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Animal models to achieve an HIV cure

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Abstract

Purpose—The introduction of effective antiretroviral therapy (ART) has transformed HIV infection from a deadly to a chronic infection. Despite its successes in reducing mortality, ART fails to cure HIV allowing HIV persists *in vivo*. HIV persistence under ART is thought to be mediated by a combination of latent infection of long-lived cells, homeostatic proliferation of latently infected cells, anatomic sanctuaries and low-level virus replication. To understand the contribution of specific cell types and anatomic sites to virus persistence *in vivo* animal models are necessary.

Recent findings—The advancements in ART and our understanding of animal models have facilitated the development of models of HIV persistence in nonhuman primates and mice. SIV or SHIV infection of rhesus and pigtail macaques followed by effective ART represents the most faithful animal model of HIV persistence. HIV infection of humanized mice also provides a useful model for answering specific questions regarding virus persistence in a uniquely mutable system.

Summary—In this review we describe the most recent findings using animals models of HIV persistence. We will first describe the important aspects of HIV infection that SIV/SHIV infection of NHP are able to recapitulate, then we will discuss some recent studies that have used these models to understand viral persistence.

Keywords

SIV; latency; HIV; Animal models; eradication strategies

Introduction

Antiretroviral therapy (ART) has transformed HIV into a life-long chronic infection. However, ART has failed to provide a cure for HIV as virus replication rapidly rebounds if ART is interrupted. Additionally, life-long ART does not fully reverse the immunological abnormalities associated with HIV infection, resulting in drug toxicities, other side effects, and creates a massive financial burden for health care systems worldwide. The major barrier to HIV cure is the persistence of latently infected long-lived $CD4^+$ memory T-cells, where latent infection is defined as the presence of stably integrated HIV DNA in absence of active viral production. The study of HIV persistence is limited due to the rarity of latent cells *in vivo* that harbor replication competent virus, estimated to be ~1 per million memory $CD4^+$

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T-cells, and difficulty in accurately modeling HIV latency and persistence *in vitro*. In addition, HIV persistence is a very complex virological and immunological phenomenon, with infection of several cell types in a wide array of anatomic tissues that are all regulated differently, including various subsets of $CD4^+$ T-cells, such as central memory T-cells (T_{CM}), follicular helper T-cells (T_{FH}), memory stem T-cells (T_{SCM}), and regulatory T-cells (T_{REG}) in blood and lymphoid tissues, effector memory T-cells (T_{EM}) and T helper-17 cells (T_{H17}) in the gut, microglial cells and astrocytes in the central nervous system and, more controversially, macrophages and dendritic cells (1–5). Therefore a cure to HIV persistence will likely require a combination of several virus elimination strategies, similar to combination ART or poly-chemotherapy for cancer, in order to eliminate the different components of the virus reservoir in different tissues and cells. Nonhuman primate (NHP) models using Indian rhesus macaques(RM, *Macaca mulatta*) or pigtail macaques (PTM, *Macaca nemestrina*) experimentally infected with simian immunodeficiency virus (SIV) or simian/human immunodeficiency virus (SHIV) and treated with ART have similar pathology and disease progression compared with ART-treated HIV infection in humans (6–8). The study of HIV/AIDS with NHP models has been instrumental in our current understanding of HIV pathogenesis, previously reviewed by Evans and Silvestri (9). In this review we will summarize the recent progress in the use of animal models to understand HIV persistence and test potential cure strategies.

Models of Nonhuman Primates for HIV infection

Historically, a key limitation of the SIV/RM model to study virus reservoirs has been the lack of optimized ART regimens that fully suppress virus replication. Today, newer ART combinations and formulations can be used in RM and PTM that are extremely effective in reducing plasma viral loads to levels equivalent to what is observed in HIV-1-infected patients on ART, thus providing an excellent *in vivo* model of HIV persistence (10–15). As summarized in Table 1, there are many benefits to using NHP models in the study of HIV persistence, including the fact that they allow us to extensively sample tissues that are not readily accessible in humans, to study rare cell populations in tissues and blood, such as T_{SCM}, T_{FH}, and T_{FR} (5,16–18), and to deplete or block specifying immune functions, including $CD8^+$ and $CD4^+$ lymphocyte depletion, manipulation of interferons or cytokines, and blockade of co-inhibitory pathways such as PD-1 (6–8,11,15,17,19–32).

Most studies of HIV cure using NHP models follow a similar protocol, detailed in Figure 1. Either RM or PTM are infected via different routes, usually intravenous or intrarectal, with macaque-adapted viruses such as SIVmac239, SIVmac251 or chimeric simian/human immunodeficiency viruses (SHIV) that are designed to include the HIV RT gene (to improve the susceptibility to certain reverse transcriptase inhibitors) or the HIV-Env gene (for studies of the anti-reservoir effects of HIV-specific broadly neutralizing antibodies). These viruses are typically used at high concentrations to induce robust levels of infection with a single dose challenge. After establishment of infection, ART is administered orally or subcutaneously, with studies reporting ART initiation anywhere from day 3 to day 60 post-infection (or later). The choice of the time of ART initiation should reflect that in HIV-infected humans delayed treatment initiation leads to a greater size of the HIV DNA reservoir (33). ART formulations used in NHP have included two nucleoside reverse

transcriptase inhibitors (typically tenofovir [PMPA] and emtricitabine [FTC]), a protease inhibitor (often Darunavir) and an integrase inhibitor (Raltegravir or Dolutegravir) with possible intensification with a CCR5 inhibitor (Maraviroc) or a non-nucleoside reverse transcriptase inhibitor in the case of RT-SHIV infection. While ART formulations differ slightly in various published studies, all ART formulations are highly effective in reducing virus replication by several logs, commonly below detectable levels in plasma, as tested using different viral RNA assays (detection ranges from 3-100 copies/ml). After 6-12 months of virus suppression, which may take variable amounts of time (1-4 months), the cure or latency reversal strategy is tested. It should be noted that while an absolute recapitulation of long-term ART-treated HIV-infected individuals would suggest the use of prolonged ART in SIV/SHIV-infected macaques, logistical and financial considerations may in fact limit the length of treatment in these animals. We propose that 6-12 months of virus suppression is a reasonable approximation of ART-treated patients that allows for more rapid evaluation of potential cure approaches. Finally, to test the efficacy of the used “anti-reservoir” interventionthe size of the virus reservoir can be measured directly using assays such as total and integrated cell-associated SIV-DNA (34, Mavigner at al., unpublished), Tat-Rev-induced limiting dilution assay (TILDA; 35), and quantitative viral outgrowth assays (QVOA;(36،37،10،38)with input cells derived from blood or multiple tissues through biopsies or at necropsy (summarized in Table 2). An alternative study design involves the functional measurement of the virus reservoirs as inferred by the kinetic of virus rebound after ART interruption (11،12،14). Overall, these types of studies involving ART-treated SIV/SHIV-infected macaques are highly informative in the field of HIV cure research and their use has increasing rapidly over the past few years. However, there are biological caveats and limitations to using NHP models for studies of HIV cure. These include the presence of species-specific differences in certain immune function (i.e., MHC genes), as well as high costs associated with animal housing and care (Table 1). As such, while these NHP models provide an excellent tool to study HIV persistence in humans and potential therapeutic strategies that can be used to disrupt persistence, all key findings must ultimately be replicated in the setting of HIV infection of humans.

The humanized mouse model provides a small-scale animal model to study HIV infection, and upon ART treatment mice control viremia and immune function recovers (39⁻41). Given recent reviews of the humanized mouse models for use in HIV persistence by by Policicchio et al. and Garciahere we will briefly describe two robust mouse models (42،43). The BLT mouse model is the best representative of a humanized mouse with latent HIV infection (44), where irradiated NOD.CB17-Prkdc^{SCID}/J (NSG) are transplanted with human bone marrow, liver and thymus to re-populate a humanized immune system including T-cells, B-cells, monocytes, macrophages and DC (45). Infection progresses similar to humans and latency is established at a similar frequency, allowing potential HIV therapeutics to be tested (Vorinostat, I-BET151 inhibitor, anti-cytotoxic-lymphocyte antigen 4 [CTLA-4]; 46). To only assess T-cell function in HIV persistence, a humanized mouse model has been developed with engraftment of only human T-cells and no myeloid cells, ToM, in a similar method to BLT engraftment(47). Importantly, the To M mice do not develop graft-versus-host disease (GVHD), have potent HIV infection, which is suppressed by ART and rebounds with ART interruption. Additionally, latency is detected at a similar frequency to humans as

measured by the QVOA (36). The same group has also developed a model with myeloid cells and no lymphoid cells, MoM (Honeycutt JCI, in press). The ToM and MoM models have allowed researchers to address important questions regarding virus persistence within T-cells or myeloid cells only, allowing differentiation of specific contribution to reservoir or control of HIV persistence. However, due to suboptimal human immune cell engraftment and the small size of mice the latent reservoir remains small and detection of rare immune cell populations, like $T_{SCM}$and T_{FH}cells, remains difficult. Furthermore, blood volumes obtained are limited leading to reduced ability to measure virus suppression below a threshold of 400 copies/ml(47).

NHP models of SIV persistence

Early establishment of latent infection

In a recent influential study Whitney et al. investigated the early kinetics of reservoir establishment in RM infected with SIVmac251 and early initiation of ART at day 3, 7, 10 and 14 post-infection (12). Interestingly, the authors found that ART initiation at day 3 resulted in detectable SIV RNA and DNA in lymph nodes (LN) and colorectal mucosa, but neither SIV DNA in peripheral blood mononuclear cells (PBMC) nor SIV RNA plasma. In the animals initiated on ART at day 7, 10 and 14 post-infection, i.e., after detection of plasma viremia, and viral RNA and DNA in PBMC, LN and colorectal mucosa, subsequent ART interruption resulted in rapid virus rebound within 7 days. Animals in which treatment was initiated before the presence of detectable SIV RNA in plasma (day 3 ART initiation), a delayed rebound (day 21 post-ART interruption) was also observed. These data are consistent with human studies suggesting that early ART initiation leads to a smaller reservoir size and slower disease progression (33). The findings by Whitney et al. highlight that a small but clearly fully functional SIV reservoir is established very early after experimental inoculation of SIV and before virus detection in plasma and PBMC, with latent virus infection of a critical mass of cells in lymphoid tissues. The implication of these findings for HIV infection in humans is that early ART initiation alone is highly unlikely to represent a potential cure for HIV, but may represent a setting in which additional interventions targeting the established but smaller reservoir are more likely to be effective.

Virus persistence in CD4+ T_{SCM}

Despite an initial observation of HIV infection of hematopoietic stem cells *in vitro* (48), most human studies suggest that hematopoietic stem cells do not contribute to the latent reservoir *in vivo* (49).In contrast, multi-potent $CD4^+$ T memory stem cells, T_{SCM}, harbor high amounts of viral DNA and contribute to the latent reservoir in $CD4^+$ memory T-cells,, in fact, this contribution increases over time in long-term ART-treated HIV-infected humans (3).In addition, studies from our lab of $CD4^+$ T_{SCM} in SIV-infected RM in the absence of ART revealed that these cells are readily infected with SIV in both blood and lymphoid tissues (16). We found that while total T_{SCM} numbers were maintained, the fraction $CD4^+$ T_{SCM} expressing CCR5 was depleted while the percentage of $CD4^+$ T_{SCM} expressing the proliferation antigen Ki-67 was expanded (16). In follow up work, SIV-infected RMs were treated with ART and we found that suppression of virus replication is associated with an improved homeostasis of the $CD4^+$ T_{SCM} compartment but no major decline of the fraction

of these cells containing SIV DNA, even though the frequency of the shorter-lived $CD4^+$ T_{TM} and T_{EM} harboring SIV DNA declined significantly under ART (Cartwright, unpublished). Interestingly, Jaafoura et al. reached similar conclusions regarding the role of $CD4^+$ T_{SCM} in virus persistence under ART by using mathematical modeling of integrated HIV DNA levels in $CD4^+$ T-cells subsets from ART-treated patients (50). Collectively, these studies show that $CD4^+$ T_{SCM} may be important contributors to life-long HIV/SIV persistence under ART, and further highlight the importance of targeting cure strategies towards elimination of latent infection in all long-lived cells.

The role of germinal centers (GC) and follicular T helper cells (T_{FH}) in viral persistence

The role of GC and T_{FH} in HIV persistence has been poorly studied until recently due to the lack of accurate *in vivo* models. Previous work has shown that human follicular dendritic cells (FDC) in GC can harbor HIV on their surface in an archival fashion, where virus on these FDCs persists for months without decay (51–54). Connick et al. found that GC harbor high levels of SIV RNA and proposed that poor $CD8^+$ T-cell infiltration in the lymph node drives persistence of SIV RNA in GC (55). Petrovas et al. was the first to characterize T_{FH} in RM and during SIV infection, showing that activated $CD4^+$ T-cells constantly differentiate into T_{FH} and upon SIV infection T_{FH} adopt a pro-inflammatory phenotype and function but are not depleted, rather they accumulate in the GC (56). In a recent influential study, Fukazawa et al. (2015) showed that low-level viremia in elite controllers originates from T_{FH} as a consequence of the limited access of SIV-specific $CD8^+$ T-cells to the GC (5). We and others have also defined a population of follicular regulatory T-cells (T_{FR}) in SIV-infected and uninfected RM, showing that a reduction in the T_{FR}/T_{FH} ratio after SIV infection was associated with T_{FH} expansion (17,29,57). While both T_{FH} and T_{FR} are known to be important in the development of a strong and balanced humoral response against HIV/SIV antigens during vaccination (and potentially therapeutic vaccination as part of a curative approach), these studies show that T_{FH} and T_{FR} infection are also important for HIV persistence in that they function as viral targets and represent a source of low-level viremia under ART (58). Collectively, these observations suggest that virus-producing T_{FH} in the B-cell area of LN form a continual source of virions that can bind to and interact with FDC, and that, in the context of limited $CD8^+$ T-cell mediated clearance and possibly low bio-distribution of drugs in LN, may crucially contribute to virus persistence under ART.

The role of $CD8^+$ T-cells in controlling viremia under ART

A number of experimental observations demonstrate the role of $CD8^+$ T-cells in controlling HIV and SIV replication *in vivo* during the natural history of infection. This evidence includes the key observation that *in vivo* depletion of $CD8^+$ lymphocytes in SIV-infected RM invariably results in increased levels of virus replication (59–61). However, the role of $CD8^+$ T-cells in suppressing virus replication under ART treated was not addressed in these earlier studies. Recently, we performed experimental $CD8^+$ lymphocyte depletion in thirteen ART-treated SIV-infected RM, and found that this procedure resulted in increased virus production in both plasma and lymphoid tissues in 13 out of 13 of the animals, with levels of viremia up to ~5000 copies/ml from plasma while ART was maintained (Cartwright, unpublished). In most animals we also observed that upon $CD8^+$ T-cell repopulation (but not NK cell repopulation), viremia returned to an undetectable level. Taken together, these data

indicate that $CD8^+$ lymphocytes are required to maintain virus suppression in ART-treated SIV-infected RM. Our data reveal a previously unrecognized antiviral function of $CD8^+$ lymphocytes that could be boosted by interventions such as therapeutic vaccination and immune-based interventions to release inhibitory stimuli from $CD8^+$ T-cells (e.g., checkpoint blockade inhibitors; Cartwright, unpublished).

SIV cure strategies

Total body irradiation, bone marrow transplant and hematopoietic stem cell engraftment in NHP

To date only one person, the Berlin patient, appears to have been cured of HIV. This patient was treated for acute myeloid lymphoma (AML) with two rounds of total body irradiation (TBI) followed by bone marrow transplant (BMT) with a HIV resistant, homozygous CCR5δ32 donor (62,63). Follow-up studies have failed to achieve a similar outcome in two HIV-infected individuals that underwent BMT from a CCR5 wild type donor (64,65). To better understand the impact of TBI and autologous hematopoietic stem cell transplant (HSCT) with HSC that were mobilized and collected prior to RT-SHIV infection we designed and conducted a pilot study in ART-treated SHIV-infected RM. We observed that virus rebounded in 2/3 SHIV infected, ART-treated animals that underwent HSCT, engraftment, and, ART interruption, while the third animal was sacrificed at 2 weeks post ART interruption with undetectable viremia, but detectable virus in lymphoid tissues (11). Together, these results and those obtained by other groups in PTMs (66), demonstrate that autologous HSCT can be performed in SHIV-infected RM and PTM, but the protocols used did not result in cure. This model can be further interrogated to ask critical questions regarding the pathogenesis of the apparent HIV cure achieved in the Berlin patient.

Purging the reservoir with latency reactivating agents (LRA)

Current HIV cure efforts are focused around the hypothesis that the latent reservoir can be disrupted and eliminated by the reactivation of latent virus. This is conceived to involved the delivery of a “shock” signal, followed by immune-mediated clearance (“kill”) of the cells expressing reactivated virus. Agents designed to reactivate latent virus (latency reversing agents, LRA), including histone de-acetylase inhibitors (HDACi) such as Vorinostat, Panobinastat, and Romidepsin, and a drug used to treat alcohol addiction Disulfiram, have been used safely in HIV-infected individuals in several pilot studies, although with limited impact on virus reactivation and virtually no effect on the size of the virus reservoirs (67–71).Ling et al. and Del Prete et al. tested Vorinostat in suppressed SIV infection of Chinese and Indian RM, respectively, and obtained results similar to those of the above mentioned human clinical trials (72,73). Del Prete et al. have also recently used a pan HDACi, Romidepsin, to reactivate SIV infection in ART-treated RMs (74). When ART was interrupted, plasma virus rebound was similar in Romidepsin treated SIV-infected RM compared to non-Romidepsin treated control animals. While these initial results with LRA strategies in both pre-clinical NHP studies and human clinical trials have not been very impressive, there is considerable optimism in the field as novel classes of LRAs are being developed and tested, including toll-like receptor agonists, and combinations of LRAs with immune enhancing strategies (i.e., therapeutic vaccines, checkpoint blockade inhibitors,

monoclonal antibodies) are being explored in studies that rely heavily on the use of ART-treated SIV-infected RM.

Mapping out HIV/SIV infection in the central nervous system under ART

A subset of ART-treated HIV-infected individuals show CNS symptoms that have been grouped under the term of HIV-associated neurocognitive disorder (HAND; (75,76,Reviewed in 77). However, it remains unclear to what extent the pathogenesis of HAND is due to direct virus replication in the CNS despite ART. Using ART-treated SIV-infected RM, it was shown that SIV-DNA can be detected in the brain tissue and cerebrospinal fluid (CSF), thus indicating that the CNS can serve as a site of SIV persistence (6،7،78،79). While it is challenging to measure neurocognitive dysfunction in NHP, investigations of the CNS in NHP are particularly useful given the extreme difficulty in sampling this anatomic compartment in HIV-infected humans. As such, a number of behavioral and biological abnormalities are currently being investigated in macaques as potential markers of brain dysfunction to be used for studies of the CNS reservoirs in SIV-infected ART-treated RM. Recently, Beck et al. showed a difference in infection efficiency of the virus infecting the CNS compared to the periphery (80). These authors characterized the viral fitness of both wild type SIV and the specific CTL escape mutant (K165) to show that the perivascular CTL response exerts selective pressure on wild type SIV in the CNS but not in the periphery, suggesting that the virus causing CNS infection may be different from its peripheral counterpart. Furthermore, in a brief report Marcondes et al. specifically depleted $CD8^+$ T-cells in the CNS of SIV-infected RMs (non ART-treated) via intra-thecal injection of $CD8^+$ depleting antibody and found that viral loads in the CNS were significantly higher than those observed in control groups, thus highlighting the importance of the $CD8^+$ T-cell response protecting CNS from viral infection (79). Further studies of the CNS-based virus reservoir in ART-treated SIV-infected RM or PTM will likely identify site-specific mechanisms of virus persistence and allow the pre-clinical testing of novel therapeutic approaches that specifically target the CNS reservoir.

Targeting HIV/SIV persistence in the gut

The gut is a unique anatomical reservoir of HIV latency as well as a site of residual immune activation in ART-treated HIV-infected individuals (1،2،81،82). Th17 and Th22 $CD4^+$ T-cells mediate protection against bacteria and fungi in gastrointestinal-associated lymphoid tissues (GALT) but are depleted during HIV/SIV infection and fail to fully reconstitute upon ART (6،8،83). Recently, Ryan et al. have described a set of changes in the Th17 and Th22 frequency and function during SIV infection prior to, during and off ART treatment in blood, LN and the colorectum (15). The authors found that ART failed to restore Th17 and Th22 function and frequency to pre-SIV infection levels, and that these markers were predictive of residual immune activation, SIV persistence, and signs of disease progression in the colorectum and blood. Ortiz et al. has shown that IL-21 and probiotic treatment lead to high frequencies of Th17 cells in the gut and improved gut recovery post ART initiation in SIV-infected RMs (38). Similarly, Micci et al. showed that IL-21 administration to ART-treated SIV-infected RM results in enhanced recovery of Th17 and Th22 cells in the gastro-intestinal tract, attenuated signs of inflammation, and reduced levels of plasma SIV RNA (23). Promisingly, these enhancements in the intestinal mucosal immune function remained

stable through treatment interruption, suggesting that IL-21 treatment may represent a promising component of HIV cure interventions.

Conclusion

The use of state-of-the-art NHP models of SIV/SHIV infection under ART in combination with new technologies to assess reservoir size and function, along with novel therapeutic "anti-reservoir" approaches represents a formidable tool for basic and translational studies to develop a cure for HIV infection. We envision that these types of *in vivo* studies will lead to major advances in our understanding of the mechanisms by which SIV/HIV latency is established and maintained, thus ultimately guiding the pre-clinical and clinical development of a feasible strategy for HIV cure.

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Key points

1. NHP and Mouse models are an important aspect in building our understanding of HIV persistence and the development of a HIV cure.
2. Extensive sampling of tissue sites in NHP has allowed the characterization of unique cellular reservoirs like follicular T-helper cells, T-memory stem cells.
3. Strong understanding of SIV pathogenesis has allowed single and combination therapeutics to successfully reverse SIV mediated pathogenesis.
4. The development of SIV persistence models will allow testing of combination therapies to eliminate multiple sources of HIV persistence can now be tested.

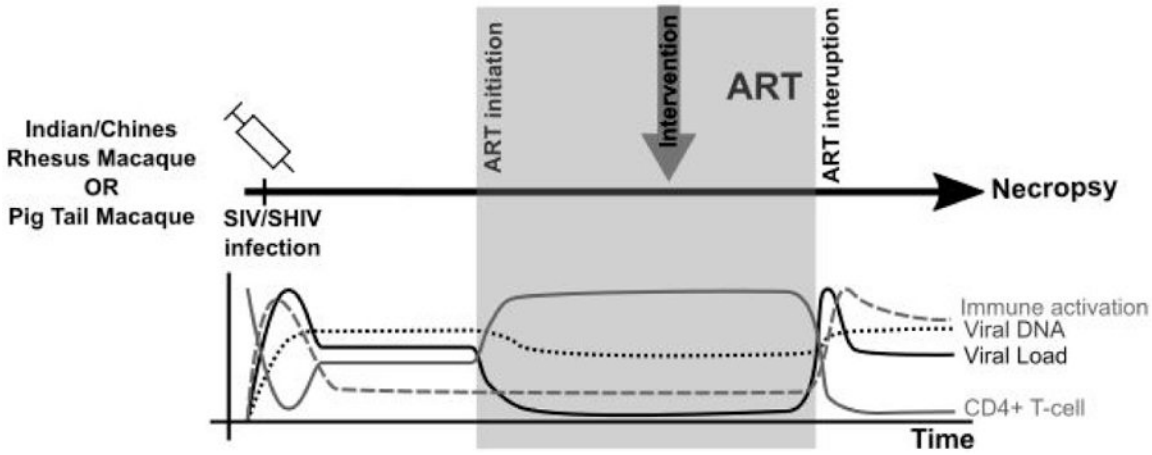

Figure 1. Schematic of Non-Human Primate Models of HIV/SIV cure

Either A. Indian Rhesus Macaques (RM, *Macaca mulatta*) or Pigtail Macaques (PTM, *Macaca nemestrina*) are experimentally infected with simian immunodeficiency virus (SIV) or simian/human immunodeficiency virus (SHIV). After peak viremia, anti-retroviral treatment (ART) is initiated, which usually includes a combination of a two-nucleoside reverse transcriptase inhibitors (NRTI), an integrase inhibitor and either a non-NRTI or a CCR5 inhibitor. Upon virus suppression, an intervention of interest is administered including; latency reversal agents (74), enhancement of immune function (23,38) or elimination of infected cells (11), and ART is continued for some time. To test success of cure strategy and any differences in reservoir size, ART is interrupted and animals are monitored for virus rebound. After viral rebound, animals are sacrificed and tissues are collected for analysis. B. Graph depicts changes in viral load, viral DNA, CD4+ T-cell counts and immune activation during infection, ART treatment and ART interruption.

Table 1
Benefits of nonhuman primate models in HIV cure studies

Benefits of NHP models	Caveats
Similar immune systems	NHP have more HLA genes, therefore more variety in Ag recognition
Similar pathogenic features including AIDS and virus control on ART	Different viruses.
Aggressive tissue sampling and invasive interventions	Need to confirm in human studies.
Deeper understanding of mechanisms	Need human in vitro models to replicate mechanisms
Normalized route of infection, clonal virus, time with productive infection, ART treatment and time of ART	High cost of animals, long experimental protocols and need for animal resources.
Controlled ART adherence	
Characterization of cellular and anatomical reservoirs	
Test pilot studies that are invasive and ethically impossible in humans.	
No barriers to analytical ART interruption.	

Table 2

Summary of selected SIV persistence models

	NHP	Sample number (n)	Virus	Virus concentration	Route of Infection	ART formulation	Time on ART (mo)	Virus suppression achieved?	Limit of detection for SIV RNA assay (copies/ml)	Intervention tested	Treatment interruption (post-ART initiation)	Viral DNA measured?	Plasma drug concentration measured
Shytaj, 2012, PloS Pathog. (10)	RM	5	$SIVmac_{251}$	10^3-10^7 RNA copies/ml	i.r or i.va	FTC, PMPA, RAL for 1.5 mo; DRV/r for +80d; MVC	5-17	Y	<40; <3	N	N	N	Y
		2	$SIVmac_{251}$	10^3-10^7 RNA copies/ml	i.r or i.va	3wks MRV/r, PMPA, FTC, RAL, DRV		Y		N	N	N	Y
		4	$SIVmac_{251}$	10^3-10^7 RNA copies/ml	i.r or i.va	FTC, PMPA, RAL, DRV/r, MVC		Y		N	N	Total DNA	Y
		2	$SIVmac_{251}$	10^3-10^7 RNA copies/ml	i.r or i.va	FTC, PMPA, RAL, DRV/r		Y		N	N	N	Y
Mavigner, 2014, PloS Pathog (11)	RM	3	RT-SHIV-TC	10^4 TCID50	i.r	PMPA, FTC, EFB, RAL	1-2	Y	<60	HSC collection + myeloablative TBI + Stem cell transplant.	Y	Total DNA	N
		3	RT-SHIV-TC	10^4 TCID50	i.r	PMPA, FTC, EFB, RAL	1-2	Y	<60	N	N	Total DNA	N
Whitney, 2014, Nature (12)	RM	20	$SIVmac_{251}$	500 TCID50	i.r	TFV, FTC, DTG	3, 5, 7, 10 days	Y	<50	N	Y	Total DNA	N
Del Prete, 2015, Aids Res and Hu Retrovirus (13)	RM	3	$SIVmac_{239}$	300 IU (TZM-bl reporter cell infection)	i.v	FTC, TDF, DTG	10	Y	<30	+romidepsin	N	N	Y
		3	$SIVmac_{239}$	300 IU (TZM-bl reporter cell infection)	i.v	FTC, TDF, DTG, DRV (DRV dropped after 4 weeks)				N	N	N	Y
Micci, 2015, JCI (14)	RM	8	$SIVmac_{239}$	300 TCID50	i.v	PMPA, FTC, RAL, DRV/r	7	Y	<60	weekly IL-21 treatment between day 67-105 and 203-241	Y	Total DNA	N
		7	$SIVmac_{239}$	300 TCID50	i.v	PMPA, FTC, RAL, DRV/r	7	Y		Control group	Y	Total DNA	N
Ryan, 2016, PloS Pathog (15)	RM	16	$SIVmac_{239}$	300 TCID50	i.v	PMPA, FTC, RAL, DRV/r	2	Y	<60	N	Y	Total DNA	N

	NHP	Sample number (n)	Virus	Virus concentration	Route of Infection	ART formulation	Time on ART (mo)	Virus suppression achieved?	Limit of detection for SIV RNA assay (copies/ml)	Intervention tested	Treatment interruption (post-ART initiation)	Viral DNA measured?	Plasma drug concentration measured
Ortiz, 2015, Muc Immun (38)	PTM	6	$SIVmac_{239}$	3000 TCID50	NR	PMPC, FTC, L8 (integrase inhibitor)	9	Y	100	Daily Probiotic + early and late IL-21 administration	N	Total DNA	N
		5	$SIVmac_{239}$	3000 TCID50	NR	PMPC, FTC, L8 (integrase inhibitor)	9	Y	100	Control group	N	Total DNA	N

Acronyms used: PloS Pathog = Plos Pathogens, JCI = Journal of clinical immunology, Aids res and Hu retrovirus = AIDS research and human retroviruses, RM = Rhesus Macaque, PTM = Pig Tail Macaque, i.r = Intra-rectal, i.va = Intra-vaginal, i.v = Intraveneous, wks = weeks, Mo= months, TCID50 = 50% Tissue culture infection dose, AID50 = 50% Animal infectious doseFTC = emtricitabine, PMPA/TFV = tenofovir, RAL = Raltegravir, DRV/r = ritonavir-boosted darunavir, MVC = maraviroc, EFV = efavirenz, DTG = dolutegravir, TDF = tenofovir disoproxil fumarate, HSC =heamopoetic stem cell, TBI = Total body irradiation, Y= Yes, N=no, NR = not reported.