Published in final edited form as:

Curr Opin HIV AIDS. 2015 January ; 10(1): 18–28. doi:10.1097/COH.0000000000000122.

How does the timing of antiretroviral therapy initiation in acute infection affect HIV reservoirs?

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Abstract

Purpose of review—The long-lived viral reservoir is a major obstacle to achieving a cure for HIV. Therapeutic strategies, such as early antiretroviral therapy (ART), may be a prerequisite to achieving long-term control of viral replication upon ART withdrawal.

Recent findings—HIV persistence is established early in acute HIV infection (AHI) with infection in long-lived memory CD4⁺ T cells. Studies conducted in nonhuman primates have suggested that this could occur as early as 3 days postinfection; however, the timing in humans is uncertain. ART during AHI significantly restricts the HIV reservoirs as compared with later treatment. Early ART, particularly prior to the detection of HIV immunoglobulin M, may also reduce the contribution of the long-lived central memory CD4⁺ T cells to the total HIV reservoir, a profile observed in individuals who naturally control HIV without ART.

Summary—It is clear that early ART has a greater impact in limiting the HIV reservoirs than later treatment. However, latently infected long-lived memory CD4⁺ T cells persist in most early treated individuals. Therefore, additional interventions will likely be required to eliminate all cells capable of producing replication-competent virus but treatment in AHI may be the critical first step in containing the HIV reservoirs.

Keywords

acute HIV infection; early antiretroviral therapy; HIV DNA; HIV reservoir; replication-competent virus

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Conflicts of interest

J.A. has received honorarium for speaking engagement and advisory board participation from Gilead and ViiV. Healthcare. Other authors declared no potential conflicts of interest relevant to this work.

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INTRODUCTION

The discovery of a small pool of latently infected cells that persist in HIV-infected individuals receiving suppressive antiretroviral therapy (ART) has considerably dampened the hope of eradicating HIV with ART alone [1–3]. This long-lived reservoir is often considered the major obstacle to achieving a cure for HIV and therapeutic strategies that could limit its size may be a prerequisite to achieving control of viral replication upon ART withdrawal, which is also called HIV remission [4,5]. Current evidence suggests that ART instituted early during acute HIV infection (AHI) is key to containing HIV reservoir establishment, particularly in long-lived CD4⁺ T cells, providing a basis for its critical role in achieving HIV remission [6[■],7,8]. Indeed, a prime example is the case of the Mississippi child who started ART by 2 days of life for 18 months and subsequently was able to remain with low HIV reservoirs and virally suppressed for 27 months in the absence of ART [9,10]. Similarly, the VISCONTI cohort of adults in France was treated within the first 2 months of infection and was able to control viremia without ART for more than 5 years [11[■]]. Through key published works, this review covers the timing and mechanisms of HIV reservoir establishment and the effects of early ART on the HIV reservoirs as well as future research directions.

The latent HIV reservoir is established early after infection

The mechanisms responsible for the establishment of HIV latency are not fully elucidated. Latently infected cells are rare *in vivo* and appear to arise when activated CD4⁺ T cells, the major targets cells for HIV infection, become infected and survive long enough to revert back to a resting memory state, which is nonpermissive for viral gene expression [12]. An alternative model proposes that HIV latency is directly established in resting CD4⁺ T cells, following interaction with dendritic cells and/or specific chemokines [13,14].

No matter how latently infected cells are generated, several lines of evidence clearly indicate that the latent reservoir is established early in infection, indicating that one or both of these mechanisms occur within the first few days or weeks after transmission. Indeed, initiation of ART as early as 10 days after the onset of symptoms of primary HIV infection (PHI) does not prevent the generation of latently infected carrying infectious HIV despite the successful control of plasma viremia shortly after institution of therapy [15[■]]. Studies in nonhuman primates (NHPs), which have the advantage of precisely defining the time and location of the reservoir establishment, have also demonstrated that lentiviruses seed their reservoir within a few days post-infection; large numbers of resting CD4⁺ T cells carry integrated simian immunodeficiency virus (SIV), and simian human immunodeficiency virus DNA [16] can be detected at the earliest stage of infection. In line with these results, Whitney *et al.* [17[■]] recently demonstrated that treatment with ART 3 days post-SIV_{mac251} infection in rhesus macaques blocks the emergence of viral RNA and proviral DNA in peripheral blood but does not prevent the establishment of a viral reservoir in tissues. After discontinuation of ART following 24 weeks of fully suppressive therapy, virus rebounded in all animals [17[■]]. These data indicate that the viral reservoir could be seeded substantially earlier than previously assumed [5]. Another study in SIV_{mac239}-infected rhesus macaques showed that ART initiated prior to peak virus replication (day 12) significantly limits

systemic virus dissemination and seeding of the reservoir in the peripheral blood and lymphoid tissues [18]. Notwithstanding the important information generated by these studies conducted in NHP, it is crucial to caution their application to HIV infection in humans as SIV and HIV may establish their reservoir in distinct cells and tissues, and with different efficiency and kinetics. For instance, resting CD4⁺ T and myeloid cells are essentially resistant to HIV infection due to the restriction factor, Sterile alpha motif domain-containing protein 1 and HD domain-containing protein 1 (SAMHD1) [19,20–22], a mechanism that is counteracted by the SIV protein vpx, which is absent in HIV-1. This suggests that SIV may be more prone to directly establishing latency in resting CD4⁺ T cells and myeloid cells when compared with HIV. Moreover, the amount of virus used to efficiently infect NHPs is usually much higher than the ones that cause natural HIV transmission in humans and ART may not be as effective in NHPs as in HIV-infected patients. Therefore, the establishment of latent viral reservoir in humans and NHPs may differ in numerous ways, and further studies are warranted to demonstrate that these experimental models can be used to investigate the mechanisms responsible for the establishment and persistence of the long-lived HIV reservoir in infected individuals.

Early antiretroviral therapy limits the size of the latent HIV reservoir

Although the reservoir is seeded very early in infection, the beneficial effect of starting ART during the first weeks or months after infection to reduce the size of the latent reservoir has been reported in numerous studies. Strain *et al.* [23[■]] reported that after 12 months of ART, replication-competent virus could not be detected in all patients who initiated treatment before seroconversion, nor in the majority of individuals who initiated ART in the first 6 months after seroconversion [23[■]]. These observations were confirmed in several studies indicating that HIV-infected individuals who start ART within the first few months of HIV infection display a reservoir of smaller magnitude compared with those who start later in infection [6[■],24[■]–26[■],27[■]]. Of note, the association between early ART initiation and a reduced size of the reservoir has been reported in multiple groups of patients using different assays including total HIV DNA [23[■],25[■],26[■],27[■]], integrated DNA [24[■], 27[■]], as well as the quantitative viral outgrowth assay [6[■],23[■],27[■]]. Importantly, there is a gradual increase in the size of the reservoir during the first few weeks after infection. Individuals who start ART at the Fiebig [28] I stage of the disease [HIV RNA⁺, p24 antigen-, HIV Immunoglobulin M (IgM-)] show a significantly lower frequency of CD4⁺ T cells harboring integrated HIV DNA when compared to Fiebig II (HIV RNA⁺, p24 antigen +, HIV IgM-) and Fiebig III (HIV IgM+, HIV IgG-) individuals [25[■]]. Therefore, the benefits of early ART may be maximal during the first few weeks of infection.

The decay of the latent HIV reservoir may also be faster in individuals who start ART early after infection [27[■],29[■]], although the limited size of the initial pool of latently infected CD4⁺ T cells has been proposed to be the most important factor contributing to a smaller size of the reservoir after several years of therapy. Archin *et al.* [6[■]] demonstrated that although the frequency of cells harboring replication-competent HIV declines after the first year of ART in most acutely infected patients, no further decay is noted when this frequency reaches very low levels (less than 0.5 cells per million). Therefore, a subset of latently infected cells may persist indefinitely, even in patients who have started ART during acute

infection. The identification of the phenotype of these cells, in which HIV may persist for decades, is currently an area of intense investigation. Indeed, the composition of the reservoir (i.e., its distribution in distinct memory CD4⁺ T cell subsets) may provide crucial information on the mechanisms of HIV persistence, and several recent studies indicate that early ART greatly influences this parameter.

Early antiretroviral therapy alters the distribution of the reservoir in CD4⁺ T-cell subsets

Resting memory CD4⁺ T cells are the predominantly infected cells in virally suppressed patients. The use of a combination of markers expressed by memory CD4⁺ T cells has led to the identification of three cellular reservoirs in which HIV persists after prolonged suppressive ART, namely central (T_{CM}), transitional (T_{TM}) and effector (T_{EM}) memory CD4⁺ T cells [24[■],30] (Fig. 1). Because of their very distinct functional and phenotypic properties [31], these three cellular reservoirs may support viral persistence through different mechanisms. For instance, the drastic differences in the activation status of T_{CM} and T_{EM} cells [24[■],32] suggest that the former might represent an ideal reservoir for latent HIV, whereas the latter may be more prone to support residual levels of viral replication in the face of ART. The relative contribution of each subset to the pool of HIV-infected cells is highly variable between HIV-infected individuals [24[■]]. Several recent studies suggest that a relatively low infection frequency of T_{CM} cells may be strongly associated with lack of disease progression in a variety of apparently unrelated clinical scenarios in untreated individuals. Long-term nonprogressors (LTNPs) display a low infection frequency of T_{CM} cells, which is associated with an increased size of the T_{CM} pool and a greater number of anti-gag CD8⁺ T cells. Of note, T_{CM} from HLAB27/B57 LTNPs are markedly less infected than T_{CM} from individuals who do not bear these protective alleles [33]. In addition, in the recently described VISCONTI cohort of posttreatment controllers (PTCs), who initiated ART early in infection and naturally control HIV replication in the absence of ART, T_{CM} contribute very minimally to the total HIV reservoir [11[■]]. Taken together, the relative protection of T_{CM} cells in LTNPs and PTCs suggests that a T_{CM} compartment devoid of HIV may be an important correlate of natural control of HIV replication.

Can early ART initiation reproduces this skewed distribution of the reservoir by protecting T_{CM} cells from infection? Preliminary data generated on samples from the RV254 study, an AHI study conducted by the Thai Red Cross AIDS Research Center and the US Military HIV Research Program, indicate that very early ART efficiently protects all memory CD4⁺ T-cell subsets from infection, including the long-lived T_{CM} cells [7]. This is in agreement with other studies investigating the frequency of infection of distinct memory CD4⁺ T-cell subsets in study participants who initiated ART early in infection [8,26[■],27[■]]. Whether the T_{CM} subset is more protected than the other subset by early ART is still unclear.

In addition to T_{CM}, T_{TM} and T_{EM} cells, the recently described stem cell memory T cells, which share functional and self-renewal properties with T_{CM} cells [34], may represent an additional relevant reservoir for HIV [35]. Importantly, early ART may significantly reduce the contribution of this extremely long-lived subset to the overall HIV reservoir [27[■]].

Clinical trials of early antiretroviral therapy and the HIV reservoirs

There are, so far, only a limited number of studies evaluating the impact of ART initiated in AHI on the HIV reservoirs. A major limitation is the difficulty in identifying people in AHI; this is a narrow window that spans the first 4 weeks of infection when the HIV IgG antibody response is not yet detected by standard assays [28,36]. The diagnosis generally requires nucleic acid testing that is costly and not part of standard testing algorithms in most settings. Thus, studies of acutely treated individuals are usually small. The immediate initiation of ART is also challenging, and in many studies, ART is initiated weeks or months after AHI is identified.

Table 1 summarizes the findings of selected studies published between 2005 and 2014 [6[■], 25[■], 27[■], 37–39, 40[■], 41, 42[■]]. Most enrolled less than 40 participants. They included individuals treated within a few weeks to 6 months after the onset of HIV with a handful including those treated during the earliest stages of AHI prior to HIV IgM detection [25[■], 42[■]]. These studies consistently demonstrated the superiority of early ART over late treatment in reducing the HIV reservoir size as defined by HIV DNA levels (total and integrated, 2-long terminal repeat (LTR) circles 2-LTR circles) and frequencies of resting CD4⁺ T cells that are capable of producing replication-competent virus. Occurrence of low-level viremia measured by single-copy HIV RNA assay is also less with early ART [6[■]]. The frequency of infected cells in the gastrointestinal tract was evaluated in two studies that observed a statistically significant decline in HIV DNA after early ART [25[■], 41]. Studies comparing standard vs. regimens intensified with integrase and entry inhibitors did not show a superior effect of the latter regimen, but these studies are greatly underpowered [42[■], 43]. Comparison between studies is limited by the highly variable timing of ART initiation, ART regimen and follow-up periods as well as the different measures of the HIV reservoir.

FUTURE RESEARCH

Most of the studies that have demonstrated the ability of early ART at reducing the size of the latent reservoir have been conducted on cells isolated from peripheral blood. Little information is known on the establishment of the reservoir in tissues (particularly the major anatomical HIV reservoirs such as the gut and lymph nodes) at the earliest stage of infection. This limitation is obviously attributable to the difficulty in accessing these samples, but also to the lack of a sensitive, reproducible and flexible assay that can measure a few latently infected cells harboring replication-competent HIV. Alleviating this technical limitation would also allow a better understanding of the relative importance of each memory T-cell subset to HIV persistence, as well as the capacity of early ART at interfering with the establishment of latency in these discrete subsets of cells.

The persistence of infection in T_{CM} after 10 years of ART in the study by Buzon *et al.* [27[■]] suggests that treatment within the first 6 months of infection is not early enough, but it remains unknown what the window of opportunity to intervene is before reaching the ‘point of no return.’ Theoretically, the earlier ART is initiated (i.e., Fiebig stages I and II) [28], the higher should be the chance for HIV remission. However, the PTCs in the VISCONTI cohort were mainly those treated in Fiebig IV and V stages [11[■]]. Understanding the precise timing of ART initiation that has the greatest impact on HIV

reservoirs will require careful characterization of patients at time of ART initiation for their history of HIV exposure and viro-logic and serologic markers of HIV. A concerted effort to evaluate and identify AHI staging system that could predict outcomes relevant to HIV cure (e.g., HIV reservoir size) would be a major advancement. Additionally, there is a dearth of data on the HIV-specific immune responses following early ART. It is possible that such immune responses are blunted with rapid treatment and viral suppression, as evidenced by the higher proportion of early treated individuals who remain HIV seronegative compared with the later treatment initiators [44,45]. Knowledge on the association between existing immune responses and HIV reservoirs after AHI treatment will inform future immune-based strategies to eliminate latently infected cells.

CONCLUSION

It is clear that early ART results in lower reservoir size which could delay the time to viral rebound when ART is interrupted, as has been demonstrated in the Mississippi child. However, reaching sustained HIV remission or cure will require not only reduction size of the HIV reservoir, but also, to modify the distribution of the replication-competent HIV reservoir in CD4⁺ T cells and tissues. ART initiated during AHI, however, may be the first critical step in the path aimed at HIV remission or cure by limiting the HIV reservoir establishment prior to employing additional interventions to eliminate all cells capable of producing HIV.

Acknowledgements

None.

Financial support and sponsorship

The given work was supported in part by grants to N.C. from the amfAR (ARCHE grants 108687-54 and 108928-56), the National Institutes of Health (1R21 AI113096-01 and 1U19AI096109 – DARE: Delaney AIDS research enterprise to find a cure) and a grant to J.A. from the National Institute of Allergy and Infectious Diseases (R01HD080435-01). This work was also partly supported by the CARE – Collaboratory of AIDS Researchers for Eradication (U19AI096113); David M. Margolis PI.

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KEY POINTS

- HIV persistence is established early in AHI in long-lived memory CD4⁺ T cells that are nonpermissive for viral gene expression, therefore, not eliminated by immune surveillance or ART.
- ART instituted during AHI can reduce the HIV reservoir size to a greater extent than when treatment is given in chronic HIV. However, infection generally persists in memory CD4⁺ T cells in most early treated individuals.
- Recent data suggest that treatment in the earliest AHI stage (Fiebig I) may protect central memory CD4⁺ T cells from infection and skew the distribution of latently infected cells to the shorter lived memory CD4⁺ T cells as is the case in individuals who control HIV without ART (elite controllers and PTCs).
- ART in AHI may be the first critical step in the path to achieve HIV remission by containing HIV reservoir seeding prior to instituting additional interventions to eliminate all latently infected cells.

FIGURE 1.

The size and composition of the latent HIV reservoir is affected by early ART. (a) Subsets of CD4 T cells can be identified based on the levels of expression of several cellular markers including CD45RA, CCR7, CD27 and CD95. The pathway of T cell differentiation, that is, the sequence of development of the different T-cell subsets, remains elusive in humans, and it is unclear if the differentiation pathway is linear or branched, one-way or reversible [31]. (b) In chronically infected patients, the contribution of TCM cells to the overall pool of infected cells is important [30]. As these cells are long lived, they survive after years of ART and represent the major latent HIV reservoir in patients who started ART during chronic infection [24[■]]. The contribution of T_{SCM} cells also increases with time [27[■]]. Patients with acute or recent infection display a small size of the reservoir (as depicted by a reduced size of the grey box). In addition, their T_{CM} cells may be relatively preserved from infection, with a greater contribution of short-lived cells. After years of ART, this may result in a reservoir of a very small magnitude. ART, antiretroviral therapy.

Table 1

Effects of early antiretroviral therapy on HIV reservoirs

Reference	Sample size	Study population	Intervention	Timing of ART initiation	Treatment duration	Effect on reservoir size (measure ^a)	Main findings and/or comments
[25■]	n = 15 3 Fiebig I 2 Fiebig II 8 Fiebig III 2 Fiebig IV	Acutely infected Thais, mostly MSM with HIV clade CRF01_AE	MegaHAART regimen during AHI, defined as: Tenofovir Emtricitabine Efavirenz Raltegravir Maraviroc	Fiebig stages I-IV	24 weeks	Significant decline in total HIV DNA ^a copies in PBMCs and gut mucosal tissue; HIV DNA undetectable in 3/15 in blood and 3/7 in gut. After megaHAART, median total HIV DNA in PBMCs declined from 1513 / 10 ⁶ CD4 ⁺ at baseline to 106 copies/10 ⁶ at week 24 (P = 0.002).	MegaHAART initiated in early Fiebig stages was associated with reduced reservoir size in blood and gut.
[6■]	n = 27	Acutely infected individuals (CHAVI cohort)	Mathematical model to analyze frequency of latently infected cells post-ART	ART <45 days of estimated date of infection	6 months	Replication-competent virus in resting CD4 ⁺ T cells serially measured by viral outgrowth assay ^a and SCA ^a . Positive correlation between the frequency of latently infected cells and the area under the pretreatment viral load curve; viremia frequently undetectable (<1 copy/ml) when resting cell infection frequency <0.3/ million in resting CD4 ⁺ T cells.	Degree of latent infection can be limited by early ART during AHI. Few latently infected cells are generated after ART initiation. This study also showed that latency was founded immediately upon HIV infection.
[27■■]	n = 9	Acutely infected individuals compared with reference cohort of chronically infected individuals (ART initiated >2 years postinfection)	ART initiation <6 months of infection (regimen not specified)	<6 months of infection	10 years	Replication-competent virus measured by viral outgrowth assay ^a retrievable in 5/8 early ART patients; estimated frequency of CD4 ⁺ T cell with replication-competent virus higher in chronically infected patients than in early ART patients (P = 0.052). Statistically significant longitudinal decreases in total, integrated and 2-LTR HIV-1 DNA ^a in early ART patients (P = 0.0008).	In patients treated during PHI, the decay of HIV-1 DNA in CD4 ⁺ T cells is faster and more pronounced than in chronically infected individuals. CD4 ⁺ T cells reservoirs in early treated patients are smaller but infection persists in long-lasting central memory and T memory stem cells. Time from HIV-1 acquisition to ART initiation correlated with total or integrated HIV-1 DNA after >10 years of ART. Levels of CD4 ⁺ T cell-associated 2-LTR HIV-1 DNA lower in early ART patients, particularly in effector memory CD4 ⁺ T cells.

Reference	Sample size	Study population	Intervention	Timing of ART initiation	Treatment duration	Effect on reservoir size (measure ^a)	Main findings and/or comments
[37]	<i>n</i> = 32	Acutely and recently infected patients, mostly MSM with HIV-1 clade B	Standard first-line ART followed by interruption	Early ART defined as 60 days of infection (<i>n</i> = 24) vs. later start >60 days of infection (<i>n</i> = 8)	18 months (median)	Average levels of cell-associated HIV-1 DNA ^a in early ART patients after treatment interruption 0.4 log ₁₀ (<i>P</i> = 0.03) lower compared with later starters.	Early ART within 60 days of HIV-1 infection resulted in lower levels of proviral HIV-1 DNA for 1-year post-ART interruption.
[38]	<i>n</i> = 38 Six post-ART controllers Seven low viremic patients 25 noncontrollers	Acutely infected patients who interrupted ART during PHI (ANRS C06 PRIMO study group)	Comparison of patient characteristics to predict HIV-1 control	ART initiated during first 3 months after HIV infection	Not specified	Post-ART controllers had lower median HIV-1 DNA ^a of 3.1 log ₁₀ copies/10 ⁶ PBMC, compared with 3.4 log ₁₀ copies/10 ⁶ PBMCs for post-ART noncontrollers (<i>P</i> = 0.09).	Lower HIV DNA levels at ART interruption were associated with post-ART HIV control, compared with patients who experienced viral rebound.
[39]	<i>n</i> is variable per number for whom available of patients data are	Patients treated during PHI in 39 health centers in 10 countries	Quadruple ART administered twice daily during PHI, defined as: (QUEST GE PROB3005 cohort) Zidovudine Lamivudine Abacavir Amprenavir	Quadruple ART initiated during PHI	48 weeks	Median cell-associated RNA ^a level decreased from 3.4 log copies/million PBMCs (<i>n</i> = 114) to 0.8 log copies/million PBMCs (<i>n</i> = 63) and median cell-associated DNA ^a level (<i>n</i> = 113) decreased from 2.8 log copies/million PBMCs (<i>n</i> = 113) to 1.6 log copies/million PBMCs (<i>n</i> = 63) after 48 weeks.	Data represent one of the largest cohorts of patients prospectively enrolled during PHI. ART initiation during PHI was associated with lower levels of cell-associated RNA and DNA than with ART initiation during chronic infection.
[40]	<i>n</i> = 34	Patients in early HIV infection (Options Project, San Francisco, USA)	Standard ART regimen	<6 months of infection, compared with later ART group (<i>n</i> = 32), untreated (<i>n</i> = 34) and HIV-negative controls (<i>n</i> = 19)	Not specified	Early ART predicted 4.8-fold lower HIV DNA ^a levels in PBMCs than with later ART initiation (<i>P</i> = 0.005). Early ART predicted lower cell-associated HIV RNA ^b levels (difference in signal-to-cutoff-ratio (S/Co) 3.2 (<i>P</i> = 0.035).	Data suggest that early ART initiated within 6 months of infection is associated with lower HIV DNA and cell-associated reservoir sizes. This study shows that timing of ART initiation is a critical determinant of the HIV reservoir size. Early ART is also associated with lower levels of CD4 ⁺ and CD8 ⁺ T-cell activation.
[41]	<i>n</i> = 8	Patients in AHL, defined as 3 bands on western blot, p24 antigen positive and/or proviral DNA positive	Raltegravir Emtricitabine Tenofovir	AHL, compared with patients treated during chronic infection (<i>n</i> = 8)	52 weeks	In acutely treated patients: Total HIV-1 DNA ^a levels in PBMCs reduced in both groups, with more pronounced decrease in PHI patients at week 52 - from 4594 copies per 10 ⁶ cells at baseline to 494 at week 52 (<i>P</i> = 0.0003). Total HIV-1 DNA in colon tissue biopsies ^a reduced from	Levels of HIV-1 DNA in PBMCs and gut tissue were lower in PHI vs. chronically infected patients at week 52.

Reference	Sample size	Study population	Intervention	Timing of ART initiation	Treatment duration	Effect on reservoir size (measure ^a)	Main findings and/or comments
[42]	n = 36 (randomized) n = 34 met criteria for primary endpoint analysis	Acutely infected patients defined as plasma HIV-1 RNA levels >5000 copies/ml with negative or indeterminate EIA	Once daily fixed combination 3-drug PI-based cART regimen vs. 5-drug regimen intensified with raltegravir and maraviroc (1:2 ratio)	ART initiated at randomization in AH	Approximately 2 years	2125 copies per 500 ng DNA at baseline to 113 at week 52 (0.001). At week 48, undetectable single-copy assay ^a in 3/11 in 3-drug arm vs. 9/21 in 5-drug arm (P = 0.46). No statistically significant differences in cell-associated HIV-1 DNA ^a in PBMCs or replication-competent virus in resting CD4 ⁺ T cells at week 96 between 3-drug vs. 5-drug arm.	No statistically significant effect on the HIV reservoir of intensified 5-drug cART vs. 3-drug cART initiated during early infection.
[11]	n = 14	PTCs who controlled viremia for several years post-ART interruption (VISCONTI cohort)	Standard ART	Combination ART initiated within 10 weeks of PHI	Approximately 3 years	PBMC-associated HIV-1 DNA ^a levels in PTCs during infection control (median 1.71 log copies/10 ⁶ PBMC) similar to those in spontaneous HIV controllers and lower than patients who started ART during chronic HIV infection. In the absence of ART, HIV-1 DNA ^a levels in PBMC declined in 5/8 and was stable in 2/8. Replication-competent virus detected in at least one T-cell subset (TCM, TTM, TEM) in 7/7 tested patients. Low frequency of infection in TCM compared with TTM.	Control of viremia post-ART interruption was associated with low HIV blood reservoir size. This finding suggests that limiting the HIV reservoir is crucial for control of viral replication off ART. Long-lived HIV-infected CD4 ⁺ T-cell subsets contributed minimally to the total resting HIV reservoir in PTCs.
[23]	n = 27, MSM	Subgroups of MSM recruited from PHI studies in San Diego and Los Angeles, CA between 1996 and 1999	Standard ART	ART initiation before or <6 months after seroconversion with viral <50 copies/ml load, compared with 17 patients who initiated ART during chronic infection and with viremia <50 copies/ml for 3 - 6 years	Duration of study	Total CAI in resting CD4 ⁺ T cells ^a was undetectable after 1 year of ART (<0.07 IUPM) in 9/9 patients who initiated ART before seroconversion and in 6/8 patients who initiated ART <6 months after seroconversion, compared with none of the 17 controls who started ART during chronic infection	This study contributed to the quantitative understanding of the dynamics of long-lived cellular reservoirs of HIV-1 in patients during PHI compared with chronic infection

Fiebig stages: Fiebig I (RNA⁺, p24 antigen–, HIV IgM–), Fiebig II (p24 antigen–, HIV IgM–), Fiebig III (HIV IgM⁺, HIV IgG–, western blot indeterminate).

AHI, acute HIV infection; ART, antiretroviral therapy; CAI, cell-associated infectivity; IUPM, infectious units/million cells; MSM, men who have sex with men; PBMC, peripheral blood mononuclear cells; PHI, primary HIV infection; PI, protease inhibitor; PTC, posttreatment controllers; SCA, single-copy assay; TCM, central memory T cells; TEM, effector memory T cells; TTM, transitional memory T cells.

^a for references of special interest.

^b for references of outstanding interest.