown that APCs from PWH who are able to control disease progression in the absence of ART, that is, NPs, are unable to trans-infect autologous and heterologous CD4+ T cells and that this phenotype is under the control of cellular cholesterol homeostasis regulation [10]. Here we tested 2 NPs who chose to initiate ART to determine if this phenotype was maintained during therapy. As shown in Figure 4, B cells derived from NPs before or after initiation of ART (NP-ART) were unable to trans-infect autologous CD4+ T cells or heterologous CD4+ T cells obtained from an HIV-1-seronegative donor (SN). B cells from the SN donor were able to trans-infect autologous CD4+ T cells from SN donor (grey bar). CD4+ T cells from NP under ART were co-cultured with B cells from a SN donor loaded with HIVBal at 10^-3 m.o.i (thatched bar) (B) Purified CD4+ T cells from NP were infected in cis with HIVBal at 10^-1 m.o.i. in parallel with CD4+ T cells cryopreserved prior to ART initiation. Supernatants were collected at the indicated time points and tested for HIV-1 Gag p24. Data are representative of 2 independent experiments and are mean values ±SE of triplicate wells. GraphPad prism 7.0 Software was used for statistical analysis. *p<0.05. Abbreviations: ART, antiretroviral therapy; NP, nonprogressor; SN, HIV-1-seronegative donor.

**DISCUSSION**

Here we show that HIV-1 trans infection of CD4+ T cells by 2 types of APCs, DCs and B lymphocytes, is insensitive to virus-suppressive levels of ART, that is, darunavir, rilpivirine, and maraviroc; rilpivirine, and to some extent tenofovir treatment in vitro, showed a limited effect. This was demonstrated both by in vitro treatment of these APCs from HIV-1 SN donors with concentrations of ART drugs sufficient to block cis infection and by inefficient HIV-1 trans infection mediated by APCs derived from the peripheral blood of HIV-1-infected individuals on suppressive ART. The concept that ART has a limited effect on trans infection by HIV-1 has been a long-standing concern regarding the efficacy of ART, primarily in terms of T-cell-to-T-cell infection [12, 13, 22, 23]. To our knowledge, there have not been comprehensive studies on the effects of ART on APC-to-T-cell trans infection [15, 24, 25], although a recent report showed that 2 formulations of current ART drugs, that is, tenofovir and raltegravir, administered in vitro failed to inhibit DC-to-T-cell trans infection [16]. Thus, our analysis is the first both to assess the effects of multiple types of ART on APC trans infection in vitro and ex vivo and to emphasize the importance of this being a stealth mode to circumvent the antiviral effects of ART.

Our study supports that APC-mediated trans infection of T cells with HIV-1 yields a high level of virus replication. The burst of virus production resulting from trans infection of target cells can be up to 1000-fold higher than that resulting from HIV-1 cis infection through passive dissemination in the extracellular fluid [26]. This has also been observed in time-lapse videos documenting the transmission of multiple viral particles at the point of contact between cells [27]. In fact, we regularly observed a 20- to 100-fold-greater level of HIV-1 replication mediated by DCs and B cells compared with direct cis infection of either autologous or heterologous CD4+ T cells, using a relatively low input multiplicity of HIV-1 (m.o.i. 10^-5) compared with the 100-fold higher amount of HIV-1 used to demonstrate cis infection (m.o.i. 10^-1). Interestingly, using colonic explant models showed that cell-associated HIV-1 in the mucosa is transmitted at a much higher efficiency compared with cell-free virus [28] and that myeloid DCs in human cervical explants are the first cells to capture HIV-1 and transfer it to mucosal CD4+ T cells [3].
The high efficiency of cell-to-cell HIV-1 infection is thought to be due to the formation of the virologic synapse, a virus-orchestrated contact between a cell carrying infectious virus and an uninfected target cell, which can overcome the target cell barriers that are active against cell-free HIV-1 infection [14, 29, 30]. Others have shown a lack of ART effect when trans infection is mediated by an APC [16, 21, 31], although these were in vitro studies. It is not clear if this phenomenon relates to HIV-1 replication in the APCs or to high virus particle transfer from these APCs to the T cells. A role for virus replication in trans infection by APCs is not likely the case for B lymphocytes, as they do not support productive HIV-1 replication [11]. However, CD40L-activated B cells can take up HIV-1 via the intercellular adhesion molecule-3–grabbing nonintegrin (DC-SIGN), which is essential for their trans infection of T cells [11]. Our data show that HIV-1 replication is not necessary in APCs for productive trans infection of CD4+ T cells, given the more efficient B-cell-mediated trans infection compared with DC-mediated trans infection, confirming previous observations [8, 11]. In contrast, HIV-1 can infect DCs through DC-SIGN or CD4 and chemokine receptors and lead to a limited, low level of virus production or no evidence of virus replication [24].

Although the efficiency of cell-to-cell transmission of HIV-1 is well established, its importance in the pathogenesis of HIV-1 is uncertain. There is conflicting evidence that viral persistence is achieved in persons on ART by ongoing virus replication in lymphoid tissues, with full HIV-1 suppression in the peripheral blood [13, 32–36]. Our current and previous results [8, 10, 11] argue for a pivotal role of APC-mediated trans infection in the control of HIV-1 disease progression and their role in the maintenance of the viral reservoir. The recognized importance of HIV-1 sequestration in B-cell follicles further supports a role for these APCs in maintaining virus infection through trans infection during the normal process of antigen presentation, thereby transferring infectious virus with high efficiency [7, 37].

Data presented here strengthen the notion that trans infection can be a potent mechanism of HIV-1 persistence in the B-cell follicles of lymphoid tissues [38], where target cells are in close proximity, allowing for the formation of APC-target cell contact [39]. Here we show for the first time that B-lymphocyte transfer of HIV-1 to CD4+ T cells is not susceptible to ART and that B cells derived from PWH under fully suppressive ART still maintain the ability to trans-infect autologous CD4+ T cells. Thus, myeloid DCs could mediate HIV-1 trans infection during their normal interaction with and signaling of immune responses in CD4+ follicular helper T cells (Tfh). Likewise, B lymphocytes normally signal Tfh to initiate antibody responses in B-cell follicles [40]. When these APCs are infected with HIV-1, this could create a constant supply of infected, virus-replicating target T cells in a privileged lymphoid compartment. There, ART drugs have limited access [32] and thus may not reach the necessary concentrations to overcome the high virus particle concentration at the site of cell-to-cell transfer [13, 14]. In tissues where target cells and APCs are in close contact, sufficiently high concentrations of HIV-1 are likely to be achieved, allowing for efficient spread and ART evasion.

Notably, APCs from HIV-1 NP s who have chosen to undergo ART maintained their inability to transfer virus to CD4+ T cells, with or without ART. In this regard, our recent data on the role of APCs’ cholesterol homeostasis in HIV-1 disease progression could inform effective strategies as an adjuvant to ART. Interestingly, a recent study showed that concomitant use of statins after treatment interruption is associated with lower risk of virologic rebound [9]. Taken together, these data highlight the pivotal importance of APC-mediated cell-to-cell spread of HIV-1 in the face of effective ART measured as viral suppression in the periphery. This stresses the need for a more comprehensive approach to the eradication of reservoirs, where interference with APC function could provide an important tool in thwarting efficient HIV-1 spread.

Acknowledgments

The authors wish to thank the participants of the MACS for their dedication to the study, without which this work would not have been possible. We thank William Buchanan and Jeffrey Toth from the Pitt Men’s Study MACS clinic for their support and Kathy Hartle for technical contribution. We also thank Drs. Jeremy Martinson and Paolo Piazza for their insightful discussions of this work.

Financial support. This work was supported by National Institutes of Health grants R01AI118403, U01-AI35041, R01AI081571 and R01GM086406.

Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Prior presentation. Part of this work was presented at the 8th HIV Persistence During Therapy Conference December 12-15, 2017; Miami, FL; and the 22nd International AIDS Conference July 23-27, 2018; Amsterdam, the Netherlands.

References

1. Wong JK, Hezareh M, Günthard HF, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 1997; 278:1291–5.
2. Archin NM, Sung JM, Garrido C, et al. Eradicating HIV-1 infection: seeking to clear a persistent pathogen. Nat Rev Microbiol 2014; 12:750–64.
3. Trifonova RT, Bollman B, Bartenueva NS, Lieberman J. Myeloid cells in intact human cervical explants capture HIV and can transmit it to CD4 T cells. Front Immunol 2018; 9:2719.
4. Cameron PU, Freudenthal PS, Barker JM, et al. Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells. Science 1992; 257:383–7.
5. Geijtenbeek TB, Kwon DS, Torensma R, et al. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 2000; 100:587–97.
6. Hammonds JE, Beeman N, Ding L, et al. Siglec-1 initiates formation of the virus-containing compartment and enhances macrophage-to-T cell transmission of HIV-1. PLoS Pathog 2017; 13:e1006181.
7. Wu L, KewalRamani VN. Dendritic-cell interactions with HIV: infection and viral dissemination. Nat Rev Immunol 2006; 6:659–68.
8. DeLucia DC, Rinaldo CR, Rappocciolo G. Inefficient HIV-1 trans infection of CD4(+) T cells by macrophages from HIV-1 nonprogressors is associated with altered membrane cholesterol and DC-SIGN. J Virol 2018; 92.
9. Drechsler H, Ayers C, Cutrell J, et al. Current use of statins reduces risk of HIV rebound on suppressive HAART. PLoS One 2017; 12:e0172175.
Rappoccio et al. Alterations in cholesterol metabolism restrict HIV-1 trans infection in nonprogressors. MBio 2014; 5:e01031–13.

10. Rappoccio G, Jais M, Piazza P, et al. DC-SIGN on B lymphocytes is required for transmission of HIV-1 to T lymphocytes. PLoS Pathog 2006; 2:e70.

11. Gupta P, Balachandran R, Ho M, et al. Cell-to-cell transmission of human immunodeficiency virus type 1 in the presence of azidothymidine and neutralizing antibody. J Virol 1989; 63:2361–5.

12. Sigal A, Kim JT, Balazs AB, et al. Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. Nature 2011; 477:95–8.

13. Sigal A, Kim JT, Balazs AB, et al. Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. PLoS Pathog 2011; 7:e1002211.

14. Sigal A, Kim JT, Balazs AB, et al. Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. J Virol 2011; 85:7169–76.

15. Hübner W, McNerney GP, Chen P, et al. Quantitative 3D video microscopy of HIV transfer across T cell virological synapses. Science 2009; 323:1743–7.

16. Kim JT, Chang E, Sigal A, Baltimore D. Dendritic cells efficiently transmit HIV to T cells. Retrovirology 2013; 10:161.

17. Duncan CJ, Williams JP, Schiffner T, et al. High-multiplicity HIV-1 infection and neutralizing antibody evasion mediated by the macrophage-T cell virological synapse. J Virol 2014; 88:2025–34.

18. Russo P, Cocchi F, Balotta C, et al. Growth of macrophage-tropic and primary human immunodeficiency virus type 1 (HIV-1) isolates in a unique CD4+ T-cell clone (PM1): failure to downregulate CD4 and to interfere with cell-line-tropic HIV-1. J Virol 1995; 69:3712–20.

19. Hunt PW, Shulman NS, Hayes TL, et al. The immunologic effects of maraviroc block cell-to-cell spread of HIV-1 between T cells. Retrovirology 2013; 10:336.

20. Dinoso JB, Kim SY, Wiegand AM, et al. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. Proc Natl Acad Sci U S A 2009; 106:9403–8.

21. Fletcher CV, Staskus K, Wietgrefe SW, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. Proc Natl Acad Sci U S A 2014; 111:2307–12.

22. Keenan MF, Wiegand A, Shao W, et al. Ongoing HIV replication during ART reconsidered. Open Forum Infect Dis 2017; 4(X):XXX–XX.

23. Lorenzo-Redondo R, Fryer HR, Bedford T, et al. Persistent HIV-1 replication maintains the tissue reservoir during therapy. Nature 2016; 530:51–6.

24. Rosenbloom DIS, Hill AL, Laskey SB, Siliciano RF. Re-evaluating evolution in the HIV reservoir. Nature 2017; 551:E6–9.

25. Sengupta S, Siliciano RF. Targeting the latent reservoir for HIV-1. Immunity 2018; 48:787–92.

26. Lert K, Smed-Sørensen A, Vasudevan J, et al. Myeloid and plasmacytoid dendritic cells transfer HIV-1 preferentially to antigen-specific CD4+ T cells. J Exp Med 2005; 201:2023–33.

27. Bronnimann MP, Skinner PJ, Connick E. The B-cell follicle in HIV infection: barrier to a cure. Front Immunol 2018; 9:20.

28. Grakoui A, Bromley SK, Sureau C, et al. The immunological synapse: a molecular machine controlling T cell activation. Science 1999; 285:221–7.

29. Nields J, Kaufmann DE. Harnessing T follicular helper cell responses for HIV vaccine development. Viruses 2018; 10:336.