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Initiation of Antiretroviral Therapy during Primary HIV Infection: Effects on the Latent HIV Reservoir, Including on Analytic Treatment Interruptions

*Eva M. Shelton[1], Daniel B. Reeves[2], and Rachel A. Bender Ignacio[2,3]**

[1]University of Washington School of Medicine, Seattle, Washington, USA; [2]Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; [3]Division of Allergy and Infectious Diseases, University of Washington, Seattle, USA

Abstract

Antiretroviral therapy (ART) inhibits HIV replication but does not eradicate the latent reservoir. The previous research suggests that earlier ART initiation provides benefit on limiting reservoir size, but timing and extent of this effect remain unclear. Analytic treatment interruption (ATI) may be used to demonstrate HIV remission, but whether early ART also improves likelihood or duration of even temporary virologic remission is unclear. This review seeks to answer both questions. We performed a systematic review and analysis following Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines and included 21 interventional or observational studies with sufficient HIV reservoir outcomes. We also aggregated reservoir outcomes and transformed data into approximate measurements of total HIV DNA per million peripheral blood mononuclear cells and analyzed the correlation between timing of ART initiation and reservoir size. People living with HIV who initiate ART in primary infection maintain smaller reservoirs on suppressive ART than those who initiate treatment during chronic infection. The reduction of reservoir is most pronounced when ART is started within 2 weeks of HIV acquisition. Across studies, we found a moderately strong association between longer time to ART initiation and reservoir size, which was strongest when measured after 1 year on ART (Pearson's r = 0.69, p = 0.0003). After ATI, larger pre-ATI reservoir size predicts shorter time to viral rebound. Early ART may also facilitate long-term control of viremia. Although achieving sustained HIV remission will require further interventions, initiating ART very early in infection could limit the extent of the reservoir and also lead to post-ATI control in rare cases. ***(AIDS Rev. 2018;20:28-39)***

Corresponding author: Rachel Bender Ignacio, Rbi13@uw.edu

Key words

Primary HIV. Acute HIV. HIV reservoir. Antiretroviral therapy. Analytic treatment interruption (ATI).

Introduction

UNAIDS estimates that there were 37.9 million persons living with HIV (PLWH) worldwide in 2019, 24.5 million of whom are currently accessing antiretroviral therapy (ART)[1]. ART inhibits viral replication but does not eradicate the latent HIV reservoir, which causes a significant barrier to achieving a functional

Correspondence to:
*Rachel Bender Ignacio
E-mail: Rbi13@uw.edu

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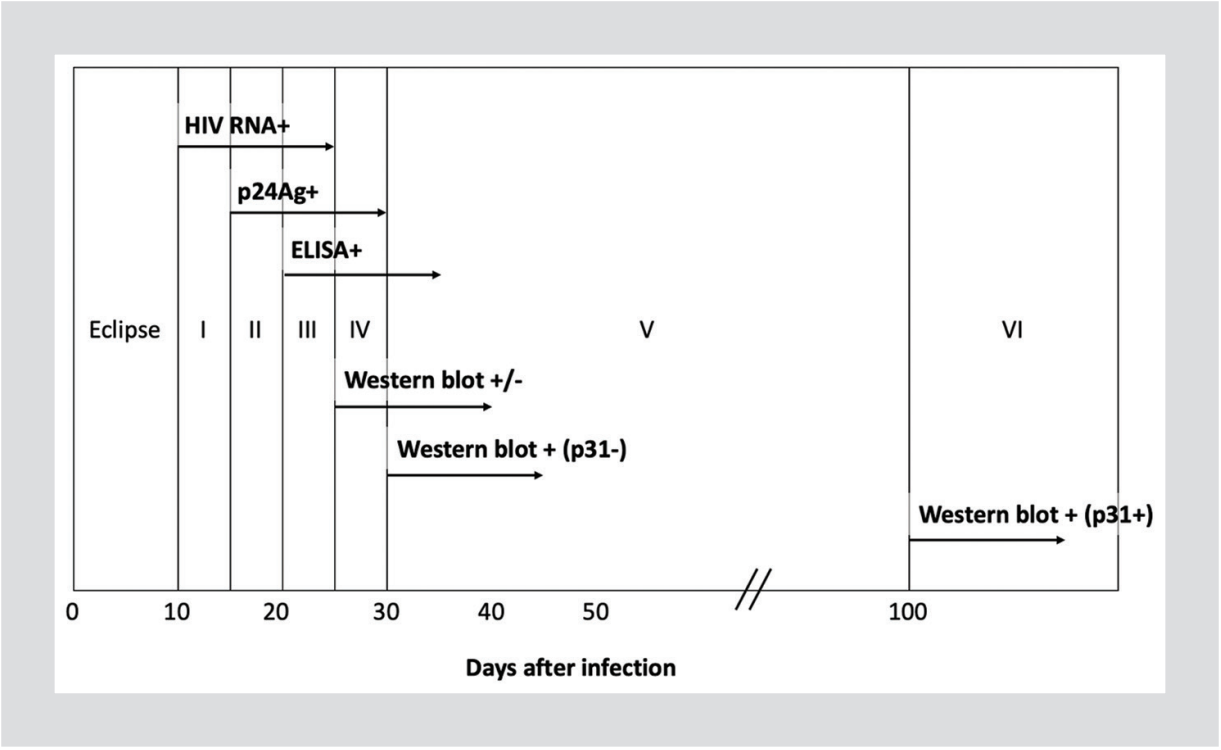

Figure 1. *Fiebig laboratory staging of early HIV infection, seroconversion occurs at Fiebig III (adapted from Fiebig et al.[10]). Acute HIV is defined either as being seronegative (Fiebig I or II) or the short duration of time (generally 1 month) after HIV acquisition. Primary or "early" HIV refers more broadly to the initial infection period, generally, the first 6 months, during which viral load stabilizes and immune responses mature.*

cure[2,3]. The latent reservoir is composed of replication-competent proviruses integrated into the host cell genome of CD4+ T cells and to a lesser extent, other cell types such as monocytes and macrophages[4]. If reactivated, these cells can produce infectious HIV virions. If ART is not present, reactivated virions propagate infection within the body causing recrudescent viremia[5]. Under suppressive ART, long-lived latently infected cells decrease slowly over time, with a half-life between 3.5 and 13 years[6,7], but do not entirely disappear[8]. Given this long observed half-life, modeling studies predict more than 70 years of ART would be required to eradicate the reservoir with ART alone[9]. Thus, it is imperative to better understand factors associated with the maintenance of the HIV reservoir such that curative interventions can be designed.

Primary HIV infection (PHI) generally refers to the first 6 months of infection, characterized initially by very high plasma viral load and activation of the immune system with ultimate development of detectable HIV antibodies and relative equilibrium between viral replication and CD4+ T cell counts (Fig. 1). These changes can be described using Fiebig staging, which estimates an average duration of infection of 17-22 days for Fiebig I/II stages (acute HIV) and 25-31 days for Fiebig III/IV[10] (Fig. 1). The HIV reservoir rapidly increases in these first few weeks of infection and then remains relatively stable[11], creating an early "set point" that may contribute to persistent long-term consequences, such as chronic inflammation and intendent non-AIDS outcomes, including increased risk of cancer and cardiovascular disease[12]. To prevent infection of target cells and limit the size of the HIV reservoir[13,14], it is thought to be beneficial to begin ART in this critical early period[15,16], in addition to the undisputed public health benefit of preventing HIV transmission by rapidly suppressing virus in plasma and body fluids[16-18].

Since 2016, the World Health Organization has recommended ART to all PLWH at any CD4 T-cell count. However, the influence of timing of ART initiation on the size of the HIV reservoir during PHI is unclear. Thus far, it has not been possible to completely circumvent the establishment of latency through use of early ART in non-human primates nor in clinical studies[19]. Although post-exposure prophylaxis (PEP), comprised a

standard three-drug ART regimen, is successful at averting infection if prescribed within 5 days of exposure and before any evidence of HIV infection, there are no known cases in which PEP or ART otherwise prescribed after documentation of HIV viremia has successfully aborted infection and prevented latency establishment. Thus, this review aims to evaluate the impact of the timing of ART in PHI on the size of the HIV reservoir.

Analytic treatment interruption (ATI) can be useful as a tool to evaluate HIV reservoir kinetics and potential efficacy of HIV remission strategies[20]. ATI involves cessation of ART while viral load and clinical status are carefully monitored, and generally done only within the context of clinical research studies. Stopping ART has many ethical and logistical challenges, including the potential to negatively impact patient safety and risk of HIV transmission while a previously suppressed participant becomes viremic again[21,22].

The goals of this systematic review were to answer the following questions: what is the effect of the timing of ART in PHI on the size of the HIV reservoir? What is the relationship between ATI and HIV reservoir size in persons treated during primary HIV?

Methods

This review followed Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. During June and July 2019, search terms "early HIV," "acute HIV," and "primary HIV" were cross-referenced with "HIV reservoir," "ART," "ART timing," and "ATI." We searched these terms in PubMed, Web of Science, and Google Scholar; manual review of citations within selected articles was also performed to improve completeness of search.

We evaluated both interventional and observational studies with clear descriptions of the intervention and sampling methods, outcomes of which included any quantitative method of evaluating the HIV reservoir and publication in English. We included studies published after 2000, containing at least 10 adult human participants, and with specified timing of ART initiation with respect to estimated HIV acquisition. We included studies with the same criteria that additionally included ATIs to answer the second question. Animal studies, case series of spontaneous virologic control, and studies not directly relevant to primary HIV or HIV reservoir were excluded (Fig. 2).

Article titles and abstracts were screened by two reviewers (EMS and RBI) to determine whether they met inclusion criteria. If the abstract was deemed potentially relevant by both reviewers, the full text was reviewed. Consensus was reached as to whether or not the article met inclusion criteria. All included articles are highlighted in table 1 (reservoir outcomes) and table 2 (ATI).

We then performed a quantitative analysis (modified meta-analysis) to assess the relationship between time to ART initiation after estimated date of infection and reservoir size. From each study, we used the aggregate reservoir outcome measurements. We transformed all measurements into approximate units of $\log_{10}$ cell-associated HIV DNA in peripheral blood mononuclear cells (PBMC), the most uniformly reported endpoint. This transformation required two assumptions: (1) in studies that only reported total HIV DNA per million $CD4^+$ T cells, we used a 1:4 ratio of $CD4^+$ T cells to PBMCs[23]; (2) if studies only reported mean values of reservoir size, we assumed normally distributed data (mean ≃ median), and calculated data spread from reported standard deviations. Using the aggregated transformed data, we quantified the correlation between time to ART initiation and reservoir size. We used $\log_{10}$ (weeks) as a predictor of $\log_{10}$ cell-associated-DNA copies (CA-DNA) per million PBMC. We performed Pearson correlations for endpoints measured at all times, as well as stratified by those measured < 1 year and > 1 year after ART initiation. This additional stratification was based on previous studies showing that reservoir clearance is multiphasic and estimates may be unstable before 1 year on ART[24].

Results and discussion

Study characteristics

Our search criteria identified 591 potentially relevant titles, with 21 articles meeting criteria for inclusion in this review (Fig. 2, PRISMA diagram).

Early ART limits size of the latent HIV reservoir

HIV reservoir seeding rapidly increases with progression of PHI and involves the peripheral blood and tissues, especially during the first 4 weeks of infection, as demonstrated by pre-ART data from the RV254/SEARCH010 cohort[25-27]. In this subset of Thais diagnosed during very early infection (n = 30), total HIV DNA was found to increase 10-fold from Fiebig I through

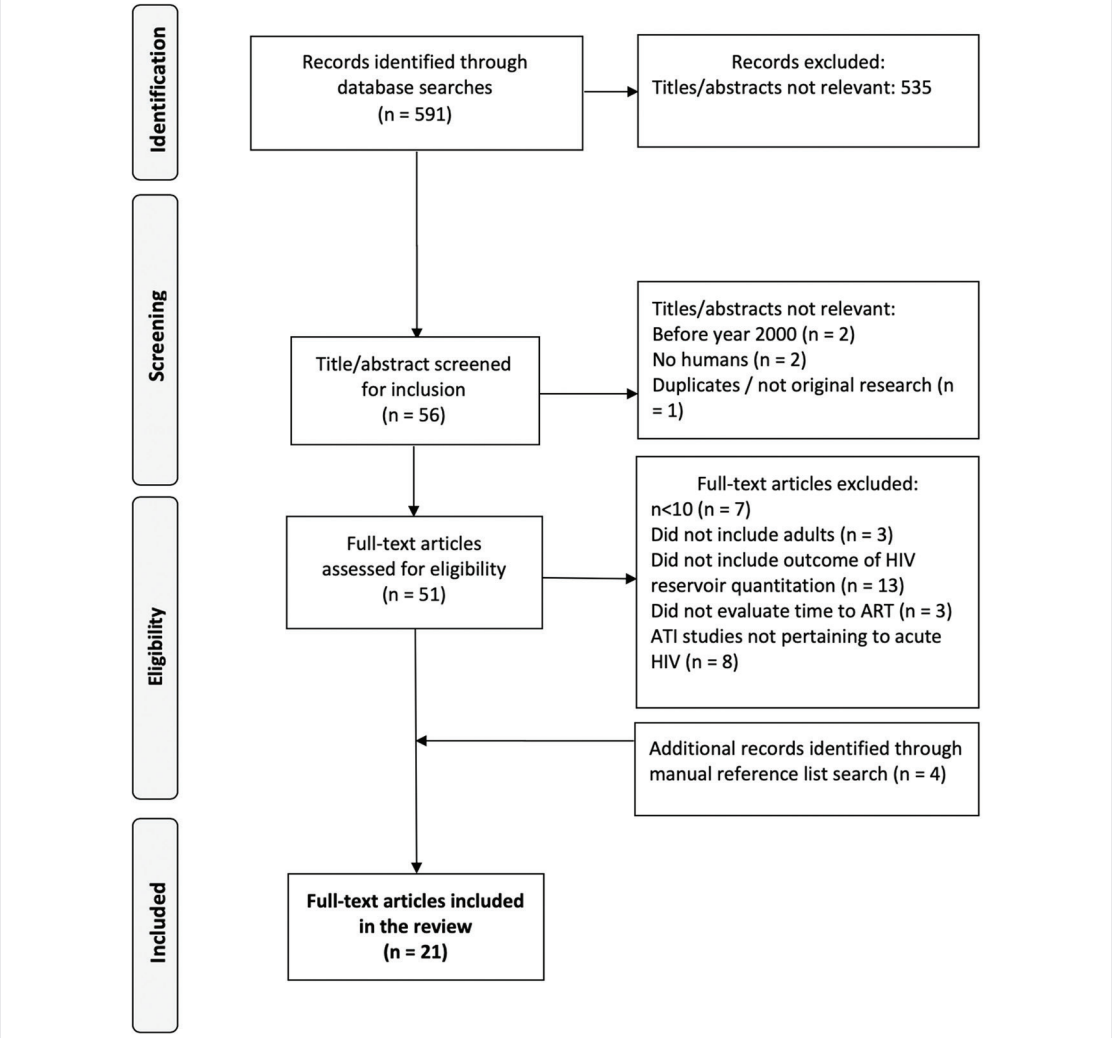

Figure 2. *Preferred reporting items for systematic reviews and meta-analyses diagram of literature search and process for selection of included manuscripts.*

Fiebig III[28]. The total HIV DNA in both CD4+ T cells and PBMCs at baseline strongly predicted reservoir size after 24 weeks of ART as well (ρ = 0.8, p = 0.0002).

Profound differences in reservoir were also found when comparing between persons who started ART in PHI with those who started in chronic infection (> 6 months after infection). Buzon et al. compared HIV DNA in PBMC from PLWH who initiated ART in Fiebig III-IV versus PLWH who initiated ART more than 2 years after diagnosis and found that the reservoir reduction was more pronounced when ART was started during PHI (log_{10} decay per 10^6 PBMC/year: acute 0.07 vs. chronic 0.01, p < 0.00001)[29]. In a similar study, Koelsch et al. conducted an open-label, non-randomized study comparing HIV DNA in PBMC from participants who began ART during Fiebig I-IV (n = 8) with those who began ART during chronic infection (n = 8). After 52 weeks of ART, the size of the reservoir was five-fold lower in PLWH who initiated ART in early infection (p = 0.003)[25,30]. In the Swiss cohort characterized by Gianella et al., persons starting ART during the first 120 days of infection had a mean level of HIV-DNA of 3.1 log_{10} copies/10^6 PBMC (95% confidence interval [CI] 2.9, 3.4) at diagnosis, which declined to a minimum of 1.9 (95% CI 1.6, 2.1) in 18 months on ART[31]. Strain et al. characterized a cohort in San Diego and Los Ange-

Table 1. Summary of studies evaluating the effect of initiating ART during primary HIV infection on the latent HIV reservoir at subsequent time points (by order cited)

Study	Study population	Reservoir endpoint(s)	Main findings (median log_{10} copies/10^6 PBMC unless otherwise noted)
Ananworanich, E Bio Medicine, 2016, RV217 (NCT00796146) and RV254 (NCT00796263)[7]	All FI-IV n = 40 megaHAART, n = 31 ART n = 19 untreated	HIV DNA in PBMC over 144 weeks of ART	Compared to untreated: 1.3 log less (20-fold) HIV DNA (1.3 log) after 2 weeks of ART and 2.5 log less (316-fold) after 144 weeks of ART
Chéret, Lancet Infect Dis, 2015, OPTIPRIM cohort (NCT01033760)[12]	n = 110, FI-IV randomized 5-drug versus 3-drug ART	HIV DNA in PMBC after 24 months ART	No additional benefit of 5-drug ART over standard (3 drugs) ART. HIV DNA 2.35 versus 2.25 log, p = 0.21
Chéret, PLoS One, 2017, ANRS 147 OPTIPRIM cohort (NCT01033760)[15]	n = 19, FI-IV randomized 5-drug versus 3-drug ART	HIV in DNA in PBMC, semen after 24 months ART	HIV DNA in PBMC fell by 1.35-fold (p < 0.0001) to 2.32 log at 24 months. Seminal HIV DNA declined similarly, by 0.31 log/ cells (p = 0.019)
Koelsch, J AIDS, 2011, PINT cohort (NCT00641641)[24]	n = 8 FI-IV versus n = 8 ART > 52 weeks	HIV DNA in PBMC after 52 weeks ART	HIV-1 DNA lower if ART in PHI versus chronic infection (2.69 vs. 3.40 log/10^6 $CD4^+$ T cells, p = 0.003)
Crowell, J AIDS, 2016, RV254/SEARCH010 (NCT00796146)[25]	n = 41, single arm FI-V	HIV DNA in PBMC, CMMC after 24 weeks ART	If detectable colonic HIV RNA at ART start, higher CMMC HIV DNA (1.2 vs. −1.0 log/10^6 CMMCs, p = 0.03) trend toward higher total HIV DNA in PBMC (1.61 vs. 0.17 log, p = 0.06)
Ananworanich, PLoS One, 2012, RV254/ SEARCH010 (NCT00796146)[27]	n = 30, single-arm FI-IV	HIV DNA in PBMC over 24 weeks megaHAART	HIV DNA higher in FIII (2.74 log, n = 15) and FII (1.98 log, n = 5) than FI (0.90 log, n = 7) at diagnosis, p = 0.01
Buzon, J Virol, 2014, PLWH in Boston, MA and Badalona, Spain[28]	n = 9 ART < 6 months versus n = 26 chronic infection > 2 years	HIV DNA in PBMC before and after > 10 years ART	HIV-1 DNA decay more pronounced if ART started in Fiebig III-IV than in chronic infection (log_{10} decay/year: acute 0.07, chronic −0.01, p < 0.00001)
Hey-Cunningham, J AIDS, 2015, PINT cohort (NCT00641641)[29]	n = 8 FI-IV versus n = 8 ART > 52 weeks	HIV DNA in PBMC after 3 years	After ART in PHI: 2.72 log/10^6 $CD4^+$ T cells versus in chronic = 3.23 log, p < 0.01
Gianella, Antivir Ther, 2011, Swiss HIV cohort (NCT00537966)[30]	n = 32, observational, single-arm ART < 120 days infection	HIV DNA in PBMC at enrollment to 18 months on ART	HIV DNA mean 3.1 log (95% CI 2.9, 3.4) at baseline, minimum 1.9 log, (1.6, 2.1) on treatment (before ATI)
Strain, J Infect Dis, 2005, MSM San Diego and Los Angeles, CA[31]	n = 27 FI-V versus n = 17 chronic	CAI (PBMC coculture with *ex vivo* stimulation) after > 1 year ART	CAI undetectable (< 0.07 IUPM) if ART start in acute infection versus detectable (1.1 IUPM) if in chronic infection
Ananworanich, J Virus Erad, 2015, RV254/ SEARCH010 (NCT 00796146) optional ART offered (NCT00796263)[32]	n = 62, ART versus megaHAART × 24 weeks, randomized	HIV DNA in PBMC and CMMC over 96 weeks	HIV DNA similar after 96 weeks in megaHAART and ART (0.84 and 0.60 log, respectively, p = 0.41)

(continues)

Table 1. Summary of studies evaluating the effect of initiating ART during primary HIV infection on the latent HIV reservoir at subsequent time points (by order cited) *(Continued)*

Hoen, Clin Infect Dis, 2007, QUEST (NCT03441906)[33]	n = 63, single-arm FI-V	HIV DNA in PBMC after 48 weeks ART	HIV-1 DNA decreased from 2.8 to 1.6 log by 48 weeks, 9% had undetectable DNA
Laanani, Clin Infect Dis, 2015, ANRS PRIMO CO6 (NCT03148964)[34]	n = 327, single-arm FI-IV	HIV DNA in PBMC at 6 months and every year on ART	Faster CA-HIV-DNA decrease in ART started at 15 days vs 3 months ($p < 0.0001$). CA-HIV-DNA after 5 years ART: 1.62 for early versus 2.24 log later ($p = 0.0006$)
Archin, Proc Natl Acad Sci, 2012, mathematical model from CHAVI study[36]	n = 27, single-arm FI-III	RCI by outgrowth assay, after 6 months ART in leukapheresis	Positive correlation ($r = 0.40$, $p = 0.04$) between RCI frequency and area under pretreatment viral load curve. Modeled RCI based on time to ART initiation ($r = 0.65$, $p = 2.5 \times 10^{[-4]}$). No further decrease in RCI below 0.5 IUPM
Leite, Front Microbiol, 2019, Brazilian MSM with acute HIV[37]	n = 10, single-arm FI-V	Single genome amplification, sequencing, PBMC, plasma > 1 year ART	HIV-1 DNA reservoir remained genetically stable, suggesting replenishment of reservoir without ongoing replication

ART: antiretroviral therapy, referring to standard 3-drug ART unless otherwise specified; PBMC: peripheral blood mononuclear cells; CMMC: colonic mucosal mononuclear cells; CA-HIV-DNA: cell-associated HIV DNA; PHI: primary HIV infection; RCI: resting cell infection frequency; IUPM: infectious units per million; MegaHAART: two additional drug classes in addition to standard three-drug ART.

les using co-culture assays to evaluate the replication-competent reservoir (n = 27). In PLWH who initiated treatment within 6 months of seroconversion, replication-competent virus was not recoverable after 1 year of ART; in contrast, virus remained recoverable after 6 years of ART in PLWH who initiated treatment during chronic infection[32]. Thus, it is clear that treatment within the first few months of infection limits the size of the HIV reservoir over longer term.

The virologic benefits of early ART were confirmed in several other studies indicating a markedly smaller reservoir size in PLWH who began treatment earlier in PHI compared to those who begin later. Several studies suggest benefits of ART to be maximal when initiated within 2 weeks of infection. The RV254/SEARCH010 cohort and the RV217 cohort found that initiation of ART during Fiebig I-IV reduced the reservoir in PLWH by 20-fold after 2 weeks of treatment and by 316-fold after 144 weeks (n = 90)[8]. This difference did not differ between PLWH who were provided standard three-drug ART versus "megaHAART," which included two additional drug classes to complete a five-drug regimen[33]; 3 of 15 persons with samples available after "megaHAART" had undetectable HIV DNA in PBMC at 24 weeks. These Thai studies were not able to provide comparisons with persons who initiated ART in chronic infection or comparative outcomes by Fiebig stage at enrollment. The QUEST cohort (n = 148) found that ART initiated before Fiebig IV decreased PBMC associated DNA levels by 94% after 48 weeks of treatment[34]. Finally, the ANRS PRIMO CO6 cohort (n = 327) concluded that after 5 years of ART, the reservoir size was 25 times smaller in those who started ART 15 days after estimated date of infection compared to those who started ART approximately 3 months after infection ($p = 0.0006$)[35]. These findings suggest that ART best prevents expansion of the HIV reservoir when initiated within 2 weeks of estimated acquisition and that this difference persists after a long duration of virologic suppression.

Our meta-analysis showed a moderately positive correlation toward between reservoir size with longer interval between HIV infection and ART initiation ($r = 0.36$, $p = 0.045$; Fig. 3A) when all aggregate endpoints were considered. When limited to endpoints measured after 1 year of ART, the positive linear relationship was much stronger ($r = 0.69$, $p = 0.0003$; Fig. 3C) and there was little correlation with reservoir size for time points measured before or early after ART initiation (Fig. 3B). The lack consistent outcomes between early endpoints cor-

Table 2. Relationship between ART initiation during primary infection, viral rebound, and latent reservoir

Study	Study population	Reservoir endpoint(s)	Main findings (median log_{10} copies/10^6 PBMC unless otherwise noted)
Gianella, Antivir Ther, 2011, Swiss HIV cohort (NCT00537966)[30]	n = 32, observational, single-arm < 120 days infection, 18 months ART then ATI	HIV DNA in PBMC during ATI	Mean ATI HIV DNA: 2.3 versus 2.7 log, p = 0.03 (ART initiation within 60 vs. 61-120 days). Max ATI HIV DNA, 2.7 versus 3.0 log, p = 0.18. 9% of early starters maintained undetectable plasma RNA for 0.8-2.5 years follow-up
Li, J AIDS, 2016, ACTG studies (A371, A5024, A5068, A5170, A5187, A5197[45]	n = 235, ART in PHI and chronic, 16 weeks ATI	HIV DNA in PBMC over 16 weeks ATI	No association between HIV DNA levels and timing of viral rebound. Higher pre-ATI RNA levels significantly associated with shorter time to viral rebound
Williams, eLife, 2014, SPARTAC trial[46]	n = 154 total (ART and no ART, randomized) n = 47 with ATI	HIV DNA in PBMC at 4, 12, and 60 weeks ATI	HIV-1 DNA above median (0.63 log) at ATI start predicted time to RNA rebound to > 400 copies/ml (high vs. low DNA: HR 2.43 (1.23-4.79), p = 0.01)
Goujard, Antivir Ther, 2012, French ANRS PRIMO Cohort (CO6)(NCT03148964)[47]	n = 164, single-arm < 120 days infection versus > 1 year ATI	HIV DNA in PBMC after 1 year ATI	Smaller reservoir after 1-year ATI (3.1 log) for low versus high pre-ATI HIV DNA levels (3.4 log; p = 0.09)
Lewin, J Acquir Immune Defic Syndr, 2008, PULSE cohort[48]	n = 19, individuals with acute HIV	HIV DNA in PBMC during ATI	PBMC associated DNA levels not significantly different for individuals who did or did not develop viral rebound
von Wyl, PLoS One, 2011, Zurich Primary HIV Infection Study (NCD00537966), Swiss HIV cohort (NCT00537966)[49]	n = 33, on ART during PHI versus untreated chronic HIV	HIV DNA in PBMC after 1 year ATI	HIV-DNA levels rebounded from 2.0 log on ART to 2.7 log after 1 year off ART, not significantly different from untreated controls (2.8 log)
Sáez-Cirión, PLoS, 2013, VISCONTI cohort, French ANRS PRIMO Cohort (CO6) (NCT03148964)[50]	n = 14, acutely infected individuals in VISCONTI cohort	HIV DNA in PBMC during ATI	Lower HIV DNA levels during ATI for ART start during PHI vs chronic infection

ATI: analytic treatment interruption; ART: antiretroviral therapy, referring to standard 3-drug ART unless otherwise specified; PBMC: peripheral blood mononuclear cells; CA-HIV-DNA: cell-associated HIV DNA; PHI: primary HIV infection; MegaHAART: two additional drug classes in addition to standard three-drug ART.

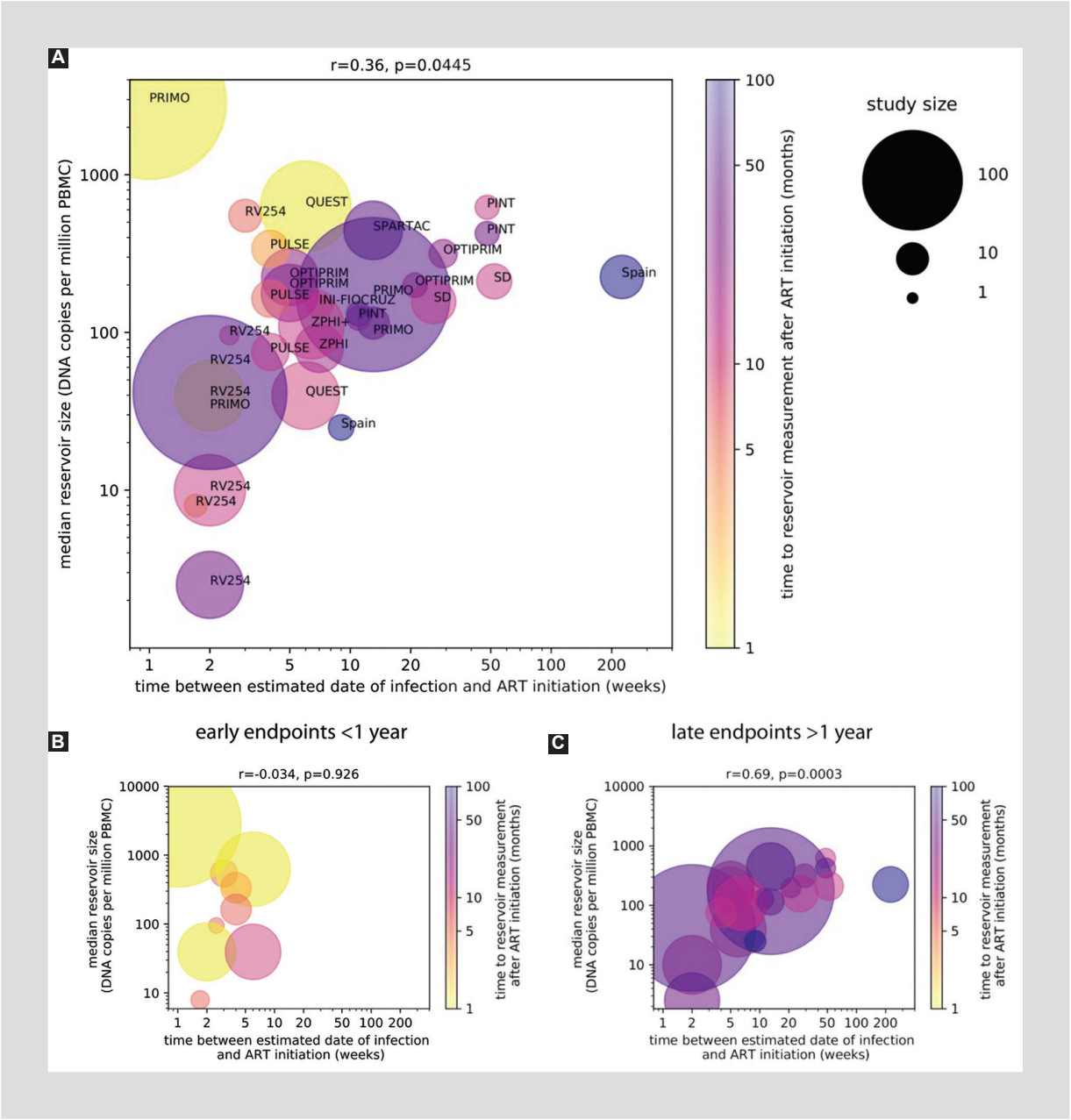

Figure 3. **A:** *Reservoir size in all studies (y-axis) plotted according to time to antiretroviral therapy initiation on x-axis and time to reservoir measurement (by color) are moderately positively correlated, r = 0.36, p = 0.045.* **B:** *Studies that measured reservoir size before 1 year are not significantly correlated.* **C:** *Studies that measured reservoir size after 1 year are strongly correlated r = 0.69, p = 0.0003.*

respond to experience that latent reservoir clearance is multiphasic so that measurements may be highly dynamic until at least 6 months after virologic suppression, or about 1 year on ART (N. Chomont, personal communication and Blankson et al., Reeves et al.[24,36]).

Data from these studies and others have shown that decay of the reservoir is extremely slow after 1 year of ART, even when initiated in PHI. Archin et al. used a mathematical model derived from measurements of HIV latency in the San Diego cohort who initiated ART during Fiebig I-III (n = 27). This model was able to predict frequency of resting cell infection (RCI) from time to ART initiation and also demonstrated minimal decrease in the frequency of RCI beyond 0.5/million after the 1st year of ART, demonstrating the persistence of the latent reservoir following initial decay[37]. Leite et al. used single genome amplification and next-generation sequencing in PBMC and plasma

from a cohort of Brazilian participants with acute HIV infection (n = 10) to reach similar conclusions. The genetic stability of the reservoir after 1 year of suppressive ART suggests that the remaining reservoir is maintained through T-cell survival and homeostatic cell proliferation rather than ongoing viral replication[38,39]. Thus, early ART limits ongoing seeding of susceptible cells, but once infected, these cells may persist and proliferate, maintaining potentially intact virus even in the absence of *de novo* infection events[36,40-45].

Larger HIV reservoir at time of ATI may predict shorter time to viral rebound

Viral rebound occurs in the majority of PLWH following ATI, often by the 4th week[46]. However, the impact of the size of the HIV reservoir on time to viral rebound remains unclear. Williams et al. studied participants in the SPARTAC trial who were randomized to receive no therapy or 48 weeks of ART (n = 154) starting in primary infection. The level of total HIV DNA at ART cessation was predictive of the timing to viral rebound in ATI (hazard ratio 2.43 for above vs. below the mean DNA level, 95% CI 1.23-4.79; p = 0.01) in those who were treated[47]. Similarly, the ANRS PRIMO (CO6) cohort (n = 164) found that PLWH whose pre-ATI HIV DNA was lower tended toward lower levels of plasma viremia after 1 year of ATI compared to persons with high pre-ATI DNA levels (p = 0.09)[48].

Li et al. studied participants who started ART during either primary or chronic infection from six ACTG clinical trials, all of whom had undetectable plasma viremia and were observed over 16 weeks of ATI after having receiving ART for at least 52 weeks (n = 235). Reservoir analyses from both acutely and chronically infected individuals found no association between magnitude of HIV DNA before ATI and time to viral rebound[46]. However, having started ART during PHI and having lower levels of cell-associated RNA were both associated with longer time to viral rebound[46]. The PULSE study found associations between integrated HIV DNA at ART initiation, plasma RNA level 12 weeks after ART initiation, and response to ATI, but not with total or integrated DNA or ultrasensitive plasma RNA at beginning of ATI[49]. Other studies have also found strong correlations between pre-ATI HIV RNA levels and time to viral rebound[46,49]. Further studies are needed to evaluate predictors of sustained virologic remission.

ART during PHI may limit HIV reservoir expansion during ATI

Gianella et al. quantified HIV DNA in PBMC from the Swiss HIV cohort (n = 32) during an ATI begun after a median of 18 months of ART and found that participants who started ART within 60 days of infection had 60% smaller mean reservoirs during the ATI than those who started between 61 and 120 days (p = 0.03)[31]. Sáez-Cirión et al. compared the HIV reservoir size in persons who started ART during PHI in the Zurich Primary HIV study to persons with untreated chronic infection from the Swiss HIV cohort and reported that, after 1 year of ATI, the size of the HIV reservoir in acutely treated individuals was not significantly different from that of individuals with chronic HIV who had never received ART[50]. These results suggest that impact of early ART on the size of the HIV reservoir could be lost if sustained viremia recurs over long durations of ATI. In addition, in a small subset of people from the Zurich Primary HIV and VISCONTI cohorts who started treatment during PHI, viral loads remained undetectable years after ATI; these participants had smaller pre-ART reservoirs than those who started ART in chronic infection[48,51]. These results suggest that ART started during early infection may also assist with immunologic control, which could contribute to functional HIV remission, although this phenomenon does not occur in most persons who initiate ART during primary infection. Factors associated with post-treatment virologic control are not well understood.

Future research

It is clear that there are personal and public health benefits of initiating ART during primary infection[15]. Findings from studies reviewed here suggest that starting ART within an estimated 2 weeks after infection confers the greatest benefit in limiting the extent of the HIV reservoir[35]. In addition to limiting HIV reservoir establishment, early ART also prevents adverse clinical outcomes, although randomized trials that addressed this question generally define “early” as having a preserved CD4 count and not during primary or incident HIV infection specifically. The INSIGHT START (n = 4685) and TEMPRANO ANRS trials (n = 2056) evaluated the effect of immediate versus delayed ART on adverse clinical events, including cancer, cardiovascular disease, renal disease, and death. Twice as many participants who initiated ART with CD4 < 350

cells/m^3 developed these outcomes than those who initiated ART with CD4 > 500 in INSIGHT START and immediate ART reduced the risk of death from any cause by 44% in TEMPRANO, including for participants with CD4 > 500[52,53]. Clinical and immunologic endpoints of the *Sabes* study, which randomized timing of ART initiation during acute and early infection have been described[54,55] and reservoir endpoints are forthcoming.

Limiting the size of the HIV reservoir through early ART seems to be only part of the solution to achieve sustained HIV remission. Takata et al. showed that potent CD8+ T cells, especially those generated before Fiebig III, contribute to the reduction of the HIV reservoir[56]. It is unclear whether providing non-specific or HIV-specific antibodies during acute infection provides additional immunologic control above and beyond ART. For example, Tiraboschi et al. added intravenous immunoglobulin to ART, which unfortunately did not add virologic benefit above ART alone[57]. Studies administering broadly neutralizing monoclonal antibodies during suppressive ART[58,59] may delay time to rebound but the precise mechanisms remain incompletely understood[60,61].

Finally, the relationship between the size of the HIV reservoir and the duration that controlled viremia can be maintained is not well established. Current studies suggest that larger HIV reservoirs are generally predictive of a shorter time to viral rebound and that the reservoir expands during ATI. The RV411 substudy (NCT02614950) within the RV254/SEARCH010 (NCT00796146) study included eight participants who initiated ART during Fiebig I; albeit not meeting our inclusion criteria due to small size, the results are intriguing. Analysis of plasma viral load and CA-DNA in the RV411 cohort (median 4 [4-12] HIV-1 DNA/10^6 PBMC cells at ATI start, 68 [24-240] HIV-1 DNA copies/10^6 PBMC cells after 24 weeks of ATI)[62] was similar to those of the ANRS PRIMO CO6 cohort (median 116 HIV-1 DNA copies/10^6 PBMC cells at ATI start, 39 HIV-1 DNA copies/10^6 cells after 6 years of ATI)[51], and reservoirs in the RV411 cohort returned as similarly low as pre-ATI levels after ART had been resumed for 6 months. Criteria to resume ART included confirmed viral load > 1000 copies/ml, similar to other ATI studies reviewed[62]. These studies in aggregate demonstrate safety of short duration of low-level viremia, notwithstanding transmission risks. Further studies should continue to investigate predictors of viral rebound which immunologic factors contribute to sustaining remission.

Conclusions

Approximately 5 years have elapsed since the last available review of this topic in the literature in 2015, which included 45 articles published before 2014[63]. Our review included eight new studies published since, including an additional six studies of ATI after treatment in primary HIV. Our goal in the current systematic review and analysis was to obtain a more complete picture of the effect of timing of ART initiation on HIV reservoir. An important distinction from previous analysis was the focus on the how the extent of the reservoir expansion is affected by precise timing of ART after infection, which was augmented by analysis of aggregate data from these published studies. While a clear pattern emerged across studies, it is important to note that heterogeneity in study design, sampling, and HIV reservoir quantification methods makes direct inference between individual studies challenging.

Based on current research, we conclude that ART initiated in PHI reduces HIV reservoir size maximally when initiated before 2 weeks after infection. Smaller pre-ATI reservoir sizes may predict longer time to viral rebound. However, reaching sustained HIV remission will require further interventions to eliminate the replication-competent HIV reservoir, as most persons who undergo treatment interruption after initiation of ART during primary infection still experience viral rebound. Nevertheless, initiating ART very early in infection is one first step toward HIV cure by limiting the HIV reservoir expansion, which could make future reservoir reduction interventions with smaller magnitude of effect more feasible. Whether a smaller HIV reservoir also translates to decreased chronic immune activation and less exhaustion has yet to be fully understood[7,64]. The study of future HIV remission strategies could take advantage of cohorts of PLWH treated since primary infection.

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References

1. Global HIV and AIDS Statistics-2019 Fact Sheet; 2019. Available from: http://www.unaids.org/en/resources/fact-sheet.
2. CDC. Statistics Overview, Statistics Center HIV/AIDS. United States: CDC; 2019.
3. Wong M, Jaworowski A, Hearps AC. The HIV reservoir in monocytes and macrophages. Front Immunol. 2019;10:1435.
4. Medicine NUSNLo. What is a Latent HIV Reservoir? Understanding HIV/AIDS. Irvine, CA: Medicine NUSNLo; 2019.

5. Pierson T, McArthur J, Siliciano RF. Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. Annu Rev Immunol. 2000;18:665-708.
6. Gandhi RT, McMahon DK, Bosch RJ, Lalama CM, Cyktor JC, Macatangay BJ, et al. Levels of HIV-1 persistence on antiretroviral therapy are not associated with markers of inflammation or activation. PLoS Pathog. 2017;13:e1006285.
7. Ananworanich J, Chomont N, Eller LA, Kroon E, Tovanabutra S, Bose M, et al. HIV DNA set point is rapidly established in acute HIV infection and dramatically reduced by early ART. EBio Medicine. 2016;11:68-72.
8. Marsden MD, Zack JA. HIV/AIDS eradication. Bioorg Med Chem Lett. 2013;23:4003-10.
9. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS. 2003;17:1871-9.
10. Brian R. Wood DH. Acute and Recent HIV Infection-screening and Diagnosis-national HIV Curriculum; 2019. Available from: https://www.hiv.uw.edu/go/screening-diagnosis/acute-recent-early-hiv/core-concept/all, 2019.
11. Lee SA, Deeks SG. The benefits of early antiretroviral therapy for HIV infection: how early is early enough? EBioMedicine. 2016;11:7-8.
12. Chéret A, Bacchus-Souffan C, Avettand-Fenoël V, Mélard A, Nembot G, Blanc C. Combined ART started during acute HIV infection protects central memory CD4+ T cells and can induce remission. J Antimicrob Chemother. 2019;70:2108-20.
13. Jain V, Hartogensis W, Bacchetti P, Hunt PW, Hatano H, Sinclair E, et al. Antiretroviral therapy initiated within 6 months of HIV infection is associated with lower T-cell activation and smaller HIV reservoir size. J Infect Dis. 2013;208:1202-11.
14. Eisinger RW, Dieffenbach CW, Fauci AS. HIV viral load and transmissibility of HIV infection: Undetectable equals untransmittable. JAMA. 2019;321:451-2.
15. Chéret A, Durier C, Mélard A, Ploquin M, Heitzmann J, Lécuroux C, et al. Impact of early cART on HIV blood and semen compartments at the time of primary infection. PLoS One. 2017;12:e0180191.
16. World Health Organization. Guideline on when to Start Antiretroviral Therapy and on Pre-exposure Prophylaxis for HIV. Geneva, Switzerland: World Health Organization; 2018.
17. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med. 2011;365:493-505.
18. Katlama C, Deeks SG, Autran B, Martinez-Picado J, van Lunzen J, Rouzioux C, et al. Barriers to a cure: new concepts in targeting and eradicating HIV-1 reservoirs. Lancet. 2013;381:2109-17.
19. Garner SA, Rennie S, Ananworanich J, Dube K, Margolis DM, Sugarman J, et al. Interrupting antiretroviral treatment in HIV cure research: scientific and ethical considerations. J Virus Erad. 2017;3:82-4.
20. Montserrat M, Plana M, Guardo AC, Andrés C, Climent N, Gallart T, et al. Impact of long-term antiretroviral therapy interruption and resumption on viral reservoir in HIV-1 infected patients. AIDS. 2017;31:1895-7.
21. Ugarte A, Romero Y, Tricas A, Casado C, Lopez-Galindez C, Garcia F, et al. Unintended HIV-1 infection during analytical therapy interruption. J Infect Dis. 2019;221:1740-2.
22. Murphy K, Weaver C. Janeway's Immunobiology. United States: W.W. Norton & Company; 2020.
23. Blankson JN, Finzi D, Pierson TC, Sabundayo BP, Chadwick K, Margolick JB, et al. Biphasic decay of latently infected CD4+ T cells in acute human immunodeficiency virus Type 1 infection. J Infect Dis. 2000;182:1636-42.
24. Koelsch K, Boesecke C, McBride K, Gelgor L, Fahey P, Natarajan V, et al. Impact of treatment with raltegravir during primary or chronic HIV infection on RNA decay characteristics and the HIV viral reservoir. AIDS. 2019;25:2069-78.
25. Crowell TA. Initiation of antiretroviral therapy before detection of colonic infiltration by HIV reduces viral reservoirs, inflammation and immune activation. J Int AIDS Soc. 2016;19:21163.
26. Crowell TA, Colby DJ, Pinyakorn S, Fletcher JL, Kroon E, Schuetz A, et al. Acute retroviral syndrome is associated with high viral burden, CD4 depletion, and immune activation in systemic and tissue compartments. Clin Infect Dis. 2018;66:1540-9.
27. Ananworanich J, Schuetz A, Vandergeeten C, Sereti I, de Souza M, Rerknimitr R, et al. Impact of multi-targeted antiretroviral treatment on gut T cell depletion and HIV reservoir seeding during acute HIV infection. PLoS One. 2012;7:e33948.
28. Buzon MJ, Martin-Gayo E, Pereyra F, Ouyang Z, Sun H, Li JZ, et al. 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. 2014;88:10056-65.
29. Hey-Cunningham WJ, Murray JM, Natarajan V, Amin J, Moore CL, Emery S, et al. Early antiretroviral therapy with raltegravir generates sustained reductions in HIV reservoirs but not lower T-cell activation levels. AIDS. 2015;29:911-9.
30. Gianella S, Fischer M, Niederoest B, Battegay M, Bernasconi E, Cavassini M, et al. Effect of early antiretroviral therapy during primary HIV-1 infection on cell-associated HIV-1 DNA and plasma HIV-1 RNA. Antivir Ther. 2011;16:535-45.
31. Strain MC, Little SJ, Daar ES, Havlir DV, Gunthard HF, Lam RY, et al. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. J Infect Dis. 2019;191:1410-8.
32. Ananworanich J, Chomont N, Fletcher JL, Pinyakorn S, Schuetz A, Sereti I, et al. Markers of HIV reservoir size and immune activation after treatment in acute HIV infection with and without raltegravir and maraviroc intensification. J Virus Erad. 2015;1:116-22.
33. Hoen B, Cooper DA, Lampe FC, Perrin L, Clumeck N, Phillips AN, et al. Predictors of virological outcome and safety in primary HIV Type 1-infected patients initiating quadruple antiretroviral therapy: QUEST GW PROB3005. Clin Infect Dis. 2007;45:381-90.
34. Laanani M, Ghosn J, Essat A, Melard A, Seng R, Gousset M, et al. Impact of the timing of initiation of antiretroviral therapy during primary HIV-1 infection on the decay of cell-associated HIV-DNA. Clin Infect Dis. 2019;60:1715-21.
35. Reeves DB, Duke ER, Wagner TA, Palmer SE, Spivak AM, Schiffer JT. A majority of HIV persistence during antiretroviral therapy is due to infected cell proliferation. Nat Commun. 2018;9:4811.
36. Archin NM, Vaidya NK, Kuruc JD, Liberty AL, Wiegand A, Kearney MF, et al. From the cover: 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. 2012;109:9523-8.
37. Leite TF, Delatorre E, Côrtes FH, Ferreira AC, Cardoso SW, Grinsztejn B, et al. Reduction of HIV-1 reservoir size and diversity after 1 year of cART among Brazilian individuals starting treatment during early stages of acute infection. Front Microbiol. 2019;10:145.
38. Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat Med. 2009;15:893-900.
39. Reeves DB, Duke ER, Hughes SM, Prlic M, Hladik F, Schiffer JT. Antiproliferative therapy for HIV cure: a compound interest approach. Sci Rep. 2017;7:4011.
40. Wagner TA, McLaughlin S, Garg K, Cheung CY, Larsen BB, Styrchak S, et al. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. Science. 2014;345:570-3.
41. Maldarelli F, Wu X, Su L, Simonetti FR, Shao W, Hill S, et al. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science. 2014;345:179-83.
42. von Stockenstrom S, Odevall L, Lee E, Sinclair E, Bacchetti P, Killian M, et al. Longitudinal genetic characterization reveals that cell proliferation maintains a persistent HIV Type 1 DNA pool during effective HIV therapy. J Infect Dis. 2015;212:596-607.
43. Kearney MF, Spindler J, Shao W, Yu S, Anderson EM, O'Shea A, et al. Lack of detectable HIV-1 molecular evolution during suppressive antiretroviral therapy. PLoS Pathog. 2014;10:e1004010.
44. Cohn LB, Silva IT, Oliveira TY, Rosales RA, Parrish EH, Learn GH, et al. HIV-1 integration landscape during latent and active infection. Cell. 2015;160:420-32.
45. Li JZ, Etemad B, Ahmed H, Aga E, Bosch RJ, Mellors JW, et al. The size of the expressed HIV reservoir predicts timing of viral rebound after treatment interruption. AIDS. 2016;30:343-53.
46. Williams JP, Hurst J, Stöhr W, Robinson N, Brown H, Fisher M, et al. HIV-1 DNA predicts disease progression and post-treatment virological control. Elife. 2014;3:e03821.
47. Goujard C, Girault I, Rouzioux C, Lécuroux C, Deveau C, Chaix ML, et al. HIV-1 control after transient antiretroviral treatment initiated in primary infection: role of patient characteristics and effect of therapy. Antivir Ther. 2012;17:1001-9.
48. Lewin S, Murray J, Solomon A, Wightman F, Cameron PU, Purcell J, et al. Virologic determinants of success after structured treatment interruptions of antiretrovirals in acute HIV-1 infection. J Acquir Immune Defic Syndr. 2019;47:140-7.
49. von Wyl V, Gianella S, Fischer M, Niederoest B, Kuster H, Battegay M, et al. Early antiretroviral therapy during primary HIV-1 infection results in a transient reduction of the viral setpoint upon treatment interruption. PLoS One. 2011;6:e27463.
50. Sáez-Cirión A, Bacchus C, Hocqueloux L, Avettand-Fenoel V, Girault I, Lecuroux C, et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI study. PLoS Pathog. 2013;9:e1003211.
51. The Insight Start Study Group. Initiation of antiretroviral therapy in early asymptomatic HIV infection. N Engl J Med. 2015;373:795-807.
52. Temprano Anrs Group. A trial of early antiretrovirals and isoniazid preventive therapy in Africa. N Engl J Med. 2015;373:808-22.
53. Lama JR, Brezak A, Dobbins JG, Sanchez H, Cabello R, Rios J, et al. Design strategy of the sabes study: diagnosis and treatment of early HIV infection among men who have sex with men and transgender women in Lima, Peru, 2013-2017. Am J Epidemiol. 2018;187:1577-85.
54. Lama JR, Bender Ignacio RA, Alfaro R, Rios J, Cartagena JG, Valdez R, Bain C, et al. Clinical and Immunologic Outcomes After Immediate or

Deferred Antiretroviral Therapy Initiation During Primary Human Immunodeficiency Virus Infection: The Sabes Randomized Clinical Study. Clin Infect Diseases. 2020;Feb 28 [Epub ahead of print].
55. Takata H, Buranapraditkun S, Kessing C, Fletcher JL, Muir R, Tardif V, et al. Delayed differentiation of potent effector CD8 T cells reducing viremia and reservoir seeding in acute HIV infection. Sci Transl Med. 2017;9:eaag1809.
56. Tiraboschi J, Ray S, Patel K, Teague A, Pace M, Phalora P, et al. The impact of immunoglobulin in acute HIV infection on the HIV reservoir: a randomized controlled trial. HIV Med. 2017;18:777-81.
57. Lynch RM, Boritz E, Coates EE, DeZure A, Madden P, Costner P, et al. Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection. Sci Transl Med. 2015;7:319ra206.
58. Bree GJ, Sanders RW. Broadly neutralising antibodies in post-treatment control. The Lancet HIV. 2019;6:E271-2.
59. Mendoza P, Gruell H, Nogueira L, Pai JA, Butler AL, Millard K, et al. Combination therapy with anti-HIV-1 antibodies maintains viral suppression. Nature. 2018;561:479-84.
60. Crowell TA, Colby DJ, Pinyakorn S, Sacdalan C, Pagliuzza A, Intasan J, et al. Safety and efficacy of VRC01 broadly neutralising antibodies in adults with acutely treated HIV (RV397): a phase 2, randomised, double-blind, placebo-controlled trial. Lancet HIV. 2019;6:e297-306.
61. Colby D, Trautmann L, Pinyakorn S, Leyre L, Pagliuzza A, Kroon E, et al. Rapid HIV RNA rebound after antiretroviral treatment interruption in persons durably suppressed in Fiebig I acute HIV infection. Nat Med. 2018;24:923-6.
62. Ananworanich J, Dubé K, Chomont N. How does the timing of antiretroviral therapy initiation in acute infection affect HIV reservoirs? Curr Opin HIV AIDS. 2015;10:18.
63. Garcia-Broncano P, Maddali S, Einkauf KB, Jiang C, Gao C, Chevalier J, et al. Early antiretroviral therapy in neonates with HIV-1 infection restricts viral reservoir size and induces a distinct innate immune profile. Sci Transl Med. 2019;11:eaax7350.
64. Massanella M, Fromentin R, Chomont N. Residual inflammation and viral reservoirs: alliance against an HIV cure. Curr Opin HIV AIDS. 2016;11:234.