Published in final edited form as:

Sci Transl Med. 2020 March 04; 12(533): . doi:10.1126/scitranslmed.aav3491.

Abundant HIV-infected cells in blood and tissues are rapidly cleared upon ART initiation during acute HIV infection

Louise Leyre¹, Eugène Kroon², Claire Vandergeeten³, Carlo Sacdalan², Donn J. Colby², Supranee Buranapraditkun⁴, Alexandra Schuetz^{5,6,7}, Nitiya Chomchey², Mark de Souza², Wendy Bakeman³, Rémi Fromentin¹, Suteeraporn Pinyakorn^{6,7}, Siriwat Akapirat⁵, Rapee Trichavaroj⁵, Suthat Chottanapund², Sopark Manasnayakorn⁴, Rungsun Rerknimitr⁴, Phandee Wattanaboonyoungcharoen⁴, Jerome H. Kim⁸, Sodsai Tovanabutra^{6,7}, Timothy W. Schacker⁹, Robert O'Connell^{5,7}, Victor G. Valcour¹⁰, Praphan Phanuphak^{2,4}, Merlin L. Robb^{6,7}, Nelson Michael⁷, Lydie Trautmann^{6,7,†}, Nittaya Phanuphak², Jintanat Ananworanich^{6,7,11,‡}, Nicolas Chomont^{1,‡,*} RV254/SEARCH010, RV304/SEARCH013 and SEARCH011 Study Groups

¹Centre de Recherche du CHUM and Department of microbiology, infectiology and immunology, Université de Montréal, Montréal, QC H2X 0A9, Canada ²SEARCH, Thai Red Cross AIDS Research Centre, Bangkok 10330, Thailand ³Vaccine and Gene therapy Institute-Florida, FL 34987, United States ⁴Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand ⁵Department of Retrovirology, Armed Forces Research Institute of Medical Sciences, Bangkok 10400, Thailand ⁶Henry M. Jackson Foundation Advancement of Military Medicine, Bethesda, MD 20817, United States ⁷U. S. Military HIV research Program, Walter Reed Army Institute of Research Silver Spring, MD 20910, United States ⁸International Vaccine Institute, Seoul 08826, Korea ⁹Department of Medicine, Medical School, University of Minnesota, Minneapolis, Minnesota 55455, United States ¹⁰University of California San Francisco, San Francisco 94117, CA, United States ¹¹Department of Global Health, University of Amsterdam, Amsterdam, The Netherlands

*To whom correspondence should be addressed. nicolas.chomont@umontreal.ca.

Author contributions: L.L. designed and performed the experiments, analyzed the data, and wrote the manuscript draft. E.K., C.S., D.J.C., N.Chomchey, N.P. managed the participant recruitment, follow-up in the studies, and provided conceptual advice. C.V., W.B. and R.F. designed and performed part of the experiments. S.B. provided pellets of LNMCs and flow analyses on lymph nodes. A.S. provided flow analyses on gut biopsies. S.P. provided help in statistical analyses. MdeS, S.A., R.T., S.T. designed and managed the laboratory analyses for the clinical study. S.C., S.M., R.R., P.W., J.H.K., T.W.S., R.O., V.G.V., P.P., M.L.R., and N.M. provided support for the clinical studies. T.W.S. and L.T. provided conceptual advice. J.A. designed the clinical study, provided conceptual advice and edited the manuscript. N.Chomont designed the experiments, analyzed the data, and wrote the manuscript draft. All authors read, edited and approved the manuscript.

†Present address: OHSU, Vaccine & Gene Therapy Institute, Beaverton, OR, United States.

‡Contributed equally to this manuscript

Competing interests: J.A. has received honoraria from ViiV Healthcare; Merck; Abbvie, Gilead Sciences; and Roche Pharmaceuticals for her participation in advisory meetings. V.V. has consulted for Merck and ViiV Healthcare. N.C. is a board member of Theravectys. For the remaining authors, none were declared.

Disclaimer: The views expressed are those of the authors. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, U.S. Army or Department of Defense, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government including the U.S. National Institutes of Health. The investigators have adhered to the policies for protection of human subjects as prescribed in AR-70.

Data and Materials Availability: All data associated with this study are present in the paper or Supplementary Materials

Abstract

The timing and location of the establishment of the viral reservoir during acute HIV infection remain unclear. Using longitudinal blood and tissue samples obtained from HIV-infected individuals at the earliest stage of infection, we demonstrate that frequencies of infected cells reach maximal values in gut-associated lymphoid tissue and lymph nodes as early as Fiebig stage II, before seroconversion. Both tissues displayed higher frequencies of infected cells than blood until Fiebig stage III, after which infected cells were equally distributed in all compartments examined. Initiation of ART at Fiebig stages I-III led to a profound decrease in the frequency of infected cells to nearly undetectable level in all compartments. The rare infected cells that persisted were preferentially found in the lymphoid tissues. Initiation of ART at later stages (Fiebig IV/V stages and chronic infection) induced only a modest reduction in the frequency of infected cells. Quantification of HIV DNA in memory CD4⁺ T cell subsets confirmed the unstable nature of the majority of infected cells at Fiebig stages I-III and the emergence of persistently infected cells during the transition to Fiebig stage IV. Our results indicate that although a large pool of cells is infected during acute HIV infection, the majority of these early targets are rapidly cleared upon ART initiation. Therefore, infected cells present post-peak viremia have a greater ability to persist.

One Sentence Summary:

Although a large pool of cells is infected during acute HIV infection, the majority of these early targets are rapidly cleared upon ART initiation.

Introduction

Although lifelong suppression of HIV replication with antiretroviral therapy (ART) now seems possible, medication side effects, the risk for drug resistance, stigma and substantial costs all contribute to the necessity of finding a cure (1, 2). ART alone does not eradicate HIV: even after more than 15 years of intensive and continuous therapy, viral rebound occurs within a few weeks upon cessation of ART in all but exceptional cases (3, 4).

HIV persists in a latent form in a small pool of long-lived memory CD4⁺ T cells (5–7) which is considered the major obstacle to eradication (8). HIV latency may be established directly in resting CD4⁺ T cells (9) or during the contraction phase of the immune response, when the antigen load decreases and activated cells transition from an effector to a memory phenotype (10). While the first model implies that latently infected cells are generated during the first hours following viral dissemination, the temporal constraints of memory T cell generation involved in the second model suggest that latently infected cells may not be established during the first days of infection. Regardless of the mechanism by which latently infected cells are generated, a persistent viral reservoir is unavoidably established rapidly both in HIV-infected humans and in SIV-infected non-human primates (NHPs) and is the source of viral rebound upon ART cessation, even when suppressive therapy is initiated at the earliest sign of infection (11, 12). This pool of infected cells harbouring replication competent HIV is maintained by survival as well as homeostatic and antigen-induced proliferation (13–19).

During the past decade, considerable efforts have been made to reduce the size of this persistent reservoir and to facilitate its immune control, with the objective of developing a functional cure for HIV infection. Unfortunately, most of these approaches have had minimal impact on the size of the reservoir (20–23) and did not result in a significant delay to viral rebound nor in a lower viral setpoint upon ART cessation (24, 25). To date, early initiation of ART is the only intervention that has a measurable and reproducible impact on the size of the HIV reservoir in humans.

During acute infection, plasma viral load increases rapidly and then falls to reach a viral set point (26–29). ART initiation early in infection leads to a rapid decay in viremia and in the frequency of circulating infected cells at all stages (30–33). However, the frequency of infected cells in blood and tissues from individuals at the earliest stages of HIV infection and how the size of this pool is affected by ART remain unclear. In the absence of ART, most infected cells contain labile forms of unintegrated viral DNA (34), which precedes integrated viral DNA, and is followed by productive infection and, usually, rapid cell death (35–37). Although the majority of viral genomes are intact at the earliest phase of infection (38), defective proviruses rapidly accumulate (39).

Individuals who start ART early in infection display a smaller reservoir compared to those initiating ART at a later stage (32, 33, 40–45). Although early ART dramatically curbs the size of the persistent reservoir during therapy, most early treated individuals experience rapid viral rebound following analytic treatment interruption (ATI) (43, 46, 47). Studies in NHPs revealed that, although early ART restricts the pool of SV-infected cells (48), a long-lived reservoir harboring replication-competent SIV is already established 3 days post infection, when plasma viremia is not yet detectable (12). In these models, lymphoid tissues appear to represent preferential reservoirs for persistent SIV during ART (49). However, because of the difficulty of accessing samples from individuals at the earliest stage of HIV infection, the tissues and cell subsets in which this reservoir is primarily established and maintained in humans remain largely unidentified. Since blood harbors relatively few T cells compared to lymphoid tissues, such as lymph nodes and gut-associated lymphoid tissue (GALT) which are known to be preferential sites for HIV persistence during ART (50–53), analyzing blood and tissues collected from individuals at the earliest phase of HIV infection is crucial to identify the compartments in which the HIV reservoir is seeded and persists during ART.

In this study, we sought to identify the tissues and cell subsets in which HIV establishes and maintains its reservoir in a cohort of acutely-infected individuals who initiated ART at the earliest stage of HIV infection. The RV254/SEARCH 010 cohort enrolls acutely infected individuals in Fiebig stages I–V stages who initiate ART within a median time of 2 days after diagnosis. In a subset of participants, lymph nodes and sigmoid colon tissue were obtained and large numbers of PBMCs were collected by leukapheresis, allowing us to study the establishment of cellular and tissue reservoirs during acute infection and their persistence during ART.

Results

The frequency of HIV-infected cells in tissues rapidly increases throughout acute infection

Most participants were male (96%) with a median age of 27 years [IQR 23–33] and the majority of them (69%) were infected with HIV-1 CRF01-AE (Table 1). As expected, HIV-1 plasma viral loads were relatively low in Fiebig I participants ($4.1 \log_{10}$ copies/mL [IQR 3.6–4.7]) and peaked at the Fiebig III stage ($6.4 \log_{10}$ copies/mL [IQR 5.7–7.0], fig. S1A). The CD4/CD8 ratio gradually decreased after the Fiebig II stage (fig. S1b), reflecting depletion of CD4+ T cells and concomitant proliferation of CD8+ T cells (fig. S1c–d).

To determine the timing and location of the establishment of the pool of infected cells during the earliest phases of HIV infection, we measured the frequency of cells harboring integrated HIV DNA in blood and tissues (inguinal lymph nodes and sigmoid colon biopsies) in recently infected individuals enrolled at different stages of acute HIV infection (Fiebig I to V) as well as in chronically infected controls. In peripheral blood, integrated HIV DNA values showed a continuous increase through all acute infection stages but remained lower than values in chronic infection, even in Fiebig IV/V participants (all comparisons to chronic $p < 0.001$, fig. 1a). Measurements of integrated HIV DNA in lymph nodes and sigmoid colon biopsies from these participants revealed very different kinetics: although infected cells were rarely detected in tissues from Fiebig I participants, maximal frequencies of lymph node and sigmoid colon cells harboring integrated HIV DNA were reached at the Fiebig II stage and remained stable through chronic infection (fig. 1b–c). This suggested that the bulk of infected cells is first established in tissues and that the virus subsequently disseminates in the blood, possibly through the recirculation of infected cells or because blood cells are infected at a later stage. Indeed, integrated HIV DNA was not detected in a substantial proportion of blood samples collected in recently infected participants (35%), but was often detected in matched lymph node or matched sigmoid colon from these individuals (79%, $p = 0.04$ and 76%, $p = 0.08$ respectively, compared to blood) (fig. 1d), in sharp contrast with chronically infected controls who displayed detectable amounts of integrated viral genomes in all compartments. We frequently observed higher frequencies of cells harboring integrated HIV DNA in lymph node or sigmoid colon compared to peripheral blood in Fiebig I, II and III participants (Fiebig I: LN vs blood $p < 0.05$; Fiebig II and III: sigmoid colon vs blood $p < 0.01$ and $p < 0.001$, respectively, fig. S2a–e). Most of these differences were still observed when only matched samples were included in the analysis (fig. S2f–g). The contribution of tissue reservoirs to the initial levels of viral replication was further suggested by positive correlations between plasma HIV viral load and the frequencies of cells harboring integrated HIV DNA in lymph node and sigmoid colon ($p < 0.0001$, $r = 0.68$ and $p = 0.002$, $r = 0.40$, fig. 1e–f, respectively). To assess the relative ability of these compartments to support high levels of HIV replication, we measured the ratio of total and integrated HIV DNA among lymph nodes, sigmoid colon and blood, since this ratio is proposed to reflect active viral replication(54). Lymph node displayed a higher total/integrated HIV DNA ratio compared to sigmoid colon and blood ($p < 0.01$ for both comparisons, fig. 1g), suggesting that the lymphoid tissue was a preferential site for HIV replication during acute infection. Collectively, these results indicate that the frequency of productively infected cells reached their maximal frequencies in tissues as early as Fiebig stage II and suggest that infected cells

in lymph node likely contribute to the high levels of plasma viremia measured in acutely infected individuals.

Initiation of ART in acute infection results in a rapid decay in the frequency of cells harboring integrated HIV DNA in blood and tissues

All participants started ART during acute infection and reached undetectable plasma viremia after a median time of 24 weeks (range 2-36 weeks for minimal and maximal time), as described in a previous study (31). To determine if the timing of ART initiation had an impact on the decay of HIV persistence markers, we measured total (which captures both integrated and unintegrated HIV genomes) and integrated HIV DNA as well as 2-LTR circles, which represent dead end products of failed infections, in longitudinal blood samples. Samples from individuals who started ART during chronic infection were compared to samples from 80 individuals who initiated ART at the earliest stages of HIV infection (including 11 Fiebig I participants). In individuals who started ART during the chronic infection, concomitant measures of integrated and total HIV DNA revealed that total HIV DNA measures paralleled integrated HIV DNA, whereas 2-LTR circles were detected at much lower levels relative to the other forms. The converse was observed in acutely treated individuals in whom total HIV DNA largely exceeded integrated HIV DNA and displayed similar kinetics to 2-LTR circles at baseline and up to 2 years of ART (fig. 2a). This indicates that the total HIV DNA assay essentially captures unintegrated, likely irrelevant genomes in acutely infected individuals before and after ART initiation. Therefore, measures of integrated HIV DNA were used to assess HIV persistence longitudinally.

Dramatic differences were observed in the dynamics of integrated HIV DNA among the different groups of individuals: Whereas the frequency of infected cells remained stable in participants who initiated ART during chronic infection, there was a sharp decay in the frequency of cells harboring integrated genomes in all acutely treated individuals during the first 12 weeks of therapy (fig. 2b). Integrated HIV DNA remained undetectable at all time points in the vast majority of blood samples collected from individuals who initiated ART at the Fiebig I stage. Collectively, these observations indicate that compared to individuals starting treatment during chronic infection, participants who started ART during acute infection displayed lower frequencies of infected cells at baseline (fig. 1a), and a faster decay upon ART initiation in the blood (fig. 2b).

To determine if HIV-infected cells predominantly persist in tissues from individuals who initiated ART early in infection, we compared the frequency of cells harboring integrated HIV DNA in blood, lymph node and sigmoid colon biopsies after 24-96 weeks of therapy (fig. 2c-e). Importantly, with the exception of an extremely low frequency of infected cells in PBMCs from one individual, ART initiation at the Fiebig I stage led to undetectable amounts of integrated HIV DNA in all blood and tissue samples available for analysis (fig. S3a). Early ART at any acute HIV infection stage was consistently associated with reduced frequencies of cells harboring integrated HIV DNA in blood, lymph node and sigmoid colon when compared to individuals who initiated ART during chronic infection, with the exception of the Fiebig IV/V who displayed similar levels of integrated HIV DNA as

chronic individuals in the sigmoid colon (fig. 2c–e). These lower frequencies of infected cells in tissues from acutely treated individuals were in sharp contrast with the measurements performed at baseline, in which Fiebig II to V were indistinguishable from chronically infected individuals (fig. 2f). Overall, the average fold decreases in the frequency of infected cells were higher in acutely treated individuals than in participants treated in chronic infection (fig. 2g). The rare proviruses that persisted following ART initiation during acute infection were more likely to be located in tissues (fig. S3a–e). Similar observations were made when measuring the frequency of blood CD4⁺ T cells producing *tat/rev* transcripts using the Tat/Rev Induced Limiting Dilution Assay (TILDA) (fig. 2h and fig. S3f), with more profound decreases in the frequency of infected cells when ART was initiated early, despite similar levels of infection measured at baseline. Using the frequency of cells harboring integrated HIV DNA in blood, lymph nodes and sigmoid colon and the proportion of CD4⁺ T cells in each compartment, we estimated the maximal and minimal absolute numbers of infected CD4⁺ T cells in the entire body of individuals at different stages of infection (fig. S3g). Before ART initiation, Fiebig I participants displayed the smallest absolute numbers of infected cells (range 8×10^5 – 5×10^6 cells). The number of infected cells rapidly increased in Fiebig II and III (reaching up to 2×10^9 and 1×10^{10} cells, respectively), before decreasing in Fiebig IV/V and in chronically infected individuals (2×10^8 and 5×10^8 cells, respectively). ART initiation during chronic infection had a minimal impact on the total number of infected cells (range 4×10^6 to 2×10^8), which was consistently higher than the total body reservoir size estimated in all acutely treated participants (ranges 1×10^5 to 4×10^6 , 5×10^5 to 6×10^6 , 7×10^5 to 3×10^7 and 8×10^5 to 9×10^6 in Fiebig I, II, III and IV/V, respectively).

Altogether, these results indicate that although the frequencies of infected cells are elevated in tissues from acutely infected individuals, only a very small fraction of these cells will persist during ART, suggesting that the early pool of cells harboring integrated HIV DNA is short-lived.

Infected memory CD4⁺ T cells are labile up to the Fiebig III stage

HIV primarily infects and persists in CD4⁺ T cells displaying a memory phenotype, with central (T_{CM}), transitional (T_{TM}) and effector memory (T_{EM}) CD4⁺ T cells being the main contributors to the pool of infected cells during ART (55–57), whereas naive cells (T_N) are rarely infected. To assess the effect of early ART initiation on the infection and persistence of infected memory cells, we sorted T_N, T_{CM}, T_{TM} and T_{EM} cells from the blood of acutely infected participants prior to and after 24–96 weeks of ART.

At baseline, Fiebig I participants displayed low to undetectable amounts of integrated HIV DNA in all subsets (fig. 3a). In contrast, infected T_{CM}, T_{TM} and T_{EM} cells were readily detected as early as the Fiebig II stage and throughout all infection stages (fig. 3b–e). Of note, there were no statistically significant differences in the infection frequencies of memory cells between Fiebig III, IV/V and chronically infected individuals at baseline, indicating that the circulating pool of infected memory cells reaches its maximal size as early as the Fiebig III stage (fig. S4a–d).

Initiation of ART led to a marked decrease in the infection frequency of T_{CM}, T_{TM} and T_{EM} cells in Fiebig II and III participants (fig. 3b–c), whereas integrated HIV DNA levels were not affected in any of these subsets both in Fiebig IV/V and in chronically treated controls (fig. 3d–e). Indeed, the average fold decrease of integrated HIV DNA levels in the three memory subsets was maximal in Fiebig III individuals and decreased in Fiebig IV/V participants and chronically infected controls in whom infection frequencies remained stable before and after ART (fig. 3f). Importantly, even by assessing large numbers of memory cells obtained by flow cytometry cell sorting, we were unable to detect integrated genomes in T_{CM}, T_{TM} and T_{EM} from 5/6 participants who initiated ART at the Fiebig I stage (fig. 3a). Whereas infection frequencies in T_{CM}, T_{TM} and T_{EM} cells were similar in individuals who initiated ART during chronic infection, the long-lived T_{CM} cells displayed lower levels of integrated HIV DNA than T_{TM} and T_{EM} cells when ART was initiated in Fiebig stages I–V (fig. 3g). Accordingly, the contribution of the T_{CM} subset to the pool of infected cells was lower in suppressed individuals who initiated ART in acute infection compared to chronic controls (28% VS 44%, respectively, fig. S4e–h). Altogether, these results indicated that the majority of CD4+ T cells harboring integrated HIV DNA are cleared when ART is initiated during the Fiebig II–III stages of acute infection. In contrast, infected memory CD4+ T cells from Fiebig IV/V and chronically infected individuals had a greater ability to persist during ART and were more likely to display the phenotype of long-lived T_{CM} cells.

Discussion

Most of the studies that investigated the early events responsible for the establishment of a viral reservoir during the first few days/weeks of infection have been conducted in non-human primates infected with SIV for logistical and technical reasons. The RV254/SEARCH 010 acute infection study provided a unique opportunity to identify the tissues and cell subsets that are targeted by HIV during the earliest stages of infection and to determine the impact of ART on these potential persistent reservoirs in humans. Consistent with the observations in macaques intrarectally infected with SIV_{MAC251} by Whitney et al. (12), we found that proviruses are more frequently detected in lymph nodes and sigmoid colon than in blood in recently infected individuals. The heightened total/integrated HIV DNA ratio we measured in lymph nodes suggests that viral replication may be prominent in this compartment, in line with previous studies indicating that the lymphoid tissues and particularly T_{fh} cells may be an important source of viremia during untreated HIV infection (52, 58, 59). This is supported by the strong correlation we observed between plasma viremia and the frequency of infected cells in this tissue. Of note, maximal infection frequencies in lymph nodes and sigmoid colon were already attained as early as Fiebig stage II, whereas the frequency of infected cells in blood gradually increased throughout all stages of acute HIV infection until the chronic phase, during which amounts of integrated DNA were comparable across the three compartments. These observations suggest that the lymphoid tissue may be a major source of HIV-infected cells at the earliest stage of infection, before recirculation and accumulation in the blood.

In this study, we used integrated HIV DNA as a marker for HIV infection and persistence in blood and tissues. The concurrent measures of total and integrated HIV DNA as well as 2-LTR circles revealed important differences in the relative proportions of these molecular

forms between acutely and chronically infected individuals. Chronically infected participants displayed low levels of 2-LTR circles and high amounts of total and integrated HIV DNA. Surprisingly, 2-LTR circles were prominent in acute HIV infection and accounted for the majority of the HIV DNA molecules, whereas integrated genomes were rare, suggesting a possible defect in proviral integration at the earliest phase of HIV infection. Since 2-LTR circles are considered as dead-end forms for HIV replication (60), our results suggest that measuring total HIV DNA may considerably overestimate the pool of infected cells in acute infection. We acknowledge that all PCR-based assays, including integrated HIV DNA, overestimates the frequency of cells carrying replication-competent virus (61), since a large fraction of viral genomes is defective (62). Although this difference may be less pronounced during the earliest stage of infection, it is still likely that our integrated assay captures many defective viral genomes. In an attempt to partially circumvent this problem, we used TILDA to measure the frequency of cells with inducible *tat/rev* RNA in a subset of the participants and observed that the majority of the samples obtained from people treated during Fiebig I to III were below the limit of detection of the assay. Therefore, we opted for the integrated DNA assay as a compromise to quantify HIV-infected cells, since it is likely that any other assay would have resulted in negative results given the relatively limited amounts of cells available from blood and tissue samples from these acutely treated individuals.

Initiation of ART at the Fiebig I-III stages was associated with a profound decrease in the frequency of cells harbouring integrated HIV DNA to nearly undetectable levels in all compartments. Strikingly, initiation of ART during chronic infection led to only a modest decrease in the levels of integrated HIV DNA. The dynamic of integrated and non-integrated viral genomes was elegantly studied by Koelsch et al. (63) who observed that ratios of total HIV DNA levels to integrated HIV DNA levels were high before initiation of therapy but diminished during therapy. Similarly, we found that initiation of ART during chronic infection led to a rapid decay in total HIV DNA, whereas levels of integrated HIV DNA remained stable. In contrast, we observed that both forms decayed rapidly in acutely treated participants. This may be explained by the fact that the majority of cells harbouring integrated HIV DNA during acute infection are productively infected, whereas a large fraction of the integrated genomes from chronically infected participants are likely to be latent or defective (39), and therefore less prone to elimination. Indeed, previous studies have shown that productively HIV-infected cells are short lived (~2 days) (35, 36), whereas latently infected CD4+ T cells have a much longer half-life than productively infected cells (57, 64). These long-lived cells are likely to be generated at a constant but very low fraction of the total infection events at all stages of infection and T cell proliferation may largely contribute to the expansion of the pool of latently infected cells during acute infection (19), as was previously demonstrated during ART (13–16). In addition, although the relative contribution of viral cytotoxicity and CTL-mediated killing to the elimination of these productively infected CD4+ T cells during acute infection remains unclear, it is possible that HIV-specific CD8+ T cells, which are still functional at this early stage, may also contribute to the observed rapid decay in the frequency of infected cells. Indeed, in this same cohort of recently infected individuals, Takata et al. (65) reported that during a very short window of time, potent HIV-specific CD8+ T cells are able to decrease the number of HIV-producing

cells and also to decrease the seeding of the persistent viral reservoir after ART initiation. The emergence of CTL dysfunction may contribute to the stability of the pool of infected cells when ART is initiated during or after the Fiebig IV stage.

Our measures performed in PBMCs are in line with the study from Buzon et al. (33), who reported a faster decay of HIV DNA in the blood of individuals who started ART in early infection, leading to substantially lower frequencies of infected cells after long-term treatment. Interestingly, we observed that whereas integrated HIV DNA was rarely detected in the blood and sigmoid colon of participants who initiated ART during Fiebig stages II and III, persistently infected cells were readily detected in lymph nodes from these individuals (5/6 Fiebig II and 8/10 Fiebig III participants). This may be attributed to the low frequency of CD4⁺ T cells in the colon compared to lymph nodes (66), which would limit the efficient detection of HIV DNA in this preferential anatomical reservoir (67). It is also possible that the few proviruses that persist in early treated individuals show greater stability in lymph nodes, which is consistent with the capacity of B cell follicles to serve as a sanctuary for HIV in the face of potent HIV-specific T cell responses (68).

Quantification of integrated HIV DNA in sorted memory CD4⁺ T cell subsets largely confirmed our observations in blood and tissues: ART had no measurable impact on the levels of integrated HIV DNA when therapy was initiated at the Fiebig IV stage or later. On the contrary, these frequencies were reduced by ART when therapy was initiated at an earlier stage, particularly before the Fiebig III stage, i.e. before the generation of HIV-specific antibodies. The distribution of integrated HIV DNA in CD4⁺ T cell subsets was also different between the acutely treated participants and the chronically treated controls, with a lower contribution of the long lived TCM cells when ART was started early. This difference may reflect differential contributions of these subsets to the pool of infected cells before ART initiation. In addition, a prolonged contraction phase of the CD4⁺ T cell responses as a result of antigen clearance following ART initiation in chronic infection and/or increased levels of expression of exhaustion markers that facilitate latency establishment (69, 70) may also contribute to these differences.

Detection of integrated HIV DNA in blood and tissues from Fiebig I participants was extremely rare, both before and after ART. Since the numbers of cells collected by colon and lymph node biopsies were limited, one cannot exclude that these tissues contained low frequencies of infected that were not detected using our approach. Nonetheless, even by sorting memory CD4⁺ T cell subsets highly enriched in HIV DNA, we failed to detect infected cells in the majority of the Fiebig I participants in this study. Our measures performed in blood, sigmoid colon, and lymph nodes obtained from these rare individuals who initiated ART at the earliest stage of infection allowed us to estimate the total body reservoir between 0.2×10^6 and 4×10^6 cells, which is 30 to 600-times lower than our estimate in chronically treated individuals. Although our results indicate that initiation of ART at the earliest possible stage dramatically restricts the pool of persistently infected cells, we recently reported rapid viral rebound in 8 Fiebig I participants who interrupted therapy after a median of 2.8 years of viral suppression (11). Therefore, although early ART has a profound impact on the size of the viral reservoir, the clinical benefit of this intervention remains to be demonstrated. Estimates from Hill et al. (71) suggest that ~2,000-

fold reductions in the frequency of infected cells would be required to permit a majority of individuals to interrupt ART for 1 year without rebound. Taken together, our results and the recent report from Colby et al. (11), indicate that although very early ART substantially limits the establishment of the reservoir, additional strategies are needed to further reduce its size in order to achieve viral control upon ART interruption, consistent with recent observations in the NHP model (72). Combining our estimates and Hill et al. (71), we predict that an additional 3 to 60-fold reduction in the size of the reservoir may be necessary to observe transient control of viremia in Fiebig I participants following ART cessation in the absence of adjunctive therapy.

Materials and Methods

Study design

The overall objective of this study was to measure the size of the pool of HIV-infected cells in blood and tissues during acute infection and to determine the impact of early ART initiation on these parameters. The RV254/SEARCH 010 cohort study ([clinicaltrials.gov NCT00796146](https://clinicaltrials.gov/ct2/show/study/NCT00796146)) enrolls participants at the earliest stages of acute HIV infection at the Thai Red Cross AIDS Research Centre in Bangkok. High-risk volunteers are screened for acute HIV infection in real time with pooled nucleic acid testing and sequential immunoassay as previously described (44). Individuals with acute HIV infection were enrolled if they had a positive HIV RNA with or without a reactive immunoassay. ART was voluntary and offered to all participants and was initiated at a median (IQR) of 2 (0-5) days after enrollment (73) under a separate protocol ([NCT00796263](https://clinicaltrials.gov/ct2/show/study/NCT00796263)). Samples from chronically infected treatment-naïve individuals from Bangkok enrolled in the previously described SEARCH 011 (74) ([NCT00782808](https://clinicaltrials.gov/ct2/show/study/NCT00782808)) and RV304/SEARCH 013 (75) ([NCT01397669](https://clinicaltrials.gov/ct2/show/study/NCT01397669)) studies were also obtained. Several markers of HIV persistence including total and integrated HIV DNA as well as 2-LTR circles were measured in blood and tissues (lymph node and colon biopsies) collected at baseline and after viral suppression by ART. Primary data are reported in data file S1.

Ethics statement

All clinical studies were approved by the Institutional Review Boards (IRBs) of Chulalongkorn University in Thailand, Walter Reed Army Institute of Research (WRAIR) and University of California at San Francisco (UCSF) in the USA, and the Centre Hospitalier de l'Université de Montréal in Canada. All participants gave written informed consent.

Fiebig staging

Participants were categorized as Fiebig stages I to V as follows: Fiebig I - positive HIV RNA, negative p24 antigen, non-reactive 3rd generation immunoassay; Fiebig II - positive HIV RNA, positive p24 antigen, non-reactive 3rd generation immunoassay; Fiebig III - positive HIV RNA, positive p24 antigen, reactive 3rd generation immunoassay, negative western blot; Fiebig IV - positive HIV RNA, positive or negative p24 antigen, reactive 3rd generation immunoassay, indeterminate western blot; Fiebig V - positive HIV RNA, positive

or negative p24 antigen, reactive 3rd generation immunoassay, positive western blot except p31(27).

Plasma viral load determination

HIV RNA in plasma was measured using the Roche Amplicor v 1.5 ultrasensitive assay with a lower quantification limit of 50 copies/mL of plasma (Roche Diagnostics) or by the COBAS TaqMan HIV-1 Test v2.0 (Roche Diagnostics) with a lower measurement limit of 20 copies/mL.

Quantification of total and integrated HIV DNA and 2-LTR circles

PBMCs were isolated from peripheral blood or leukapheresis by Lymphoprep density gradient centrifugation. Cryopreserved PBMCs were used to quantify the frequency of cells harboring HIV DNA (total, integrated and 2-LTR circles) using previously described real-time PCR assays (76) with LTR-gag (for total), Alu-gag (for integrated) and 2-LTR junction (2-LTR) specific primers. Results were normalized to the number of copies of the CD3 gene (2 copies per cell). To facilitate the comparisons between anatomic compartments, we assumed that each infected cell carries a single copy of HIV DNA, as previously described in circulating CD4⁺ T cells from both acutely and chronically untreated HIV-infected individuals (77). HIV DNA quantification was also performed in sigmoid colon biopsies stored in RNAlater and in lymph node mononuclear cells (LNMCs) isolated from inguinal lymph nodes, respectively. The sigmoid colon biopsies (n=2 for each measure) were washed twice with PBS and resuspended in a fixed volume of 250µL of proteinase K lysis buffer, as previously described (76). Lymph nodes were processed as follows: after removal of surrounding fat, the lymph nodes were minced and transferred through a 70 µm nylon cell strainer inserted into the top of 50ml conical tube. Cells were washed, counted and cryopreserved in FBS/10% DMSO. For DNA quantification, 1x10⁶ LNMCs were transferred to a microtube, washed and digested with proteinase K overnight. Only undetectable samples for which at least 100,000 cells in the blood and 65,000 cells in sigmoid/lymph node biopsies (as measured by CD3 copy numbers in the HIV DNA quantification assays) were included in the analysis (fig. S5). In all analyses, negative values were considered as “0”, except in the average fold decay calculations where limits of detection were taken into account.

The total number of infected cells in the entire body was calculated for each Fiebig stage by using the integrated HIV DNA values in each compartment and applying estimates from Ganusov et al. (78). We first calculated the infection frequencies of CD4⁺ T cells in each compartment by using the average percentage of CD4⁺ T cells (measured by flow cytometry) in each tissue and for each Fiebig stage and our integrated HIV DNA measures in each participant. Infection frequencies measured in lymph nodes were used to estimate the contribution of all secondary lymphoid organs. Infection frequencies measured in the sigmoid colon were used to estimate the contribution of mucosal tissues. Infection frequencies measured in the blood were used to estimate the contribution blood and spleen. We then calculated the average values for each Fiebig stage and for each compartment and added the absolute numbers of infected cells in lymphoid tissues, mucosal tissues and blood to obtain the total body reservoir.

Tat/rev Induced Limiting Dilution Assay (TILDA)

CD4⁺ T cells were isolated from cryopreserved PBMCs using magnetic depletion as per the manufacturer's protocol (Stem Cell Technologies). The frequency of CD4⁺ T cells producing multiply spliced HIV RNA (*tat/rev*) after PMA/ionomycin stimulation for 12h was assessed using TILDA as previously described (79), with modified primers and probes for efficient amplification of HIV genomes of the A/E clade. The following primers were used for reverse transcription and pre-amplification: tat1.4AE (TGG CAG GAA GAA GCG GAA G) and revAE (TGT CTC TGY CTT GCT CKC CAC C). For quantitative PCR, the following primers and probe were used: tat2AE (GCA GTA AGG ATC ATC AAA ATC CTA TAC CAG AGC A), revAE and probe MSHIVFAMzenAE (56-FAM/TTC YTT CGG/ZEN/GCC TGT CGG GTT CC/3IABkFQ).

Flow cytometry cell sorting

CD4⁺ T cell subsets were sorted on a BD FACS Aria II cell sorter. The following antibodies were used: CD3-FITC (clone HIT3a, BD #555339), CD4-APC (clone SK3, BD #340443), CD45RA-APCH7 (clone HI100, BD #560674), CCR7-PE-Cy7 (clone 3D12, BD #557648), CD27-PE (clone M-T271, BD #555441) and LIVE/DEAD fixable Aqua (Invitrogen #L34957). Frequencies of memory CD4⁺ T cell subsets were determined as previously described after the exclusion of dead cells (LIVE/DEAD) (55). Four CD4⁺ T cell subsets were defined based on the expression of CD45RA, CCR7 and CD27: naïve (T_N: CD45RA⁺, CCR7⁺, CD27⁺), central memory (T_{CM}: CD45RA⁺, CCR7⁺, CD27⁺), transitional memory (T_{TM}: CD45RA⁺, CCR7⁺, CD27⁺) and effector memory (T_{EM}: CD45RA⁺, CCR7⁺, CD27⁺). Integrated HIV DNA was measured in the sorted populations. All subsets for which insufficient number of cells were analyzed (<40,000 sorted cells as measured in the PCR assay) were excluded from the analysis.

Statistical Analysis

All data were analyzed using Graphpad Prism v6.0h. HIV DNA and TILDA values were transformed in $\log_{10}([HIV \text{ copies}/10^6 \text{ cells}] + 1)$. Undetectable values with a limit of detection equal or greater than 10 copies/10⁶ PBMCs, reflecting analysis of <10⁵ cells, were excluded from the analysis. Undetectable tissue values with a limit of detection equal or greater than 15 copies/10⁶ cells were also excluded. Results were represented as median or mean values, with interquartile range or minimum and maximum values, as indicated in the figure legends. Mann Whitney U test was used to compare participants between Fiebig stages. Correlations were determined using nonparametric Spearman's test. One-way ANOVA was used to compare infection frequencies in different CD4⁺ T cell subsets and in different compartments. P values of less or equal to 0.05 were considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

The study team is grateful to the individuals who volunteered to participate in this study and the staff at the Thai Red Cross AIDS Research Centre and the Department of Retrovirology, U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences (AFRIMS). We thank the RV254/SEARCH 010, RV304/SEARCH 013 and SEARCH 011 Protocols team members. We also thank the flow cores at VGTI Florida (Yu Shi and Kim Kusser) and at the CRCHUM (Dominique Gauchat and Annie Gosselin) for cell sorting as well as the NC3 core at the CRCHUM (Olfa Debbeche). The authors thank Rafick-Pierre Sekaly and Marta Massanella for advice and helpful discussions and Amélie Pagliuzza and Esther Merlini for technical assistance. We also thank the RV254/SEARCH010, RV304/SEARCH013 and SEARCH011 study group members.

Funding: The empirical component of this study was funded by the US Military HIV Research Program, Walter Reed Army Institute of Research, Rockville, Maryland, USA, under a cooperative agreement (W81XWH-07-2-0067) with the Henry M. Jackson Foundation for the Advancement of Military Medicine Inc. at the US Department of Defense and R01 NS061696 (VV). The scientific component of this study was supported by the Foundation for AIDS Research (amfAR Research Consortium on HIV Eradication 108687-54-RGRL and 108928-56-RGRL) and by the Canadian Institutes for Health Research (#364408). NC is supported by a Research Scholar Career Award of the Quebec Health Research Fund (FRQ-S, #253292). Investigators were partially supported by R01AI125127 (TWS and JA) and R01AI108433 (LT and JA).

References and Notes

- Deeks SG, Lewin SR, Ross AL, Ananworanich J, Benkirane M, Cannon P, Chomont N, Douek D, Lifson JD, Lo YR, Kuritzkes D, Margolis D, Mellors J, Persaud D, Tucker JD, Barre-Sinoussi F, Alter G, Auerbach J, Autran B, Barouch DH, Behrens G, Cavazzana M, Chen Z, Cohen EA, Corbelli GM, Eholie S, Eyal N, Fidler S, Garcia L, Grossman C, Henderson G, Henrich TJ, Jefferys R, Kiem HP, McCune J, Moodley K, Newman PA, Nijhuis M, Nsubuga MS, Ott M, Palmer S, Richman D, Saez-Cirion A, Sharp M, Siliciano J, Silvestri G, Singh J, Spire B, Taylor J, Tolstrup M, Valente S, van Lunzen J, Walensky R, Wilson I, Zack J, International AIDS Society global scientific strategy: towards an HIV cure 2016. *Nature medicine* 22, 839–850 (2016); published online EpubAug (10.1038/nm.4108).
- Durand CM, Blankson JN, Siliciano RF, Developing strategies for HIV-1 eradication. *Trends Immunol* 33, 554–562 (2012); published online EpubNov (10.1016/j.it.2012.07.001). [PubMed: 22867874]
- Chun TW, Justement JS, Murray D, Hallahan CW, Maenza J, Collier AC, Sheth PM, Kaul R, Ostrowski M, Moir S, Kovacs C, Fauci AS, Rebound of plasma viremia following cessation of antiretroviral therapy despite profoundly low levels of HIV reservoir: implications for eradication. *AIDS*, (2010); published online EpubOct 19 (10.1097/QAD.0b013e328340a239).
- Hocqueloux L, Prazuck T, Avettand-Fenoel V, Lafeuillade A, Cardon B, Viard JP, Rouzioux C, Long-term immunovirologic control following antiretroviral therapy interruption in patients treated at the time of primary HIV-1 infection. *AIDS* 24, 1598–1601 (2010); published online EpubJun 19 ([PubMed: 20549847]
- Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, Quinn TC, Chadwick K, Margolick J, Brookmeyer R, Gallant J, Markowitz M, Ho DD, Richman DD, Siliciano RF, Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278, 1295–1300 (1997); published online EpubNov 14 ([PubMed: 9360927]
- Wong JK, Hezareh M, Gunthard HF, Havlir DV, Ignacio CC, Spina CA, Richman DD, Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 278, 1291–1295 (1997); published online EpubNov 14 ([PubMed: 9360926]
- Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, Lloyd AL, Nowak MA, Fauci AS, Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci U S A* 94, 13193–13197 (1997); published online EpubNov 25 ([PubMed: 9371822]
- A. S. S. W. G. o. H. I. V. C. International, Deeks SG, Autran B, Berkhout B, Benkirane M, Cairns S, Chomont N, Chun TW, Churchill M, Di Mascio M, Katlama C, Lafeuillade A, Landay A, Lederman M, Lewin SR, Maldarelli F, Margolis D, Markowitz M, Martinez-Picado J, Mullins JI, Mellors J, Moreno S, O'Doherty U, Palmer S, Penicaud MC, Peterlin M, Poli G, Routy JP, Rouzioux C, Silvestri G, Stevenson M, Telenti A, Van Lint C, Verdin E, Woolfrey A, Zaia J, Barre-Sinoussi F,

Towards an HIV cure: a global scientific strategy. *Nat Rev Immunol* 12, 607–614 (2012); published online EpubAug (10.1038/nri3262). [PubMed: 22814509]

9. Saleh S, Solomon A, Wightman F, Xhila M, Cameron PU, Lewin SR, CCR7 ligands CCL19 and CCL21 increase permissiveness of resting memory CD4⁺ T cells to HIV-1 infection: a novel model of HIV-1 latency. *Blood* 110, 4161–4164 (2007); published online EpubDec 15 (blood-2007-06-097907 [pii] 10.1182/blood-2007-06-097907). [PubMed: 17881634]
10. Shan L, Deng K, Gao H, Xing S, Capoferri AA, Durand CM, Rabi SA, Laird GM, Kim M, Hosmane NN, Yang HC, Zhang H, Margolick JB, Li L, Cai W, Ke R, Flavell RA, Siliciano JD, Siliciano RF, Transcriptional Reprogramming during Effector-to-Memory Transition Renders CD4⁺ T Cells Permissive for Latent HIV-1 Infection. *Immunity* 47, 766–775 e763 (2017); published online EpubOct 17 (10.1016/j.immuni.2017.09.014). [PubMed: 29045905]
11. Colby DJ, Trautmann L, Pinyakorn S, Leyre L, Pagliuzza A, Kroon E, Rolland M, Takata H, Buranapraditkun S, Intasan J, Chomchey N, Muir R, Haddad EK, Tovanabutra S, Ubolyam S, Bolton DL, Fullmer BA, Gorelick RJ, Fox L, Crowell TA, Trichavaroj R, O'Connell R, Chomont N, Kim JH, Michael NL, Robb ML, Phanuphak N, Ananworanich J, R. V. s. group, Rapid HIV RNA rebound after antiretroviral treatment interruption in persons durably suppressed in Fiebig I acute HIV infection. *Nat Med*, (2018); published online EpubJun 11 (10.1038/s41591-018-0026-6).
12. Whitney JB, Hill AL, Sanisetty S, Penaloza-MacMaster P, Liu J, Shetty M, Parenteau L, Cabral C, Shields J, Blackmore S, Smith JY, Brinkman AL, Peter LD, Mathew SI, Smith KM, Borducchi EN, Rosenbloom DI, Lewis MG, Hattersley J, Li B, Hesselgesser J, Geleziunas R, Robb ML, Kim JH, Michael NL, Barouch DH, Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. *Nature* 512, 74–77 (2014); published online EpubAug 7 (10.1038/nature13594). [PubMed: 25042999]
13. Maldarelli F, Wu X, Su L, Simonetti FR, Shao W, Hill S, Spindler J, Ferris AL, Mellors JW, Kearney MF, Coffin JM, Hughes SH, HIV latency. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. *Science* 345, 179–183 (2014); published online EpubJul 11 (10.1126/science.1254194). [PubMed: 24968937]
14. Wiegand A, Spindler J, Hong FF, Shao W, Cyktor JC, Cillo AR, Halvas EK, Coffin JM, Mellors JW, Kearney MF, Single-cell analysis of HIV-1 transcriptional activity reveals expression of proviruses in expanded clones during Art. *Proc Natl Acad Sci U S A* 114, E3659–E3668 (2017); published online EpubMay 2 (10.1073/pnas.1617961114). [PubMed: 28416661]
15. Wang Z, Gurule EE, Brennan TP, Gerold JM, Kwon KJ, Hosmane NN, Kumar MR, Beg SA, Capoferri AA, Ray SC, Ho YC, Hill AL, Siliciano JD, Siliciano RF, Expanded cellular clones carrying replication-competent HIV-1 persist, wax, and wane. *Proc Natl Acad Sci U S A* 115, E2575–E2584 (2018); published online EpubMar 13 (10.1073/pnas.1720665115). [PubMed: 29483265]
16. Simonetti FR, Sobolewski MD, Fyne E, Shao W, Spindler J, Hattori J, Anderson EM, Watters SA, Hill S, Wu X, Wells D, Su L, Luke BT, Halvas EK, Besson G, Penrose KJ, Yang Z, Kwan RW, Van Waes C, Uldrick T, Citrin DE, Kovacs J, Polis MA, Rehm CA, Gorelick R, Piatak M, Keele BF, Kearney MF, Coffin JM, Hughes SH, Mellors JW, Maldarelli F, Clonally expanded CD4⁺ T cells can produce infectious HIV-1 in vivo. *Proc Natl Acad Sci U S A* 113, 1883–1888 (2016); published online EpubFeb 16 (10.1073/pnas.1522675113). [PubMed: 26858442]
17. Wagner TA, McLaughlin S, Garg K, Cheung CY, Larsen BB, Styrchak S, Huang HC, Edlefsen PT, Mullins JI, Frenkel LM, HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. *Science* 345, 570–573 (2014); published online EpubAug 1 (10.1126/science.1256304). [PubMed: 25011556]
18. De Scheerder MA, Vrancken B, Dellicour S, Schlub T, Lee E, Shao W, Rutsaert S, Verhofstede C, Kerre T, Malfait T, Hemelsoet D, Coppens M, Dhondt A, De Looze D, Vermassen F, Lemey P, Palmer S, Vandekerckhove L, HIV Rebound Is Predominantly Fueled by Genetically Identical Viral Expansions from Diverse Reservoirs. *Cell Host Microbe*, (2019); published online EpubAug 27 (10.1016/j.chom.2019.08.003).
19. Coffin JM, Wells DW, Zerbato JM, Kuruc JD, Guo S, Luke BT, Eron JJ, Bale M, Spindler J, Simonetti FR, Hill S, Kearney MF, Maldarelli F, Wu X, Mellors JW, Hughes SH, Clones of

- infected cells arise early in HIV-infected individuals. *JCI Insight* 4, (2019); published online EpubJun 20 (10.1172/jci.insight.128432).
20. Archin NM, Liberty AL, Kashuba AD, Choudhary SK, Kuruc JD, Crooks AM, Parker DC, Anderson EM, Kearney MF, Strain MC, Richman DD, Hudgens MG, Bosch RJ, Coffin JM, Eron JJ, Hazuda DJ, Margolis DM, Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* 487, 482–485 (2012); published online EpubJul 26 (10.1038/nature11286). [PubMed: 22837004]
 21. Elliott JH, McMahon JH, Chang CC, Lee SA, Hartogensis W, Bumpus N, Savic R, Roney J, Hoh R, Solomon A, Piatak M, Gorelick RJ, Lifson J, Bacchetti P, Deeks SG, Lewin SR, Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. *Lancet HIV* 2, e520–529 (2015); published online EpubDec (10.1016/S2352-3018(15)00226-X). [PubMed: 26614966]
 22. Rasmussen TA, Tolstrup M, Brinkman CR, Olesen R, Erikstrup C, Solomon A, Winckelmann A, Palmer S, Dinarello C, Buzon MJ, Lichterfeld M, Lewin SR, Ostergaard L, Sogaard OS, Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. *Lancet HIV* 1, 13–21 (2014).
 23. Elliott JH, Wightman F, Solomon A, Ghneim K, Ahlers J, Cameron MJ, Smith MZ, Spelman T, McMahon J, Velayudham P, Brown G, Roney J, Watson J, Prince MH, Hoy JF, Chomont N, Fromentin R, Procopio FA, Zeidan J, Palmer S, Odeval L, Johnstone RW, Martin BP, Sinclair E, Deeks SG, Hazuda DJ, Cameron PU, Sekaly RP, Lewin SR, Activation of HIV transcription with short-course vorinostat in HIV-infected patients on suppressive antiretroviral therapy. *PLoS Pathog* 10, e1004473 (2014); published online EpubOct (10.1371/journal.ppat.1004473). [PubMed: 25393648]
 24. Bar KJ, Sneller MC, Harrison LJ, Justement JS, Overton ET, Petrone ME, Salantes DB, Seamon CA, Scheinfeld B, Kwan RW, Learn GH, Proschan MA, Kreider EF, Blazkova J, Bardsley M, Refsland EW, Messer M, Clarridge KE, Tustin NB, Madden PJ, Oden K, O'Dell SJ, Jarocki B, Shiakolas AR, Tressler RL, Doria-Rose NA, Bailer RT, Ledgerwood JE, Capparelli EV, Lynch RM, Graham BS, Moir S, Koup RA, Mascola JR, Hoxie JA, Fauci AS, Tebas P, Chun TW, Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption. *N Engl J Med* 375, 2037–2050 (2016); published online EpubNov 24 (10.1056/NEJMoa1608243). [PubMed: 27959728]
 25. Gay CL, DeBenedette MA, Tcherepanova IY, Gamble A, Lewis WE, Cope AB, Kuruc JD, McGee KS, Kearney MF, Coffin JM, Archin NM, Hicks CB, Eron JJ, Nicolette CA, Margolis DM, Immunogenicity of AGS-004 Dendritic Cell Therapy in Patients Treated During Acute HIV Infection. *AIDS Res Hum Retroviruses* 34, 111–122 (2018); published online EpubJan (10.1089/aid.2017.0071). [PubMed: 28636433]
 26. Little SJ, McLean AR, Spina CA, Richman DD, Havlir DV, Viral dynamics of acute HIV-1 infection. *J Exp Med* 190, 841–850 (1999); published online EpubSep 20 (10.1084/jem.190.6.841). [PubMed: 10499922]
 27. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, Heldebrandt C, Smith R, Conrad A, Kleinman SH, Busch MP, Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS* 17, 1871–1879 (2003); published online EpubSep 5 (10.1097/01.aids.0000076308.76477.b8). [PubMed: 12960819]
 28. Robb ML, Eller LA, Kibuuka H, Rono K, Maganga L, Nitayaphan S, Kroon E, Sawe FK, Sinei S, Sriplienchan S, Jagodzinski LL, Malia J, Manak M, de Souza MS, Tovanabutra S, Sanders-Buell E, Rolland M, Dorsey-Spitz J, Eller MA, Milazzo M, Li Q, Lewandowski A, Wu H, Swann E, O'Connell RJ, Peel S, Dawson P, Kim JH, Michael NL, Team RVS, Prospective Study of Acute HIV-1 Infection in Adults in East Africa and Thailand. *N Engl J Med* 374, 2120–2130 (2016); published online EpubJun 2 (10.1056/NEJMoa1508952). [PubMed: 27192360]
 29. Cohen MS, Gay CL, Busch MP, Hecht FM, The detection of acute HIV infection. *J Infect Dis* 202 Suppl 2, S270–277 (2010); published online EpubOct 15 (10.1086/655651). [PubMed: 20846033]
 30. Ananworanich J, Chomont N, Eller LA, Kroon E, Tovanabutra S, Bose M, Nau M, Fletcher JL, Tipsuk S, Vanderveeten C, O'Connell RJ, Pinyakorn S, Michael N, Phanuphak N, Robb ML, Rv,

- R. S. s. groups, HIV DNA Set Point is Rapidly Established in Acute HIV Infection and Dramatically Reduced by Early ART. *EBioMedicine* 11, 68–72 (2016); published online EpubSep (10.1016/j.ebiom.2016.07.024). [PubMed: 27460436]
31. Crowell TA, Phanuphak N, Pinyakorn S, Kroon E, Fletcher JL, Colby D, Tipsuk S, Karnsomlap P, Laopraynak N, O'Connell RJ, Robb ML, Ananworanich J, Group RSS, Virologic failure is uncommon after treatment initiation during acute HIV infection. *AIDS* 30, 1943–1950 (2016); published online EpubJul 31 (10.1097/QAD.0000000000001148). [PubMed: 27163706]
 32. Archin NM, Vaidya NK, Kuruc JD, Liberty AL, Wiegand A, Kearney MF, Cohen MS, Coffin JM, Bosch RJ, Gay CL, Eron JJ, Margolis DM, Perelson AS, Immediate antiviral therapy appears to restrict resting CD4+ cell HIV-1 infection without accelerating the decay of latent infection. *Proc Natl Acad Sci U S A* 109, 9523–9528 (2012); published online EpubJun 12 (10.1073/pnas.1120248109). [PubMed: 22645358]
 33. Buzon MJ, Martin-Gayo E, Pereyra F, Ouyang Z, Sun H, Li JZ, Piovoso M, Shaw A, Dalmau J, Zangger N, Martinez-Picado J, Zurakowski R, Yu XG, Telenti A, Walker BD, Rosenberg ES, Lichterfeld M, Long-term antiretroviral treatment initiated at primary HIV-1 infection affects the size, composition, and decay kinetics of the reservoir of HIV-1-infected CD4 T cells. *J Virol* 88, 10056–10065 (2014); published online EpubSep 1 (10.1128/JVI.01046-14). [PubMed: 24965451]
 34. Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, Chen IS, HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell* 61, 213–222 (1990); published online EpubApr 20 (10.1016/0092-8674(90)90802-1). [PubMed: 2331748]
 35. Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, Lifson JD, Bonhoeffer S, Nowak MA, Hahn BH, et al., Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 373, 117–122 (1995); published online EpubJan 12 (10.1038/373117a0). [PubMed: 7529365]
 36. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M, Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 373, 123–126 (1995); published online EpubJan 12 (10.1038/373123a0). [PubMed: 7816094]
 37. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD, HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science* 271, 1582–1586 (1996); published online EpubMar 15 (10.1126/science.271.5255.1582). [PubMed: 8599114]
 38. Lee GQ, Reddy K, Einkauf KB, Gounder K, Chevalier JM, Dong KL, Walker BD, Yu XG, Ndung'u T, Lichterfeld M, HIV-1 DNA sequence diversity and evolution during acute subtype C infection. *Nature communications* 10, 2737 (2019); published online EpubJun 21 (10.1038/s41467-019-10659-2).
 39. Bruner KM, Murray AJ, Pollack RA, Soliman MG, Laskey SB, Capoferri AA, Lai J, Strain MC, Lada SM, Hoh R, Ho YC, Richman DD, Deeks SG, Siliciano JD, Siliciano RF, Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat Med* 22, 1043–1049 (2016); published online EpubSep (10.1038/nm.4156). [PubMed: 27500724]
 40. Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS, Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. *Proc Natl Acad Sci U S A* 95, 8869–8873 (1998); published online EpubJul 21 ([PubMed: 9671771]
 41. Strain MC, Little SJ, Daar ES, Havlir DV, Gunthard HF, Lam RY, Daly OA, Nguyen J, Ignacio CC, Spina CA, Richman DD, Wong JK, Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. *J Infect Dis* 191, 1410–1418 (2005); published online EpubMay 1 ([PubMed: 15809898]
 42. Hocqueloux L, Saez-Cirion A, Rouzioux C, Immunovirologic Control 24 Months After Interruption of Antiretroviral Therapy Initiated Close to HIV Seroconversion. *JAMA internal medicine* 173, 475–477 (2013); published online EpubMar 25 (10.1001/jamainternmed.2013.2176).
 43. Ananworanich J, Sacdalan CP, Pinyakorn S, Chomont N, de Souza M, Luekasemsuk T, Schuetz A, Krebs SJ, Dewar R, Jagodzinski L, Ubolyam S, Trichavaroj R, Tovanabutra S, Spudich S, Valcour V, Sereti I, Michael N, Robb M, Phanuphak P, Kim JH, Phanuphak N, Virological and immunological characteristics of HIV-infected individuals at the earliest stage of infection. *J Virus Erad* 2, 43–48 (2016). [PubMed: 26889497]

44. Ananworanich J, Schuetz A, Vandergeeten C, Sereti I, de Souza M, Rerknimitr R, Dewar R, Marovich M, van Griensven F, Sekaly R, Pinyakorn S, Phanuphak N, Trichavaroj R, Rutvisuttinunt W, Chomchey N, Paris R, Peel S, Valcour V, Maldarelli F, Chomont N, Michael N, Phanuphak P, Kim JH, R. S. S. Group, Impact of multi-targeted antiretroviral treatment on gut T cell depletion and HIV reservoir seeding during acute HIV infection. *PLoS One* 7, e33948 (2012) (10.1371/journal.pone.0033948). [PubMed: 22479485]
45. Deleage C, Schuetz A, Alvord WG, Johnston L, Hao XP, Morcock DR, Rerknimitr R, Fletcher JL, Puttamaswin S, Phanuphak N, Dewar R, McCune JM, Sereti I, Robb M, Kim JH, Schacker TW, Hunt P, Lifson JD, Ananworanich J, Estes JD, Impact of early cART in the gut during acute HIV infection. *JCI Insight* 1, (2016); published online EpubJul 7 (10.1172/jci.insight.87065).
46. Colby DJ, Trautmann L, Pinyakorn S, Leyre L, Pagliuzza A, Kroon E, Rolland M, Takata H, Buranapraditkun S, Intasan J, Chomchey N, Muir R, Haddad EK, Tovanabutra S, Ubolyam S, Bolton DL, Fullmer BA, Gorelick RJ, Fox L, Crowell TA, Trichavaroj R, O'Connell R, Chomont N, Kim JH, Michael NL, Robb ML, Phanuphak N, Ananworanich J, R. V. s. group, Rapid HIV RNA rebound after antiretroviral treatment interruption in persons durably suppressed in Fiebig I acute HIV infection. *Nat Med* 24, 923–926 (2018); published online EpubJul (10.1038/s41591-018-0026-6). [PubMed: 29892063]
47. Henrich TJ, Hatano H, Bacon O, Hogan LE, Rutishauser R, Hill A, Kearney MF, Anderson EM, Buchbinder SP, Cohen SE, Abdel-Mohsen M, Pohlmeier CW, Fromentin R, Hoh R, Liu AY, McCune JM, Spindler J, Metcalf-Pate K, Hobbs KS, Thanh C, Gibson EA, Kuritzkes DR, Siliciano RF, Price RW, Richman DD, Chomont N, Siliciano JD, Mellors JW, Yukl SA, Blankson JN, Liegler T, Deeks SG, HIV-1 persistence following extremely early initiation of antiretroviral therapy (ART) during acute HIV-1 infection: An observational study. *PLoS medicine* 14, e1002417 (2017); published online EpubNov (10.1371/journal.pmed.1002417).
48. Okoye AA, Hansen SG, Vaidya M, Fukazawa Y, Park H, Duell DM, Lum R, Hughes CM, Ventura AB, Ainslie E, Ford JC, Morrow D, Gilbride RM, Legasse AW, Hesselgesser J, Geleziunas R, Li Y, Oswald K, Shoemaker R, Fast R, Bosche WJ, Borate BR, Edlefsen PT, Axthelm MK, Picker LJ, Lifson JD, Early antiretroviral therapy limits SIV reservoir establishment to delay or prevent post-treatment viral rebound. *Nat Med* 24, 1430–1440 (2018); published online EpubSep (10.1038/s41591-018-0130-7). [PubMed: 30082858]
49. Estes JD, Kityo C, Ssali F, Swainson L, Makamdop KN, Del Prete GQ, Deeks SG, Luciw PA, Chipman JG, Beilman GJ, Hoskuldsson T, Khoruts A, Anderson J, Deleage C, Jasurda J, Schmidt TE, Hafertepe M, Callisto SP, Pearson H, Reimann T, Schuster J, Schoephoerster J, Southern P, Perkey K, Shang L, Wietgreffe SW, Fletcher CV, Lifson JD, Douek DC, McCune JM, Haase AT, Schacker TW, Defining total-body AIDS-virus burden with implications for curative strategies. *Nat Med* 23, 1271–1276 (2017); published online EpubNov (10.1038/nm.4411). [PubMed: 28967921]
50. Chun TW, Nickle DC, Justement JS, Meyers JH, Roby G, Hallahan CW, Kottlil S, Moir S, Mican JM, Mullins JI, Ward DJ, Kovacs JA, Mannon PJ, Fauci AS, Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy. *J Infect Dis* 197, 714–720 (2008); published online EpubMar 1 (10.1086/527324). [PubMed: 18260759]
51. Yukl SA, Gianella S, Sinclair E, Epling L, Li Q, Duan L, Choi AL, Girling V, Ho T, Li P, Fujimoto K, Lampiris H, Hare CB, Pandori M, Haase AT, Gunthard HF, Fischer M, Shergill AK, McQuaid K, Havlir DV, Wong JK, Differences in HIV burden and immune activation within the gut of HIV-positive patients receiving suppressive antiretroviral therapy. *J Infect Dis* 202, 1553–1561 (2010); published online EpubNov 15 (10.1086/656722). [PubMed: 20939732]
52. Perreau M, Savoye AL, De Crignis E, Corpataux JM, Cubas R, Haddad EK, De Leval L, Graziosi C, Pantaleo G, Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. *J Exp Med* 210, 143–156 (2013); published online EpubJan 14 (10.1084/jem.20121932). [PubMed: 23254284]
53. Svicher V, Ceccherini-Silberstein F, Antinori A, Aquaro S, Perno CF, Understanding HIV Compartments and Reservoirs. *Current HIV/AIDS Reports* 11, 186–194 (2014); published online EpubJune 01 (10.1007/s11904-014-0207-y). [PubMed: 24729094]
54. Mexas AM, Graf EH, Pace MJ, Yu JJ, Papasavvas E, Azzoni L, Busch MP, Di Mascio M, Foulkes AS, Migueles SA, Montaner LJ, O'Doherty U, Concurrent measures of total and integrated HIV

- DNA monitor reservoirs and ongoing replication in eradication trials. *AIDS* 26, 2295–2306 (2012); published online EpubNov 28 (10.1097/QAD.0b013e32835a5c2f). [PubMed: 23014521]
55. Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, Boucher G, Boulassel MR, Ghattas G, Brechley JM, Schacker TW, Hill BJ, Douek DC, Routy JP, Haddad EK, Sekaly RP, HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med* 15, 893–900 (2009); published online EpubAug (10.1038/nm.1972). [PubMed: 19543283]
 56. Douek DC, Brechley JM, Betts MR, Ambrozak DR, Hill BJ, Okamoto Y, Casazza JP, Kuruppu J, Kunstman K, Wolinsky S, Grossman Z, Dybul M, Oxenius A, Price DA, Connors M, Koup RA, HIV preferentially infects HIV-specific CD4+ T cells. *Nature* 417, 95–98 (2002); published online EpubMay 2 ([PubMed: 11986671]
 57. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, Kovacs C, Gange SJ, Siliciano RF, Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat Med* 9, 727–728 (2003); published online EpubJun ([PubMed: 12754504]
 58. Pantaleo G, Graziosi C, Butini L, Pizzo PA, Schnittman SM, Kotler DP, Fauci AS, Lymphoid organs function as major reservoirs for human immunodeficiency virus. *Proc Natl Acad Sci U S A* 88, 9838–9842 (1991); published online EpubNov 1 ([PubMed: 1682922]
 59. Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, Fox CH, Orenstein JM, Kotler DP, Fauci AS, HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* 362, 355–358 (1993); published online EpubMar 25 (10.1038/362355a0). [PubMed: 8455722]
 60. Sloan RD, Wainberg MA, The role of unintegrated DNA in HIV infection. *Retrovirology* 8, 52 (2011); published online EpubJul 1 (10.1186/1742-4690-8-52). [PubMed: 21722380]
 61. Eriksson S, Graf EH, Dahl V, Strain MC, Yukl SA, Lysenko ES, Bosch RJ, Lai J, Chioma S, Emad F, Abdel-Mohsen M, Hoh R, Hecht F, Hunt P, Somsouk M, Wong J, Johnston R, Siliciano RF, Richman DD, O'Doherty U, Palmer S, Deeks SG, Siliciano JD, Comparative analysis of measures of viral reservoirs in HIV-1 eradication studies. *PLoS Pathog* 9, e1003174 (2013); published online EpubFeb (10.1371/journal.ppat.1003174). [PubMed: 23459007]
 62. Ho Y-C, Shan L, Hosmane NN, Wang J, Laskey SB, Rosenbloom DIS, Lai J, Blankson JN, Siliciano JD, Siliciano RF, Replication-Competent Noninduced Proviruses in the Latent Reservoir Increase Barrier to HIV-1 Cure. *Cell* 155, 540–551 (2013). [PubMed: 24243014]
 63. Koelsch KK, Liu L, Haubrich R, May S, Havlir D, Gunthard HF, Ignacio CC, Campos-Soto P, Little SJ, Shafer R, Robbins GK, D'Aquila RT, Kawano Y, Young K, Dao P, Spina CA, Richman DD, Wong JK, Dynamics of total, linear nonintegrated, and integrated HIV-1 DNA in vivo and in vitro. *J Infect Dis* 197, 411–419 (2008); published online EpubFeb 1 (10.1086/525283). [PubMed: 18248304]
 64. Crooks AM, Bateson R, Cope AB, Dahl NP, Griggs MK, Kuruc JD, Gay CL, Eron JJ, Margolis DM, Bosch RJ, Archin NM, Precise Quantitation of the Latent HIV-1 Reservoir: Implications for Eradication Strategies. *J Infect Dis* 212, 1361–1365 (2015); published online EpubNov 1 (10.1093/infdis/jiv218). [PubMed: 25877550]
 65. Takata H, Buranapraditkun S, Kessing C, Fletcher JL, Muir R, Tardif V, Cartwright P, Vandergeeten C, Bakeman W, Nichols CN, Pinyakorn S, Hansasuta P, Kroon E, Chalermchai T, O'Connell R, Kim J, Phanuphak N, Robb ML, Michael NL, Chomont N, Haddad EK, Ananworanich J, Trautmann L, Rv254/Search, R. V. S. S. G. the, Delayed differentiation of potent effector CD8+ T cells reducing viremia and reservoir seeding in acute HIV infection. *Sci Transl Med* 9, (2017); published online EpubFeb 15 (10.1126/scitranslmed.aag1809).
 66. Sathaliyawala T, Kubota M, Yudanin N, Turner D, Camp P, Thome JJ, Bickham KL, Lerner H, Goldstein M, Sykes M, Kato T, Farber DL, Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets. *Immunity* 38, 187–197 (2013); published online EpubJan 24 (10.1016/j.immuni.2012.09.020). [PubMed: 23260195]
 67. Khoury G, Fromentin R, Solomon A, Hartogensis W, Killian M, Hoh R, Somsouk M, Hunt PW, Girling V, Sinclair E, Bacchetti P, Anderson JL, Hecht FM, Deeks SG, Cameron PU, Chomont N, Lewin SR, Human Immunodeficiency Virus Persistence and T-Cell Activation in Blood, Rectal, and Lymph Node Tissue in Human Immunodeficiency Virus-Infected Individuals Receiving

- Suppressive Antiretroviral Therapy. *J Infect Dis* 215, 911–919 (2017); published online EpubMar 15 (10.1093/infdis/jix039). [PubMed: 28453847]
68. Fukazawa Y, Lum R, Okoye AA, Park H, Matsuda K, Bae JY, Hagen SI, Shoemaker R, Deleage C, Lucero C, Morcock D, Swanson T, Legasse AW, Axthelm MK, Hesselgesser J, Geleziunas R, Hirsch VM, Edlefsen PT, Piatak M Jr., Estes JD, Lifson JD, Picker LJ, B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers. *Nat Med* 21, 132–139 (2015); published online EpubFeb (10.1038/nm.3781). [PubMed: 25599132]
 69. Fromentin R, Bakeman W, Lawani MB, Khoury G, Hartogensis W, DaFonseca S, Killian M, Epling L, Hoh R, Sinclair E, Hecht FM, Bacchetti P, Deeks SG, Lewin SR, Sekaly RP, Chomont N, CD4+ T Cells Expressing PD-1, TIGIT and LAG-3 Contribute to HIV Persistence during ART. *PLoS Pathog* 12, e1005761 (2016); published online EpubJul (10.1371/journal.ppat.1005761). [PubMed: 27415008]
 70. Fromentin R, DaFonseca S, Costiniuk CT, El-Far M, Procopio FA, Hecht FM, Hoh R, Deeks SG, Hazuda DJ, Lewin SR, Routy JP, Sekaly RP, Chomont N, PD-1 blockade potentiates HIV latency reversal ex vivo in CD4(+) T cells from ART-suppressed individuals. *Nature communications* 10, 814 (2019); published online EpubFeb 18 (10.1038/s41467-019-08798-7).
 71. Hill AL, Rosenbloom DI, Fu F, Nowak MA, Siliciano RF, Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1. *Proc Natl Acad Sci U S A* 111, 13475–13480 (2014); published online EpubSep 16 (10.1073/pnas.1406663111). [PubMed: 25097264]
 72. Borducchi EN, Cabral C, Stephenson KE, Liu J, Abbink P, Ng'ang'a D, Nkolola JP, Brinkman AL, Peter L, Lee BC, Jimenez J, Jetton D, Mondesir J, Mojta S, Chandrashekar A, Molloy K, Alter G, Gerold JM, Hill AL, Lewis MG, Pau MG, Schuitemaker H, Hesselgesser J, Geleziunas R, Kim JH, Robb ML, Michael NL, Barouch DH, Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. *Nature* 540, 284–287 (2016); published online EpubDec 08 (10.1038/nature20583). [PubMed: 27841870]
 73. Ananworanich J, Chomont N, Fletcher JL, Pinyakorn S, Schuetz A, Sereti I, Rerknimitr R, Dewar R, Kroon E, Vandergeeten C, Trichavaroj R, Chomchey N, Chalermchai T, Michael NL, Kim JH, Phanuphak P, Phanuphak N, Markers of HIV reservoir size and immune activation after treatment in acute HIV infection with and without raltegravir and maraviroc intensification. *J Virus Erad* 1, 116–122 (2015). [PubMed: 26835516]
 74. Chalermchai T, Valcour V, Sithinamsuwan P, Pinyakorn S, Clifford D, Paul RH, Tipsuk S, Fletcher JL, Degruittola V, Ratto-Kim S, Hutchings N, Shikuma C, Ananworanich J, Trail Making Test A improves performance characteristics of the International HIV Dementia Scale to identify symptomatic HAND. *Journal of neurovirology* 19, 137–143 (2013); published online EpubApr (10.1007/s13365-013-0151-4). [PubMed: 23483520]
 75. Schuetz A, Deleage C, Sereti I, Rerknimitr R, Phanuphak N, Phuang-Ngern Y, Estes JD, Sandler NG, Sukhumvittaya S, Marovich M, Jongrakthaitae S, Akapirat S, Fletscher JL, Kroon E, Dewar R, Trichavaroj R, Chomchey N, Douek DC, RJ OC, Ngaay V, Robb ML, Phanuphak P, Michael NL, Excler JL, Kim JH, de Souza MS, Ananworanich J, Rv254/Search, R. S. S. Groups, Initiation of ART during early acute HIV infection preserves mucosal Th17 function and reverses HIV-related immune activation. *PLoS Pathog* 10, e1004543 (2014); published online EpubDec (10.1371/journal.ppat.1004543). [PubMed: 25503054]
 76. Vandergeeten C, Fromentin R, Merlini E, Lawani MB, DaFonseca S, Bakeman W, McNulty A, Ramgopal M, Michael N, Kim JH, Ananworanich J, Chomont N, Cross-clade ultrasensitive PCR-based assays to measure HIV persistence in large-cohort studies. *J Virol* 88, 12385–12396 (2014); published online EpubNov (10.1128/JVI.00609-14). [PubMed: 25122785]
 77. Josefsson L, King MS, Makitalo B, Brannstrom J, Shao W, Maldarelli F, Kearney MF, Hu WS, Chen J, Gaines H, Mellors JW, Albert J, Coffin JM, Palmer SE, Majority of CD4+ T cells from peripheral blood of HIV-1-infected individuals contain only one HIV DNA molecule. *Proc Natl Acad Sci U S A* 108, 11199–11204 (2011); published online EpubJul 5 (10.1073/pnas.1107729108). [PubMed: 21690402]
 78. Ganusov VV, De Boer RJ, Do most lymphocytes in humans really reside in the gut? *Trends Immunol* 28, 514–518 (2007); published online EpubDec (10.1016/j.it.2007.08.009). [PubMed: 17964854]

79. Procopio FA, Fromentin R, Kulpa DA, Brehm JH, Bebin AG, Strain MC, Richman DD, O'Doherty U, Palmer S, Hecht FM, Hoh R, Barnard RJ, Miller MD, Hazuda DJ, Deeks SG, Sekaly RP, Chomont N, A Novel Assay to Measure the Magnitude of the Inducible Viral Reservoir in HIV-infected Individuals. *EBioMedicine* 2, 874–883 (2015); published online EpubAug (10.1016/j.ebiom.2015.06.019). [PubMed: 26425694]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1. Rapid establishment of a large pool of infected cells in blood and tissues during acute HIV infection.

A, Frequency of peripheral blood mononuclear cells (PBMCs) harboring integrated HIV DNA according to the Fiebig stage before antiretroviral therapy (ART) initiation (Fiebig I n=14, Fiebig II n=31, Fiebig III n=40, Fiebig IV/V n=12 and chronic n=37). **B**, Frequency of lymph node mononuclear cells (LNMCs) harboring integrated HIV DNA according to the Fiebig stage before ART initiation (Fiebig I n=7, Fiebig II n=7, Fiebig III n=10, Fiebig IV/V n=6 and chronic n=3). **C**, Frequency of colon cells harboring integrated HIV DNA according to the Fiebig stage before ART initiation (Fiebig I n=3, Fiebig II n=12, Fiebig III n=31, Fiebig IV/V n=10 and chronic n=7). In **A-C**, each symbol represents an individual sample. Undetectable samples are plotted as “zero” and are represented as open circles. Columns represent the median values with interquartile range. Values were transformed in $\log_{10}[(\text{copies}/10^6 \text{ cells})+1]$. Nonparametric Mann Whitney U tests were performed to compare Fiebig I to all other stages and chronic to all other stages: ns $p > 0.05$, * $p < 0.05$, ** $p < 0.009$, *** $p < 0.0009$, **** $p < 0.0001$. **D**, Percentages of lymph node, sigmoid colon

biopsies and blood matched samples with detectable and undetectable integrated HIV DNA in acutely infected individuals. Chi-Square tests were performed to compare blood to colon, blood to lymph nodes and colon to lymph nodes **E**, **F**, Correlations between plasma viremia and frequencies of cells harboring integrated HIV DNA in lymph nodes and sigmoid colon biopsies, respectively. Values were transformed in $\log_{10}([\text{copies}/10^6 \text{ cells}]+1)$ and the nonparametric Spearman's tests were used to calculate p- and r values. **G**, Ratio between total and integrated HIV DNA values in lymph nodes, sigmoid colon biopsies and blood during acute infection. The ratio between total HIV DNA and integrated HIV DNA was quantified in each sample and represented by individual symbols. Samples with undetectable integrated HIV DNA (denominator = 0) were excluded from the analysis. Columns represent the median values with interquartile range. A one-way ANOVA test was performed to compare the ratio between the 3 compartments: ns $p>0.05$, * $p \leq 0.05$, ** $p \leq 0.009$, *** $p \leq 0.0009$, **** $p \leq 0.0001$.

Fig. 2. ART initiation during acute infection leads to profound decreases in the frequency of infected cells in blood and tissues.

A, Longitudinal measures of total, integrated HIV DNA and 2-LTR circles in PBMCs from acutely infected participants (n=81). Samples from chronically infected participants were used as controls (total n=16, integrated n=31, 2-LTR n=13). Measurements were performed before treatment initiation and thereafter at 2, 12, 24, 36, 48, 72 and 96 weeks of ART for acutely treated participants and at week 0, 24 and 96 for chronically treated individuals. **B**, Longitudinal quantifications of integrated HIV DNA in participants treated in acute vs

chronic infection (Fiebig I n=11, Fiebig II n=27, Fiebig III n=34, Fiebig IV/V n=9 and chronic n=31). Nonparametric paired t tests were performed to compare baseline to 12 weeks measurements in acutely treated individuals and baseline to 48 and 96 weeks measurements in chronically treated participants. Quantifications were performed at the same time points as in **A**. In **A** and **B**, mean values and 95% confidence interval are represented. **C**, Frequency of PBMCs harboring integrated HIV DNA after >24 weeks of ART (Fiebig I n=34, Fiebig II n=59, Fiebig III n=82, Fiebig IV/V n=30 and chronic n=41). **D**, Frequency of LNMCs harboring integrated HIV DNA after >24 weeks of ART (Fiebig I n=6, Fiebig II n=6, Fiebig III n=10, Fiebig IV/V n=7 and chronic n=5). **E**, Frequency of sigmoid colon cells harboring integrated HIV DNA after >24 weeks of ART (Fiebig I n=7, Fiebig II n=11, Fiebig III n=22, Fiebig IV/V n=7 and chronic n=7). In **C-E**, each symbol represents an individual sample. Open circles denote measurements below the limit of detection of the assay and are plotted as zero. Columns represent medians with interquartile ranges. Values are transformed in $\log_{10}([\text{copies}/10^6 \text{ cells}] + 1)$. Nonparametric Mann Whitney t tests were performed to compare Fiebig I and chronic controls to all other stages: ns $p > 0.05$, * $p < 0.05$, ** $p < 0.009$, *** $p < 0.0009$, **** $p < 0.0001$. **F**, Quantity of integrated HIV DNA in acutely infected and treated individuals was compared to chronic controls (black bars). Columns represent median values. Nonparametric Mann Whitney t tests were performed to compare acutely infected participants from different Fiebig stages to chronic controls: ns $p > 0.05$, * $p < 0.05$, ** $p < 0.009$, *** $p < 0.0009$, **** $p < 0.0001$. **G**, Average fold decrease in the frequencies of cells harboring integrated HIV DNA in blood, sigmoid colon biopsies and lymph nodes according to the Fiebig stage after >24 weeks of ART. Columns represent mean values. **H**, Average fold decrease in the frequency of circulating CD4+ T cells producing *tat/rev* RNA after PMA/ionomycin stimulation according to the stage of infection. Columns represent mean values. In **G** and **H** the limit of detection was used to calculate the ratio for undetectable samples.

Fig. 3. HIV reservoir in CD4⁺ T cells subsets before and after ART initiation in acute infection. **A-E** Frequency of sorted naïve, T_{CM}, T_{TM} and T_{EM} harboring integrated HIV DNA before and after >24 weeks of ART according to the Fiebig stage. **A**, Fiebig I baseline n= 7, ART n=6 **B**, Fiebig II baseline n= 8, ART n=6 **C**, Fiebig III baseline n=8, ART n=16 **D**, Fiebig IV/V baseline n=6, ART n=6 **E**, Chronic baseline n=6, ART n=8. Nonparametric Mann Whitney t tests were performed to compare infection frequencies before and after ART in each CD4⁺ T cell subset according to the Fiebig stage: ns p>0.05, * p 0.05, ** p 0.009, *** p 0.0009, **** p 0.0001. Each point represents an individual sample. Open circles denote measurements below the limit of detection of the assay and are plotted as zero. Columns represent medians with interquartile range. Values were transformed in log₁₀[(copies/10⁶ cells)+1]. **F**, Average fold decrease in the frequencies of cells harboring integrated HIV DNA in T_{CM}, T_{TM} and T_{EM} cells according to Fiebig stage. Columns represent mean values. Limits of detection were used for the undetectable samples. **G**, Frequencies of naïve, T_{CM}, T_{TM} and T_{EM} cells harboring integrated HIV DNA after >24 weeks of ART in acutely treated individuals compared to chronically treated individuals.

Columns represent median values. Open symbols denote measurements below the limit of detection of the assay and are plotted as zero. Values were transformed in $\log_{10}([\text{copies}/10^6 \text{ cells}]+1)$ and a one-way ANOVA was performed to compare the different CD4+ T cells subsets individuals treated in acute and chronic infection: ns $p>0.05$, * $p \leq 0.05$, ** $p \leq 0.009$, *** $p \leq 0.0009$, **** $p \leq 0.0001$.

Table 1.

Clinical data of the participants at baseline

	Acute	Fiebig I	Fiebig II	Fiebig III	Fiebig IV/V	Chronic
Age, years median [IQR]	27 [23-33]	27 [25-29]	27 [18-53]	28 [22-30]	26 [25-36]	31 [26-36]
Sex						
Male, n (%)	164 (96)	22 (92)	46 (98)	67 (97)	29 (97)	24 (65)
Female, n (%)	6 (4)	2 (8)	1 (2)	2 (3)	1 (3)	13 (35)
HIV - Clade						
CRF01_AE, n (%)	117 (69)	19 (79)	34 (72)	42 (61)	22 (73)	80 (30)
B, n (%)	4 (2)	0 (0)	2 (4)	1 (1)	1 (3)	0 (0)
CRF01_AE/B Recombinant, n (%)	14 (8)	2 (8)	5 (11)	6 (9)	1 (3)	0 (0)
Other or indeterminate, n (%)	35 (21)	3 (13)	6 (13)	20 (29)	6 (21)	20 (7)
Risk						
MSM, n (%)	160 (94)	21 (88)	45 (96)	66 (96)	28 (93)	30 (80)
Hetero, n (%)	10 (6)	3 (12)	2 (4)	3 (4)	2 (7)	6 (15)
IV drug use, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)
Estimated time between HIV exposure and enrollment, (days) median [IQR]	18 [14-23]	15 [11-18]	16 [12-23]	19 [15-23]	22 [19-32]	868 [502-2337]
Estimated time between HIV exposure and initiation of ART, (days) median [IQR]	19 [14-25]	18 [14-19]	18 [13-25]	20 [15-24]	25 [19-32]	291 [130-410]
Baseline CD4 T cell count, (cells/L) median [IQR]	375 [278-504]	556 [431-665]	412 [309-529]	350 [265-463]	289 [214-494]	323 [219-386]
ART CD4 T cell count,	634 [529-802]	602 [560-748]	655 [565-849]	635 [505-780]	588 [468-856]	533 [404-691]

	Acute	Fiebig I	Fiebig II	Fiebig III	Fiebig IV/V	Chronic
(cells/L) median[IQR]						
Baseline CD8 T cell count, (cells/L) median [IQR]	445 [81-4556]	453 [189-2518]	257 [81-1127]	570 [139-3269]	1180 [426-4556]	914 [661-1208]
ART CD8 T cell count, (cells/L) median [IQR]	573 [438-745]	575 [402-761]	509 [422-615]	576 [410-762]	633 [491-767]	698 [548-751]
Baseline Plasma viral load, (HIV RNA copies/mL) median [IQR]	680232 [218141-5429036]	11732 [4313-46108]	580357 [253478-2803595]	2656900 [549732-9871790]	788111 [322510-4698648]	57487 [22381-106914]
ART Plasma viral load, (HIV RNA copies/ mL)median [IQR] **	20 [20-50]	20 [20-50]	20 [20-50]	20 [20-50]	20 [20-50]	20 [20-50]
ART Treatment						
RTI, n (%)	113 (67)	18 (75)	29 (62)	44 (63)	22 (78)	37 (100)
INI ENI, n (%)	53 (31)	6 (25)	16 (34)	26 (37)	5 (18)	0 (0)
Unknown, n (%)	3 (2)	0 (0)	2 (4)	0 (0)	1 (4)	0 (0)
Baseline						
HIV quantification in blood, n	107	18	32	43	14	37
HIV quantification in gut biopsies, n	70	8	15	35	12	7
HIV quantification in lymph node, n	30	7	7	10	6	3
ART						
HIV quantification in blood, n	205	34	59	82	30	41
HIV quantification in gut biopsies, n	51	8	15	22	6	7

	Acute	Fiebig I	Fiebig II	Fiebig III	Fiebig IV/V	Chronic
HIV quantification in lymph node, n	29	6	6	10	7	5

Roche Amplicor HIV-1 Monitor Test version 1.5 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) LOD<50
Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v2.0 (Roche Diagnostics, Branchburg, NJ, USA) LOD<20

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript