

**Second European Round Table on the Future
Management of HIV: 10-11 October 2014, Barcelona,
Spain**

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Second European Round Table on the Future Management of HIV 10–11 October 2014, Barcelona, Spain

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Abstract

The Second European Round Table on the Future Management of HIV took place in Barcelona, 10–11 October 2014 and focused on the HIV-1 reservoir, strategies for HIV cure and primary HIV infection (PHI). Important issues in the HIV-1 reservoir research field are the validity of reservoir measurement techniques and the potential of new drugs to target latently infected cells. Current HIV-1 cure concepts are based on theoretical assumptions of biologically plausible mechanisms, supported by several clinical observations. Three main potential strategies are under investigation in order to achieve a sterilising cure or maintain HIV-1 remission: latency reversal resulting in antigen expression and viral cytolysis or immune targeted cell-death; immunological control of the reservoir; or replacement of the complete autologous haematopoietic and lymphoid stem-cell repertoire by transplantation.

An interesting opportunity for restricting the size of the reservoir entails the early initiation of antiretroviral treatment (ART) during PHI. In terms of the reservoir, early treatment limits its size, alters its composition, and restricts the genetic variability of integrated proviral HIV-1 DNA. The challenges ahead involve the identification of patients undergoing seroconversion to HIV-1 and the prompt initiation of treatment. How the seemingly beneficial impact of early treatment will make cure more feasible, and whether the positive effects of the cure efforts outweigh the potentially negative impact of life-long ART, are important aspects of future collaborative research prospects.

Keywords: HIV-1, primary HIV-1 infection, HIV-1 latency, HIV reservoir, inflammation, ART, pharmacokinetics, animal models, gene therapy, HIV-1 cure

Introduction

The latent HIV-1 reservoir, new strategies for HIV-1 eradication and cure and the opportunity for early therapeutic intervention during primary HIV-1 infection (PHI) were the main topics at the Second Round Table on the Future Management of HIV (Barcelona, 10–11 October 2014). At present, the ‘Berlin patient’ [1] remains the only case of HIV-1 cure and the post-treatment viraemia controllers from the Visconti cohort [2] represent cases of apparent HIV-1 remission. In other instances, such as the ‘Mississippi baby’ [3,4] and the ‘Boston transplant patients’ [5], viraemia relapse was delayed. These cases demonstrate both the feasibility and the difficulties in obtaining a sterilising cure or maintaining HIV-1 remission. None the less, these reports represent the foundation for ongoing research to characterise HIV-1 latency and efforts to substantially decrease viral reservoirs in order to achieve durable HIV-1 remission or a sterilising cure.

This year’s round table brought together key investigators from specialties such as virology, immunology and pharmacology as well as clinicians and funders, to discuss the most recent scientific developments in HIV-1 cure and debate future research strategies. This review summarises the main advances in the research field and highlights the key challenges ahead to achieve an HIV-1 cure through future collaborative research activities.

Obstacles and opportunities to target the latent HIV reservoir for eradication strategies

Ongoing research activities have greatly expanded our knowledge on the latent HIV-1 reservoir. Douglas Richman (University of California, USA) provided an overview of the prospects in reservoir and eradication research. The two principal aims are: (1) the identification of cellular drug targets to safely activate HIV-1; and (2) the use of reliable reservoir measurements. HIV-1 latency is maintained by multiple restriction factors preventing the exposure of the viral long-terminal repeat (LTR) promoter region at nucleosome 1, blocking HIV transcription and subsequent mRNA formation. The best-studied latency-reversing agents (LRAs) in humans are inhibitors of histone deacetylases (HDACi). Histone deacetylases (HDACs) promote DNA chromatin folding and prevent transcriptional factors and polymerases from targeting the DNA promoter regions. The targets include, but are not restricted to, integrated replication-competent but transcriptionally silent proviral HIV-1 DNA in latently infected cells. Of the four HDAC classes, HDAC subtypes 2 and 3 of class I are predominantly involved in maintaining HIV-1 latency [6]. The inhibition of HDAC disrupts chromatin architecture and exposes HIV-1 promoter regions for transcription. The maintenance of an HIV-1 repressive transcriptional environment is complex. The Merck corporation has screened over 2.9 million compounds to reverse latency, 66.5% of which affect unknown targets, 17.4% were farnesyltransferase inhibitors and 16.1% were HDACi [7]. Combining LRAs may have antagonistic, additive or synergistic effects *in vitro*, but whether these can be achieved *in vivo* remains unknown (Figure 1).

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Validated measurements of the HIV-1 reservoir will be critical for interpreting the impact of candidate LRAs. Current methods are limited as they usually measure virus found only in blood and measurements have not yet been standardised. It is uncertain whether measures of virus in blood alone reflect other, more inaccessible and heterogeneous reservoirs. Additionally, while total HIV-1 DNA may reflect the size of the reservoir, it does not differentiate between integrated and unintegrated virus. Measurements of integrated (proviral) and 2-LTR circular HIV-1 DNA are the most sensitive methods for detecting presence of viral genome, but do not differentiate replication-competent from replication-incompetent integrated HIV-1 DNA [8]. Plasma single-copy and cell-associated HIV-1 RNA assays can provide confirmation of latency reversal, but do not discriminate between a situation where a few cells each produce high quantities of viral RNA or many cells a small amount. Cell-associated HIV-1 RNA measurements performed in limiting dilution assays might overcome this problem. Measuring the production of infectious virus using a limiting dilution viral outgrowth assay (VOA) is the current standard for quantifying the reactivation of viral replication-competent virus. However, the results of this labour-intensive, expensive and imprecise procedure are difficult to reproduce due to donor-cell dependency and the insensitivity of the assay at the detection limit. Therefore, VOAs probably miss some non-induced but replication-competent provirus. Most of the proviral populations in subjects initiating treatment during chronic infection contain defective viruses due to internal deletions, hypermutations [9] and this indicates a potential for underestimation of the amount of intact and replication-competent provirus by VOA. The size of the reservoir has been

modelled to predict the time to rebound after treatment interruption [10] and even with a continuous decline in the replication-competent HIV-1 reservoir through the use of anti-latency drugs, predicting the time to plasma HIV-1 RNA rebound following treatment interruption remains stochastic.

Animal models for use in reservoir studies and the testing of cure concepts

Non-human primate (NHP) models are very useful tools for reservoir eradication research as host responses and simian immunodeficiency virus (SIV) replication profiles can be monitored under controlled conditions and allow use of intensive tissue analysis in pilot studies of new therapeutic interventions [11]. Their importance has recently been highlighted in a pivotal study of 20 rectally SIV-infected rhesus monkeys of Indian origin [in the absence of protective major histocompatibility complex (MHC) class I alleles *Mamu-A*01*, *Mamu-B*08* or *Mamu-B*17*] [12]. These and previous results suggest a very early reservoir seeding prior to the detection of plasma viraemia that could not be prevented by the prompt initiation of suppressive ART [13]. Interestingly, proviral SIV DNA was absent in blood peripheral blood mononuclear cells (PBMCs) but detectable in lymph nodes and gut mucosa, and inversely correlated with local antiviral drug concentrations.

Work by Michaela Müller-Trutwin's group (Institut Pasteur, Paris, France) has focused on SIV natural hosts and controllers in order to study the requirements for reservoir formation and inflammation control [14]. Natural NHP hosts do not have chronic inflammation, increased T cell activation, gastrointestinal T helper (Th) type 17 depletion or progression to AIDS. They also harbour reduced levels of SIV DNA in their secondary lymphoid organs and long-lived T lymphocytes despite high plasma viraemia (Figure 2). Research on regulation of inflammation control in SIV natural hosts centres on early innate immune responses such as natural killer (NK) cells, plasmacytoid dendritic cells (pDC) and myeloid dendritic cells in African green monkeys (AGM). These animals have pDCs with normal SIV-sensing capacity that produce a strong but rapidly controlled interferon (IFN) type I response during acute SIV infection. This downregulates IFN-stimulated genes and the activation of the adaptive immune [15]. How these and other immunological factors, in combination with possible epigenetic mechanisms, protect specific immune components and prevent chronic inflammation is the subject of ongoing research. Natural SIV controllers will be crucial to help decipher the development of

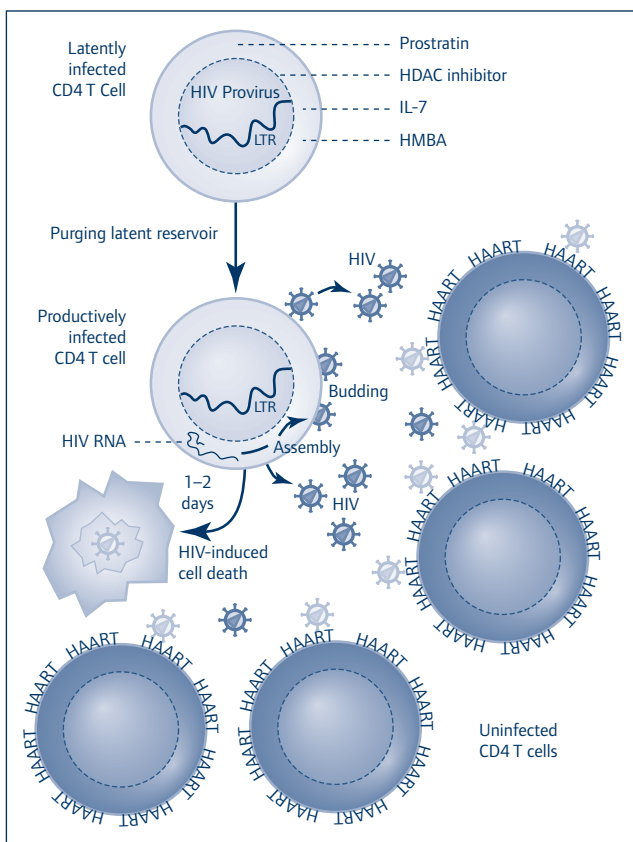


Figure 1. The use of histone deacetylase (HDAC) inhibitors alone or in combination with other latency reversing agents [IL7, prostratin or hexamethylbisacetamide (HMBA)] to purge the persistent proviral HIV infection by activating latent HIV from CD4 T cells. The use of antiretroviral treatment (HAART) is necessary to prevent HIV spread to uninfected CD4+ T cells

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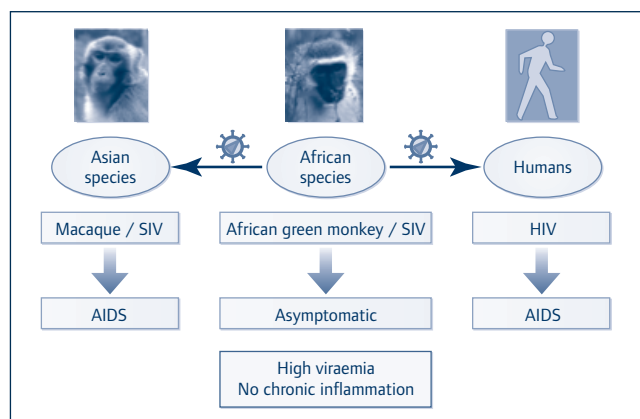


Figure 2. Primates with human or simian immunodeficiency virus (HIV/SIV) infections can be divided in to natural hosts (for example, the African green monkey) and non-natural hosts (humans, macaques). Natural hosts have the ability to tolerate high plasma viraemia without aberrant chronic inflammation or progression to AIDS

immunological factors underlying the formation and control of viral reservoirs in tissues, particularly during early infection.

Guido Silvestri's group (Emory University, USA) uses NHP models to study immunological aspects of cure strategies. They have recently explored the feasibility of autologous haematopoietic stem cell transplantation (HSCT) following myelo-ablative total body irradiation in three SIV-infected rhesus macaques [16]. Despite successful HSCT under ART and undetectable PBMCs SIV DNA levels, rapid viraemia rebounds were observed in two of three animals following ART interruption. Necropsy of the third animal revealed SIV DNA in circulating CD4 T cells, spleen and LN. Thus, drastic haematopoietic reset alone is probably not sufficient to achieve a cure. Additional immunological approaches to HIV-1 cure that consider the complex differentiation pathways and activation stages of CD4+ T cells might be necessary (Figure 3). This group's ongoing research focuses on the immunological effects of three possible cure strategies in NHP models. The 'shock and kill' concept which assumes cytotoxic killing of latently infected cells after viral reactivation [17]. The importance of CD8+ mediated responses is stressed by the observation that their depletion in ART-treated SIVmac239-infected rhesus macaques resulted in viraemia rebound within days or weeks after depletion. Recovery of the CD8+ cells resulted in resuppression of viraemia [18].

Another experimental avenue involves preventing the activation of the latently infected memory T cell pool. Without viral production from these cells, the reseeding of the reservoir would be blocked and immune activation diminished. This hypothesis is supported by the fact that infusion of the immuno-regulatory cytokine interleukin (IL)-21 in eight SIVmac239-infected rhesus macaques on ART resulted in improved viraemic control over time and increased Th17 recovery compared to controls on ART alone. This improved Th17 recovery could limit gastrointestinal microbial translocation and its associated immune activation.

The persistence of a stable latent reservoir is facilitated by the homeostatic proliferation of central memory (T_{CM}) and stem-cell memory (T_{SCM}) T cell subsets [19]. T_{SCM} harbour high per-cell HIV-1 DNA levels, are archived during PHI and contribute progressively to the HIV-1 reservoir over time (from 1.0% during PHI to 24.0% in individuals on long-term ART). Testing using VOAs has confirmed the ability of T_{SCM} from individuals on long-term ART to produce replication-competent virions [20].

The third type of cure concept involves producing a shift for long-lived memory T cells into shorter-lived transitional memory (T_{TM}) or effector memory (T_{EM}) T cells, thereby increasing the reservoir decay rate (i.e. 'push and vanish'). This may be achieved by combining proliferative-type cytokines (IL-7, IL-15) with co-inhibitors (PD1, LAG-3, TIGIT), by blocking T_{SCM} self-maintenance differentiation pathways or by exploiting the higher CCR5 levels on T_{EM} compared to T_{CM} with the use of maraviroc. This general concept was tested in SIV-infected sooty mangabeys, natural hosts, which have minimal SIV DNA in T_{SCM} and T_{CM} [21,22]. However, 6 months of rapidly suppressive ART in two monkeys did not result in persistent viraemia control following ART interruption. Important caveats in this concept are the optimal identification of T_{CM} and T_{SCM} with replication-competent virus and the influence of the non-CD4 expressing reservoir (macrophages).

Gene therapy

One of the main challenges for obtaining a cure is the identification of HIV-specific, efficacious, safe and scalable interventions. Gene therapy could offer such a specific approach

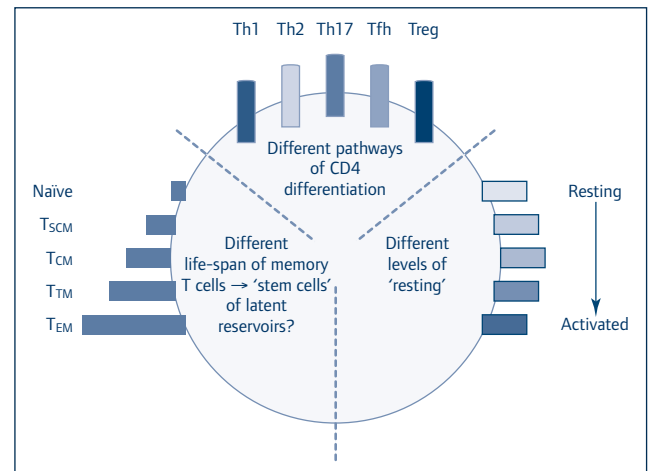


Figure 3. The complexity of the CD4+ T cell, considering the various subsets (Th1, Th2, Th17, Tfh, Treg), differentiations (naive, T_{SCM} , T_{CM} , T_{TM} , T_{EM}) and levels of activation. Integrated proviral HIV DNA may be present in all these CD4+ T cells during an HIV infection, which complicates the search for a sterilising cure

and Jan van Lunzen (University of Hamburg, Germany) provided an overview on the subject. The use of a limited number of gene-modified cells might be sufficient due to their beneficial effect on other cell types (bystander effect) or improved survival through natural selection *in vivo*. HIV-1 can be targeted by gene therapy at various stages, such as pre-integration, post-transcription or during viral assembly. Intervening at a pre-integrational stage could promote the preferential accumulation of gene-modified cells that do not carry HIV-1 DNA. The infusion of *ex vivo* expanded CD4+ T cells modified with the peptide M87o/macC46, which inhibits gp41 fusion, was shown to be safe and resulted in temporary CD4+ T cell increases in a proof-of-concept study using multiple treatment-experienced patients with drug resistance [23]. *Ex vivo* CCR5 gene disruption upstream of the naturally occurring CCR5-Δ32 mutation by zinc finger nucleases (ZFN) has produced preferential expansion of infused autologous CCR5-modified CD4+ T cells (SB-728-T) in 12 ART-suppressed patients. However, treatment interruption resulted in viral rebound in all patients; one individual, heterozygous for CCR5-Δ32 deletion, re-suppressed before ART re-initiation [24]. Despite promising initial findings, the off-target effects of ZFN remain unknown [25]. The more-specific artificial restriction enzymes, TALENs (transcription activator-like effector nucleases), were shown to induce a dose-dependent specific and efficient transient T cell receptor modification *in vitro* after mRNA-electroporation. T cells modified by TALENs showed reduced infectivity without a significant impact on other cell functions [26]. The selective CCR5 disruption might also promote a tropism shift towards CXCR4 usage, which has occurred in a CCR5Δ32 homozygous stem-cell transplant recipient upon treatment interruption [27]. A combination of CCR5 gene disruption with M87o/macC46 modification of T cells could theoretically prevent this phenomenon. Another approach includes the excision of integrated HIV-1 DNA by Tre-recombinases. These enzymes detect HIV-1 integrated LTRs and have been used successfully *in vitro* [28] and in humanised mice with transplanted Tre-transduced human CD4+ T cells [29]. Future studies using this approach are planned.

In the absence of safe gene therapies to disrupt CCR5, the allogeneic stem-cell transplant of an HLA identical homozygous CCR5Δ32-deleted donor remains the only documented cure for HIV-1. Notably, the 'Berlin patient' is the only HIV-1 patient among seven others, who has survived a homozygous CCR5Δ32 allogeneic stem-cell transplant. Apart from the stem-cell

transplant-associated mortality, this therapy is restricted by the limited availability of adult donors with homozygous CCR5 Δ 32 deletions and the necessity of HLA matching. Other stem-cell transplant-based approaches have failed to establish a cure. The use of wild type CCR5 stem-cell transplant in the two long-term (>20 years) HIV-1-infected Boston patients after reduced-intensity conditioning and the use of ART for 2–5 years post-transplantation, could not prevent viral rebound upon ART interruption. In these patients, no HIV-1 DNA was isolated from PBMCs and rectal tissue, VOA were negative and anti-HIV antibodies absent. These results indicate that new treatment combination approaches will be necessary to be tested in proof-of-concept studies. A multicentre collaborative project has therefore been initiated to investigate the potential for HIV-1 cure in patients requiring allogeneic stem-cell transplant for haematological disorders (EPISTEM), and is supported by the AmfAR Research Consortium on HIV Eradication (ARCHE).

Pharmacokinetic and clinical challenges of eradication strategies

Knowledge of the pharmacokinetic challenges required to purge the HIV-1 reservoir *in vivo* is essential for eradication research. Saye Khoo (University of Liverpool, UK) has stressed the need for research on ART and LRA penetration in HIV-1 sanctuary sites in order to: define target concentrations in light of the significant inter-patient variability of current methods; assess viral dynamic discrepancies between compartments; and track the evolution of drug resistance in separate compartments. Compartmental drug exposure depends not only on the ability of the drug to penetrate a compartment but also on the compartment-specific metabolic pathways and pharmacogenomics. The main tissue compartments discussed were the central nervous system (CNS), genital tract, breast milk, lungs and lymphoid tissues.

The effect of ART on the HIV-1 reservoir in the CNS remains incompletely understood. Although lower CNS penetration effectiveness (CPE) correlates with higher cerebrospinal fluid viral load and HIV-1 genetic diversity, the potential of ART with a high CPE score to improve cognitive function is disputed [30], and its exposure in microglia and macrophages needs to be better defined. Targeting the CNS reservoir may further be limited by ART neurotoxicity or the selection of drug resistance due to plasma/CNS viral discordance as was found in participants with viral blips in PARTITION (Penetration of Antiretroviral Therapy into the Nervous System study) [31]. Single nucleotide polymorphisms in enzymes involved in drug metabolism (e.g. CYP2B6 G516T for efavirenz) could also influence CNS drug exposure [32].

ART penetration into the genital tract is partially dependent on the variable expression of transporters, including P-glycoprotein, multidrug resistance protein (MRP)-2 and MRP-4, which are drug specific rather than class specific. However, results from the PARTNER study suggest that suppressed plasma HIV-1 RNA might be an adequate marker for HIV-1 RNA in the genital tract [33].

Despite the importance of breast milk in viral transmission [34] and its association with multiclass resistance in postpartum mother-to-child transmission, ART pharmacology in this compartment remains incompletely characterised. Treatment does not fully suppress cell-associated viral replication in CD4 T cells and macrophages in breast milk [35,36], and cell-to-cell HIV-1 transmission in the early postpartum period could be an important factor. HIV-1 transmission via breastfeeding can occur even when the mother is seemingly aviraemic. In addition, HIV-1

resistance mutations not present in the mother have been transmitted to the child [37], suggesting that the development of mutations might be driven by sub-therapeutic ART levels in breast-milk.

The relevance of the lungs in harbouring HIV-1 latently infected cells is unclear, although it has been shown that alveolar macrophages are preferentially infected compared to T cells and have irreversible impaired phagocytic function [38]. Macrophages and lymphoid tissue have shown variable drug levels with persistence of HIV-1 replication. Viral decay rates have been directly correlated with intracellular drug levels [39,40]. Taken together, modern ART does not adequately control HIV-1 replication in all body compartments and improved formulation as well as new compounds with better penetration (more potent pro-drugs, for example TAF and cell-targeted nanoformulations) are necessary to promote reservoir eradication.

Clinical trials

Several clinical trials have been conducted in humans in an attempt to purge latent HIV-1 from long-lived resting CD4+ T cells by LRAs, and were discussed by Mathias Lichterfeld (Harvard University, USA). Various drug classes (HDACi, protein kinase C activators, DNA methylation inhibitors) have been characterised or are already being tested *in vivo* as LRAs. HDACi are currently the most extensively studied LRAs. The anticonvulsant and HDACi, valproic acid, combined with intensified ART was the first compound shown to decrease the number of infected resting CD4+ T cells in four patients [41]. Studies with vorinostat, panobinostat and romidepsin have followed. A single orally administered dose of vorinostat was able to increase biomarkers of cellular acetylation and HIV-1 RNA expression in resting CD4+ T cells [42]. Repeated vorinostat exposure did not result in increased expression of cell-associated HIV-1 RNA [43]. Panobinostat is also administered orally and is active against HDAC subtypes 1, 2 and 3 with greater potency than vorinostat *in vitro* [44]. The administration of panobinostat, dosed three times weekly every second week for 8 weeks in a Phase I non-randomised trial in 15 aviraemic HIV-1 patients on ART showed an acceptable safety profile. This resulted in an increased level of cell-associated unspliced HIV-1 RNA and intermittent increases in plasma viraemia were observed in most patients [45]. Overall, no reduction in total and integrated HIV-1 DNA or in the VOA was observed. An interesting observation was that of nine patients who consented to analytical treatment interruptions, three patients with sustained HIV-1 DNA reductions during panobinostat treatment had the longest time to viral rebound. This may result from an observed increase in magnitude and breadth of HIV-1 specific cytotoxic T lymphocyte (CTL) responses during panobinostat treatment. However, CTL function did not correlate with changes in HIV-1 DNA, nor did protective HLA class I alleles. Other explanations could be the increased expression of interferon-stimulated genes (preferentially in IL-28B CC carriers), or the effect on innate immunity such as NK cells. Romidepsin was able to reactivate HIV-1 *ex vivo* [46], and is being tested as an intravenous drug in a randomised clinical trial (ACTG 5315). A non-randomised trial of several romidepsin treatments (day 0, 7, 14) in six patients has shown increases in cell-associated HIV-1 RNA and plasma viraemia after drug administration [47]. This compound was associated with a total of 36 drug-related adverse events during the 3 weeks of the study. Altogether, firm conclusions are difficult to draw from these preliminary and small human trials. These observations should be confirmed in larger groups of patients in order to exclude stochastic phenomena. It is important to stress

that these current treatment strategies do not target HIV-1 alone. The potential of oncogene activation by the HDACi class stresses the need for pharmacovigilance programmes.

The immunological and virological implications of treating primary HIV-1 infection

The evolving viro-immunological events and host dynamics in PHI are distinct from the later stage of the infection. PHI may represent a unique opportunity to interrupt immunopathogenesis, preserve vital host functions and prevent reservoir formation in long-lived and genetically diverse CD4+ memory T cells. Marcus Altfeld (Heinrich-Pette-Institut, Germany) presented data on early immunological responses to HIV-1 and treatment opportunities in PHI. Fiebig stages [48] divide PHI into windows of opportunity and encompass a 1-week eclipse phase post-infection; 4 weeks of progressively measurable disseminating virus with emerging antibody responses (Fiebig stages 1 to 4); and a potential point of no return in which a viraemia set-point is reached by partial immunological control after the massive initial CD4+ T cell destruction (Fiebig stages 5 and 6) [49].

Early intervention may help limit immunological dysfunction with subsequent lower levels of T cell activation (including IP-10 and TNF- α), which predicts long-term clinical outcome for the individual [50–52]. Host factors, innate immunity, HIV-1 specific CTL responses and CD4+ T cells are all important factors in controlling viraemia during early PHI. Autologous virus-specific broadly neutralising antibodies (bNAbs) become increasingly important thereafter. NK cells, triggered by IFN- α , expand prior to the decline of peak plasma viraemia as the first cellular immune effector cells. These cells are able to recognise infected CD4+ T cells depending on the expression of activating and inhibitory NK immunoglobulin-like receptors [53,54]. In a large international cohort of 615 patients with PHI (35.6% of whom were in Fiebig stage 4 or earlier) the presence of the HLA class I alleles B27 and B57 was associated with lower viral set-points [55]. The immunodominance of HLA-B27- or B57-restricted CTL responses (both in magnitude and breadth) in PHI is associated with fewer symptoms, persists during chronic infection and can decrease the contribution of T_{CM} to the size of the reservoir [56–59]. Apart from CTL, observations suggest that CD4+ T cells also demonstrate HIV-1-specific cytolytic responses during PHI [60]. In particular the emergence of Gag-specific granzyme A-positive CD4+ T cell responses was associated with better clinical outcomes [61]. Another subset of CD4+ T cells (Tfh) can provide immunological help to activate HIV-1-specific B cells, responsible for the production of neutralising antibodies; however, bNAbs are shown to develop after PHI in only a subset of patients. The preservation of CD4+ T cells and CXCR5+ PD-1+ CD4+ Tfh cells during PHI may be associated with better CTL function and higher levels of B cell activation and bNAB production [62].

The initiation of ART during early PHI preserves immunological functions that could increase the probability of HIV-1 control in patients in the absence of therapy. The existence of HIV-1 post-treatment controllers following ART initiation in PHI was first reported around 2000 [63,64]. However, for the majority of patients who have initiated treatment during PHI, viral control has not been achieved on treatment interruption [65]. The reason for this limited durability of viraemic control and its rarity remains under investigation. The expression of protective HLA class I alleles (B57 in particular) has been linked to sustained control of viraemia post-treatment [66]. An important observation is that the majority of the 14 post-treatment HIV-1 controllers of the

Visconti cohort did not carry these protective HLA class I alleles [2], indicating a role for other viro-immunological factors in viral control as observed in these individuals.

The impact of HIV-1 on the pathogenesis and the impact of ART during PHI were described by Carlo Federico Perno (University of Rome, Italy). Despite advances in PHI diagnosis, intense viral replication and dissemination occur before current laboratory methods can detect the virus [67]. Several viral factors have a role in PHI dynamics and outcomes. Co-usage of the CXCR4 receptor in PHI might increase integrated HIV-1 DNA in naïve (CD45RA+ CCR7+) CD4+ T cells of patients in Fiebig stages 3/4. This was associated with rapid disease progression, despite a comparable amount of HIV-1 DNA in T_{CM} (CD45RA- CCR7+) and T_{EM} (CD45RA- CCR7-) in slow progressors [68]. Viral replication capacity (associated with CTL escape mutations such as T242N, or resistance mutations affecting viral fitness)[69] and *nef* function [70] were found to be decreased during PHI in HIV-1 controllers. During PHI, a maraviroc-sensitive CCR5 conformation of CD4+ T cells is more advantageous to the virus, with reduced glycosylation of the conserved envelope viral protein [71]. Both CTL pressure and early ART initiation might prevent the selection of the most replication-competent HIV-1 strains with the highest infectivity and fewest antibody targets. Gastrointestinal lymphoid tissue and blood in early Fiebig stages have been shown to contain more CCR5+ CD4+ T cells with lower levels of HIV-1 DNA and cell-associated HIV-1 RNA [72,73]. Early ART initiation has been associated with reduced HIV-1 DNA and CD4+ T cell depletion. Some patients treated during Fiebig 1 reached HIV-1 DNA levels comparable to those of elite controllers (EC). Four perinatally infected children who initiated ART early (between 0.5 and 2.6 months of age) showed the absence of low level viraemia after a maximum of 18 years of follow up. HIV-1 sero-reconversion was frequent, the amount of HIV-1 DNA in PBMCs decays over time, and no replication-competent virus was recovered from three of the four children [74]. Virological control prior to the age of 1 in perinatally infected infants correlated with a limited proviral reservoir size in PBMCs, a negative serostatus [75] and improved clinical outcome [76–78]. Furthermore, ART during PHI prevented viral evolution in the gut lymphoid tissue [79] and limited viral diversification of low-level viraemia, which suggests the presence of an early archived HIV-1 reservoir [80]. Early ART has been shown to benefit the CNS, with reduced levels of markers of inflammation (e.g. neopterin, white blood cells) [81]. Together, a limited homogeneous viral reservoir and a more intact immune system following ART initiation during PHI rather than later during chronic infection may be an important prerequisite for successful future interventions in HIV-1 eradication. Additional interventions such as enhancing HIV-1-specific T cell immunity [82] or neutralising antibody production may be necessary. These issues highlight the need for additional hypothesis-driven studies on HIV-1 pathogenesis and the impact of early ART.

Inflammation and ageing in treated HIV-1 infection

Peter Hunt (University of California, USA) and Paddy Mallon (University College Dublin, Ireland) discussed ongoing inflammation in the context of ageing HIV-1 patients (Figure 4). The predicted life expectancy of patients in the modern ART era has been increasing, particularly among those who have initiated ART at higher CD4 cell counts [83]. However, an overestimation may occur due to a survivor bias as the oldest patients in contemporary cohorts are most likely to have protective behavioural or genetic factors that have allowed them to survive

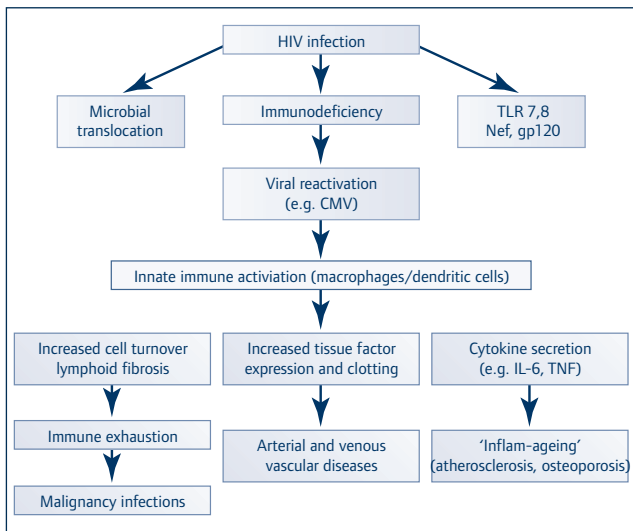


Figure 4. The relationship between HIV infection and important factors driving ongoing inflammation, ageing with HIV and immunosenescence
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advanced AIDS in the pre-ART era. A major challenge ahead will be the ageing HIV-1 population in sub-Saharan Africa. Many people in this group will have initiated ART at a more advanced disease stage [84]. HIV-1 is associated with increased rates of age-related morbidities, including cardiovascular disease (CVD), cancer, venous thromboembolism, type II diabetes, cognitive dysfunction and frailty, despite suppressive ART. Persistent inflammation has been increasingly recognised over the last few years as a possible contributor to these risks [85]. Its importance in HIV-1 pathogenesis is suggested by NHP models in which clinical progression is more closely related to the ongoing massive aberrant immune activation than to viraemia levels [86]. Prolonged, effective ART is to some extent able to reduce immune activation as sustained virological suppression decreases levels of activated CD38+ HLA-DR+ CD8+ T cells, although not to normal levels [87,88]. Observational studies have also found increased markers of inflammation (hsCRP, IL-6, D-dimer, cystatin-C) in treated HIV-1 patients compared with uninfected controls [89]. A nested case-control study of ART-suppressed participants in the SOCA cohort has shown that elevated markers of gut epithelial barrier dysfunction, innate immune activation and inflammation (IL-6, D-dimer, hsCRP, sCD14, i-FABP) all strongly predicted subsequent mortality [90]. Similarly, a pooled analysis of ART-suppressed control arms of the SMART, ESPRIT and SILCAAT studies has shown that a single IL-6 and D-dimer measurement continues to predict morbidity for the following decade [91]. This suggests the presence of an inflammatory set-point that drives the long-term clinical risk of disease.

The mechanisms underlying persistent innate and adaptive immune activation are incompletely understood. Low-level viraemia arising from the stable reservoir while being treated with ART [92] combined with microbial translocation resulting from the disrupted intestinal epithelial barrier and loss of mucosal immunity (CD4+ T cells and Th17 cells), as well as co-infections (e.g. CMV) are all suggested to contribute to persistent inflammation [93]. Some innate immune activation pathways may also exhibit feedback loops that perpetuate this inflammatory state [94]. For example, pro-inflammatory cytokines and lipopolysaccharide induce indoleamine 2,3-dioxygenase-1 (IDO-1) production in dendritic cells and macrophages, which in turn promotes tryptophan catabolism. These catabolites are neurotoxic (quinolinic acid), can impair T cell proliferation (kynurenine, picolinic acid) or cause Th17 depletion (3-hydroxyanthralinic

acid), which in turn causes lipopolysaccharide increases and results in a negative feedback loop of ongoing inflammation. Indeed, higher baseline kynurenine/tryptophan ratios have been associated with increased mortality in individuals starting ART [95].

Several treatment strategies have been suggested to decrease inflammation in HIV-1 patients, ART-suppressed viraemia remaining a pivotal factor. Spontaneous HIV-1 controllers also display microbial translocation [96], increased rates of monocyte activation (sCD163), atherosclerosis [97] and lymphoid scarring [98]. Remarkably, EC have even higher rates of CTL activation than ART-suppressed HIV patients. Treating EC with ART decreases their levels of immune activation, demonstrating that even very low levels of viral replication are sufficient to drive this phenomenon [99]. The initiation of ART within 6 months of HIV-1 seroconversion has led to modest decreases in CD4+ T cells (1.4%) and CTL activation (6.7%) compared with later ART initiation in the OPTIONS cohort [100]. Anti-inflammatory drugs (cyclosporine, inhibitors of toll-like receptor 4/pro-inflammatory cytokines) may reduce inflammation. Rosuvastatin was shown to decrease monocyte activation in the 48-week blinded placebo-controlled randomised SATURN-HIV trial [101]. A clinical endpoint study (REPRIEVE) with pitavastatin is intended to start in 2015. Restricting microbial translocation may be beneficial but recent studies with sevelamer [102], mesalamine [103], rifaximin [104] and probiotics [105] have been unsuccessful in reducing microbial translocation or immune activation in HIV-1 patients. Whether treating co-infections reduces CD38+ HLA-DR+ CD8 activation and is clinically meaningful remains unclear; trials have observed both beneficial effects of valganciclovir in cytomegalovirus infections [106], but also an absence of effect of valacyclovir in HSV-2 infections [107]. Finally, medical interventions could be irrelevant if the necessity of maintaining a healthy lifestyle (smoking cessation, exercise, diets) is overlooked [108]. As a conclusion, control of viraemia is not sufficient to prevent morbidity and inflammation. Strategies to reduce inflammation and prevent immune activation, or even produce immune responses that are beneficial without deleterious effects, should be further explored.

The relationship between ongoing inflammation and premature immunosenescence is especially of concern for the ageing HIV-1 population. ART and associated CD4+ T cell recovery >500 cells/mm³ has led to mortality rates in suppressed HIV-1-positive individuals comparable to those who are uninfected [109,110]. The current causes of mortality for HIV-1-positive individuals are non-AIDS related in 97% of cases. Non-AIDS-related morbidity is, however, increasing, especially in those over 50 years of age [111]. Three factors associated with accelerated ageing were highlighted by Paddy Mallon: immune dysfunction, bone disease and CVD. First, the T cell subsets are strikingly similar between populations of treated HIV-1-positive adults and HIV-1 uninfected elderly individuals [112]. The lower CD4/CD8 T cell ratio found in these populations has been correlated with a greater number of effector memory CD4+ and CD8+ T cells and fewer naïve CD4+ and CD8+ T cells [113], and could predict non-AIDS-related morbidity [114].

Secondly, bone mineral density (BMD) in HIV-1-positive patients is lower. Low BMD was much more prevalent in patients in the UPBEAT study, a prospective cohort of HIV-1-positive and -negative subjects from similar demographic backgrounds, regardless of the site of BMD measurement [115]. This lower BMD in HIV-1 patients seems multifactorial and higher levels of bone-turnover markers (osteocalcin, procollagen type 1 N-terminal, collagen type 1 cross-linked C-telopeptide) have

been seen [116]. Not only HIV-1 infection itself, but also exposure to ART has been shown to reduce BMD, with most of the loss observed in the first year after ART initiation. Reductions in BMD have been observed with both first [117] and second-line ART therapy in unsuppressed patients after first-line failure, with relatively greater BMD losses observed with the use of tenofovir disoproxil fumarate (DF) [118]. In virologically suppressed patients, switching from tenofovir-DF to tenofovir-DF-sparing ART in the TROP and OsteoTDF studies [119,120], or vice versa in the SWAP and PREPARE studies [121,122], significant increases and decreases in BMD were seen, respectively. Calcium/25-OH vitamin D supplementation for mild-moderate vitamin D deficiency in patients initiating efavirenz/tenofovir-DF/emtricitabine prevented some BMD reduction over 48 weeks following treatment initiation [123]. Placebo-controlled randomised trials of bisphosphonates are ongoing.

Thirdly, CVD-related mortality is the predominant cause of death in virologically suppressed HIV-1 patients with good CD4 responses [124]. The pathogenesis of CVD in HIV-1 infection is complex with contributions from age, HIV-1 and lifestyle. However, there is 10% discrepancy between the predicted and actual CVD mortality rates in HIV-1-positive individuals [125], and factors other than associations between CVD and exposure to abacavir or lopinavir, or dyslipidaemia, for example, are likely to be involved [126,127]. One biologically plausible mechanism for the association between abacavir and CVD is that abacavir has been found to alter platelet function, as noted in a change in the soluble glycoprotein-6 level, which is involved in platelet activation [128]. Furthermore, patients with HIV-1 have increased rates of dyslipidaemia, characterised by low levels (<1.0 mmol/L) of the cardioprotective high-density lipoprotein cholesterol (HDL-C) [129]. Whether ART-related dyslipidaemia [130] and the use of interventions that increase levels of HDL-C influence clinical endpoints is unclear. Finally, HIV-1-related ongoing inflammation (particularly monocyte activation) affects CVD risk. Recent research has shown that platelet activation and endothelial dysfunction markers, but not the monocyte activation markers sCD163 or sCD14, were decreased after ART initiation [131]. The latter have been associated with changes in carotid intima media thickness [132] and non-calcified coronary plaques [133,134]. Future challenges will be to study ART benefits in older patients, identify clinically useful markers of inflammation and identify key interventions in order to prevent HIV-1-related accelerated age-associated disease risks [135].

Identification and treatment of patients with PHI

PHI identification is currently restricted by both physician (insufficient knowledge of either the clinical identification of the acute stage of the infection or lack of suitable tests to ascertain diagnosis) and patient-related factors (knowledge, shame or active denial of high risk). Jürgen Rockstroh (University of Bonn, Germany) emphasised the similarities about raising awareness for PHI and acute hepatitis C virus (HCV) infection and described the lessons learned in HCV. The acute HCV epidemic among HIV-1-infected MSM warranted a collaborative effort to halt onward transmission of the infection [136]. As a result, the European collaborative network (NEAT) introduced a joint definition for acute HCV in 2011 [137]. As is the case for PHI, screening with antibody tests is not diagnostic of HCV infection shortly after high-risk transmission events and therefore HCV RNA tests should be used [138]. Acute HCV screening is cost-effective [139] and early treatment is recommended as it

benefits patients [140]. However, for prevention of the HCV epidemic, the unique opportunity for early intervention with new direct-acting antivirals (DAAs) has been missed. This is attributed to the high and non-reimbursed costs of DAAs (indicated for only advanced chronic HCV in Europe) and the increasing unwillingness of patients, or the lack of urgency for pegylated interferon- (peg-IFN) based treatment. Studies on peg-IFN- α -free DAA-based treatment strategies in acute HCV are planned. Several trials are ongoing to evaluate the efficacy of DAAs (boceprevir, telaprevir, sofosbuvir, ledipasvir) for 6, 8 or 12 weeks, with or without ribavirin or peg-IFN, and for various HCV genotypes. Despite the high sustained virological response rates of all-oral DAAs (>95.0%), the previously mentioned factors combined with ineffective screening continues to fuel the MSM epidemic. Without changes in behaviour, the risk for re-infection in this particular patient group remains as high as 25.0% within 2 years of the first acute HCV episode [141,142].

In contrast to peg-IFN-based HCV treatment, current ART is well tolerated, reimbursed and patients are in general willing to receive treatment. Successful PHI identification and prompt linkage to care are major goals and were highlighted by Anton Pozniak (Chelsea and Westminster Hospital, London). Although the symptoms of PHI are well described, they are not specific and the personal context (i.e. sexual preference, origin, substance abuse) is frequently unknown [143]. An important consideration in increasing PHI screening efforts and initiating early treatment is the prevention of amplified transmission during PHI. HIV-1 infectivity is highest during PHI [144] and accounts for approximately half of all transmissions [145]. Patients who are unaware of their infection represent the minority of those who are infected but cause approximately half of all new HIV cases [146]. Relevant testing leading to a diagnosis of PHI is performed by physicians in relatively few patients despite the fact that most patients with PHI will already have had frequent voluntary antibody testing [147]. Furthermore, routine screening for HIV-1 in high-risk settings has been shown to be beneficial [148]. Together this argues for more active screening and supports targeting high-risk social environments (gay clubs, sex parties) together with sexual-contact tracing.

Another important factor is the use of proper algorithms for the necessary tests to diagnose or exclude PHI. The window of opportunity is short and the utility of certain tests depends on the Fiebig stage [149]. The current fourth generation combined p24 antigen/HIV antibody ELISA can detect seroconversion approximately 2 weeks post-infection, which can be further reduced to 10 days by using RT-qPCR. A negative/indeterminate fourth generation ELISA in the setting of a recent high-risk event or clinical suspicion of PHI should be followed by RT-qPCR. A negative PCR in the eclipse phase warrants a repeated PCR after 1 week. Rapid point-of-care RT-qPCR tests can detect PHI when HIV-1 RNA typically exceeds 50,000 copies/mL [150].

Although the above mentioned virological and immunological effects of early ART are of potential benefit to patients, no long-term trials are available to evaluate the benefits for an individual patient against the potential harm of life-long early ART [151]. In trials conducted to date, PHI was defined as HIV-1 seroconversion within 6 months, which makes conclusions about outcomes difficult to generalise. The subgroup analysis of patients on ART within 12 weeks of seroconversion in the SPARTAC trial showed improved clinical outcome [152]. Overall, trials have shown that ART administration has led to improved short-term CD4+ T cell recovery, slowing the rate of CD4 T cell decline and may even lower viral set-point after treatment discontinuation. Some trials have shown absent levels of HIV-1

DNA in CD4+ T cells with improved immune reconstitution in patients treated during Fiebig 1 [153]. Very early ART may provide the opportunity for a successful treatment interruption in some patients, although most do not achieve spontaneous control after treatment interruption. Furthermore, the factors predicting HIV-1 control post-treatment interruption remain unknown. No evidence exists to show that immunomodulators may improve immune responses during PHI and the optimal ART regimen to be initiated at this stage has yet to be determined. Importantly, none of the randomised trials have provided evidence that early ART has had an impact on AIDS, death or serious adverse events. Together, although ART during PHI decreases onward HIV-1 transmission and has probable biological benefits, definite clinical advantage at the individual level remains to be assessed. Nevertheless, screening efforts for PHI should continue to be improved and more therapeutic clinical trials, especially for patients in Fiebig 1 and 2 are needed.

Conclusion

A network of scientists, clinicians, funders and committed members of the HIV community continues to develop the HIV research field. An opportunity for collaborative European research lies ahead, which will increase the probability of producing breakthrough data on the HIV reservoir, cure strategies and PHI. The first steps towards an intensified collaboration have taken place and the commitment to fight for a common cause should benefit our joint research activities.

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