

Review

Correspondence
Chi Ngai Chan
c.chan.1@research.gla.ac.uk

Recent developments in human immunodeficiency virus-1 latency research

Chi Ngai Chan, Isabelle Dietrich, Margaret J. Hosie and Brian J. Willett

MRC-University of Glasgow Centre for Virus Research, Bearsden Road, Glasgow G61 1QH, UK

Almost 30 years after its initial discovery, infection with the human immunodeficiency virus-1 (HIV-1) remains incurable and the virus persists due to reservoirs of latently infected CD4⁺ memory T-cells and sanctuary sites within the infected individual where drug penetration is poor.

Reactivating latent viruses has been a key strategy to completely eliminate the virus from the host, but many difficulties and unanswered questions remain. In this review, the latest developments in HIV-persistence and latency research are presented.

Introduction

Before the introduction of highly active anti-retroviral therapy (HAART), a diagnosis of HIV/AIDS would have been a death sentence for most patients. However, modern anti-retroviral regimes are able to preserve the health of the patient and routinely reduce the plasma viral load to less than 50 copies of HIV-1 RNA ml⁻¹ (Volberding & Deeks, 2010). Although HAART is very effective at blocking HIV-1 spread within the body, it is not a cure, as viral loads readily rebound when treatment is interrupted (Chun *et al.*, 1999; Davey *et al.*, 1999). Furthermore, ultrasensitive detection assays have shown that in most HAART-treated patients, a low-level viraemia of less than 5 copies ml⁻¹ persists even after years of therapy (Chun *et al.*, 2005; Palmer *et al.*, 2008; Tobin *et al.*, 2005). This low-level persistent viraemia is a major obstacle to the complete elimination of HIV-1 from the body.

HAART cannot fully restore the health of an infected individual. Long-term-treated HIV-1 patients have reduced lifespans and increased susceptibilities to non-AIDS related conditions such as cardiovascular disease, cancer, liver and kidney dysfunctions as well as neurological decline, which may be a consequence of the toxicity of the drugs or the chronic inflammation caused by HIV-1 infection (Deeks, 2011; d'Arminio *et al.*, 2004). The financial cost of life-long treatment, especially in resource-poor settings, is prohibitive (Hecht *et al.*, 2010). Until the discovery of an effective vaccine, or other interventions that can halt the continuing spread of HIV-1, it will become increasingly difficult for high disease burden countries in the developing world to control the epidemic using only current anti-retroviral regimes (Lewin *et al.*, 2011). Thus, an effective cure of HIV/AIDS would not only alleviate the suffering of the millions of infected persons, it may be the only way to check the progress of the HIV-1 epidemic. In this article, we provide an overview of HIV-1 latency and address some of the major gaps in our understanding of the phenomenon. We examine recent advances in translational research

aiming to find a sterilizing (complete eradication of the virus) or a functional (virus replication is on-going but does not lead to clinical problems) cure.

The question over the source of the persistent viraemia

The half-life of the HIV-1 virion in the plasma is very short (Ho *et al.*, 1995; Ramratnam *et al.*, 2000) and it is generally believed that the persistent viraemia is either the result of the reactivation of latently infected resting T-cells or on-going virus replication in 'sanctuary' sites within the body (Lewin *et al.*, 2011; Palmer *et al.*, 2011). The existence of a latently infected population of CD4⁺ T-cells was first indicated by the discovery that the number of cells expressing HIV-1 mRNA *in vivo* was lower than the number of cells carrying proviral DNA (Schnittman *et al.*, 1989). Subsequent studies have demonstrated that a small number (approx. one million cells) of resting CD4⁺ T-cells in HAART-treated individuals harboured replication-competent latent viruses that could be reactivated by stimulation of the cells with mitogens (Chun *et al.*, 1995, 1997; Finzi *et al.*, 1997). While dormant, the virus is hidden from the host immune response and it has been shown that the decay rate of these latently infected resting CD4⁺ T-cells is very low, requiring an estimated period of 73.4 years for complete eradication using the current anti-retroviral regime (Siliciano *et al.*, 2003). Alternatively, on-going low-level virus replication may be responsible for the persistent viraemia. Persistent virus production has been found within sanctuary sites such as the central nervous system (Canestri *et al.*, 2010; Churchill *et al.*, 2006; González-Scarano & Martín-García, 2005), the gastrointestinal tract (Chun *et al.*, 2008) and the male and female genital tract (Halfon *et al.*, 2010; Launay *et al.*, 2011). Recent studies have indicated that anti-retroviral drug-penetration is site- and compound-specific, and drugs that penetrate poorly may allow virus replication at that site even when plasma viral load is below 50 copies ml⁻¹ (Best *et al.*, 2012; Di Mascio *et al.*, 2009; Else *et al.*, 2011; Halfon *et al.*,

2010; Kwara *et al.*, 2008; Launay *et al.*, 2011). Since the rate of reactivation of latent viruses in resting T-cells is unknown *in vivo* (Siliciano & Siliciano, 2010), it is unclear whether it occurs frequently enough to maintain the low-level viraemia that is detected in patients. Thus, the most probable origin of the low-level viraemia may be the sanctuary sites where productive infection is expected to be occurring constantly.

In order to determine the contribution of each of these factors to the low-level viraemia in the body, phylogenetic studies were performed on the viral sequences isolated from the residual viraemia. The results were contradictory: while some studies showed a lack of evolution among the sequences found, suggesting that the progeny virions came from one stable reservoir among CD4⁺ T-cells (Bailey *et al.*, 2006; Joos *et al.*, 2008; Ruff *et al.*, 2002), others found viral sequences that were not detected among the resting T-cell population, indicating another cellular source for the residual viraemia (Bailey *et al.*, 2006; Brennan *et al.*, 2009; Sahu *et al.*, 2009). Another indication of on-going productive infection would be if treatment intensification (the addition of a fourth anti-retroviral to the standard three-drug regime) reduced the basal level of viraemia further. The majority of treatment intensification studies using the HIV-integrase inhibitor Raltegravir (RGV) showed no significant reduction of the residual plasma viraemia (Dinosa *et al.*, 2009b; Gandhi *et al.*, 2010; Hatano *et al.*, 2011; McMahan *et al.*, 2010). However, in one study RGV increased the number of '2-LTR (long terminal repeat) circles' found in the PBMCs of 29% of the treated subjects (Buzón *et al.*, 2010). Since RGV blocks the integration of linear viral DNA from a productive infection and encourages the formation of 2-LTR circles (Middleton *et al.*, 2004; Svarovskaia *et al.*, 2004), these data indicate the presence of an on-going infection. In a separate study, intensification with RGV reduced unspliced HIV-1 RNA within the ileum, but caused no significant reduction in plasma viraemia (Yukl *et al.*, 2010), illustrating the possibility of on-going infection occurring in a compartment other than the blood (Table 1).

Most of the CD4⁺ T-cells in the body reside within the gastrointestinal tract and the lymphatic tissues rather than

within peripheral blood (Mowat & Viney, 1997). In contrast, the majority of studies on HIV-1 replication dynamics and CD4⁺ T-cell depletion have been performed in peripheral blood because it is the easiest compartment to access. It has been shown that the gastrointestinal tract is the major site of HIV-1 replication and CD4⁺ T-cell depletion during all stages of HIV/AIDS (Brenchley *et al.*, 2004a; Chun *et al.*, 2008), and that the destruction of the CD4⁺ T-cell population within the gastrointestinal tract leads to the translocation of microbial products to the circulatory system and contributes to the chronic inflammation and immune exhaustion that are associated with HIV/AIDS (Douek *et al.*, 2009). Thus, we may be overlooking vital pieces of the jigsaw if we focus solely on the peripheral blood compartment.

Apart from CD4⁺ T-cells, HIV-1 can also infect cells of the monocytic lineage (Coleman & Wu, 2009; Gartner *et al.*, 1986; Le Douce *et al.*, 2010). HIV-1 infection of macrophages tends to be less cytopathic than infection of activated T-cells (Ho *et al.*, 1986, 1995; Nicholson *et al.*, 1986). Also, infected monocytes can migrate to the central nervous system and the gastrointestinal tract before maturing into macrophages, potentially sheltering the virus from the full potency of HAART (Le Douce *et al.*, 2010). The contribution of infected macrophages to HIV-related neurological decline is well documented (González-Scarano & Martín-García, 2005). It is also well known that dendritic cells can transport whole virions to lymph nodes where susceptible activated CD4⁺ T-cells reside. Moreover, dendritic cells themselves can become infected under certain circumstances (Coleman & Wu, 2009). However, it is not clear whether proviral clones or individual infected cells within the monocytic population can survive for long enough to function as long-lived latency reservoirs (Eisele & Siliciano, 2012). It is possible that within the safety of sanctuary sites and with continuous replenishment of susceptible cells, continuous productive infection may be maintained by macrophages and dendritic cells. In addition it has been proposed that infection of immature CD4⁺/CD8⁺ 'double positive' thymocytes during thymopoiesis may generate a

Table 1. Summary of the evidence for and against the hypothesis that the persistent residual viraemia in HAART-treated patients originates from a single source

Persistent viraemia originated from one source (resting T-cells)	References
<ul style="list-style-type: none"> ● Viral genomes recovered from persistent viraemia show little variation ● HAART intensification does not reduce residual viraemia 	Bailey <i>et al.</i> (2006); Joos <i>et al.</i> (2008); Ruff <i>et al.</i> (2002) Dinosa <i>et al.</i> (2009b); Gandhi <i>et al.</i> (2010); Hatano <i>et al.</i> (2011); McMahan <i>et al.</i> (2010)
Multiple sources contribute to persistent viraemia	
<ul style="list-style-type: none"> ● Persistent HIV-1 infection has been found within different parts of the body ● Viral sequences distinct from those isolated from resting T-cells are found ● HAART intensification increased 2-LTR circles from PBMCs ● HAART intensification reduced HIV-1 RNA within the ileum 	See text Bailey <i>et al.</i> (2006); Brennan <i>et al.</i> (2009); Sahu <i>et al.</i> (2009) Buzón <i>et al.</i> (2010) Yukl <i>et al.</i> (2010)

population of latently infected naïve T-cells (Brooks *et al.*, 2001). Despite the continuing debate over the true origin of the low-level viraemia, it can be agreed that a viable therapeutic intervention to cure HIV/AIDS should involve the elimination of all these proven and potential reservoirs.

Haematopoietic stem cells (HSCs) are a viral reservoir?

HSCs are a population of primitive, self-renewing precursor cells that reside in the bone marrow (Cabrita *et al.*, 2003). HSCs can proliferate and differentiate into all the cell types found in peripheral blood. HSCs and other more mature precursor cell types such as the multipotent progenitor cells (MPPs) can express the HIV-1 receptors CD4, CCR5 and CXCR4; thus in theory these cells can be infected by HIV-1 (Alexaki & Wigdahl, 2008; McNamara & Collins, 2011). However, whether this is the case *in vivo* is controversial, with contradictory evidence emerging from different studies (Davis *et al.*, 1991; Folks *et al.*, 1988; Neal *et al.*, 1995; Stanley *et al.*, 1992).

If HSCs and other progenitor cells are proven to be latent reservoirs of HIV-1, it would make the difficult task of curing HIV/AIDS even more challenging as these cells are very long-lived, can self-propagate and the provirus in these cells may not be affected by HAART or any novel therapies that target latently infected CD4⁺ T-cells. Recently, it has been shown that CD34⁺ progenitor cells (that include HSCs and MPPs) are susceptible to latent infection *ex vivo*, and that integrated provirus was detected among CD34⁺ cells from HAART-treated patients (Carter *et al.*, 2010). A follow-up study showed that only X4 or dual R5/X4-tropic viruses could efficiently infect these CD34⁺ progenitor cells (Carter *et al.*, 2011). Furthermore, this study also showed that human HSCs infected with a GFP-reporter virus could be successfully engrafted into irradiated non-obese diabetic (NOD)/SCID IL-2R γ null mice, leading to the detection of human leukocytes in the peripheral blood that were carrying the reporter virus 14–18 weeks post-engraftment (Carter *et al.*, 2011). These results indicate that infected haematopoietic progenitor cells are a reservoir for HIV-1. However, other studies have failed to detect HIV-1 amongst the FACS-sorted CD34⁺ progenitor cells of HAART-treated patients (Durand *et al.*, 2012; Josefsson *et al.*, 2012) and it was suggested that the positive selection of CD34⁺ cells by magnetic beads as used in the studies by Carter *et al.* may be insufficient to remove all the contaminating CD4⁺ T-cells. Also, are the results from the engraftment experiment, which used a reporter virus and a highly artificial small animal model, relevant to the situation in the human body? The existence of an HSCs reservoir remains a controversy and requires further study.

Latent HIV-1 infection of resting CD4⁺ T-cells

Although HIV-1 can persistently replicate within sanctuary sites, improvements in drug penetration or HAART intensification may overcome this barrier to eradication in

the future. However, enhancing the effectiveness of HAART will not affect the latent viruses hiding within the resting CD4⁺ T-cell populations of the body. Thus, the latent infection within resting T-cells remains the biggest proven obstacle to a sterilizing cure of HIV-1 infection. The majority of the circulating CD4⁺ T-cells in the body at any given time are in a resting state (Berard & Tough, 2002). These cells are typically defined by the lack of activation marker expression (CD25, CD69 and HLA-DR), as well as the maintenance of the cells in the G₀ phase (Chun *et al.*, 1997). They can be broadly divided into those that have not undergone antigen-stimulated expansion (naïve T-cells) and those that have remained behind after the end of an immune response (memory T-cells) (Berard & Tough, 2002). Among infected resting T-cells, HIV-1 gene expression is largely suppressed (Hermankova *et al.*, 2003). However, some transcription of HIV mRNA can be detected within the resting T-cells of HAART-treated patients, although full virus production is inhibited by inefficiencies at various stages of the viral life cycle (Lassen *et al.*, 2004, 2006; Vatakis *et al.*, 2010) (Fig. 1). Since most of these latently infected resting CD4⁺ T-cells are CD45RO⁺ memory cells (Brenchley *et al.*, 2004b; Chomont *et al.*, 2009; Chun *et al.*, 1997; Pierson *et al.*, 2000), it is hypothesized that the majority of the latently infected T-cells come from activated CD4⁺ T-cells that were infected and then reverted back to a resting memory state before the start of virus replication (Han *et al.*, 2007). Latent provirus can be maintained within the memory T-cell population by the homeostatic proliferation of the infected host cells, driven by IL-7 (Bosque *et al.*, 2011; Chomont *et al.*, 2009).

Virus may infect resting T-cells directly and latent infection of naïve T-cells has been observed in patients, albeit at a lower frequency than memory T-cells (Chomont *et al.*, 2009; Pierson *et al.*, 2000). However, direct infection of resting T-cells is very inefficient (Pierson *et al.*, 2000; Stevenson *et al.*, 1990), with defects in reverse transcription and delays in integration in comparison with infection of activated CD4⁺ T-cells (Vatakis *et al.*, 2007, 2009). Recently, two studies have implicated the innate restriction factor SAMHD1 in the inhibition of reverse transcription in resting CD4⁺ T-cells. Initially shown to be absent in transformed CD4⁺ T-cell lines, SAMHD1 was found to be expressed in both resting and activated primary CD4⁺ T-cells. In resting T-cells, SAMHD1 restricted reverse transcription by depleting the cellular pool of dNTPs (Baldauf *et al.*, 2012; Descours *et al.*, 2012). Nevertheless, integration of the viral genome can still occur in resting T-cells (Vatakis *et al.*, 2009) and no method described to date has been able to distinguish between latently infected memory T-cells that were infected either during activation or during quiescence (Vatakis *et al.*, 2010). Furthermore, a recent study showed that in patients receiving HAART treatment, the amount of HIV DNA in memory T-cells declined over time while the amount of HIV DNA in naïve cells remained constant, suggesting that direct infection of resting T-cells may be replenishing the latent viral reservoir

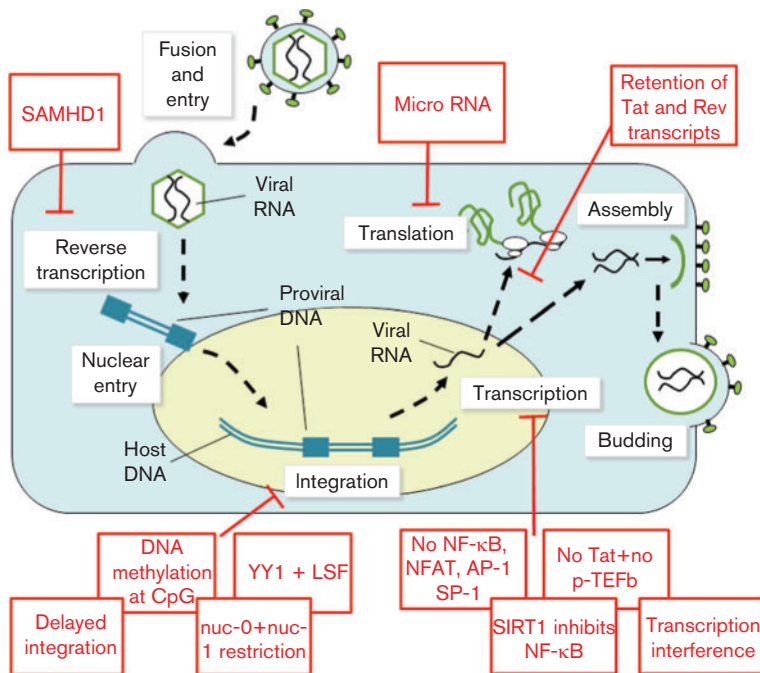


Fig. 1. A summary of the multiple obstacles blocking productive HIV-1 infection of resting CD4⁺ T-cells. Inhibition of virus replication occurs at multiple steps during the viral life cycle. Transcription interference refers to promoter occlusion and the collision of RNA polymerases that hinder efficient viral gene expression.

as the disease progresses (Wightman *et al.*, 2010). These observations are consistent with the finding that R5 tropic viruses [which are associated with acute infection (Choe *et al.*, 1996; Feng *et al.*, 1996; Zhu *et al.*, 1993)] preferentially infect CCR5-expressing memory T-cells whereas X4 tropic viruses [which are associated with late disease progression (Connor *et al.*, 1997)] exhibit a preference for CCR5- CXCR4⁺ naïve T-cells (Bleul *et al.*, 1997; Ostrowski *et al.*, 1999; Wu *et al.*, 1997). The stimulatory effects of HIV-1 gp120 may enable the direct infection of resting T-cells by activating calcium flux and NFAT signalling down the CCR5 signalling pathway, as well as upregulating inositol triphosphate-mediated signalling and the expression of the IL-2 receptor (Cicala *et al.*, 2006; Kornfeld *et al.*, 1988; Weissman *et al.*, 1997). Stimulation of CXCR4 signalling by HIV-1 gp120 induces cytoskeleton-remodelling activity in resting T-cells, increasing the efficiency of subsequent infection with HIV-1 (Yoder *et al.*, 2008). Thus, it is possible that the direct infection of resting T-cells plays an increasingly important role in maintaining the viral reservoir as the disease progresses.

The molecular mechanisms of latency in HIV-1 infection have been reviewed extensively (Coiras *et al.*, 2009; Colin & Van Lint, 2009; Marcello, 2006; Marsden & Zack, 2009; Richman *et al.*, 2009; Siliciano & Greene, 2011). In general, HIV-1 latency may be divided into pre-integration or post-integration latency. Pre-integration latency refers to the partial or complete inhibition of the viral life cycle before the integration of the virus into the host genome (see above). Most of the HIV-1 DNA found in resting T-cells is unintegrated (Chun *et al.*, 1997; Sloan & Wainberg, 2011). Although it has been shown that linear unintegrated viral DNA within resting CD4⁺ T-cells is able to complete

integration after the activation of the cell (Bukrinsky *et al.*, 1991), pre-integration latency is not thought to be relevant to the establishment of the reservoir of latently infected resting T-cells due to the labile nature of viral DNA in the cytoplasm of the cell (Pierson *et al.*, 2002). Accordingly, the unintegrated viral DNA may no longer be replication competent after a protracted period inside the host cell (Han *et al.*, 2007; Zhou *et al.*, 2005). Post-integration latency is the failure of expression of the viral genome after it has been integrated into the host genome. While less than 0.05%, or approximately 10⁶ to 10⁷ cells, carry integrated provirus, it is these integrated proviruses that are thought to constitute the latent viral reservoir (Chun *et al.*, 1997).

Even after a successful integration event, there are still multiple barriers to productive HIV-1 replication within resting CD4⁺ T-cells (Fig. 1). Although HIV-1 genomes are generally integrated into genes that are actively expressed within resting T-cells (Han *et al.*, 2004), viral gene expression may be downregulated by promoter occlusion (if the provirus is integrated in the same orientation as the host gene) (Greger *et al.*, 1998) or by collisions between RNA Pol II molecules that are travelling in opposite directions (if the provirus is integrated in the opposite orientation as the host gene) (Han *et al.*, 2008). Two nucleosomes, named nuc-0 and nuc-1, are frequently associated with the HIV-1 5'LTR and regulate the basal transcriptional activity of the viral genome by controlling the access of transcription factors to the LTR (Verdin *et al.*, 1993). The remodelling of the nucleosomes is regulated by the acetylation status of their constituent histones, which is in turn controlled by enzymes such as histone acetyltransferases and histone deacetylases (Van Lint *et al.*, 1996). This allows the manipulation of HIV-1 transcriptional activity

by pharmacological means, potentially leading to a viable method to eliminate the virus reservoir from resting CD4⁺ T-cells (see Novel drug discovery). The presence of cellular transcriptional repressors, for example YY1 and LSF, as well as the methylation of the two CpG islands at the HIV-1 transcription start site, can recruit histone deacetylases to the HIV-1 LTR and reinforces latency (Blazkova *et al.*, 2009; Coull *et al.*, 2000; Kauder *et al.*, 2009), while the binding of the transcription factor NF- κ B stimulates proviral reactivation by recruiting histone acetyltransferases to the LTR and initiating early HIV-Tat production (Lusic *et al.*, 2003; Williams *et al.*, 2007). The lack of NF- κ B, as well as transcription factors NFAT, SP-1 and AP-1 prevents the synthesis of Tat and the subsequent Tat-dependent, high level viral gene expression (Coiras *et al.*, 2009; Mbonye & Karn, 2011; Williams & Greene, 2007). The transcriptional activity of Tat is highly dependent on interacting with the cellular factor P-TEFb, which triggers effective RNA Pol II elongation (Parada & Roeder, 1996), and the negative regulation of P-TEFb activity in resting T-cells further impairs the expression of HIV-1 genes (Ghose *et al.*, 2001). Tat also interacts with several other cellular factors such as the histone acetyltransferases p300 and P/CAF to promote transactivation of viral genes (Benkirane *et al.*, 1998). The acetylation of the RelA subunit of NF- κ B by p300 increases its transcriptional activity (Chen *et al.*, 2002) and this is countered by the cellular deacetylase SIRT1 (Yeung *et al.*, 2004). SIRT1 activity is in turn blocked by HIV-1 Tat (Kwon *et al.*, 2008). In addition, it has been demonstrated that HIV Tat and Rev transcripts are retained in the nuclei of resting CD4⁺ T-cells (Lassen *et al.*, 2006) and that numerous host microRNAs can directly or indirectly downregulate HIV-1 gene expression, contributing to the maintenance of proviral latency (Chiang & Rice, 2012).

Due to the involvement of so many cellular factors, it has been proposed that there are different degrees of latency within the T-cell population, depending on the cell type and the extracellular environment (Pace *et al.*, 2011). A recent *in vitro* study of HIV-1 latency using a central memory T-cell model system has shown that IL-7-driven homeostatic replication of infected cells can induce partial virus reactivation, while stimulation of the T-cell receptor signalling pathway with anti-CD3/anti-CD28 antibody induced full reactivation (Bosque *et al.*, 2011). This supports the hypothesis of a dynamic reservoir of infected T-cells at various levels of cellular and viral activation.

An area of research which has, as yet, escaped the attention of the HIV-latency field is the molecular mechanism behind CD4⁺ T-cell quiescence. It has been known for some time that the quiescence state is actively maintained by factors such as LKLF, Tob, Foxo3a and Foxj1 (Coffer & Burgering, 2004; Tzachanis *et al.*, 2004; Yusuf & Fruman, 2003). The role of these factors in HIV-latency has been explored by few laboratories so far (Haaland *et al.*, 2005; van Grevenynghe *et al.*, 2008) and further research may provide new insights into the mechanism of latency as well as potential therapeutic targets.

***In vitro* and *in vivo* models of latency**

The latently infected CD4⁺ T-cell population within the patient is very small, thus making *ex vivo* experiments very difficult. The use of *in vitro* and *in vivo* models of latency has been and will continue to be vital to the understanding of HIV-1 latency and drug discovery. Early studies of lentiviral latency using cell lines such as ACH-2, U1 and J-Lat showed the involvement of host cytokine signalling pathways and chromatin reorganization in modulating latency (Folks *et al.*, 1987, 1989; Jordan *et al.*, 2003), but their transformed nature means their responses to treatments may not be physiologically relevant. For example, in the latently infected J-Lat cell line, HIV-1 preferentially integrates near the heterochromatin where transcriptional activity is low (Jordan *et al.*, 2003). However, this preference is not observed within the latently infected resting CD4⁺ T-cells from HAART-treated patients, rather the provirus overwhelmingly favours integration into active transcriptional regions (Han *et al.*, 2004; Schröder *et al.*, 2002).

Most of the current *in vitro* models of HIV-1 latency involve the use of primary cells (Yang, 2011). These experiments are technically challenging, often taking weeks or months to complete in order to mimic the transition of activated T-cells to quiescent memory T-cells *in vivo* (Marini *et al.*, 2008). Generating enough cells for experiments, especially in high-throughput screening of compounds, is another problem. Strategies such as the transduction of a survival gene into primary cells (Yang *et al.*, 2009), using low levels of cytokines such as IL-2 or IL-7 (Bosque & Planelles, 2009; Marini *et al.*, 2008) or co-culture with a feeder cell line (Sahu *et al.*, 2006; Tyagi *et al.*, 2010) have been described. Protocols to directly infect purified resting T-cells *ex vivo* have also been developed. To overcome the inefficient nature of infecting resting T-cells, methods such as spinoculation (O'Doherty *et al.*, 2000) or stimulation with the chemokines CCL19 and CCL21 (Saleh *et al.*, 2007, 2011) were used. The pros and cons of these *in vitro* model systems have been reviewed elsewhere (Pace *et al.*, 2011; Wightman *et al.*, 2012; Yang, 2011). Any model of latency would have to balance multiple conflicting demands such as maintaining the viability of the cells, while preserving a resting state and allowing viral integration without stimulating full-blown virus replication. A further complication is the fact that there are multiple types of cells that can be latently infected, such as central memory T-cells, transitional memory T-cells and naïve T-cells (Chomont *et al.*, 2009; Wightman *et al.*, 2010); any future treatments to reactivate the latent proviruses would have to be effective in all of these subsets of latently infected T-cells.

Non-human primates, in particular rhesus macaques infected with the simian immunodeficiency virus (SIV) or chimeric SIVs containing HIV-1 reverse transcriptase have been used to model HIV-1 latency in HAART-treated patients (Dinosa *et al.*, 2009a; North *et al.*, 2010; Shen *et al.*,

2003). The major advantage of using non-human primates is that the locations of the persistent viral reservoirs mirror those in humans (North *et al.*, 2010), which allows comparative *in vivo* studies. Also the progression of SIV in macaques resembles HIV-1 infection in humans, with distinctive acute and chronic phases of infection that may lead to immunodeficiency (Hirsch *et al.*, 1996). However, there are significant differences between SIV infection of non-human primates and HIV-1 infection in humans. For example, the residual viraemia for SIV in rhesus macaques during chronic infection is higher than the levels seen in humans (Brenchley & Paiardini, 2011). The progression to AIDS appears to be more rapid in rhesus macaques than in humans (North *et al.*, 2010). In African green monkeys or sooty mangabeys, although high levels of virus replication are observed during the chronic phase of infection, this is not accompanied by the destructive chronic immune activation seen in rhesus macaques or humans (Brenchley & Paiardini, 2011; Chahroudi *et al.*, 2012). Also different strains of SIV can produce different pathologies in the same host (Hirsch *et al.*, 2000).

The complexity of finding the correct host and SIV strain combination that mimics HIV-1 latent infection most closely, together with issues such as ethical concerns and high cost have led to the development of other, non-primate animal models for HIV-1 infection such as humanized SCID (SCID-hu) mouse models (Boberg *et al.*, 2008; Brooks *et al.*, 2001; Van Duyne *et al.*, 2009). SCID-hu mice are created by transplanting SCID mice with human fetal thymus and liver tissues or peripheral blood lymphocytes to form SCID-hu Thy/Liv and SCID-hu PBL mice, respectively (Van Duyne *et al.*, 2009). For example an *in vitro* model of latently infected immature CD4⁺/CD8⁺ thymocytes has been generated using SCID-hu Thy/Liv mice (Brooks *et al.*, 2001; Burke *et al.*, 2007). A major drawback of using SCID-mouse-based models is the failure to fully reconstitute the human immune system within the transplanted animals (Rossi *et al.*, 2001; Van Duyne *et al.*, 2009). Further improvement to efficiency of engraftment was achieved with the generation of the NOD/SCID mouse model (Hesselton *et al.*, 1995) and later with the double knockout of the common cytokine receptor γ C and the recombinae activating gene 2 (Rag2) (Goldman *et al.*, 1998). The transplantation of human CD34⁺ stem cells into Rag2^{-/-} γ C^{-/-} mice leads to the development of a functional model of the human immune system in the bodies of the mice (Traggiai *et al.*, 2004) and forms the basis of a recent murine model of HIV-1 latency that contains infected resting T-cells in the peripheral blood and lymphoid tissues (Choudhary *et al.*, 2009; 2012). Viable small animal models are vital in the preclinical evaluation of latency reversing therapies, especially if they can replicate latent infection compartments other than peripheral blood.

The feline model of HIV-1 latency

Feline immunodeficiency virus (FIV) was discovered in 1986 in California (Pedersen *et al.*, 1987). Both HIV-1 and

FIV target activated CD4⁺ T-cells (Yamamoto *et al.*, 1988; Zagury *et al.*, 1986), but whereas the primary receptor for HIV-1 is CD4 (Dalglish *et al.*, 1984), the primary receptor for FIV is CD134 (OX40) (Shimojima *et al.*, 2004). HIV-1 utilizes CCR5 and CXCR4 as its secondary receptors (Choe *et al.*, 1996; Deng *et al.*, 1996; Feng *et al.*, 1996), while FIV uses CXCR4 alone as its sole secondary receptor (Willett *et al.*, 1997). FIV is transmitted mainly by biting (Yamamoto *et al.*, 1989) and causes clinical signs in cats that are similar to AIDS in humans (Ackley *et al.*, 1990; Barlough *et al.*, 1991; Novotney *et al.*, 1990).

Since FIV has a similar cell tropism to HIV-1, it is expected that the host response to FIV and its pathogenesis will be comparable to HIV-1. Cats mount both humoral and cytotoxic T-cell responses to FIV infection (Beatty *et al.*, 1996; Egberink *et al.*, 1992; Flynn *et al.*, 2002). However, the hosts usually fail to clear the infection and may succumb to immunodeficiency. The mechanisms of pathogenesis of HIV-1 and FIV are remarkably similar. Both viruses cause massive depletion of the gastrointestinal tract CD4⁺ T-cell population (Brenchley *et al.*, 2004a; Howard *et al.*, 2010). The low fidelity of the HIV-1 and FIV reverse transcriptases results in the generation of a diverse pool of viral variants within the host, encouraging immune escape (Bebenek *et al.*, 1993; Hosie *et al.*, 2011; Kraase *et al.*, 2010; Mansky & Temin, 1995). All of these factors promote chronic immune activation, eventually leading to the breakdown of the host immune system (Douek *et al.*, 2009; Tompkins & Tompkins, 2008).

Previous studies of FIV in cats have shown that activated (CD4⁺ CD25⁺) and resting (CD4⁺ CD25⁻) CD4⁺ T-cells from peripheral blood can be latently infected *ex vivo* and that FIV replication can be reactivated by the application of ConA or IL-2 (Joshi *et al.*, 2005, 2004), mirroring the crucial role of IL-2 in productive infection with HIV-1 (Oswald-Richter *et al.*, 2004). In a separate study, cats challenged with a low-dose exposure to FIV-infected T-cells showed an aviraemic infection, and when cells from multiple tissues were stimulated by PMA, FIV gp120 production was detected (Assogba *et al.*, 2007). More recently it has been shown that FIV establishes a latent infection within activated and resting T-cells of cats during the asymptomatic phase of infection, similar to the latent infection of the resting T-cell population by HIV-1 in humans (Murphy *et al.*, 2012). These cells contained detectable FIV DNA but no FIV RNA. Furthermore, virus replication from these latently infected cells could be reactivated *ex vivo* by the mitogens PHA and PMA as well as the histone deacetylase inhibitor SAHA (McDonnell *et al.*, 2012) (see Novel drug discovery). These findings support the proposal of using FIV-infected cats as an alternative small animal model for HIV-1 latency.

Stem cell transplantation and gene therapy approaches to curing HIV/AIDS

Recently, a HIV-1-positive patient who developed acute myeloid leukaemia was apparently cured of HIV-1

infection after receiving an HSC transplant from a donor who was homozygous for the CCR5 $\Delta 32$ allele (Hütter *et al.*, 2009). The patient underwent intensive chemotherapy and radiotherapy to prepare for the transplant, which presumably also eliminated almost all the infected CD4⁺ T-cells within the body. In addition, the patient developed graft-versus-host disease after transplantation, indicating that the transplanted cells had replaced the host immune system. HAART treatment was then stopped and in the follow-up study the patient was shown to remain free from the virus (Allers *et al.*, 2011). To subject otherwise healthy HAART-treated patients to this potentially lethal procedure is ethically questionable and practically not viable, especially in resource-poor settings. However, this unique case has raised an interesting question regarding the kind of intervention necessary to clear the body of HIV-1: was the cure achieved by the intensive chemotherapy and radiotherapy or by the transplant of the $\Delta 32$ HSCs, which gave rise to HIV-1 resistant CD4⁺ T-cells? Treatment of HIV-1-positive lymphoma patients with autologous stem cell transplants failed to eliminate the virus from the body (Cillo *et al.*, 2012), which indicated the presence of residual virus or infected cells within the extracted autologous cell population or within the host. It also demonstrated the need to make the host CD4⁺ T-cells immune to HIV-1 infection before transplantation.

The CCR5 $\Delta 32$ mutation abrogates infection of CD4⁺ T-cells by R5 HIV-1 viruses (Dean *et al.*, 1996), the strains most frequently associated with early stage infection and which are transmitted preferentially between individuals (Margolis & Shattock, 2006). Thus, the nascent CD4⁺ T-cells from the transplant would be resistant to new infection. Disruption of the CCR5 gene has no apparent undesirable effects on the normal functioning of HSCs (Bai *et al.*, 2000; O'Brien & Moore, 2000). Various techniques have been developed to disrupt the CCR5 gene *ex vivo*, including the use of CCR5-specific siRNAs, ribozymes, intrabodies and zinc-finger nucleases (ZFNs) (Anderson *et al.*, 2007; Bai *et al.*, 2000; Holt *et al.*, 2010; Kumar *et al.*, 2008; Swan *et al.*, 2006). Each of these treatments has been tested in mouse models and led to the production of modified HSCs which give rise to CD4⁺ T-cells that are resistant to R5 HIV-1 infection. ZFNs, which are engineered endonucleases containing zinc finger domains that recognize specific DNA sequences (Urnov *et al.*, 2005), have also been used to disrupt the CCR5 gene in CD4⁺ T-cells in a mouse model of HIV-1 infection (Perez *et al.*, 2008). More recently, ZFNs targeting CD4⁺ T-cells have been successfully tested in a phase I clinical trial, in which the treatment was well tolerated by patients, the modified CD4⁺ T-cells were able to persist in the body and there were improvements on the CD4⁺ T-cell count and CD4⁺:CD8⁺ T-cell ratio (June *et al.*, 2012). ZFNs that can disrupt the CXCR4 gene in CD4⁺ T-cells have also been developed and it has been demonstrated that they confer resistance to cells against the X4-tropic HIV-1 strains associated with late-stage infection (Wilén *et al.*, 2011; Yuan *et al.*, 2012). Combining the disruption of CCR5 and CXCR4 may provide a viable gene

therapy approach to a functional cure, in which the patient's CD4⁺ T-cells are made resistant to HIV-1 *ex vivo* and are reintroduced back into the body. There may still be residual viraemia but the virus would not cause disease after the withdrawal of HAART. Potential problems with the use of the ZFNs include the possibility of non-specific cleavage of host DNA (Gabriel *et al.*, 2011; Pattanayak *et al.*, 2011) and the possibility of adverse effects from disrupting CXCR4, which has not been well studied at the time of writing.

Novel drug discovery

The main strategy that is currently being pursued by many laboratories to eradicate HIV-1 from the body is to reactivate the latent virus reservoir within resting CD4⁺ T-cells (Marsden & Zack, 2009; Richman *et al.*, 2009). Early attempts at reactivation using powerful cytokines such as IL-2 and TNF- α stimulated virus production (Chun *et al.*, 1998) but also caused dangerous side effects such as the non-specific, global activation of T-cells (Prins *et al.*, 1999). In contrast, the cytokine IL-7 has also been shown to have potent anti-HIV latency effects without inducing T-cell activation (Levy *et al.*, 2009; Scripture-Adams *et al.*, 2002; Wang *et al.*, 2005). IL-7 is well-tolerated *in vivo* (Levy *et al.*, 2012) and it has been used in a clinical trial to reduce the latent reservoir size (ERAMUNE 01, due to finish in January 2013. <http://clinicaltrials.gov>). However, a potential problem with the use of IL-7 to deplete the latent reservoir is that at low concentrations, IL-7 can promote the survival or induce the homeostatic proliferation of the latently infected memory T-cells without triggering activation of the virus, thus inadvertently expanding the reservoir of infected cells (Chomont *et al.*, 2009; Marini *et al.*, 2008).

Compounds that stimulate protein kinase C and NF- κ B such as the phorbol ester prostratin and 5-hydroxynaphthalene-1,4-dione (5HN) have been shown to reactivate latent infection *in vitro* (Kulkosky *et al.*, 2001; Yang *et al.*, 2009). Intriguingly, prostratin also has anti-HIV-1 replication effects (Biancotto *et al.*, 2004; Rullas *et al.*, 2004) and a similar dual effect of the compound on FIV replication has been described *in vitro* (Chan *et al.*, 2013). Histone deacetylase inhibitors such as valproic acid and suberoylanilide hydroxamic acid (SAHA or Vorinostat) have been shown to reverse HIV latency by remodelling the HIV-repressive nucleosome nuc-1 (Archin *et al.*, 2009; Contreras *et al.*, 2009; Van Lint *et al.*, 1996; Ylisastigui *et al.*, 2004). SAHA is a selective class I and II histone deacetylase inhibitor and is approved as a clinical treatment for cutaneous T-cell lymphoma. It has been used in a number of *ex vivo* and clinical studies of the latent reservoir (Archin *et al.*, 2012; Shan *et al.*, 2012). However, further research is required to investigate fully the long-term side effects of SAHA in terms of its potential as a mutagen and its ability to reactivate other latent viruses (Archin *et al.*, 2012; Kerr *et al.*, 2010; Wightman *et al.*, 2012). Using a siRNA screen, a novel HIV replication-inhibiting host factor has been identified recently (Zhu *et al.*, 2012). This factor, named

bromodomain containing 4, can be inhibited by a small molecule known as JQ1 (Filippakopoulos *et al.*, 2010) and JQ1 has been shown to have HIV-1 latency reversing activity by several laboratories (Banerjee *et al.*, 2012; Li *et al.*, 2013; Zhu *et al.*, 2012). Other compounds and small molecules that have been shown recently to reactivate latent HIV-1 infection include a bacterial protein named HIV-1-reactivating factor (Wolschendorf *et al.*, 2010), the aldehyde dehydrogenase inhibitor disulfiram (Xing *et al.*, 2011) and a number of quinolin-8-ol derivatives (Xing *et al.*, 2012). Curiously, a recent clinical trial has demonstrated that intensification of HAART with the CCR5 antagonist Maraviroc (MVC) caused a reduction in the size of the latently infected T-cell reservoir (Gutiérrez *et al.*, 2011). The mechanism behind this effect of MVC is unknown, but the binding of MVC to CCR5 may lead to the stimulation of cell signalling analogous to the stimulation of the other major HIV-1 co-receptor, CXCR4, by the binding of HIV-1 Env (Wu & Yoder, 2009; Yoder *et al.*, 2008). If the findings of this study are confirmed, a new way of reactivating latently infected cells may have been identified. Moreover, it has been shown recently that fever enhances the activity of Tat, a phenomenon mediated by the heat-shock protein Hsp-90 (Roesch *et al.*, 2012). Using a J-Lat model, this study demonstrated that although hyperthermia by itself cannot reactivate latency, it can enhance the reactivation effect of other treatments such as the co-cultivation of the J-Lat cells with IL-2 supplemented PBMCs (Roesch *et al.*, 2012). This suggests that the artificial induction of fever may be used to boost the effectiveness of any future latency reversing therapies.

Borrowing from the concept of HAART, a combination of different latency reversing agents may be used synergistically to enhance the effect of reactivation therapies (Burnett *et al.*, 2010; Deeks *et al.*, 2012; Reuse *et al.*, 2009). The number of compounds identified is likely to increase thanks to on-going and future high-throughput screens that look for molecules which can stimulate latent HIV-1 to reactivate. One novel screening method described recently can measure the expression of cell-associated viral RNA among latently infected T-cells as soon as the cells are extracted from the patient without the need for further co-culturing (Archin *et al.*, 2012). Using this assay, an increase in viral RNA can be demonstrated amongst the resting T-cells from patients after being treated with a single dose of SAHA. However, an important caveat to this type of experiment is that stimulation of viral transcription, viral protein synthesis or even virion production may not necessarily lead to the destruction of the latently infected cell (see below).

Stimulation of latent virus replication may not lead to the depletion of the viral reservoir

It has been assumed that once the latent provirus is reactivated inside a resting T-cell, the cell would die by HIV-induced cytopathic effects or be killed by the host

immune response (Richman *et al.*, 2009). However, this view has recently been challenged (Shan *et al.*, 2012). Stimulation of resting CD4⁺ T-cells from HAART-treated patients with the SAHA did not reduce the size of the latent reservoir (Shan *et al.*, 2012). Furthermore, latently infected resting T-cells reactivated by SAHA were killed neither by viral cytopathic effects, nor by autologous CD8⁺ T-cells isolated from the same patients. Only after antigen-specific stimulation of the autologous CD8⁺ T-cells was efficient killing of the SAHA-reactivated, infected resting CD4⁺ T-cells restored. The transduction of the survival gene Bcl-2 into the resting T-cells during the establishment of the latent infection assay, as well as the use of modified reporter viruses may have increased the survival rate during the study. However, reactivation by SAHA of the latent wild-type virus within unmodified resting T-cells isolated from patients also did not lead to a contraction of the latent virus reservoir. These findings showed that any future therapeutic regime to eliminate the latent reservoir would require the boosting of anti-HIV cytotoxic T-lymphocyte (CTL) responses, which would likely be in a state of exhaustion after years of chronic activation (Trautmann *et al.*, 2006). In addition to stimulating the CD8⁺ T-cells with viral antigens and cytokines, inhibiting the function of immunoregulatory molecule such as PD-1 may be another option for the restoration of full CTL function in the patient against HIV-1 (Eichbaum, 2011). Also, it is known that resting T-cells are less vulnerable to cell death compared with their activated counterparts (van Leeuwen *et al.*, 2009). Would the use of drugs that stimulate cellular activation, such as prostratin, lead to the death of the reactivated infected T-cells? Alternatively, is it possible to use novel technologies such as nanoparticles (Peer *et al.*, 2007), intrabodies (Pérez-Martínez *et al.*, 2010) or RNA aptamers (Burnett & Rossi, 2012) to target the reactivated infected T-cells for destruction (Fig. 2)?

Another observation that may be a cause for concern among the ever growing literature on latency-reversing compounds is that even the most promising molecules such as prostratin, SAHA and JQ1 cannot reliably stimulate productive infection from all HAART-treated patients' samples, despite being very successful in reactivating latent viruses from *in vitro* models (Contreras *et al.*, 2009; Kulkosky *et al.*, 2001; Zhu *et al.*, 2012). The variable performances of these compounds may be due to sampling errors as a result of the fact that there are so few latently infected cells within the patient (hence the need for *in vitro* model systems), or the underlying activation status of the cells, as in the case for prostratin (Chan *et al.*, 2012; Kulkosky *et al.*, 2001). Alternatively, this may indicate that the current *in vitro* models does not represent all the subset of CD4⁺ T-cells that are latently infected. Also can we assume that our current methods of handling CD4⁺ T-cells accurately reproduce *in vivo* conditions? Nevertheless, the potential for false negatives and false positives in the current assays demands further research into the basic molecular biology of HIV-1, T-cell biology and improvements to existing HIV-latency models.

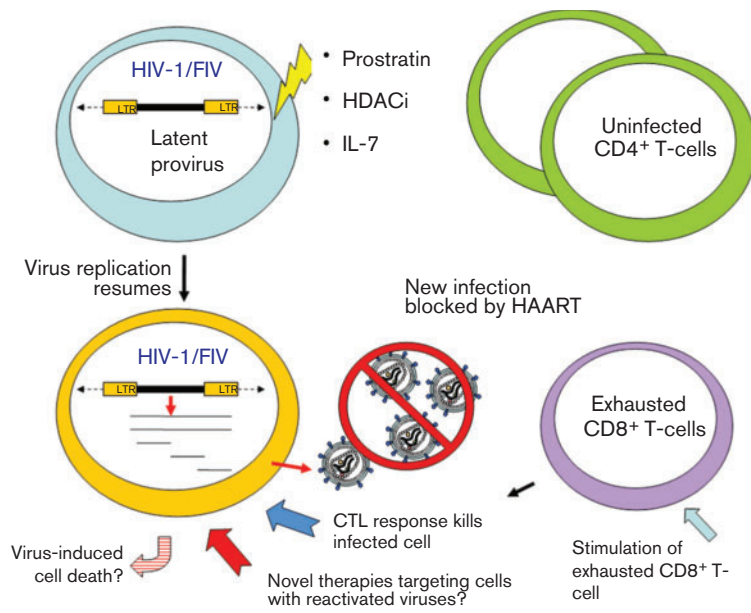


Fig. 2. A theoretical scheme to eliminate the latent HIV-1 reservoir within the resting T-cell population. Virus replication in latently infected cells could be reactivated by, for example, treatments with prostratin, histone deacetylase inhibitors (HDACi) or IL-7. Meanwhile the CTL responses of the patient could be restored by stimulation with viral antigens and cytokines. Although the reactivation of the latent virus may not lead to the apoptosis of the infected cell as previously assumed, the restored CTL response or the use of novel drug-delivery technologies may allow the specific targeting of the infected cells for destruction.

Conclusion

After more than two decades of research we are only beginning to appreciate the full complexity of the problem of HIV-1 persistence and latency. Recent research suggests that there are multiple reservoirs of replication-competent virus which contribute to viral persistence. To achieve a sterilizing cure of HIV-1 requires significant disruption or even elimination of all these reservoirs. In addition, there are still many unanswered questions regarding HIV-1 latency remaining. For example, what is the source of the persistent low-level viraemia? What is the contribution of direct infection of resting T-cells to the overall size of the viral reservoir? Can gene therapy lead to a functional cure of HIV-1? How do we eliminate the infected T-cells once they are reactivated? Research into novel small animal models of HIV-1 latency such as the Rag2^{-/-}γC^{-/-} mouse or HIV-infection of cats may speed up the drug development process but their relevance to the clinic needs to be established. Further research into these issues is needed urgently in order to stop the global HIV/AIDS epidemic, which continues to be a serious global threat to public health almost 30 years after its discovery.

Acknowledgements

The authors would like to thank the School of Veterinary Medicine, University of Glasgow and the Wellcome Trust for funding.

References

- Ackley, C. D., Yamamoto, J. K., Levy, N., Pedersen, N. C. & Cooper, M. D. (1990). Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus. *J Virol* **64**, 5652–5655.
- Alexaki, A. & Wigdahl, B. (2008). HIV-1 infection of bone marrow hematopoietic progenitor cells and their role in trafficking and viral dissemination. *PLoS Pathog* **4**, e1000215.
- Allers, K., Hütter, G., Hofmann, J., Loddenkemper, C., Rieger, K., Thiel, E. & Schneider, T. (2011). Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation. *Blood* **117**, 2791–2799.
- Anderson, J., Li, M.-J., Palmer, B., Remling, L., Li, S., Yam, P., Yee, J.-K., Rossi, J., Zaia, J. & Akkina, R. (2007). Safety and efficacy of a lentiviral vector containing three anti-HIV genes—CCR5 ribozyme, tat-rev siRNA, and TAR decoy—in SCID-hu mouse-derived T cells. *Mol Ther* **15**, 1182–1188.
- Archin, N. M., Espeseth, A., Parker, D., Cheema, M., Hazuda, D. & Margolis, D. M. (2009). Expression of latent HIV induced by the potent HDAC inhibitor suberoylanilide hydroxamic acid. *AIDS Res Hum Retroviruses* **25**, 207–212.
- Archin, N. M., Liberty, A. L., Kashuba, A. D., Choudhary, S. K., Kuruc, J. D., Crooks, A. M., Parker, D. C., Anderson, E. M., Kearney, M. F. & other authors (2012). Administration of Vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* **487**, 482–485.
- Assogba, B. D., Leavell, S., Porter, K. & Burkhard, M. J. (2007). Mucosal administration of low-dose cell-associated feline immunodeficiency virus promotes viral latency. *J Infect Dis* **195**, 1184–1188.
- Bai, J., Gorantla, S., Banda, N., Cagnon, L., Rossi, J. & Akkina, R. (2000). Characterization of anti-CCR5 ribozyme-transduced CD34⁺ hematopoietic progenitor cells in vitro and in a SCID-hu mouse model *in vivo*. *Mol Ther* **1**, 244–254.
- Bailey, J. R., Sedaghat, A. R., Kieffer, T., Brennan, T., Lee, P. K., Wind-Rotolo, M., Haggerty, C. M., Kamireddi, A. R., Liu, Y. & other authors (2006). Residual human immunodeficiency virus type 1 viremia in some patients on antiretroviral therapy is dominated by a small number of invariant clones rarely found in circulating CD4⁺ T cells. *J Virol* **80**, 6441–6457.
- Baldauf, H. M., Pan, X., Erikson, E., Schmidt, S., Daddacha, W., Burggraf, M., Schenkova, K., Ambiel, I., Wabnitz, G. & other authors (2012). SAMHD1 restricts HIV-1 infection in resting CD4⁺ T cells. *Nat Med* **18**, 1682–1689.
- Banerjee, C., Archin, N., Michaels, D., Belkina, A. C., Denis, G. V., Bradner, J., Sebastiani, P., Margolis, D. M. & Montano, M. (2012). BET bromodomain inhibition as a novel strategy for reactivation of HIV-1. *J Leukoc Biol* **92**, 1147–1154.

- Barlough, J. E., Ackley, C. D., George, J. W., Levy, N., Acevedo, R., Moore, P. F., Rideout, B. A., Cooper, M. D. & Pedersen, N. C. (1991). Acquired immune dysfunction in cats with experimentally induced feline immunodeficiency virus infection: comparison of short-term and long-term infections. *J Acquir Immune Defic Syndr* **4**, 219–227.
- Beatty, J. A., Willett, B. J., Gault, E. A. & Jarrett, O. (1996). A longitudinal study of feline immunodeficiency virus-specific cytotoxic T lymphocytes in experimentally infected cats, using antigen-specific induction. *J Virol* **70**, 6199–6206.
- Bebenek, K., Abbotts, J., Wilson, S. H. & Kunkel, T. A. (1993). Error-prone polymerization by HIV-1 reverse transcriptase. Contribution of template-primer misalignment, miscoding, and termination probability to mutational hot spots. *J Biol Chem* **268**, 10324–10334.
- Benkirane, M., Chun, R. F., Xiao, H., Ogryzko, V. V., Howard, B. H., Nakatani, Y. & Jeang, K. T. (1998). Activation of integrated provirus requires histone acetyltransferase. p300 and P/CAF are coactivators for HIV-1 Tat. *J Biol Chem* **273**, 24898–24905.
- Berard, M. & Tough, D. F. (2002). Qualitative differences between naïve and memory T cells. *Immunology* **106**, 127–138.
- Best, B. M., Letendre, S. L., Koopmans, P., Rossi, S. S., Clifford, D. B., Collier, A. C., Gelman, B. B., Marra, C. M., McArthur, J. C. & other authors (2012). Low cerebrospinal fluid concentrations of the nucleotide HIV reverse transcriptase inhibitor, tenofovir. *J Acquir Immune Defic Syndr* **59**, 376–381.
- Biancotto, A., Grivel, J.-C., Gondois-Rey, F., Bettendorfer, L., Vigne, R., Brown, S., Margolis, L. B. & Hirsch, I. (2004). Dual role of prostratin in inhibition of infection and reactivation of human immunodeficiency virus from latency in primary blood lymphocytes and lymphoid tissue. *J Virol* **78**, 10507–10515.
- Blazkova, J., Trejbalova, K., Gondois-Rey, F., Halfon, P., Philibert, P., Guiguen, A., Verdin, E., Olive, D., Van Lint, C. & other authors (2009). CpG methylation controls reactivation of HIV from latency. *PLoS Pathog* **5**, e1000554.
- Bleul, C. C., Wu, L. J., Hoxie, J. A., Springer, T. A. & Mackay, C. R. (1997). The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. *Proc Natl Acad Sci U S A* **94**, 1925–1930.
- Boberg, A., Bråve, A., Johansson, S., Wahren, B., Hinkula, J. & Rollman, E. (2008). Murine models for HIV vaccination and challenge. *Expert Rev Vaccines* **7**, 117–130.
- Bosque, A. & Planelles, V. (2009). Induction of HIV-1 latency and reactivation in primary memory CD4⁺ T cells. *Blood* **113**, 58–65.
- Bosque, A., Famiglietti, M., Weyrich, A. S., Goulston, C. & Planelles, V. (2011). Homeostatic proliferation fails to efficiently reactivate HIV-1 latently infected central memory CD4⁺ T cells. *PLoS Pathog* **7**, e1002288.
- Brenchley, J. M. & Paiardini, M. (2011). Immunodeficiency lentiviral infections in natural and non-natural hosts. *Blood* **118**, 847–854.
- Brenchley, J. M., Schacker, T. W., Ruff, L. E., Price, D. A., Taylor, J. H., Beilman, G. J., Nguyen, P. L., Khoruts, A., Larson, M. & other authors (2004a). CD4⁺ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* **200**, 749–759.
- Brenchley, J. M., Hill, B. J., Ambrozak, D. R., Price, D. A., Guenaga, F. J., Casazza, J. P., Kuruppu, J., Yazdani, J., Migueles, S. A. & other authors (2004b). T-cell subsets that harbor human immunodeficiency virus (HIV) *in vivo*: implications for HIV pathogenesis. *J Virol* **78**, 1160–1168.
- Brennan, T. P., Woods, J. O., Sedaghat, A. R., Siliciano, J. D., Siliciano, R. F. & Wilke, C. O. (2009). Analysis of human immunodeficiency virus type 1 viremia and provirus in resting CD4⁺ T cells reveals a novel source of residual viremia in patients on antiretroviral therapy. *J Virol* **83**, 8470–8481.
- Brooks, D. G., Kitchen, S. G., Kitchen, C. M. R., Scripture-Adams, D. D. & Zack, J. A. (2001). Generation of HIV latency during thymopoiesis. *Nat Med* **7**, 459–464.
- Bukrinsky, M. I., Stanwick, T. L., Dempsey, M. P. & Stevenson, M. (1991). Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. *Science* **254**, 423–427.
- Burke, B., Brown, H. J., Marsden, M. D., Bristol, G., Vatakis, D. N. & Zack, J. A. (2007). Primary cell model for activation-inducible human immunodeficiency virus. *J Virol* **81**, 7424–7434.
- Burnett, J. C. & Rossi, J. J. (2012). RNA-based therapeutics: current progress and future prospects. *Chem Biol* **19**, 60–71.
- Burnett, J. C., Lim, K. I., Calafi, A., Rossi, J. J., Schaffer, D. V. & Arkin, A. P. (2010). Combinatorial latency reactivation for HIV-1 subtypes and variants. *J Virol* **84**, 5958–5974.
- Buzón, M. J., Massanella, M., Llibre, J. M., Esteve, A., Dahl, V., Puertas, M. C., Gatell, J. M., Domingo, P., Paredes, R. & other authors (2010). HIV-1 replication and immune dynamics are affected by Raltegravir intensification of HAART-suppressed subjects. *Nat Med* **16**, 460–465.
- Cabrita, G. J. M., Ferreira, B. S., da Silva, C. L., Gonçalves, R., Almeida-Porada, G. & Cabral, J. M. S. (2003). Hematopoietic stem cells: from the bone to the bioreactor. *Trends Biotechnol* **21**, 233–240.
- Canestri, A., Lescure, F.-X., Jaureguiberry, S., Moulignier, A., Amiel, C., Marcelin, A. G., Peytavin, G., Tubiana, R., Pialoux, G. & Katlama, C. (2010). Discordance between cerebral spinal fluid and plasma HIV replication in patients with neurological symptoms who are receiving suppressive antiretroviral therapy. *Clin Infect Dis* **50**, 773–778.
- Carter, C. C., Onafuwa-Nuga, A., McNamara, L. A., Riddell, J., IV, Bixby, D., Savona, M. R. & Collins, K. L. (2010). HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. *Nat Med* **16**, 446–451.
- Carter, C. C., McNamara, L. A., Onafuwa-Nuga, A., Shackleton, M., Riddell, J., IV, Bixby, D., Savona, M. R., Morrison, S. J. & Collins, K. L. (2011). HIV-1 utilizes the CXCR4 chemokine receptor to infect multipotent hematopoietic stem and progenitor cells. *Cell Host Microbe* **9**, 223–234.
- Chahroudi, A., Bosinger, S. E., Vanderford, T. H., Paiardini, M. & Silvestri, G. (2012). Natural SIV hosts: showing AIDS the door. *Science* **335**, 1188–1193.
- Chan, C. N., McMonagle, E. L., Hosie, M. J. & Willett, B. J. (2013). Prostratin exhibits both replication enhancing and inhibiting effects on FIV infection of feline CD4⁺ T-cells. *Virus Res* **171**, 121–128.
- Chen, L. F., Mu, Y. & Greene, W. C. (2002). Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF- κ B. *EMBO J* **21**, 6539–6548.
- Chiang, K. & Rice, A. P. (2012). MicroRNA-mediated restriction of HIV-1 in resting CD4⁺ T cells and monocytes. *Viruses* **4**, 1390–1409.
- Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. D., Wu, L., Mackay, C. R., LaRosa, G. & other authors (1996). The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85**, 1135–1148.
- Chomont, N., El-Far, M., Ancuta, P., Trautmann, L., Procopio, F. A., Yassine-Diab, B., Boucher, G., Boulassel, M.-R., Ghattas, G. & other authors (2009). HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med* **15**, 893–900.
- Choudhary, S. K., Rezk, N. L., Ince, W. L., Cheema, M., Zhang, L., Su, L., Swanstrom, R., Kashuba, A. D. & Margolis, D. M. (2009). Suppression of human immunodeficiency virus type 1 (HIV-1) viremia with reverse transcriptase and integrase inhibitors, CD4⁺

- T-cell recovery, and viral rebound upon interruption of therapy in a new model for HIV treatment in the humanized Rag2^{-/-}γc^{-/-} mouse. *J Virol* **83**, 8254–8258.
- Choudhary, S. K., Archin, N. M., Cheema, M., Dahl, N. P., Garcia, J. V. & Margolis, D. M. (2012).** Latent HIV-1 infection of resting CD4⁺ T cells in the humanized Rag2^{-/-}γc^{-/-} mouse. *J Virol* **86**, 114–120.
- Chun, T. W., Finzi, D., Margolick, J., Chadwick, K., Schwartz, D. & Siliciano, R. F. (1995).** In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nat Med* **1**, 1284–1290.
- Chun, T. W., Carruth, L., Finzi, D., Shen, X., DiGiuseppe, J. A., Taylor, H., Hermankova, M., Chadwick, K., Margolick, J. & other authors (1997).** Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* **387**, 183–188.
- Chun, T. W., Engel, D., Mizell, S. B., Ehler, L. A. & Fauci, A. S. (1998).** Induction of HIV-1 replication in latently infected CD4⁺ T cells using a combination of cytokines. *J Exp Med* **188**, 83–91.
- Chun, T. W., Davey, R. T., Jr, Engel, D., Lane, H. C. & Fauci, A. S. (1999).** Re-emergence of HIV after stopping therapy. *Nature* **401**, 874–875.
- Chun, T. W., Nickle, D. C., Justement, J. S., Large, D., Semerjian, A., Curlin, M. E., O'Shea, M. A., Hallahan, C. W., Daucher, M. & other authors (2005).** HIV-infected individuals receiving effective antiviral therapy for extended periods of time continually replenish their viral reservoir. *J Clin Invest* **115**, 3250–3255.
- Chun, T.-W., Nickle, D. C., Justement, J. S., Meyers, J. H., Roby, G., Hallahan, C. W., Kottilli, S., Moir, S., Mican, J. M. & other authors (2008).** Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy. *J Infect Dis* **197**, 714–720.
- Churchill, M. J., Gorry, P. R., Cowley, D., Lal, L., Sonza, S., Purcell, D. F. J., Thompson, K. A., Gabuzda, D., McArthur, J. C. & other authors (2006).** Use of laser capture microdissection to detect integrated HIV-1 DNA in macrophages and astrocytes from autopsy brain tissues. *J Neurovirol* **12**, 146–152.
- Cicala, C., Arthos, J., Censoplano, N., Cruz, C., Chung, E., Martinelli, E., Lempicki, R. A., Natarajan, V., VanRyk, D. & other authors (2006).** HIV-1 gp120 induces NFAT nuclear translocation in resting CD4⁺ T-cells. *Virology* **345**, 105–114.
- Cillo, A., Krishnan, A., Mitsuyasu, R., McMahon, D., Li, S., Rossi, J., Zaia, J. & Mellors, J. W. (2012).** Plasma viremia and cellular HIV-1 DNA persist despite autologous hematopoietic stem cell transplantation for AIDS-related lymphoma. In *CROI*. Seattle, USA.
- Coffer, P. J. & Burgering, B. M. (2004).** Forkhead-box transcription factors and their role in the immune system. *Nat Rev Immunol* **4**, 889–899.
- Coiras, M., López-Huertas, M. R., Pérez-Olmeda, M. & Alcamí, J. (2009).** Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. *Nat Rev Microbiol* **7**, 798–812.
- Coleman, C. M. & Wu, L. (2009).** HIV interactions with monocytes and dendritic cells: viral latency and reservoirs. *Retrovirology* **6**, 51.
- Colin, L. & Van Lint, C. (2009).** Molecular control of HIV-1 postintegration latency: implications for the development of new therapeutic strategies. *Retrovirology* **6**, 111.
- Connor, R. I., Sheridan, K. E., Ceradini, D., Choe, S. & Landau, N. R. (1997).** Change in coreceptor use correlates with disease progression in HIV-1-infected individuals. *J Exp Med* **185**, 621–628.
- Contreras, X., Schwenecker, M., Chen, C.-S., McCune, J. M., Deeks, S. G., Martin, J. & Peterlin, B. M. (2009).** Suberoylanilide hydroxamic acid reactivates HIV from latently infected cells. *J Biol Chem* **284**, 6782–6789.
- Coull, J. J., Romero, F., Sun, J. M., Volker, J. L., Galvin, K. M., Davie, J. R., Shi, Y., Hansen, U. & Margolis, D. M. (2000).** The human factors YY1 and LSF repress the human immunodeficiency virus type 1 long terminal repeat via recruitment of histone deacetylase 1. *J Virol* **74**, 6790–6799.
- d'Arminio, A., Sabin, C. A., Phillips, A. N., Reiss, P., Weber, R., Kirk, O., El-Sadr, W., De Wit, S., Mateu, S. & other authors (2004).** Cardio- and cerebrovascular events in HIV-infected persons. *AIDS* **18**, 1811–1817.
- Dalgleish, A. G., Beverley, P. C., Clapham, P. R., Crawford, D. H., Greaves, M. F. & Weiss, R. A. (1984).** The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312**, 763–767.
- Davey, R. T., Jr, Bhat, N., Yoder, C., Chun, T. W., Metcalf, J. A., Dewar, R., Natarajan, V., Lempicki, R. A., Adelsberger, J. W. & other authors (1999).** HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. *Proc Natl Acad Sci U S A* **96**, 15109–15114.
- Davis, B. R., Schwartz, D. H., Marx, J. C., Johnson, C. E., Berry, J. M., Lyding, J., Merigan, T. C. & Zander, A. (1991).** Absent or rare human immunodeficiency virus infection of bone marrow stem/progenitor cells in vivo. *J Virol* **65**, 1985–1990.
- Dean, M., Carrington, M., Winkler, C., Huttley, G. A., Smith, M. W., Allikmets, R., Goedert, J. J., Buchbinder, S. P., Vittinghoff, E. & other authors (1996).** Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. *Science* **273**, 1856–1862.
- Deeks, S. G. (2011).** HIV infection, inflammation, immunosenescence, and aging. *Annu Rev Med* **62**, 141–155. .
- Deeks, S. G., Autran, B., Berkhout, B., Benkirane, M., Cairns, S., Chomont, N., Chun, T. W., Churchill, M., Di Mascio, M. & other authors (2012).** Towards an HIV cure: a global scientific strategy. *Nat Rev Immunol* **12**, 607–614.
- Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhardt, M., Di Marzio, P., Marmon, S., Sutton, R. E. & other authors (1996).** Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381**, 661–666.
- Descours, B., Cribier, A., Chable-Bessia, C., Ayinde, D., Rice, G., Crow, Y., Yatim, A., Schwartz, O., Laguette, N. & Benkirane, M. (2012).** SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4⁺ T-cells. *Retrovirology* **9**, 87.
- Di Mascio, M., Srinivasula, S., Bhattacharjee, A., Cheng, L., Martiniova, L., Herscovitch, P., Lertora, J. & Kiesewetter, D. (2009).** Antiretroviral tissue kinetics: in vivo imaging using positron emission tomography. *Antimicrob Agents Chemother* **53**, 4086–4095.
- Dinosa, J. B., Rabi, S. A., Blankson, J. N., Gama, L., Mankowski, J. L., Siliciano, R. F., Zink, M. C. & Clements, J. E. (2009a).** A simian immunodeficiency virus-infected macaque model to study viral reservoirs that persist during highly active antiretroviral therapy. *J Virol* **83**, 9247–9257.
- Dinosa, J. B., Kim, S. Y., Wiegand, A. M., Palmer, S. E., Gange, S. J., Cranmer, L., O'Shea, A., Callender, M., Spivak, A. & other authors (2009b).** Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc Natl Acad Sci U S A* **106**, 9403–9408.
- Doek, D. C., Roederer, M. & Koup, R. A. (2009).** Emerging concepts in the immunopathogenesis of AIDS. *Annu Rev Med* **60**, 471–484.
- Durand, C. M., Ghiaur, G., Siliciano, J. D., Rabi, S. A., Eisele, E. E., Salgado, M., Shan, L., Lai, J. F., Zhang, H. & other authors (2012).** HIV-1 DNA is detected in bone marrow populations containing CD4⁺ T cells but is not found in purified CD34⁺ hematopoietic progenitor cells in most patients on antiretroviral therapy. *J Infect Dis* **205**, 1014–1018.
- Egberink, H. F., Keldermans, C. E., Koolen, M. J. & Horzinek, M. C. (1992).** Humoral immune response to feline immunodeficiency virus

- in cats with experimentally induced and naturally acquired infections. *Am J Vet Res* 53, 1133–1138.
- Eichbaum, Q. (2011).** PD-1 signaling in HIV and chronic viral infection—potential for therapeutic intervention? *Curr Med Chem* 18, 3971–3980.
- Eisele, E. & Siliciano, R. F. (2012).** Redefining the viral reservoirs that prevent HIV-1 eradication. *Immunity* 37, 377–388.
- Else, L. J., Taylor, S., Back, D. J. & Khoo, S. H. (2011).** Pharmacokinetics of antiretroviral drugs in anatomical sanctuary sites: the male and female genital tract. *Antivir Ther* 16, 1149–1167.
- Feng, Y., Broder, C. C., Kennedy, P. E. & Berger, E. A. (1996).** HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 272, 872–877.
- Filippakopoulos, P., Qi, J., Picaud, S., Shen, Y., Smith, W. B., Fedorov, O., Morse, E. M., Keates, T., Hickman, T. T. & other authors (2010).** Selective inhibition of BET bromodomains. *Nature* 468, 1067–1073.
- Finzi, D., Hermankova, M., Pierson, T., Carruth, L. M., Buck, C., Chaisson, R. E., Quinn, T. C., Chadwick, K., Margolick, J. & other authors (1997).** Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278, 1295–1300.
- Flynn, J. N., Dunham, S., Mueller, A., Cannon, C. & Jarrett, O. (2002).** Involvement of cytolytic and non-cytolytic T cells in the control of feline immunodeficiency virus infection. *Vet Immunol Immunopathol* 85, 159–170.
- Folks, T. M., Justement, J., Kinter, A., Dinarello, C. A. & Fauci, A. S. (1987).** Cytokine-induced expression of HIV-1 in a chronically infected promonocyte cell line. *Science* 238, 800–802.
- Folks, T. M., Kessler, S. W., Orenstein, J. M., Justement, J. S., Jaffe, E. S. & Fauci, A. S. (1988).** Infection and replication of HIV-1 in purified progenitor cells of normal human bone marrow. *Science* 242, 919–922.
- Folks, T. M., Clouse, K. A., Justement, J., Rabson, A., Duh, E., Kehrl, J. H. & Fauci, A. S. (1989).** Tumor necrosis factor alpha induces expression of human immunodeficiency virus in a chronically infected T-cell clone. *Proc Natl Acad Sci U S A* 86, 2365–2368.
- Gabriel, R., Lombardo, A., Arens, A., Miller, J. C., Genovese, P., Kaepfel, C., Nowrouzi, A., Bartholomae, C. C., Wang, J. & other authors (2011).** An unbiased genome-wide analysis of zinc-finger nuclease specificity. *Nat Biotechnol* 29, 816–823.
- Gandhi, R. T., Zheng, L., Bosch, R. J., Chan, E. S., Margolis, D. M., Read, S., Kallungal, B., Palmer, S., Medvik, K. & other authors (2010).** The effect of Raltegravir intensification on low-level residual viremia in HIV-infected patients on antiretroviral therapy: a randomized controlled trial. *PLoS Med* 7, e1000321.
- Gartner, S., Markovits, P., Markovitz, D. M., Kaplan, M. H., Gallo, R. C. & Popovic, M. (1986).** The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* 233, 215–219.
- Ghose, R., Liou, L. Y., Herrmann, C. H. & Rice, A. P. (2001).** Induction of TAK (cyclin T1/P-TEFb) in purified resting CD4⁺ T lymphocytes by combination of cytokines. *J Virol* 75, 11336–11343.
- Goldman, J. P., Blundell, M. P., Lopes, L., Kinnon, C., Di Santo, J. P. & Thrasher, A. J. (1998).** Enhanced human cell engraftment in mice deficient in RAG2 and the common cytokine receptor gamma chain. *Br J Haematol* 103, 335–342.
- González-Scarano, F. & Martín-García, J. (2005).** The neuropathogenesis of AIDS. *Nat Rev Immunol* 5, 69–81.
- Greger, I. H., Demarchi, F., Giacca, M. & Proudfoot, N. J. (1998).** Transcriptional interference perturbs the binding of Sp1 to the HIV-1 promoter. *Nucleic Acids Res* 26, 1294–1301.
- Gutiérrez, C., Díaz, L., Vallejo, A., Hernández-Novoa, B., Abad, M., Madrid, N., Dahl, V., Rubio, R., Moreno, A. M. & other authors (2011).** Intensification of antiretroviral therapy with a CCR5 antagonist in patients with chronic HIV-1 infection: effect on T cells latently infected. *PLoS ONE* 6, e27864.
- Haaland, R. E., Yu, W. & Rice, A. P. (2005).** Identification of LKLF-regulated genes in quiescent CD4⁺ T lymphocytes. *Mol Immunol* 42, 627–641.
- Halfon, P., Giorgetti, C., Khiri, H., Pénaranda, G., Terriou, P., Porcu-Buisson, G. & Chabert-Orsini, V. (2010).** Semen may harbor HIV despite effective HAART: another piece in the puzzle. *PLoS ONE* 5, e10569.
- Han, Y., Lassen, K., Monie, D., Sedaghat, A. R., Shimoji, S., Liu, X., Pierson, T. C., Margolick, J. B., Siliciano, R. F. & Siliciano, J. D. (2004).** Resting CD4⁺ T cells from human immunodeficiency virus type 1 (HIV-1)-infected individuals carry integrated HIV-1 genomes within actively transcribed host genes. *J Virol* 78, 6122–6133.
- Han, Y., Wind-Rotolo, M., Yang, H. C., Siliciano, J. D. & Siliciano, R. F. (2007).** Experimental approaches to the study of HIV-1 latency. *Nat Rev Microbiol* 5, 95–106.
- Han, Y., Lin, Y. B., An, W., Xu, J., Yang, H. C., O'Connell, K., Dordai, D., Boeke, J. D., Siliciano, J. D. & Siliciano, R. F. (2008).** Orientation-dependent regulation of integrated HIV-1 expression by host gene transcriptional readthrough. *Cell Host Microbe* 4, 134–146.
- Hatano, H., Hayes, T. L., Dahl, V., Sinclair, E., Lee, T. H., Hoh, R., Lampiris, H., Hunt, P. W., Palmer, S. & other authors (2011).** A randomized, controlled trial of Raltegravir intensification in antiretroviral-treated, HIV-infected patients with a suboptimal CD4⁺ T cell response. *J Infect Dis* 203, 960–968.
- Hecht, R., Stover, J., Bollinger, L., Muhib, F., Case, K. & de Ferranti, D. (2010).** Financing of HIV/AIDS programme scale-up in low-income and middle-income countries, 2009–31. *Lancet* 376, 1254–1260.
- Hermankova, M., Siliciano, J. D., Zhou, Y., Monie, D., Chadwick, K., Margolick, J. B., Quinn, T. C. & Siliciano, R. F. (2003).** Analysis of human immunodeficiency virus type 1 gene expression in latently infected resting CD4⁺ T lymphocytes in vivo. *J Virol* 77, 7383–7392.
- Hesselton, R. M., Greiner, D. L., Mordes, J. P., Rajan, T. V., Sullivan, J. L. & Shultz, L. D. (1995).** High levels of human peripheral blood mononuclear cell engraftment and enhanced susceptibility to human immunodeficiency virus type 1 infection in NOD/LtSz-scid/scid mice. *J Infect Dis* 172, 974–982.
- Hirsch, V. M., Fuerst, T. R., Sutter, G., Carroll, M. W., Yang, L. C., Goldstein, S., Piatak, M., Jr, Elkins, W. R., Alvord, W. G. & other authors (1996).** Patterns of viral replication correlate with outcome in simian immunodeficiency virus (SIV)-infected macaques: effect of prior immunization with a trivalent SIV vaccine in modified vaccinia virus Ankara. *J Virol* 70, 3741–3752.
- Hirsch, V. M., Lifson, J. D. & Kuah-Teh, J. (2000).** Simian immunodeficiency virus infection of monkeys as a model system for the study of AIDS pathogenesis, treatment, and prevention. In *Advances in Pharmacology*, vol. 49, pp. 437–477. Academic Press.
- Ho, D. D., Rota, T. R. & Hirsch, M. S. (1986).** Infection of monocyte/macrophages by human T lymphotropic virus type III. *J Clin Invest* 77, 1712–1715.
- Ho, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M. & Markowitz, M. (1995).** Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 373, 123–126.
- Holt, N., Wang, J., Kim, K., Friedmann, G., Wang, X., Taupin, V., Crooks, G. M., Kohn, D. B., Gregory, P. D. & other authors (2010).** Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. *Nat Biotechnol* 28, 839–847.

- Hosie, M. J., Pajek, D., Samman, A. & Willett, B. J. (2011). Feline immunodeficiency virus (FIV) neutralization: a review. *Viruses* 3, 1870–1890.
- Howard, K. E., Reckling, S. K., Egan, E. A. & Dean, G. A. (2010). Acute mucosal pathogenesis of feline immunodeficiency virus is independent of viral dose in vaginally infected cats. *Retrovirology* 7, 2.
- Hütter, G., Nowak, D., Mossner, M., Ganepola, S., Müssig, A., Allers, K., Schneider, T., Hofmann, J., Kücherer, C. & other authors (2009). Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med* 360, 692–698.
- Joos, B., Fischer, M., Kuster, H., Pillai, S. K., Wong, J. K., Böni, J., Hirschel, B., Weber, R., Trkola, A., Günthard, H. F. & Swiss HIV Cohort Study (2008). HIV rebounds from latently infected cells, rather than from continuing low-level replication. *Proc Natl Acad Sci U S A* 105, 16725–16730.
- Jordan, A., Bisgrove, D. & Verdin, E. (2003). HIV reproducibly establishes a latent infection after acute infection of T cells in vitro. *EMBO J* 22, 1868–1877.
- Josefsson, L., Eriksson, S., Sinclair, E., Ho, T., Killian, M., Epling, L., Shao, W., Lewis, B., Bacchetti, P. & other authors (2012). Hematopoietic precursor cells isolated from patients on long-term suppressive HIV therapy did not contain HIV-1 DNA. *J Infect Dis* 206, 28–34.
- Joshi, A., Vahlenkamp, T. W., Garg, H., Tompkins, W. A. F. & Tompkins, M. B. (2004). Preferential replication of FIV in activated CD4⁺CD25⁺T cells independent of cellular proliferation. *Virology* 321, 307–322.
- Joshi, A., Garg, H., Tompkins, M. B. & Tompkins, W. A. (2005). Different thresholds of T cell activation regulate FIV infection of CD4⁺CD25⁺ and CD4⁺CD25⁻ cells. *Virology* 335, 212–221.
- June, C., Pablo, T., Stein, D., Mitsuyasu, R., Lalezari, J., Wang, S., Lee, G. & Levine, B., Tang, W. & other authors (2012). Induction of acquired CCR5 deficiency with zinc finger nuclease-modified autologous CD4 T cells (SB-728-T) correlates with increases in CD4 count and effects on viral load in HIV-infected subjects. In *19th Conference on Retroviruses and Opportunistic Infections (CROI)*.
- Kauder, S. E., Bosque, A., Lindqvist, A., Planelles, V. & Verdin, E. (2009). Epigenetic regulation of HIV-1 latency by cytosine methylation. *PLoS Pathog* 5, e1000495.
- Kerr, J. S., Galloway, S., Lagrutta, A., Armstrong, M., Miller, T., Richon, V. M. & Andrews, P. A. (2010). Nonclinical safety assessment of the histone deacetylase inhibitor Vorinostat. *Int J Toxicol* 29, 3–19.
- Kornfeld, H., Cruikshank, W. W., Pyle, S. W., Berman, J. S. & Center, D. M. (1988). Lymphocyte activation by HIV-1 envelope glycoprotein. *Nature* 335, 445–448.
- Kraase, M., Sloan, R., Klein, D., Logan, N., McMonagle, L., Biek, R., Willett, B. J. & Hosie, M. J. (2010). Feline immunodeficiency virus env gene evolution in experimentally infected cats. *Vet Immunol Immunopathol* 134, 96–106.
- Kulkosky, J., Culnan, D. M., Roman, J., Dornadula, G., Schnell, M., Boyd, M. R. & Pomerantz, R. J. (2001). Prostratin: activation of latent HIV-1 expression suggests a potential inductive adjuvant therapy for HAART. *Blood* 98, 3006–3015.
- Kumar, P., Ban, H.-S., Kim, S.-S., Wu, H., Pearson, T., Greiner, D. L., Laouar, A., Yao, J., Haridas, V. & other authors (2008). T cell-specific siRNA delivery suppresses HIV-1 infection in humanized mice. *Cell* 134, 577–586.
- Kwara, A., Delong, A., Rezk, N., Hogan, J., Burtwell, H., Chapman, S., Moreira, C. C., Kurpewski, J., Ingersoll, J. & other authors (2008). Antiretroviral drug concentrations and HIV RNA in the genital tract of HIV-infected women receiving long-term highly active antiretroviral therapy. *Clin Infect Dis* 46, 719–725.
- Kwon, H. S., Brent, M. M., Getachew, R., Jayakumar, P., Chen, L. F., Scholzer, M., McBurney, M. W., Marmorstein, R., Greene, W. C. & Ott, M. (2008). Human immunodeficiency virus type 1 Tat protein inhibits the SIRT1 deacetylase and induces T cell hyperactivation. *Cell Host Microbe* 3, 158–167.
- Lassen, K. G., Bailey, J. R. & Siliciano, R. F. (2004). Analysis of human immunodeficiency virus type 1 transcriptional elongation in resting CD4⁺ T cells in vivo. *J Virol* 78, 9105–9114.
- Lassen, K. G., Ramyar, K. X., Bailey, J. R., Zhou, Y. & Siliciano, R. F. (2006). Nuclear retention of multiply spliced HIV-1 RNA in resting CD4⁺ T cells. *PLoS Pathog* 2, e68.
- Launay, O., Tod, M., Tschöpe, I., Si-Mohamed, A., Bélarbi, L., Charpentier, C., Goujard, C., Taburet, A. M., Lortholary, O. & other authors (2011). Residual HIV-1 RNA and HIV-1 DNA production in the genital tract reservoir of women treated with HAART: the prospective ANRS EP24 GYNODYN study. *Antivir Ther* 16, 843–852.
- Le Douce, V., Herbein, G., Rohr, O. & Schwartz, C. (2010). Molecular mechanisms of HIV-1 persistence in the monocyte-macrophage lineage. *Retrovirology* 7, 32.
- Levy, Y., Lacabartz, C., Weiss, L., Viard, J. P., Goujard, C., Lelièvre, J. D., Boué, F., Molina, J. M., Rouzioux, C. & other authors (2009). Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. *J Clin Invest* 119, 997–1007.
- Levy, Y., Sereti, I., Tambussi, G., Routy, J. P., Lelievre, J. D., Delfraissy, J. F., Molina, J. M., Fischl, M., Goujard, C. & other authors (2012). Effects of recombinant human interleukin 7 on T-cell recovery and thymic output in HIV-infected patients receiving antiretroviral therapy: results of a phase I/IIa randomized, placebo-controlled, multicenter study. *Clin Infect Dis* 55, 291–300.
- Lewin, S. R., Evans, V. A., Elliott, J. H., Spire, B. & Chomont, N. (2011). Finding a cure for HIV: will it ever be achievable? *J Int AIDS Soc* 14, 4.
- Li, Z., Guo, J., Wu, Y. & Zhou, Q. (2013). The BET bromodomain inhibitor JQ1 activates HIV latency through antagonizing Brd4 inhibition of Tat-transactivation. *Nucleic Acids Res* 41, 277–287.
- Lusic, M., Marcello, A., Cereseto, A. & Giacca, M. (2003). Regulation of HIV-1 gene expression by histone acetylation and factor recruitment at the LTR promoter. *EMBO J* 22, 6550–6561.
- Mansky, L. M. & Temin, H. M. (1995). Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. *J Virol* 69, 5087–5094.
- Marcello, A. (2006). Latency: the hidden HIV-1 challenge. *Retrovirology* 3, 7.
- Margolis, L. & Shattock, R. (2006). Selective transmission of CCR5-utilizing HIV-1: the ‘gatekeeper’ problem resolved? *Nat Rev Microbiol* 4, 312–317.
- Marini, A., Harper, J. M. & Romerio, F. (2008). An in vitro system to model the establishment and reactivation of HIV-1 latency. *J Immunol* 181, 7713–7720.
- Marsden, M. D. & Zack, J. A. (2009). Eradication of HIV: current challenges and new directions. *J Antimicrob Chemother* 63, 7–10.
- Mbonye, U. & Karn, J. (2011). Control of HIV latency by epigenetic and non-epigenetic mechanisms. *Curr HIV Res* 9, 554–567.
- McDonnel, S. J., Sparger, E. E., Luciw, P. A. & Murphy, B. G. (2012). Pharmacologic reactivation of latent feline immunodeficiency virus ex vivo in peripheral CD4⁺ T-lymphocytes. *Virus Res* 170, 174–179.
- McMahon, D., Jones, J., Wiegand, A., Gange, S. J., Kearney, M., Palmer, S., McNulty, S., Metcalf, J. A., Acosta, E. & other authors (2010). Short-course raltegravir intensification does not reduce persistent low-level viremia in patients with HIV-1 suppression

- during receipt of combination antiretroviral therapy. *Clin Infect Dis* 50, 912–919.
- McNamara, L. A. & Collins, K. L. (2011). Hematopoietic stem/precursor cells as HIV reservoirs. *Curr Opin HIV AIDS* 6, 43–48.
- Middleton, T., Lim, H. B., Montgomery, D., Rockway, T., Tang, H., Cheng, X. H., Lu, L. J., Mo, H. M., Kohlbrenner, W. E. & Molla, A. (2004). Inhibition of human immunodeficiency virus type I integrase by naphthamides and 2-aminobenzimidazoles. *Antiviral Res* 64, 35–45.
- Mowat, A. M. & Viney, J. L. (1997). The anatomical basis of intestinal immunity. *Immunol Rev* 156, 145–166.
- Murphy, B., Vapniarsky, N., Hillman, C., Castillo, D., McDonnell, S., Moore, P., Luciw, P. A. & Sparger, E. E. (2012). FIV establishes a latent infection in feline peripheral blood CD4⁺ T lymphocytes in vivo during the asymptomatic phase of infection. *Retrovirology* 9, 12.
- Neal, T. F., Holland, H. K., Baum, C. M., Villinger, F., Ansari, A. A., Saral, R., Wingard, J. R. & Fleming, W. H. (1995). CD34⁺ progenitor cells from asymptomatic patients are not a major reservoir for human immunodeficiency virus-1. *Blood* 86, 1749–1756.
- Nicholson, J. K. A., Cross, G. D., Callaway, C. S. & McDougal, J. S. (1986). In vitro infection of human monocytes with human T lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV). *J Immunol* 137, 323–329.
- North, T. W., Higgins, J., Deere, J. D., Hayes, T. L., Villalobos, A., Adamson, L., Shacklett, B. L., Schinazi, R. F. & Luciw, P. A. (2010). Viral sanctuaries during highly active antiretroviral therapy in a nonhuman primate model for AIDS. *J Virol* 84, 2913–2922.
- Novotny, C., English, R. V., Housman, J., Davidson, M. G., Nasisse, M. P., Jeng, C. R., Davis, W. C. & Tompkins, M. B. (1990). Lymphocyte population changes in cats naturally infected with feline immunodeficiency virus. *AIDS* 4, 1213–1218.
- O'Brien, S. J. & Moore, J. P. (2000). The effect of genetic variation in chemokines and their receptors on HIV transmission and progression to AIDS. *Immunol Rev* 177, 99–111.
- O'Doherty, U., Swiggard, W. J. & Malim, M. H. (2000). Human immunodeficiency virus type 1 spinoculation enhances infection through virus binding. *J Virol* 74, 10074–10080.
- Ostrowski, M. A., Chun, T. W., Justement, S. J., Motola, I., Spinelli, M. A., Adelsberger, J., Ehler, L. A., Mizell, S. B., Hallahan, C. W. & Fauci, A. S. (1999). Both memory and CD45RA⁺/CD62L⁺ naive CD4⁺ T cells are infected in human immunodeficiency virus type 1-infected individuals. *J Virol* 73, 6430–6435.
- Oswald-Richter, K., Grill, S. M., Leelawong, M. & Unutmaz, D. (2004). HIV infection of primary human T cells is determined by tunable thresholds of T cell activation. *Eur J Immunol* 34, 1705–1714.
- Pace, M. J., Agosto, L., Graf, E. H. & O'Doherty, U. (2011). HIV reservoirs and latency models. *Virology* 411, 344–354.
- Palmer, S., Maldarelli, F., Wiegand, A., Bernstein, B., Hanna, G. J., Brun, S. C., Kempf, D. J., Mellors, J. W., Coffin, J. M. & King, M. S. (2008). Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci U S A* 105, 3879–3884.
- Palmer, S., Josefsson, L. & Coffin, J. M. (2011). HIV reservoirs and the possibility of a cure for HIV infection. *J Intern Med* 270, 550–560.
- Parada, C. A. & Roeder, R. G. (1996). Enhanced processivity of RNA polymerase II triggered by Tat-induced phosphorylation of its carboxy-terminal domain. *Nature* 384, 375–378.
- Pattanayak, V., Ramirez, C. L., Joung, J. K. & Liu, D. R. (2011). Revealing off-target cleavage specificities of zinc-finger nucleases by in vitro selection. *Nat Methods* 8, 765–770.
- Pedersen, N. C., Ho, E. W., Brown, M. L. & Yamamoto, J. K. (1987). Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* 235, 790–793.
- Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R. & Langer, R. (2007). Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol* 2, 751–760.
- Perez, E. E., Wang, J., Miller, J. C., Jouvenot, Y., Kim, K. A., Liu, O., Wang, N., Lee, G., Bartsevich, V. V. & other authors (2008). Establishment of HIV-1 resistance in CD4⁺ T cells by genome editing using zinc-finger nucleases. *Nat Biotechnol* 26, 808–816.
- Pérez-Martínez, D., Tanaka, T. & Rabbitts, T. H. (2010). Intracellular antibodies and cancer: new technologies offer therapeutic opportunities. *Bioessays* 32, 589–598.
- Pierson, T., Hoffman, T. L., Blankson, J., Finzi, D., Chadwick, K., Margolick, J. B., Buck, C., Siliciano, J. D., Doms, R. W. & Siliciano, R. F. (2000). Characterization of chemokine receptor utilization of viruses in the latent reservoir for human immunodeficiency virus type 1. *J Virol* 74, 7824–7833.
- Pierson, T. C., Zhou, Y., Kieffer, T. L., Ruff, C. T., Buck, C. & Siliciano, R. F. (2002). Molecular characterization of preintegration latency in human immunodeficiency virus type 1 infection. *J Virol* 76, 8518–8531.
- Prins, J. M., Jurriaans, S., van Praag, R. M., Blaak, H., van Rij, R., Schellekens, P. T., ten Berge, I. J., Yong, S. L., Fox, C. H. & other authors (1999). Immuno-activation with anti-CD3 and recombinant human IL-2 in HIV-1-infected patients on potent antiretroviral therapy. *AIDS* 13, 2405–2410.
- Ramratnam, B., Mittler, J. E., Zhang, L., Boden, D., Hurley, A., Fang, F., Macken, C. A., Perelson, A. S., Markowitz, M. & Ho, D. D. (2000). The decay of the latent reservoir of replication-competent HIV-1 is inversely correlated with the extent of residual viral replication during prolonged anti-retroviral therapy. *Nat Med* 6, 82–85.
- Reuse, S., Calao, M., Kabeya, K., Guiguen, A., Gatot, J. S., Quivy, V., Vanhulle, C., Lamine, A., Vaira, D. & other authors (2009). Synergistic activation of HIV-1 expression by deacetylase inhibitors and prostratin: implications for treatment of latent infection. *PLoS ONE* 4, e6093.
- Richman, D. D., Margolis, D. M., Delaney, M., Greene, W. C., Hazuda, D. & Pomerantz, R. J. (2009). The challenge of finding a cure for HIV infection. *Science* 323, 1304–1307.
- Roesch, F., Mezziane, O., Kula, A., Nisole, S., Porrot, F., Anderson, I., Mammano, F., Fassati, A., Marcello, A. & other authors (2012). Hyperthermia stimulates HIV-1 replication. *PLoS Pathog* 8, e1002792.
- Rossi, M. I., Medina, K. L., Garrett, K., Kolar, G., Comp, P. C., Shultz, L. D., Capra, J. D., Wilson, P., Schipul, A. & Kincade, P. W. (2001). Relatively normal human lymphopoiesis but rapid turnover of newly formed B cells in transplanted nonobese diabetic/SCID mice. *J Immunol* 167, 3033–3042.
- Ruff, C. T., Ray, S. C., Kwon, P., Zinn, R., Pendleton, A., Hutton, N., Ashworth, R., Gange, S., Quinn, T. C. & other authors (2002). Persistence of wild-type virus and lack of temporal structure in the latent reservoir for human immunodeficiency virus type 1 in pediatric patients with extensive antiretroviral exposure. *J Virol* 76, 9481–9492.
- Rullas, J., Bermejo, M., García-Pérez, J., Beltán, M., González, N., Hezareh, M., Brown, S. J. & Alcamí, J. (2004). Prostratin induces HIV activation and downregulates HIV receptors in peripheral blood lymphocytes. *Antivir Ther* 9, 545–554.
- Sahu, G. K., Lee, K., Ji, J. X., Braciale, V., Baron, S. & Cloyd, M. W. (2006). A novel in vitro system to generate and study latently HIV-1-infected long-lived normal CD4⁺ T-lymphocytes. *Virology* 355, 127–137.

- Sahu, G. K., Paar, D., Frost, S. D. W., Smith, M. M., Weaver, S. & Cloyd, M. W. (2009). Low-level plasma HIVs in patients on prolonged suppressive highly active antiretroviral therapy are produced mostly by cells other than CD4⁺ T-cells. *J Med Virol* **81**, 9–15.
- Saleh, S., Solomon, A., Wightman, F., Xhilara, M., Cameron, P. U. & Lewin, S. R. (2007). CCR7 ligands CCL19 and CCL21 increase permissiveness of resting memory CD4⁺ T cells to HIV-1 infection: a novel model of HIV-1 latency. *Blood* **110**, 4161–4164.
- Saleh, S., Wightman, F., Ramanayake, S., Alexander, M., Kumar, N., Khoury, G., Pereira, C., Purcell, D., Cameron, P. U. & Lewin, S. R. (2011). Expression and reactivation of HIV in a chemokine induced model of HIV latency in primary resting CD4⁺ T cells. *Retrovirology* **8**, 80.
- Schnittman, S. M., Psallidopoulos, M. C., Lane, H. C., Thompson, L., Baseler, M., Massari, F., Fox, C. H., Salzman, N. P. & Fauci, A. S. (1989). The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. *Science* **245**, 305–308.
- Schröder, A. R., Shinn, P., Chen, H., Berry, C., Ecker, J. R. & Bushman, F. (2002). HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* **110**, 521–529.
- Scripture-Adams, D. D., Brooks, D. G., Korin, Y. D. & Zack, J. A. (2002). Interleukin-7 induces expression of latent human immunodeficiency virus type 1 with minimal effects on T-cell phenotype. *J Virol* **76**, 13077–13082.
- Shan, L., Deng, K., Shroff, N. S., Durand, C. M., Rabi, S. A., Yang, H. C., Zhang, H., Margolick, J. B., Blankson, J. N. & Siliciano, R. F. (2012). Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. *Immunity* **36**, 491–501.
- Shen, A. D., Zink, M. C., Mankowski, J. L., Chadwick, K., Margolick, J. B., Carruth, L. M., Li, M., Clements, J. E. & Siliciano, R. F. (2003). Resting CD4⁺ T lymphocytes but not thymocytes provide a latent viral reservoir in a simian immunodeficiency virus-Macaca nemestrina model of human immunodeficiency virus type 1-infected patients on highly active antiretroviral therapy. *J Virol* **77**, 4938–4949.
- Shimojima, M., Miyazawa, T., Ikeda, Y., McMonagle, E. L., Haining, H., Akashi, H., Takeuchi, Y., Hosie, M. J. & Willett, B. J. (2004). Use of CD134 as a primary receptor by the feline immunodeficiency virus. *Science* **303**, 1192–1195.
- Siliciano, R. F. & Greene, W. C. (2011). HIV Latency. Cold Spring Harbor Perspectives in Medicine **1**.
- Siliciano, J. D. & Siliciano, R. F. (2010). Biomarkers of HIV replication. *Curr Opin HIV AIDS* **5**, 491–497.
- Siliciano, J. D., Kajdas, J., Finzi, D., Quinn, T. C., Chadwick, K., Margolick, J. B., Kovacs, C., Gange, S. J. & Siliciano, R. F. (2003). Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4⁺ T cells. *Nat Med* **9**, 727–728.
- Sloan, R. D. & Wainberg, M. A. (2011). The role of unintegrated DNA in HIV infection. *Retrovirology* **8**, 52.
- Stanley, S. K., Kessler, S. W., Justement, J. S., Schnittman, S. M., Greenhouse, J. J., Brown, C. C., Musongela, L., Musey, K., Kapita, B. & Fauci, A. S. (1992). CD34⁺ bone marrow cells are infected with HIV in a subset of seropositive individuals. *J Immunol* **149**, 689–697.
- Stevenson, M., Stanwick, T. L., Dempsey, M. P. & Lamonica, C. A. (1990). HIV-1 replication is controlled at the level of T cell activation and proviral integration. *EMBO J* **9**, 1551–1560.
- Svarovskaia, E. S., Barr, R., Zhang, X. C., Pais, G. C. G., Marchand, C., Pommier, Y., Burke, T. R., Jr & Pathak, V. K. (2004). Azido-containing diketo acid derivatives inhibit human immunodeficiency virus type 1 integrase in vivo and influence the frequency of deletions at two-long-terminal-repeat-circle junctions. *J Virol* **78**, 3210–3222.
- Swan, C. H., Bühler, B., Tschan, M. P., Barbas, C. F. & Torbett, B. E. (2006). T-cell protection and enrichment through lentiviral CCR5 intrabody gene delivery. *Gene Ther* **13**, 1480–1492.
- Tobin, N. H., Learn, G. H., Holte, S. E., Wang, Y., Melvin, A. J., McKernan, J. L., Pawluk, D. M., Mohan, K. M., Lewis, P. F. & other authors (2005). Evidence that low-level viremia during effective highly active antiretroviral therapy result from two processes: expression of archival virus and replication of virus. *J Virol* **79**, 9625–9634.
- Tompkins, M. B. & Tompkins, W. A. (2008). Lentivirus-induced immune dysregulation. *Vet Immunol Immunopathol* **123**, 45–55.
- Traggiai, E., Chicha, L., Mazzucchelli, L., Bronz, L., Piffaretti, J. C., Lanzavecchia, A. & Manz, M. G. (2004). Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science* **304**, 104–107.
- Trautmann, L., Janbazian, L., Chomont, N., Said, E. A., Gimmig, S., Bessette, B., Boulassel, M.-R., Delwart, E., Sepulveda, H. & other authors (2006). Upregulation of PD-1 expression on HIV-specific CD8⁺ T cells leads to reversible immune dysfunction. *Nat Med* **12**, 1198–1202.
- Tyagi, M., Pearson, R. J. & Karn, J. (2010). Establishment of HIV latency in primary CD4⁺ cells is due to epigenetic transcriptional silencing and P-TEFb restriction. *J Virol* **84**, 6425–6437.
- Tzachanis, D., Lafuente, E. M., Li, L. & Bousiotis, V. A. (2004). Intrinsic and extrinsic regulation of T lymphocyte quiescence. *Leuk Lymphoma* **45**, 1959–1967.
- Urnov, F. D., Miller, J. C., Lee, Y.-L., Beausejour, C. M., Rock, J. M., Augustus, S., Jamieson, A. C., Porteus, M. H., Gregory, P. D. & Holmes, M. C. (2005). Highly efficient endogenous human gene correction using designed zinc-finger nucleases. *Nature* **435**, 646–651.
- Van Duyne, R., Pedati, C., Guendel, I., Carpio, L., Kehn-Hall, K., Saifuddin, M. & Kashanchi, F. (2009). The utilization of humanized mouse models for the study of human retroviral infections. *Retrovirology* **6**, 76.
- van Grevenynghe, J., Procopio, F. A., He, Z., Chomont, N., Riou, C., Zhang, Y., Gimmig, S., Boucher, G., Wilkinson, P. & other authors (2008). Transcription factor FOXO3a controls the persistence of memory CD4⁺ T cells during HIV infection. *Nat Med* **14**, 266–274.
- van Leeuwen, E. M. M., Sprent, J. & Surh, C. D. (2009). Generation and maintenance of memory CD4⁺ T cells. *Curr Opin Immunol* **21**, 167–172.
- Van Lint, C., Emiliani, S., Ott, M. & Verdin, E. (1996). Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. *EMBO J* **15**, 1112–1120.
- Vatakis, D. N., Bristol, G., Wilkinson, T. A., Chow, S. A. & Zack, J. A. (2007). Immediate activation fails to rescue efficient human immunodeficiency virus replication in quiescent CD4⁺ T cells. *J Virol* **81**, 3574–3582.
- Vatakis, D. N., Kim, S., Kim, N., Chow, S. A. & Zack, J. A. (2009). Human immunodeficiency virus integration efficiency and site selection in quiescent CD4⁺ T cells. *J Virol* **83**, 6222–6233.
- Vatakis, D. N., Nixon, C. C. & Zack, J. A. (2010). Quiescent T cells and HIV: an unresolved relationship. *Immunol Res* **48**, 110–121.
- Verdin, E., Paras, P., Jr & Van Lint, C. (1993). Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. *EMBO J* **12**, 3249–3259.
- Volberding, P. A. & Deeks, S. G. (2010). Antiretroviral therapy and management of HIV infection. *Lancet* **376**, 49–62.
- Wang, F. X., Xu, Y., Sullivan, J., Souder, E., Argyris, E. G., Acheampong, E. A., Fisher, J., Sierra, M., Thomson, M. M. & other authors (2005). IL-7 is a potent and proviral strain-specific inducer

- of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. *J Clin Invest* **115**, 128–137.
- Weissman, D., Rabin, R. L., Arthos, J., Rubbert, A., Dybul, M., Swofford, R., Venkatesan, S., Farber, J. M. & Fauci, A. S. (1997). Macrophage-tropic HIV and SIV envelope proteins induce a signal through the CCR5 chemokine receptor. *Nature* **389**, 981–985.
- Wightman, F., Solomon, A., Khoury, G., Green, J. A., Gray, L., Gorry, P. R., Ho, Y. S., Saksena, N. K., Hoy, J. & other authors (2010). Both CD31⁺ and CD31⁻ naive CD4⁺ T cells are persistent HIV type 1-infected reservoirs in individuals receiving antiretroviral therapy. *J Infect Dis* **202**, 1738–1748.
- Wightman, F., Ellenberg, P., Churchill, M. & Lewin, S. R. (2012). HDAC inhibitors in HIV. *Immunol Cell Biol* **90**, 47–54.
- Wilen, C. B., Wang, J., Tilton, J. C., Miller, J. C., Kim, K. A., Rebar, E. J., Sherrill-Mix, S. A., Patro, S. C., Secreto, A. J. & other authors (2011). Engineering HIV-resistant human CD4⁺ T cells with CXCR4-specific zinc-finger nucleases. *PLoS Pathog* **7**, e1002020.
- Willett, B. J., Picard, L., Hosie, M. J., Turner, J. D., Adema, K. & Clapham, P. R. (1997). Shared usage of the chemokine receptor CXCR4 by the feline and human immunodeficiency viruses. *J Virol* **71**, 6407–6415.
- Williams, S. A. & Greene, W. C. (2007). Regulation of HIV-1 latency by T-cell activation. *Cytokine* **39**, 63–74.
- Williams, S. A., Kwon, H., Chen, L. F. & Greene, W. C. (2007). Sustained induction of NF- κ B is required for efficient expression of latent human immunodeficiency virus type 1. *J Virol* **81**, 6043–6056.
- Wolschendorf, F., Duverger, A., Jones, J., Wagner, F. H., Huff, J., Benjamin, W. H., Saag, M. S., Niederweis, M. & Kutsch, O. (2010). Hit-and-run stimulation: a novel concept to reactivate latent HIV-1 infection without cytokine gene induction. *J Virol* **84**, 8712–8720.
- Wu, Y. T. & Yoder, A. (2009). Chemokine coreceptor signaling in HIV-1 infection and pathogenesis. *PLoS Pathog* **5**, e1000520.
- Wu, L. J., Paxton, W. A., Kassam, N., Ruffing, N., Rottman, J. B., Sullivan, N., Choe, H., Sodroski, J., Newman, W. & other authors (1997). CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, in vitro. *J Exp Med* **185**, 1681–1692.
- Xing, S., Bullen, C. K., Shroff, N. S., Shan, L., Yang, H. C., Manucci, J. L., Bhat, S., Zhang, H., Margolick, J. B. & other authors (2011). Disulfiram reactivates latent HIV-1 in a Bcl-2-transduced primary CD4⁺ T cell model without inducing global T cell activation. *J Virol* **85**, 6060–6064.
- Xing, S. F., Bhat, S., Shroff, N. S., Zhang, H., Lopez, J. A., Margolick, J. B., Liu, J. O. & Siliciano, R. F. (2012). Novel structurally related compounds reactivate latent HIV-1 in a bcl-2-transduced primary CD4⁺ T cell model without inducing global T cell activation. *J Antimicrob Chemother* **67**, 398–403.
- Yamamoto, J. K., Sparger, E., Ho, E. W., Andersen, P. R., O'Connor, T. P., Mandell, C. P., Lowenstine, L., Munn, R. & Pedersen, N. C. (1988). Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats. *Am J Vet Res* **49**, 1246–1258.
- Yamamoto, J. K., Hansen, H., Ho, E. W., Morishita, T. Y., Okuda, T., Sawa, T. R., Nakamura, R. M. & Pedersen, N. C. (1989). Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission. *J Am Vet Med Assoc* **194**, 213–220.
- Yang, H. C. (2011). Primary cell models of HIV latency. *Curr Opin HIV AIDS* **6**, 62–67.
- Yang, H. C., Xing, S. F., Shan, L., O'Connell, K., Dinoso, J., Shen, A. D., Zhou, Y., Shrum, C. K., Han, Y. F. & other authors (2009). Small-molecule screening using a human primary cell model of HIV latency identifies compounds that reverse latency without cellular activation. *J Clin Invest* **119**, 3473–3486.
- Yeung, F., Hoberg, J. E., Ramsey, C. S., Keller, M. D., Jones, D. R., Frye, R. A. & Mayo, M. W. (2004). Modulation of NF- κ B-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J* **23**, 2369–2380.
- Ylisastigui, L., Archin, N. M., Lehrman, G., Bosch, R. J. & Margolis, D. M. (2004). Coaxing HIV-1 from resting CD4 T cells: histone deacetylase inhibition allows latent viral expression. *AIDS* **18**, 1101–1108.
- Yoder, A., Yu, D. Y., Dong, L., Iyer, S. R., Xu, X. H., Kelly, J., Liu, J., Wang, W. F., Vorster, P. J. & other authors (2008). HIV envelope-CXCR4 signaling activates cofilin to overcome cortical actin restriction in resting CD4 T cells. *Cell* **134**, 782–792.
- Yuan, J., Wang, J., Crain, K., Fearn, C., Kim, K. A., Hua, K. L., Gregory, P. D., Holmes, M. C. & Torbett, B. E. (2012). Zinc-finger nuclease editing of human *cxcr4* promotes HIV-1 CD4⁺ T cell resistance and enrichment. *Mol Ther* **20**, 849–859.
- Yuki, S. A., Shergill, A. K., McQuaid, K., Gianella, S., Lampiris, H., Hare, C. B., Pandori, M., Sinclair, E., Günthard, H. F. & other authors (2010). Effect of Raltegravir-containing intensification on HIV burden and T-cell activation in multiple gut sites of HIV-positive adults on suppressive antiretroviral therapy. *AIDS* **24**, 2451–2460.
- Yusuf, I. & Fruman, D. A. (2003). Regulation of quiescence in lymphocytes. *Trends Immunol* **24**, 380–386.
- Zagury, D., Bernard, J., Leonard, R., Cheynier, R., Feldman, M., Sarin, P. S. & Gallo, R. C. (1986). Long-term cultures of HTLV-III-infected T cells: a model of cytopathology of T-cell depletion in AIDS. *Science* **231**, 850–853.
- Zhou, Y., Zhang, H., Siliciano, J. D. & Siliciano, R. F. (2005). Kinetics of human immunodeficiency virus type 1 decay following entry into resting CD4⁺ T cells. *J Virol* **79**, 2199–2210.
- Zhu, T., Mo, H., Wang, N., Nam, D. S., Cao, Y., Koup, R. A. & Ho, D. D. (1993). Genotypic and phenotypic characterization of HIV-1 patients with primary infection. *Science* **261**, 1179–1181.
- Zhu, J., Gaiha, G. D., John, S. P., Pertel, T., Chin, C. R., Gao, G., Qu, H., Walker, B. D., Elledge, S. J. & Brass, A. L. (2012). Reactivation of latent HIV-1 by inhibition of BRD4. *Cell Rep* **2**, 807–816.