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HIV cure research: advances and prospects

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Abstract

Thirty years after the identification of HIV, a cure for HIV infection is still to be achieved. Advances of combined antiretroviral therapy (cART) in recent years have transformed HIV infection into a chronic disease when treatment is available. However, in spite of the favorable outcomes provided by the newer therapies, cART is not curative and patients are at risk of developing HIV-associated disorders. Moreover, universal access to antiretroviral treatment is restricted by financial obstacles. This review discusses the most recent strategies that have been developed in the search for an HIV cure and to improve life quality of people living with HIV.

Keywords: HIV cure, eradication, remission of HIV infection, HIV reservoirs, reactivation of latent virus, repression of provirus, control of infection.

Highlights:

Some cases of cure or remission of infection have boosted the search for an HIV cure

cART intensification has not shown significant impact in the reservoirs, but early cART may limit them

Strategies to purge the reservoirs face difficulties linked to the complexity of latency mechanisms and drug non-specificity

Repression of reservoirs or cell manipulation to render them less permissive to HIV may facilitate HIV remission

HIV cure/remission may require boosting immune responses while keeping inflammation in check

Preamble

The introduction of improved combined antiretroviral therapy (cART) has dramatically improved the clinical outcome and life expectancy of HIV infected patients. The development of drugs that inhibit different steps of viral replication allows clinicians to successfully manage the disease, improving immunologic parameters, and avoiding drug resistance and side effects. Due to advances in antiretroviral development, HIV infection can nowadays be handled as a complex chronic disease (Deeks et al., 2013). Nonetheless, despite all of the clinical benefits provided by drug therapy, cART alone is not able to eradicate the virus, which persists in reservoirs that are thought to be the source for viral reemergence after treatment interruption (Chun et al., 2010; Chun et al., 1997). In addition, cART does not fully restore health – it increases the risk of non-AIDS disorders such as cardiovascular, kidney, liver and neurological diseases. Moreover, persistent immune dysfunction and inflammation increase the risk of non-AIDS morbidity and mortality (Clifford and Ances, 2013; Lake and Currier, 2013). Undoubtedly, a cure for HIV infection is needed to bypass the limitations of the current therapy and restore health. Although a vaccine against HIV remains an unachieved aspiration, several recent findings suggest that either a cure or a durable remission of infection might be possible.

Is a cure for HIV possible?

Since the isolation and characterization of the HIV as the etiologic agent of AIDS (Barre-Sinoussi et al., 1983), the search for a cure has been considered a major research priority. The description of a unique case of HIV cure after hematopoietic stem cell (HSC) transplantation has boosted remarkable optimism in the field and rekindled the hope that a cure for HIV infection is possible. In 2007, the so-called

“Berlin patient” - HIV positive and diagnosed with acute myeloid leukemia - received double allogeneic HSC transplant from an HLA-matched and unrelated donor screened for homozygosity for the *CCR5* Δ 32 allele (Hutter et al., 2009). This patient discontinued ART the day before the first transplantation and, after 6 years of follow up in the absence of therapy, he shows no trace of HIV in blood and tissue samples (Figure 1A), revealing no evidence for persistent HIV. Levels of HIV-specific antibodies have also declined, suggesting that HIV antigen stimulation was very low or absent after transplantation (Allers et al., 2011; Hutter et al., 2009; Yukl et al., 2013).

Several studies have aimed to use autologous or allogeneic HSC transplantation in association with antiretroviral therapy as a strategy to eradicate HIV in seropositive patients diagnosed with leukemia and/or lymphomas (Table 1). Nevertheless, in most of these studies HIV was detected after transplantation, either following therapy withdrawal or because the therapeutic regimen was not able to completely eliminate the viral reservoirs. In addition, in several cases, the patients died after transplantation. The most recent study investigating the impact of cART on viral reservoirs in two patients who received HSC transplantation – known as “the Boston patients” – described an important reduction of HIV DNA in long term, reaching undetectable levels (Henrich et al., 2013). These observations are in agreement with another study which described that HIV DNA levels at month 24 post transplantation in HSC transplanted patients are lower than those observed at baseline (Simonelli et al., 2010). Altogether, these data suggest that HSC transplantation in association with cART may reduce viral reservoirs in HIV infected patients. However, a recent report during the 6th International Workshop on HIV Persistence, Reservoirs and

Eradication Strategies revealed that the Boston patients experienced a strong rebound in plasma viremia several months after analytic treatment interruption (ATI) (Figures 1B and 1C)(<http://es.scribd.com/doc/189931630/12-06-13-Statement-From-Dr-Henrich-Regarding-HIV-Patients>). These results suggest that HIV hides in yet unidentified sanctuaries or at levels that are not detectable by current available techniques.

So far, HSC transplantation to eradicate the virus was successful only in the Berlin patient. The mechanisms involved in HIV eradication in this patient are not yet fully understood. This patient underwent severe particular transplant conditions (Hutter et al., 2009) (Figure 1), such as double HSC transplantation from the same *CCR5* $\Delta 32$ donor, conditioning regimen (total body irradiation) and the development of graft-versus-host disease (GVHD), which may have contributed to cure. Comparison with the protocols applied in the case of the Boston patients (Henrich et al., 2013) suggests that total body irradiation or the engraftment with cells from a *CCR5* $\Delta 32$ donor may have been critical differential aspects in the case of the Berlin patient. As the homozygous *CCR5* $\Delta 32$ deletion offers a natural resistance to HIV infection (Dean et al., 1996), this unique successful experience has encouraged the search for new *CCR5*-based therapeutic approaches for HIV cure interventions (see below). It is interesting to notice that the Berlin patient and both Boston patients were originally heterozygous for the *CCR5* $\Delta 32$ allele, but this did not confer any advantage to the Boston patients once treatment was interrupted (Henrich et al., 2013; Hutter et al., 2009). A recent effort to cure HIV infection in a 12-year old boy with acute lymphoblastic leukemia consisted of an allogenic transplantation of HSC obtained from cord blood. Similar to the Berlin patient, the donor was screened for

homozygosity for the *CCR5* $\Delta 32$ allele. This procedure was performed at the University of Minnesota in April 2013, but the pediatric patient died two months after transplantation by a severe GVHD (<http://www.healthtalk.umn.edu/2013/07/13/pediatric-patient-dies-after-undergoing-historic-transplant-at-u-of-m/>).

Despite the impact of HSC transplantation on reducing HIV reservoirs, this kind of treatment is not a viable option for the majority of HIV infected patients, since it is a risky and expensive procedure that is recommended only for those who develop cancer. Furthermore, *CCR5* $\Delta 32$ homozygous donors are rare (1% of caucasians) (Lucotte, 2002), what makes the search for a compatible donor with the protective genotype an additional challenge.

How can HIV infected patients achieve durable remission of infection?

“HIV controllers” or “elite controllers” correspond to a small percentage of HIV infected patients that can naturally control viral replication below the levels of detection with standard clinical assays. Consequently, this group of patients is considered an important model to understand the mechanisms underlying control of infection in the absence of treatment (Hamimi et al., 2013; Saez-Cirion and Pancino, 2013). The aim of a so-called “functional cure” would be similarly to HIV controllers, that is, to allow HIV infected patients to achieve viral remission in which HIV remains in the body at low levels and it is controlled by the host in the absence of cART. This status may be achieved by 5-15% of patients treated very early during primary HIV infection (PHI) for long periods of time who experience treatment interruption afterwards (Goujard et al., 2012b; Hocqueloux et al., 2010; Lisziewicz et al., 1999;

Lodi et al., 2012; Saez-Cirion et al., 2013; Salgado et al., 2011; Steingrover et al., 2008). These patients are known as “post-treatment controllers”.

The VISCONTI study (Viro-Immunological Sustained CONTROL after Treatment Interruption) investigated whether the characteristics of 14 post-treatment controllers were similar to those observed in HIV controllers that spontaneously control HIV replication (Saez-Cirion et al., 2013). In this study, cART duration at after primary infection was 3 years, and after treatment interruption, post-treatment controllers presented a sustained control for a median of 7 years. During acute phase, post-treatment controllers had higher viremia levels and lower CD4+ T cell counts than patients who naturally control infection afterwards. In addition, these groups of patients have a different genetic background – while HIV controllers cohorts are enriched by the protective HLA-B*27 and B*57 (Migueles et al., 2000; Pereyra et al., 2008; Saez-Cirion et al., 2007), the risk alleles B*07 and B*35 (Carrington et al., 1999; Itescu et al., 1992; Pereyra et al., 2010; Peterson et al., 2013b) were prevalent among the 14 post-treatment controllers. HIV-1 specific CD8+ T cell responses and immune activation differ between post-treatment controllers and HIV controllers (post-treatment controllers have weak CD8+ T cell responses and a low level of immune activation during the control phase).

Another recent incident that suggests that a very early treatment may lead to HIV remission is the case identified as “the Mississippi baby” (Persaud et al., 2013). This infant born to a seropositive mother started receiving cART 30 hours after birth. HIV-1 RNA was detectable at 31 hours, days 6, 11 and 19, and reached undetectable levels at day 29. cART was discontinued sometime between month 18 to 23. At 36

months of age and after therapy withdrawal, HIV-1 RNA, proviral DNA and HIV-1 antibodies remain consistently undetectable or extremely low in blood and tissues in this pediatric patient.

The study of post-treatment controllers may give valuable information to guide the search for a successful strategy for inducing viral remission. However, some open questions remain. For instance: which are the mechanisms underlying control? and how can we increase the probability of HIV infected patients to become a post-treatment controller? Recent studies suggest that both the timing to initiate therapy (Ananworanich et al, 20th Conference on Retroviruses and Opportunistic Infections, 2013, San Francisco, USA) (Ananworanich et al., 2012; Gianella et al., 2011; Hecht et al., 2006) and the duration of cART (Fidler et al., 2013; Stohr et al., 2013) might play a key role in the achievement of a durable HIV control. In addition, baseline viral load and immune activation may predict success (Volberding et al., 2009). Whether the therapeutic regimen used during PHI may impact the outcome is still to be investigated. In any case, in the absence of markers predicting success after treatment interruption, this procedure is not recommended outside structured protocols for patients undergoing suppressive cART.

Treatment during PHI seems to result in broad and strong HIV-1 specific immune responses (Hecht et al., 2006; Kaufmann et al., 2004; Lisziewicz et al., 1999; Moir et al., 2010; Ortiz et al., 1999; Oxenius et al., 2000; Rosenberg et al., 2000), reduced immune activation (Volberding et al., 2009), immune restoration in the gastrointestinal mucosa (Guadalupe et al., 2006) and limited viral evolution (Chamberland et al., 2011). Additionally, initiation of cART during PHI may limit the

establishment of viral reservoirs (Archin et al., 2012b; Chun et al., 2010; Gianella et al., 2011; Pires et al., 2004; Schmid et al., 2010; Wyl et al., 2011). Central memory CD4⁺ T cells (T_{CM}) are a key component of the long-lasting HIV reservoir (Chomont et al., 2009), and recent studies have demonstrated that very early cART limits the seeding of the HIV reservoir in long-lived T_{CM} (Figure 2) (Ananworanich et al, 20th Conference on Retroviruses and Opportunistic Infections, 2013, San Francisco, USA). Reservoirs levels in post-treatment controllers during the control phase were very low and it was mostly associated to the transitional memory CD4⁺ T cell subset, due to a skewed distribution of quiescent CD4⁺ T cell subsets in these patients (Saez-Cirion et al., 2013)(Figure 2).

Treatment interruption in patients who started cART during chronic phase leads to viral rebound within weeks, with viral loads frequently reaching pretreatment set-point levels (Fernandez Guerrero et al., 2005; Steingrover et al., 2008; Wit et al., 2005). Notwithstanding, some patients that started cART during chronic phase were also described to maintain controlled levels of viremia after therapy discontinuation (Van Gulck et al., 2011). For all these studies describing cases of a potential “functional cure”, long period follow-up is needed to evaluate whether long HIV control can be maintained and the mechanisms involved in the long-term suppression after treatment interruption. Collectively, these studies suggest that HIV remission is possible to be achieved with the help of therapeutic interventions in patients without a favorable genetic background to naturally control HIV infection. Certainly, additional studies will be needed to understand the mechanisms that may lead to a long-lasting viral remission in post-treatment controllers.

Is it better if we intensify ART?

Therapeutic intervention during PHI appears to be valuable in inducing viral remission in a certain number of HIV infected patients. However, just a small percentage of patients are diagnosed during PHI. Most of them will know their seropositive status only during the chronic phase of HIV infection. For the great majority of HIV infected patients, other strategies to achieve viral remission must be developed. Combined antiretroviral therapy dramatically reduces viremia to levels below the detection limit of current assays (<50 copies/mL), but low-level residual viremia persists and is usually detectable by ultra-sensitive tests in patients undergoing successful ART (Palmer et al., 2008). Residual viremia may partially be the cause of persistent immune activation (Corbeau and Reynes, 2011), which increases the risk of non-AIDS morbidity and mortality in treated patients (Clifford and Ances, 2013; Lake and Currier, 2013). There are two probable sources of residual viremia (Doyle and Geretti, 2012): (i) long-lived, latently infected cells whose provirus became transcriptionally active, with intermittent or continuous viral release; (ii) ongoing low-levels of HIV replication with *de novo* viral infection, due to partial suppression and/or to inadequate drug penetration. In the latter case, therapy intensification should reduce the residual viremia and associated persistent inflammation. Moreover, in the long term, it would be expected to decrease HIV reservoir levels.

Previous studies of therapy intensification with protease and reverse transcriptase inhibitors were contradictory in determining whether or not ongoing replication is the source of persistent viremia (Dinso et al., 2009; Havlir et al., 2003; Ramratnam et al., 2004). The development of new classes of antiretroviral drugs allowed the

exploration of the impact of treatment intensification with drugs targeting other steps of HIV replication cycle. Treatment intensification with Raltegravir (RAL), a potent integrase inhibitor, offered a unique opportunity to study the dynamic of ongoing low-level replication. As RAL blocks HIV integration, residual replication could be assessed by the increase of 2-LTR circles (Schacker, 2010). Most studies of RAL treatment intensification showed no impact on residual viremia, immune activation or in promoting CD4+ T cell reconstitution. Collectively, these data suggested that residual viremia does not originate from ongoing cycles of HIV replication (Gandhi et al., 2012; Gandhi et al., 2010; Hatano et al., 2011; McMahon et al., 2010; Negredo et al., 2013). In contrast, the study of Buzon and colleagues (Buzon et al., 2010) identified a transient increase in the levels of 2-LTR HIV DNA within two to four weeks after intensification, but no significant decrease in low-level viremia was observed. In addition, only those patients with high levels of immune activation at baseline presented a decrease in these markers. These findings strongly suggested that RAL intensification was blocking new rounds of infection in these patients and that consequently ongoing replication was taking place. Nonetheless, other studies failed to detect 2-LTR increase in RAL intensified-treated patients (Besson et al., 2012; Gandhi et al., 2012). It is possible that differences in patients' characteristics, cART regimens at baseline, and/or the time of therapy intensification with RAL had an impact in the discrepancies observed among these studies. An important observation in the Buzon et al, study was the fact that 61% of the patients with detectable 2-LTR were taking a protease inhibitor-based regimen (PI). More recently, Hatano and colleagues also detected the transient increase of 2-LTR, which was also more accentuated in patients taking PI (Hatano et al., 2013). In addition, a significant

reduction in D-Dimer levels, a coagulation biomarker, was observed, indicating that RAL intensification may lead to reduction in inflammation/activation markers.

Because HIV-1 might replicate in tissue reservoirs where drug concentrations are suboptimal (Fletcher et al., 2014), such as lymph nodes, gut associated lymphoid tissues, bone marrow and the central nervous system, some RAL intensification studies assessed the immunologic and virologic responses in tissues. Yukl et al showed reduction in unspliced HIV RNA, T cell activation and a trend towards CD4+ T cell increases in ileum, suggesting that this tissue may be an important site for ongoing replication in some patients on cART (Yukl et al., 2010). In contrast, therapy intensification with RAL did not impact cerebrospinal viral loads (Dahl et al., 2011) or isolated HIV semen shedding (Osborne et al., 2013).

Based on previous observations that Maraviroc (MVC), a CCR5 antagonist, promotes a gain of CD4+ T cells in viremic HIV-infected patients (Cooper et al., 2010; Wilkin et al., 2010), it was hypothesized that the immunomodulatory effects of this drug could decrease immune activation and improve CD4+ T cell counts in patients under suppressive cART with insufficient immunological restoration. As observed in RAL intensification trials, no impact in residual viremia or HIV reservoir was achieved. Data regarding CD4+ T cell gain was also controversial in these trials (Cuzin et al., 2012; Hunt et al., 2013; Rusconi et al., 2013; Wilkin et al., 2012). Some unexpected effects, however, were observed in MVC intensification trials, such as increased activation levels in blood and rectal mucosa (Hunt et al., 2013; Rusconi et al., 2013), increase in plasma LPS, sCD14 and sCD163 (Gutierrez et al., 2011; Hunt et al., 2013) and increased 2-LTR levels (Gutierrez et al., 2011), suggesting that MVC

intensification might induce viral replication. The mechanisms underlying these effects are still unclear, but some evidences suggest that MVC promotes an increase in the circulating levels of CCL3 (MIP-1 α), CCL4 (MIP-1 β) and CCL5 (RANTES), natural ligands of CCR5, which may activate T cells and monocytes/neutrophils through CCR3/CCR4 and CCR1 signaling, respectively (Guan et al., 2002; Sarau et al., 1997). Further studies are necessary to carefully evaluate the immunomodulatory effects of MVC *in vivo* and the benefits of therapy intensification with CCR5 antagonists in HIV-infected patients.

Despite some evidence that therapy intensification may have positive effects on residual replication, its potential to eradicate the virus appears limited. These results highlight the importance of designing new strategies to eliminate persistent HIV reservoirs.

HIV persistence as a challenge for a cure

HIV replication and persistence depend on the integration of the viral DNA in the host genome. Although most proviruses integrate in transcriptionally active genomic regions, in most cases these proviruses remained repressed (Ho et al., 2013). Our knowledge of the complex mechanisms underlying establishment and maintenance of HIV latency has been enormously improved in the last years (see (Mbonye and Karn, 2014) in this issue of Virology for a thorough review). Repressed HIV proviruses integrated in long-lived cells constitute the persistent HIV reservoir, which is not recognized by immune responses, not eliminated under cART, and is the main obstacle to achieving an HIV cure. Since the description of HIV reservoirs despite long-term cART, attempts to purge this reservoir have been tried. The first one consisted in the infusion in three patients under cART of the anti-CD3 OKT3 antibody

in the presence of IL2, in an effort to mimic in vivo a well-established in vitro reactivation protocol (Prins et al., 1999). OKT3+IL2 treatment was accompanied by very strong activation of T cells, and transient increases of HIV RNA levels were observed in plasma or lymph nodes of these patients. However, no decrease in the frequency of infected cells could be reported and the treatment had many undesirable effects, associated with exacerbated immune activation and cytokine release, induction of anti-OKT3 antibodies and severe long-lasting depletion of CD4+ T cells (Prins et al., 1999; van Praag et al., 2001).

HDAC inhibitors were next proposed as optimal candidate to flush HIV reservoirs due to their potential to activate HIV transcription without inducing generalized immune activation. Chromatin condensation is regulated by the level of acetylation/deacetylation of the histones that constitute the core of the nucleosomes. Histone acetyl transferases (HAT) mediate the addition of acetyl groups to histones, reducing chromatin condensation and promoting transcription. Histone deacetylases (HDAC) remove the acetyl groups and repress transcription. The balance of HAT/HDAC activities is thus thought to be a key component of HIV latency. The first molecule with HDAC inhibitory activity to be assayed in vivo was valproic acid (VPA). A first study reported a decrease in the frequency of circulating resting CD4+ T cells carrying replication competent HIV-1 in four patients receiving cART after 16 weeks of additional treatment with VPA (Lehrman et al., 2005). However, subsequent randomized clinical studies could not confirm these results and no significant effects of VPA were evidenced (Archin et al., 2010; Routy et al., 2012; Sagot-Lerolle et al., 2008; Steel et al., 2006). Failure of VPA was eventually associated to a poor capacity of this molecule to inhibit HDAC3, the HDAC isoform that is thought to play a

preponderant role in HIV latency (Huber et al., 2011). In a proof of concept study, the more potent HDAC inhibitor vorinostat has shown capacity to increase levels of HIV mRNA transcripts after a single dose, without affecting overall HIV DNA cellular levels (Archin et al., 2012a). Preliminary results of the NCT01365065 trial presented in the 20th Conference on Retrovirus and Opportunistic Infections (Lewin S, et al 2013, San Francisco, USA) also reported an increase of cell associated unspliced HIV mRNAs in 15/17 patients on cART who received daily doses of vorinostat for 14 days. Another clinical trial (NCT01680094) is evaluating the impact on HIV reservoirs of panobinostat, which has been described as an even more potent HDAC inhibitor (Rasmussen et al., 2013).

The use of HDAC inhibitors in the context of HIV cure face several hurdles. Among them, the lack of specificity of HDAC inhibitors may impact the transcription of a large fraction of all cellular genes (Glaser et al., 2003). Also, it has been recently shown (Shan et al., 2012) that viral reactivation with HDAC inhibitors will not necessarily be followed by viral-induced cell death, and that immune responses may need to be boosted in parallel to recognize and eliminate these new viral-producing cells (see below). It is also unlikely that HDAC inhibition by itself will be able to induce efficient viral reactivation in the absence of the factors necessary to ensure full viral transcription. The current belief is that it will be necessary to combine treatment of HDAC inhibitors with other molecules able to provide the additional stimuli required for HIV transcription. PKC agonists, such as prostratin (Kulkosky et al., 2001; Reuse et al., 2009) and bryostatin (Perez et al., 2010), or TLR agonists (Novis et al., 2013), that activate NF- κ B have been proposed as good potential combinatorial candidates.

Purging viral reservoirs is undoubtedly the only solution to achieve HIV eradication. However, letting aside the mechanistic issues, activating every single latently infected cell in the organism still appears a gargantuan endeavor. Taking the example of natural HIV controllers or the post-treatment controllers from the VISCONTI study, durable HIV remission may be a more reasonable goal in the medium term. In this setting, limiting and controlling the viral reservoirs would be the main objectives. With this in mind, our current knowledge, albeit incomplete, of HIV latency and transcription might be turned to devise strategies aiming to repress, rather than activate, viral transcription. As alternatives to HDAC inhibitors, HAT inhibitors such as garcinol derivatives (Mantelingu et al., 2007) and curcumin (Zhang et al., 2011) have been shown to inhibit HIV transcription in vitro. Transcriptional elongation of the viral promoter depends on the recruitment of the HIV transactivator factor Tat to the transactivation-responsive element (TAR) located at the end of initial HIV transcripts (D'Orso and Frankel, 2010). Compounds such as Celastrol (Narayan et al., 2011) directly block Tat function by altering its structure. An analogue of Cortistatin A, a natural steroidal alkaloid, has shown to block HIV transcription initiation and elongation by binding specifically to the TAR-binding domain of Tat and impairing the interaction of Tat with viral RNAs (Mousseau et al., 2012). This promising molecule showed potent inhibitory capacity in vitro at very low concentrations (nM-pM). Zinc-finger domains, small protein domains that can be designed to bind specific DNA sequences, can be fused to appropriate effector domains of transcription factors to specifically regulate the expression of targeted genes. Zinc-finger transcription factors targeting different regions of the LTR promoter and carrying the repression domain of the KOX1 protein were able to repress transcription from the LTR in infected cells in vitro (Reynolds et al., 2003). Overall these results suggest that

different opportunities exist and may be exploited to specifically repress HIV transcription.

Gene therapy

The apparent cure of the Berlin patient after receiving HSCT from a *CCR5* Δ 32 homozygous donor and the impossibility of applying this protocol in a large scale have inspired attempts to generate HIV-resistant cells through gene therapy (Peterson et al., 2013a). One of the most promising gene therapy strategies against HIV infection aims to disrupt the *CCR5* gene by expressing an engineered zinc-finger nuclease (ZFN). Evidence that ZFN could inactivate *CCR5* in primary human CD4⁺ T cells and in CD34⁺ hematopoietic stem cells limiting HIV replication was first obtained in mouse models (Holt et al., 2010; Perez et al., 2008). The results of a Phase I clinical trial showed that HIV-infected patients treated with ZFN *CCR5* modified CD4⁺ T cells (SB-728-T) have sustained increases in the CD4⁺ T cells, mostly T_{CM} and T_{TM} with low PD-1 expression. Viremia was controlled after treatment interruption for some patients, but cART was resumed by most of them due to dual-tropic infection or viral load rebound (Ando D. et al 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, 2013, Denver, CO, USA). Phase II clinical trials are currently ongoing to evaluate the efficacy of SB-728-T. In a recent development of these studies, ZFNs have been designed to be combined and simultaneously target *CCR5* and *CXCR4*. This approach resulted in human primary CD4⁺ T cells that were resistant to R5 and X4 viruses in vitro and also in vivo when introduced in humanized mice (Didigu et al., 2014). This strategy would tackle the risk of tropism switching.

Other strategies consist of transducing CD34+ or CD4+ T cells with vectors expressing (i) shRNA interfering with *CCR5* or other key factors associated with the HIV life cycle (Kiem et al., 2012), including its promoter (Suzuki et al., 2013); (ii) small peptides with capacity to inhibit HIV entry into the cells (Younan et al., 2013). Another encouraging gene therapy approach directly targets the viral reservoir and is based on the ability of a tailored site-specific recombinase (Tre) to excise HIV provirus from host genome (Sarkar et al., 2007). Recently, successful expression of Tre-recombinase in human cells was demonstrated. In addition, an important antiviral effect was observed in humanized mice, suggesting that this approach may be valuable for future HIV eradication strategies (Hauber et al., 2013).

Innovative approaches to eliminate HIV reservoirs

Several experimental and proof-of-concept studies aim to interfere with cell cycle and survival of HIV infected cells to facilitate their elimination or avoid their persistence by homeostatic proliferation. Rapamycin is an immunosuppressor that blocks the mammalian target of rapamycin (mTOR), a protein kinase that control cell cycle, proliferation and survival, and plays a critical role in the differentiation of T cells. Rapamycin has been shown to be able to block HIV infection in vitro (Donia et al., 2010) by decreasing the expression of *CCR5*, and also interferes with the synthesis of HIV transcripts (Roy et al., 2002). Through its capacity to suppress activation and proliferation of T cells, rapamycin has also the potential to block HIV infection by reducing the number of target cells and to dodge the maintenance of viral reservoirs through homeostatic proliferation. Rapamycin is commonly used to avoid organ rejection in kidney transplantation when there are complications associated with

calcineurin inhibitors. Interestingly, a clinical report on a group of 14 HIV infected patients who received kidney transplantation showed that the patients who switched to rapamycin monotherapy after transplantation were able to better control HIV replication than those who kept calcineurin inhibitors (Di Benedetto et al., 2010). Another recent study, which analyzed a larger group of HIV-infected kidney recipients, has shown that those who received rapamycin experienced, two years after transplantation, some reduction in the levels of cell associated DNA when compared to patients receiving other immunosuppressors (Stock P, et al, 19th International AIDS Society conference, 2013, Kuala Lumpur, Malaysia).

Another compound with cytostatic activities that has been tried in the context of HIV infection is hydroxyurea. Hydroxyurea is an inhibitor of ribonucleotide reductase (RNR) (Koc et al., 2004), a key enzyme responsible for dNTP synthesis that has been shown to play an important role in regulating HIV infection (Allouch et al., 2013). Hydroxyurea, other than maintaining cells in a quiescent state, reduces the levels of intracellular dNTPs thus inhibiting HIV DNA synthesis during reverse transcription. Although the impact of hydroxyurea in HIV reservoirs has not been evaluated, hydroxyurea was shown to act synergically with the nucleoside analogue didanosine to reduce HIV viral loads in patients (Lori et al., 2005; Lori and Lisziewicz, 2000). Despite its promises, hydroxyurea treatment was shown to provoke complications, such as blunted CD4+ T cell recovery and severe toxicity, in HIV infected patients (Bloch et al., 2006; Zala et al., 2002). Recent studies suggest that these undesirable effects were due to a too high dose of the drug, and that both the antiviral and the cytostatic activities of hydroxyurea may contribute to control HIV when used at lower concentrations (Lori et al., 2012).

JQ1 is an agonist of the bromodomain protein BRD4, which promotes cell cycle progression (Yang et al., 2008). BRD4 is also a positive regulator of P-TEFb that competes with Tat for binding to this complex at the HIV promoter (Bisgrove et al., 2007). JQ1 dissociates BRD4 from the HIV promoter and allows Tat recruitment and subsequent HIV elongation (Li et al., 2013). In addition, JQ1 appears also to alter chromatin organization and downregulates T cell activation genes (Banerjee et al., 2012).

A different strategy has been explored with auranofin, a gold-based compound that has been evaluated in vivo in SIV infected macaques. This compound was shown in vitro to promote differentiation of CD27+ memory CD4+ T cells to more terminal short-lived stages (Lewis et al., 2011) preceding increased cell death. Administration of auranofin to a group of SIVmac251 infected rhesus macaques with viral loads suppressed by cART, induced indeed a decreased in the frequency of T_{CM} and T_{TM} CD4+ T cells, the cells thought to contribute the most to persistent reservoirs (Chomont et al., 2009). Interestingly, auranofin treatment was accompanied by a transient decrease of viral reservoirs and a delayed rebound of viremia after interruption of auranofin and antiretroviral treatment (Lewis et al., 2011). In a follow-up study, auranofin treatment in two macaques combined with cART and buthionine sulfoximine, a glutathione-depleting agent that has been proposed to favor selective death of infected cells in vitro (Savarino et al., 2009), favored control of infection at relatively low levels after treatment interruption (Shytaj et al., 2013).

How these interventions may impact (positively or negatively) T cell immune defenses against HIV has not been thoroughly addressed, and the translation of current formulations of these drugs to clinical intervention is unlikely due to toxicity and possible harmful unspecific effects. However, the proof-of-principle studies described above suggest that targeting T cell proliferation and survival deserves further attention in the search for an HIV cure.

Immunotherapies to treat HIV infection

There is a consensus in the scientific community on the need to potentiate immune responses in the context of combinatory approaches to reach an HIV cure. Such responses would contribute to either control or eliminate HIV infected cells. A study performed by the Siliciano group showed that reactivation of latent viruses would not automatically entail the elimination of the infected cells by viral-replication-driven apoptosis (Shan et al., 2012). Efficient CD8+ T cell responses, such as those found in HIV controllers, react to very small amounts of antigens and eliminate HIV producing cells (Almeida et al., 2007; Saez-Cirion et al., 2007; Shan et al., 2012). In contrast, responses from non-controller patients would not have such capacity. Anti-exhaustion strategies try to reverse negative signaling provided by immunoregulatory molecules such as PD-1 to restore T cell function, in particular, the capacity to kill infected cells (Porichis and Kaufmann, 2012). Blockage of PD-1 interactions with PD-1 ligands on SIV-infected macaques resulted in the expansion of CD8+ T cells with improved functionality, longer survival, lower viral loads and delayed viral rebound after antiretroviral treatment interruption (Velu et al., 2009). However, another report suggests that mere expansion of polyfunctional CD8+ T cell responses through PD-1 blockade may not suffice to sustainably decrease viremia (Amancha et al., 2013). A

key aspect may be the association of the efficient responses found in HIV controllers with the selection of particular TCR clonotypes in these cells. In this sense, PD-1 blockade may be a useful adjuvant in vaccination protocols (Finnefrock et al., 2009) helping to induce efficient responses. This review will not summarize therapeutic vaccine strategies currently in development. However, recent results showing that (i) non-conventional CD8⁺ T cell responses were associated with control of infection and viral clearance in a group of macaques vaccinated with a cytomegalovirus vector (Hansen et al., 2013a; Hansen et al., 2013b) despite profound viral dissemination during primary infection; and that (ii) passive transfer of broadly neutralising antibodies allows control of infection in chronically SIV-infected macaques (Barouch et al., 2013), suggest that therapeutic vaccines should be an important element in the global plan to achieve HIV cure or remission.

So far, interventions based on the administration of gamma-chain cytokines such as IL-2, IL-7 or IL-15, which tried to improve T cell function and restore T cell homeostasis (Leone et al., 2009; Levy et al., 2009; Levy et al., 2012; Vanham and Van Gulck, 2012), have not shown, at least by themselves, significant benefits in HIV treatment. Indeed, improved immune reconstitution with IL-7 is also accompanied by a significant increase in the total number of HIV-harboring CD4⁺ T cells (Levy et al., 2009; Vandergeeten et al., 2013). It is not excluded, however, that these cytokines may have an important adjuvant effect in vaccine strategies. Therapies based on IFN α administration have been shown to decrease viremia during chronic infection and reduce viral reservoirs after treatment interruption (Asmuth et al., 2010; Azzoni et al., 2013; Pillai et al., 2012). However, these effects were not observed in all

situations (Boue et al., 2011; Goujard et al., 2012a), which may be due to the dual role that IFN α (and immune activation) may play at different stages of infection.

Immune activation is a major determinant of HIV pathogenesis, and increased levels of inflammation markers are associated with faster progression to disease and CD4+ T cell loss (Liovat et al., 2012) including in HIV controllers with undetectable viremia (Hunt et al., 2008; Noel et al., 2014). Therefore, targeting the harmful immune activation appears to be a necessary element in ensuring a long-life remission of HIV infection. Some of the molecules targeting HIV reservoirs described in this review have also immunosuppressive effects, and their impact is being evaluated. Other molecules such as chloroquine analogues or statins specifically target inflammation. So far, studies employing these and other anti-inflammatory molecules have rendered contrasting results (Hatano, 2013). Thus, chloroquine administration has been shown to decrease levels of T cell activation (Murray et al., 2010) but produce faster CD4+ T cell loss when used for longer periods of time (Paton et al., 2012). A better knowledge of the best timing to implement interventions aimed at decreasing persistent immune activation is warranted.

Concluding remarks

Achieving HIV cure at a global scale through the eradication of HIV reservoirs seems still far off. In contrast, durable HIV remission with low levels of infected cells being controlled by host mechanisms seems more plausible at medium-term horizon. The first step in such strategy would be to limit the size of the viral reservoirs. Early treatment initiation has been shown to provide immunological and virological benefits to HIV infected patients, and early treated patients may be more prone to positively

respond to cure therapies. Moreover, treatment is prevention (Cohen et al., 2011) and, thus, early diagnosis and treatment initiation appears as a priority in the global fight against HIV. Approaches trying to purge the viral reservoirs, including activation with HDAC inhibitors or PKC agonists face the difficulty of trying to circumvent complex mechanisms of repression with non-specific drugs that may cause undesirable effects. An alternative strategy may be to further reinforce latency and repression mechanisms with specific molecules targeting Tat-dependent transcription of HIV products. Gene therapy offers also the possibility to target specific genes to render cells resistant to HIV infection and even to excise integrated provirus. Enhancing susceptibility to apoptosis of HIV infected cells or impairing homeostatic proliferation and persistence of these cells are also proposed as potential strategies to tackle HIV reservoirs. However, such approaches need to carefully evaluate the impact they may have in immune responses of treated individuals. HIV remission will require efficient host responses to control infected cells and to prevent harmful HIV-related inflammation. These areas of research need to be explored in parallel to strategies targeting the reservoirs.

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Figure legends

Figure 1: Schematic representation of the clinical course before and after allogeneic hematopoietic stem cell transplantation (HSCT) in the Berlin (A) and the Boston patients (B and C) as described in (Henrich et al., 2013; Hutter et al., 2009). The most important events associated to the HSCT and HIV disease are indicated. Blue arrows represent the periods of radio/chemotherapy. Red arrows represent total body irradiation. Periods of detectable viremia are represented by orange boxes. The period that patients were under antiretroviral treatment are indicated in grey below the timeline. The period between allogeneic HSCT and ATI in Boston patients are indicated by brackets. AML: acute myeloid leukemia; ATI: analytical treatment interruption; cART: combined antiretroviral therapy; HL: Hodgkin lymphoma; HSCT: hematopoietic stem cell transplantation; GVHD: graft-versus-host disease; WT: wild type.

Figure 2: Schematic representation of the HIV acute infection and the contribution of each T cell subset to the establishment of the HIV reservoir. Early treatment during Fiebig stages I-III may limit the number of infected cells and protect T_{CM} cells from infection. Treatment during Fiebig stages IV-V may decrease the contribution of long-lived T_N and T_{CM} cells to the reservoir due to low relative abundance of these cell subsets. Frequency of T_{CM} normalizes during chronic infection increasing the contribution of these cells to the reservoir.

Table 1: Hematopoietic stem cell transplantation in HIV positive patients

Study Reference	Patients			Diagnosis	Graft type	Strategy against HIV infection	Effect on HIV persistence	Clinical Outcome
	N	Gender	Age					
(Holland et al., 1989)	1	M	41	NHL	Allogeneic (bone marrow)	High-dose zidovudine for 2 weeks before transplantation. Lower maintenance dose zidovudine after transplantation.	No detectable HIV RNA and DNA at day 32 after transplantation and at autopsy.	Death 47 days after transplantation.
(Contu et al., 1993)	1	F	25	NA	Allogeneic (bone marrow)	Zidovudine, IFN-alpha 2 and anti-HIV-1-specific T cell clones.	No detectable HIV RNA at day 30 after transplantation and at autopsy.	Death 10 months after transplantation.
(Sora et al., 2002)	1	F	33	AML	Allogeneic (bone marrow)	cART before and after transplantation, with interruptions due to side-effects.	No detectable HIV RNA on cART from day 210 after transplantation.	Alive after 42 months of follow up.
(Gabarre et al., 2004)	14	M/F	27-53	BL, HL, NHL	Autologous	cART before and after transplantation.	No detectable HIV RNA on cART in three patients who survived.	Five patients were alive.
(Resino et al., 2007)	4	NA	31-58	BL, HL, NHL	Autologous	cART before and after transplantation.	On cART No detectable HIV RNA after transplantation (two patients), viral load rebound (two patients). Detectable HIV DNA at month 12 after transplantation for all patients.	The four patients were alive after 12 months of follow up.
(Avettand-Fenoel et al., 2007)	1	M	17	BL,AML	Allogeneic (bone marrow)	cART before and after transplantation, with interruptions due to side-	Undetectable RNA and DNA on cART. Detectable HIV RNA and DNA at day 16	Death 191 days after transplantation.

						effects.	after TI.	
(Hutter et al., 2009)	1	M	40	AML	Allogeneic (bone marrow)	Donor homozygous for CCR5 Δ 32.	No cART. No trace of HIV after 6 years of follow-up.	Alive after 6 years of follow up. Considered the first case of AIDS cure.
(Simonelli et al., 2010)	24	M/F	<45 (n=15) ≥45 (n=9)	HL, NHL	Autologous	cART before and after transplantation, with interruptions due to side-effects (n=8).	On cART Detectable HIV RNA and DNA after transplantation. HIV DNA significantly lower at month 24 than those at baseline.	Alive, immunologic characteristics comparable to HIV negative patients.
(Cillo et al., 2013)	10	M	24-60	BL, HL, NHL	Autologous	cART before and after transplantation, with interruptions due to side-effects (n=3).	On cART. Detectable HIV RNA (9/10 patients) and DNA (10/10 patients) after transplantation.	Alive with undetectable VL by conventional methods, but with detectable proviral DNA.
(Henrich et al., 2013)	2	M	NI	HL	Allogeneic (bone marrow)	cART before and after transplantation.	Undetectable HIV RNA on cART, detectable after TI. Detectable HIV DNA early after transplantation and undetectable in long term follow-up.	Alive 5 and 3 years after transplantation, but viremia rebounded after TI.
University of Minnesota (not published)	1	M	12	ALL	Allogeneic (cord blood)	Donor homozygous for CCR5 Δ 32 deletion.	No detectable HIV after treatment discontinuation.	Died 2 months after transplantation by a severe graft-versus-host disease.

ALL: acute lymphoblastic leukemia; AML: Acute myeloid leukemia; BL: Burkitt lymphoma; BM: bone marrow; F: Female; HL: Hodgkin lymphoma; M: male; NHL: non-Hodgkin lymphoma; NA: not available; PBMC: Peripheral blood mononuclear cells; PCR: polymerase chain reaction; TI: treatment interruption; VL: viral load.