

# The Search for a Cure for Persistent HIV Reservoirs

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## Abstract

**The persistence of prolonged HIV reservoirs in patients on effective antiretroviral therapy is the main hurdle to HIV eradication. However, major advances have been made over the last few years, both in basic and clinical science of HIV reservoirs. Consequently, the scientific community no longer banishes the term “cure”. Despite such renewed hope, there is little investment by both public and private groups in the field. It is therefore imperative to make this a priority and allocate sufficient resources, especially financial support, to aid in finding the cure since there is no effective preventive vaccine. This article discusses the main scientific aspects and strategies to address and build an international task force tackling issues associated with HIV reservoirs. (AIDS Rev. 2011;13:63-6)**

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## Key words

**HIV reservoirs. HIV persistence. HIV eradication. HIV cure.**

## Introduction

The advent of combination antiretroviral therapy (ART) has improved quality and expectancy of life quite considerably among the victims of HIV/AIDS globally. However, current ART does not have the ability to eradicate HIV infection completely. Persistent proviral infection in a small pool of cells provides a long-lived source of rebound viremia, which is the “reservoir” of HIV infection<sup>1</sup>. The main consequence is that, once initiated, ART is life-long, which implies that the problems of adherence, toxicity, and cost associated with it must be taken care of by the patient. Despite a deep knowledge of HIV transmission routes, the HIV pandemic remains very active, with more than 7,000 new cases of infection daily. In fact, for each new patient on ART, two to three new cases of infection occur in the world.

Most of such cases are commonly in developing countries where there is either little or no treatment access. Nevertheless, the developed countries also face a problem of sustainability of ART due to its huge financial demands. It is therefore paramount that in the absence of an effective preventive vaccine, funding the cure initiatives should be given a priority.

Considerable progress has been achieved over the past few years in understanding the mechanisms of HIV latency and designing new strategies to attack it<sup>2,3</sup>. Nevertheless, it has been ascertained that the level of involvement of governmental agencies and private investors in the field of HIV eradication is minimal, with no proper coordination. Faced with the urgency of the situation, we call for the setting up of an international, coordinated, multidisciplinary task force against HIV reservoirs.

## Mechanisms of HIV persistence

Despite sustained viral suppression with ART, the replication ability of HIV persists. Such persistence has been found to occur in a pool of cells although very low levels of HIV RNA are detected in plasma. In addition, some anatomical sites have been established to act as sanctuaries for the virus. The mechanisms of HIV latency maintenance in CD4<sup>+</sup> lymphocytes are very

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important discoveries. Stable post-integration latency is the most relevant aspect of HIV persistence. Such a pool contains about  $10^7$  cells in an infected individual and the situation is stable and long-lived<sup>4</sup>.

Most mechanisms maintaining HIV latency operate at the transcriptional level. Hypo-acetylation of histones by histone deacetylase correlates with transcription repression, although histone methylation can be associated with both activation and silencing. Several features of the metabolism of resting CD4<sup>+</sup> cells are also important for the establishment of latency. Resting cells lack co-activation factors such as nuclear factor-kappa B or nuclear factor of activated T-cells; positive transcription elongation factor-b can be at reduced levels or sequestered by inhibitors<sup>5</sup>. Finally, another regulation step is situated at the level of cellular microRNA and RNA interference. HIV proviral latency is therefore an active phenomenon and an unstable state of HIV infection amenable to therapeutic attack<sup>1</sup>. At the anatomic level, gut-associated lymphoid tissue remains the major viral reservoir on ART, while the brain is both a reservoir and a sanctuary site where drug diffusion is limited<sup>4</sup>.

### Critical questions

Several issues still require investigation, but two questions seem top priority. The first is to determine the nature of non-lymphocytic HIV reservoirs. Chomont, et al.<sup>6</sup> established that the large majority of the reservoir resides in CD4<sup>+</sup> T lymphocytes, particularly in the central memory and transitional memory subpopulations. However, the circulating viral variants in half of the patients on long-term effective ART are composed of genetically indistinguishable viral clones, which are rarely found in resting CD4<sup>+</sup> T-cells in the peripheral blood<sup>7</sup>. It has been proposed that multipotent hematopoietic progenitor cells and monocytes could contribute to the reservoir<sup>8</sup>. Their characterization is of utmost importance in designing strategies that are not uniquely oriented towards the lymphocytic reservoir.

The second priority is to determine whether there is a “threshold”, below which we can drive the HIV reservoir so that the body will be able to control it. The recent observation by Chun, et al.<sup>9</sup> of a patient with undetectable HIV DNA both in blood and in tissue, who experienced viremia rebound after ART cessation, tends to show that such limit does not exist. If it is proved that one remaining latent, infected cell is sufficient to reignite a whole infection, most eradication approaches will have to be complemented by an immunological intervention.

### New hope ahead

The year 2010 was marked by the confirmation of the first case of HIV cure in a patient who had been transplanted with stem cells from a donor homozygous for the CCR5Δ32 deletion<sup>10</sup>. Although this will probably remain an isolated case<sup>11</sup>, it was a proof of concept that HIV can be cured and it became a major boost to optimism in the field. The confirmation that when ART is initiated very early at the time of acute HIV infection, a significant proportion of patients can remain aviremic when ART is subsequently stopped is very important<sup>3,12</sup>. This “functional cure” is an attractive approach to give patients drug-free time. However, it cannot be the ultimate goal since even “elite controllers” still experience disease activity like persistent inflammation.

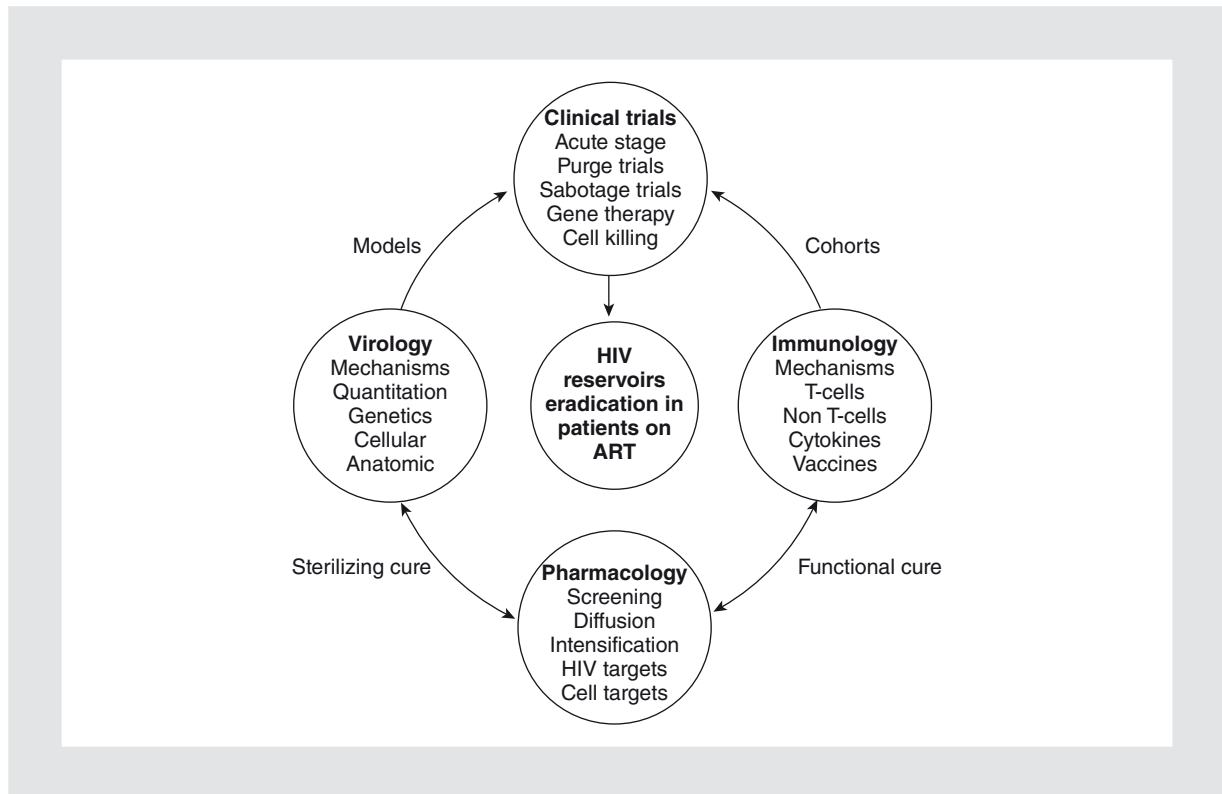
### Need for better antiretroviral therapy

It is generally admitted that current ART combinations are able to block HIV replication totally<sup>13</sup>. However, what is established *in vitro* is probably wrong *in vivo* due to the limited penetration of antiretrovirals in body systems and organs like the central nervous system. Furthermore, very little is known of the pharmacology of these drugs in tissue cells, like in the gut, compared to circulating lymphocytes. All intensification trials have failed to show any effect on persistent viremia < 50 copies/ml, with the exception of only one study<sup>14</sup>, which addressed the evolution of tissue viral load and found changes in some regions such as the terminal ileum. Moreover, in one intensification trial with raltegravir, 30% of patients experienced a transient increase in 2-long terminal repeat circles in peripheral lymphocytes, which is an indication that ongoing viral replication was present<sup>15</sup>.

There is therefore need for new antiretroviral drugs that have the ability to target the HIV reservoirs. They certainly will not be able to eradicate HIV by themselves, but developing latent HIV reactivation strategies without a robust ART in each compartment could induce more harm than good<sup>16</sup>.

### Purge of HIV reservoirs

The first type of strategy includes reactivating latent reservoirs in the presence of effective ART with the “oncologic” intention to deplete residual disease<sup>1</sup>. Initial attempts with cytokines or valproic acid were ineffective and sometimes toxic<sup>17</sup>. Histone deacetylase inhibitors remain the most promising compounds, with new molecules isolated regularly. Products like vorinostat



**Figure 1.** The strategy towards HIV eradication in patients on antiretroviral therapy (ART) should contain four coordinated modules. Basic and clinical science should be closely related to drug discovery and development. Testing in adequate models and studying special cohorts of patients should lead to clinical trials trying both functional and sterilizing cure approaches.

(suberoylanilide hydroxamic acid), which are already in the market in some places, are both more potent and specific than valproic acid<sup>1</sup>. Some molecules have been identified to have the ability to induce proviral expression via induction of the protein kinase C signaling pathway, as prostratin, or by releasing positive transcription elongation factor-b, as hexamethylene bisacetamide<sup>3</sup>. Interleukin-7 (IL-7) has been proposed to “purge” HIV reservoirs, but could also contribute to its homeostatic proliferation<sup>6</sup>. However, in a recent study, HIV-1 RNA blips following one administration of IL-7 were closely related to previous circulating viruses and did not come from the reservoir<sup>18</sup>.

It is likely that different kinds of drugs will have to be combined or sequentially given in order to purge each compartment<sup>19</sup>. This strategy, which aims at obtaining a “sterilizing cure”, can be followed by a last killing step<sup>20</sup>, with immunotoxins for instance. Beside the previously mentioned risk of activating HIV in the brain without correct ART diffusion<sup>16</sup>, these strategies could also theoretically reactivate other ancestral retroviral sequences present in the human genome and promote the emergence of lymphomas as well<sup>21</sup>.

## Sabotage of HIV infection

These approaches try to impair the normal pathophysiological mechanisms of HIV infection. Drugs can be selected to induce lethal mutations in the HIV genome, leading to progressive viral ablation. This concept of “error catastrophe” has already been tested in cell cultures with KP-1461<sup>22</sup>. Gene therapy is another promising way to hijack the damages induced by HIV on the immune system. Hematopoietic stem cells have been treated by zinc finger nucleases to disrupt CCR5 expression<sup>23</sup>. Once transplanted in an animal model of HIV infection, the hematopoietic stem cells resistant to infection were rapidly selected and almost completely replaced CD4<sup>+</sup>CCR5<sup>+</sup> in the gut<sup>24</sup>. Trials are ongoing in humans using CD4<sup>+</sup> T lymphocytes modified in a similar manner.

## Task force commitment

Tackling HIV persistence in viral reservoirs needs innovation, ingenuity, and rationale. Eradication of HIV-1 reservoirs requires a prolonged scientific commitment

to understanding the molecular mechanisms of HIV persistence and the immunological factors at play. In addition, it requires safe and effective new drugs, new design of therapeutic approaches, as well as testing them in adequate models before clinical application (Fig. 1). Involvement of governmental agencies, research agencies, funding groups, and pharmaceutical companies at their highest level is fundamental for building this multidisciplinary task force for HIV eradication. The decisive step must be taken immediately and with necessary urgency.

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