

# Treatment ISSUES

## Adenovirus-based vaccines against HIV: A look at how the immune system could be trained to combat HIV

Dr. Pablo Penaloza-MacMaster

The first cases of HIV infection were documented in the early 1980s and quickly gained overt attention from the public, not only because of HIV's potential life-threatening symptoms, but also because of its associated social stigma. Nowadays, HIV infection is gradually becoming a more treatable condition, similar to diabetes or high blood pressure, but still represents a major cause of illness and death in many parts of the developing world. Hence, the quest for a vaccine still remains a holy grail in the field of vaccinology. Finding an HIV vaccine has been one of the greatest challenges in vaccine research. Almost three decades of arduous investigation have taught the scientific community many lessons about the immune system and how vaccines typically work. Here we outline the basic principles of vaccination, and explain the rationale behind the next generation of promising HIV vaccine candidates based on the use of alternative (rare) adenoviral vectors.

The history of vaccines started back in 1796 when an English physician, sir Edward Jenner, used pus scraped from cowpox blisters to vaccinate people against smallpox. The out-of-the-box idea was used to prime the immune system with a less virulent, genetically related virus, in order to induce immune memory to the deadly smallpox virus in humans. This was the first vaccine ever created, and shortly after, using a less virulent or attenuated form of a pathogen was popularly applied to prevent other diseases, including polio, measles, yellow fever, and rabies. This resulted in effective, life-saving vaccines that drastically increased life expectancy in the human population. However, in the case of HIV, high mutation rates are associated with spontaneous increase in virulence, rendering the approach of using live or attenuated viruses inadequate and dangerous for a vaccine.<sup>1,2</sup>

Currently, a great deal of focus in vaccine research has been centered on the elicitation of immune responses using viral vectors that serve as a "Trojan horse" to deliver vaccine antigens to the immune system.<sup>3,4</sup> The Merck

(STEP) vaccine trial consisted of a modified cold virus (adenovirus 5 or Ad5) expressing several proteins derived from HIV.<sup>5</sup> It is thought by many scientists in the field that the failure of the STEP trial may have been at least partially due to the presence of pre-existing immunity to Ad5, which is widely distributed among humans.<sup>6</sup> According to this hypothesis, when volunteers were vaccinated with an Ad5 vector expressing HIV genes, pre-existing Ad5-specific immune responses quickly neutralized the Ad5 vector before it had a chance to deliver and express its cargo of HIV antigens to the immune system.

In layman terms, imagine a hypothetical village that has been previously attacked by hostile colonizers on white horses. After continuous assaults, the village would have trained an army of highly specialized soldiers acting as gatekeepers to quickly obliterate anything that resembles a white horse.

As a result, it would be very difficult to deliver an important message to this village if an amicable emissary rides a white horse into the village. The emissary on a white horse would be confused with a hostile invader, and both the white horse and the emissary would be quickly annihilated before the message could be released to the village. So it would be wise to utilize a novel, alternative transport system, perhaps a black horse, to allow the emissary to infiltrate the village in order to express an important message (thus bypassing the pre-existing defenses geared against white horses). Now, think of immune defenses as the village's soldiers, and the seroprevalent Ad5 viral vector as the white horse from the example above. Up to 90% of people in some populations have been exposed to Ad5,<sup>6</sup> so most individuals have pre-existing immune responses that quickly counteract Ad5, limiting the efficacy of this viral vector to deliver vaccines antigens.

Adenoviruses are a class of virus that most commonly cause respiratory illness, with symptoms ranging from the common cold to bronchitis. However, some adenoviruses cause infections in the tonsils and intestines with

*In the case of HIV, high mutation rates are associated with spontaneous increase in virulence, rendering the approach of using live or attenuated viruses inadequate and dangerous for a vaccine.*

no symptoms at all. These viruses constitute an excellent vector delivery system with potential use in vaccines and gene therapy due to their high insert capacity. In other words, scientists can stuff many foreign genes inside adenoviruses without losing viral viability. As a result, one can clone many HIV genes inside the adenoviral genome in order to elicit a potent and broad anti-HIV response. We like to think of adenoviral vectors as computers with a lot of memory capacity. They allow us to install many programs at once without the fear of failing or crashing. Adenoviruses are quite diverse. There are 65 different serotypes of adenoviruses, which display distinct ranges of seroprevalence in the human population, and many of these serotypes are currently being explored for their potential use as vaccine vectors. Thus, the toolkit for making adenoviral-based vaccines is quite ample, so even if the population acquires immunity to one vector, we may be able to substitute with another vector.

The reason why most people have higher pre-existing immunity to Ad5 compared to alternative or rare Ads is not fully understood. One possibility is that Ad5 vectors are able to cause a prolonged infection in the host, compared to rare Ads, which may cause a more transient infection. Persistent infection normally results in constant shedding of the virus, and greater chance of transmission to new hosts, thus increasing the prevalence of the virus in the community.

A great portion of vaccine research is focused on identifying novel viral vectors with low seroprevalence, which would circumvent the issue of neutralization by pre-existing immunity, mostly in the form of antibody responses.<sup>6-8</sup> Some rare adenovirus serotypes that have been identified are Ad26, Ad35, and Ad48 (and the list is growing). Due to their low seroprevalence in the population, vaccines based on these vectors may be able to efficiently deliver HIV antigens to jump-start the immune system without being immediately cleared from the body upon vaccination. It is possible that specific adenoviral vaccines would have to be tailored based on the prevalence of that adenovirus among particular populations. Most importantly, the immune system would then be able to develop immune responses to HIV antigens without actually being exposed to HIV.

There is growing evidence that rare adenovirus-based vaccines may offer additional advantages over Ad5 at triggering immune responses beyond the issue of pre-existing immunity.<sup>8-12</sup> This could be because different viral vectors engage the cells in the body in very distinct ways.<sup>13-18</sup> Their tropism and life cycles may explain their distinct elicitation of immune responses and the observed biases in the location of immune responses following vaccination.<sup>14,19,20</sup> Ad5 enter cells using a surface receptor called CAR (Cocksackievirus and Adenovirus Receptor), which is expressed in epithelial cells and red blood cells. On the other hand, rare Ads such as Ad26, Ad35, and Ad48 target CD46, which is an immune receptor important for complement-mediated immune responses.

Also, every virus has specific pathogen-associated molecular patterns, referred to as PAMPs, which trigger particular “danger signals” in the body. Some of the danger signals that are induced by specific vaccine vectors may be more suitable for generating a response against HIV. Because of these reasons, different viral vectors “tickle” the immune system in unique ways, stimulating specific receptors that influence the generation of immune responses.<sup>20,21</sup> Consistent with this notion, we have shown that rare Ads can offer protection upon a challenge with SIV (Simian Immunodeficiency Virus) in monkeys.<sup>9,11</sup> In addition, it was reported that a vaccine using rare Ads (but not Ad5) as viral vectors protected monkeys against an Ebola challenge.<sup>8</sup> Despite all the evidence showing enhanced responses with rare Ad vaccines, currently there are no approved vaccines that use adenoviral expressing vectors in humans. This is because we first need to better understand these viral vectors to make sure they are safe in humans.

Interestingly, we have preliminary data in naïve mice (devoid of pre-existing Ad5 immunity) showing that vaccination using the common Ad5 serotype as a viral vector generates cytotoxic T cells with impaired recall responses in many tissues, including the gut mucosa and lymphoid organs. Intriguingly, vaccination of mice with alternative adenoviral vectors results in a more potent recall response at these sites, which are niches of active HIV replication in humans.<sup>22,23</sup> HIV typically enters the body at mucosal surfaces, so recruiting an ample army of anti-HIV immune defenses to these sites by vaccination could offer preemptive sterilizing protection upon viral challenge. So at least when it comes to a vaccine, location and size do matter significantly.

In conclusion, vaccines consisting of alternative (rare) adenoviral vectors may offer substantial benefits over common Ad5 vectors to prime immune responses. Differences in vaccine efficacy may be explained not only by pre-existing anti-vector immunity, but also by the intrinsic biology of the viral vectors. The use of alternative adenoviral vectors has thus re-invigorated the field of HIV research, and has provided new hopes for an HIV vaccine. But regardless of the outcomes of the only HIV trial currently under way, it goes without saying that safe sexual practices and responsible adherence to antiretroviral therapy will remain critical in minimizing the incidence of infection, morbidity, and mortality associated with HIV infection in our community.

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***Vaccines based on adenoviral vectors may be able to efficiently deliver HIV antigens to jump-start the immune system...without actually being exposed to HIV.***

# Curing HIV infection, an effort renewed

Nancie M. Archin and David M. Margolis

## Introduction

A cure for HIV infection has long been a distant wish for millions of patients across the world. Thirty-one years ago, when the first case of what was to become one of the worst human health crises in history was reported,<sup>1</sup> researchers raced to find a treatment and a vaccine to stop the spread of this deadly disease. Although it was briefly hoped that antiviral therapy could cure infection, a sober and methodical quest for approaches to eradicate infection has now begun in earnest. This article will examine previous attempts to eradicate infection, challenges inherent in finding a cure for HIV, and the promise in a new approach using a drug called vorinostat.

## Early attempts at finding a cure

The introduction of combination anti-retroviral therapy (ART) in the mid-1990s heralded in a new era in treatment of HIV infection. Initially, it was postulated that a patient receiving ART could be cured by a few years of intensive ART.<sup>2</sup> However, in 1997, with the discovery that a reservoir of infected resting CD4+ T-cells persists despite effective ART, it was estimated that it would require decades of therapy to cure an individual of HIV.<sup>3</sup> With over 50,000 new cases of HIV infection a year in the U.S. alone, and greater than 30 million people living with HIV world-wide, the challenge of providing lifelong ART to all those that need it has reinvigorated interest within the scientific community in the quest to eradicate HIV infection.

HIV preferentially infects and replicates in activated T-cells, leading to the depletion of these cells over time. Without ART to block viral replication, CD4 cell depletion culminates in the development of AIDS. Within days of an individual acquiring HIV infection, a population of T-cells known as resting CD4+ memory T-cells becomes infected with latent or dormant virus [3]. In large part, this is believed to happen when an activated T-cell is infected and reverts to a resting state, which must occur before viral replication within the cell can result in the death and clearance of the infected cell. This “dormant” virus then exists as an integrated pro-viral DNA within the genome of resting CD4+ T-cells, essentially a foreign viral gene hidden within the human DNA, not susceptible to any currently available therapy, and invisible to immune surveillance. Therapy interruption, even in individuals with years of viral suppression, then results in a rebound of virus, new rounds of infection, and eventually, clinical disease.<sup>4,5</sup> Thus, any therapies that seek to eradicate HIV infection must target this long-lived population of latently infected T-cells. However, targeting this population of cells is not an easy task, as there are no markers on the cell surface

that identify infected resting T-cells and differentiate them from the vast pool of uninfected cells.

## Vorinostat and other experimental HIV latency treatments

The discovery of vorinostat (suberoylanilide hydroxamic acid, SAHA) spanned three decades. Initially a compound called DMSO, commonly used in the laboratory as an anti-freeze during long-term storage of cells, was noted to cause cancer cells to terminally differentiate and die.<sup>6</sup> Researchers discovered that chemicals derived from DMSO were potent anti-tumor agents, eventually resulting in the synthesis of SAHA or vorinostat. This drug is a member of a class of drugs called histone deacetylase (HDAC) inhibitors and was recently approved for the treatment of cutaneous T-cell lymphoma, a type of cancer.<sup>6</sup> HDACs are natural human enzymes that repress the expression of genes, causing them to become dormant. HDAC inhibitors block the action of HDACs and allow genes to be expressed. Our laboratory and others have discovered that by treating latently infected resting T-cells that were taken from people who are HIV-positive with an HDAC inhibitor such as vorinostat, we could induce the expression of HIV from dormancy. We therefore theorized that

vorinostat could be used in a clinical setting to force HIV out of dormancy as an initial step to try to flush out virus that remained latent in T-cells.

At the University of North Carolina, Chapel Hill we recently performed a

small pilot study in patients successfully treated with ART. It was first measured whether vorinostat could induce virus expression from the cells of these patients in the laboratory. The patients donated blood and CD4 T-cells were isolated from their blood and treated with vorinostat. Once it was established that the cells of these patients responded to vorinostat treatment in the laboratory, the patients were brought into the clinic and given a small dose of vorinostat to assess safety and tolerability of the drug. This safety dose was followed by a standard cancer therapy dose several weeks later, and we compared detectable virus expression in their resting CD4+ T-cell reservoir before and after the vorinostat dose. A significant increase in virus expression by the resting CD4+ T-cells after this single dose of vorinostat was observed. This suggests that vorinostat could indeed disrupt latency in a clinical setting. Whether or not multiple doses of vorinostat could lead to a decline in the pool of latently infected cells in these patients remains to be determined and is an active area of research.<sup>7</sup>

Recently, it has been suggested that although vorinostat may induce the expression of virus from latency, the cells expressing virus may not die or be cleared by the immune response, as one would hope. In response to this potential challenge, our laboratory and others are beginning to study the potential role of various strategies aimed at enhancing the anti-HIV immune response. Thus, one can envision a curative strategy in which a patient is given

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GMHC Treatment Issues

446 West 33 Street, New York, NY 10001

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multiple doses of an agent to disrupt latency, such as vori-  
nostat, along with an immune-enhancing therapy to insure  
the clearance of all infected cells.

### Obstacles to curing HIV

In a substantial proportion of treated patients, very low  
levels of viral RNA can be detected by research assays.<sup>8–11</sup>  
This low-level viremia does not appear to lead to drug  
resistance or failure of therapy, and appears to represent  
expression of viral particles without effective rounds of  
new replication.<sup>12,13</sup> but is nevertheless a potential addi-  
tional obstacle to viral eradication.

Additionally, other reservoirs of persistent infection  
despite ART have been reported that could reignite HIV  
infection. These reservoirs have not been defined as well  
in patients on successful, suppressive ART. Naïve T-cells  
have been suggested to harbor persistent replication-  
competent HIV, but the frequency of these cells appears  
low.<sup>14</sup> Macrophages have long been identified as another  
cell type capable of supporting persistent infection  
despite ART. Macrophages and monocytes are long-lived  
cells that may serve as potential sites of persistent viral  
expression, surviving with ongoing low levels of virus  
release in patients on ART.<sup>15,16</sup> A minor subset of CD16+  
monocytes have been shown to be more permissive to  
HIV-1 replication compared with the major CD14<sup>high</sup>CD16<sup>-</sup>  
compartment, and HIV-1 was identified within the CD16+  
monocytes of patients after full suppression on HAART.<sup>17</sup>

However, it has yet to be clearly documented that  
these cells carry quiescent provirus *in vivo* for many  
months, as can resting CD4+ T-cells. This is an important  
distinction, as viral persistence in a cell that is expressing  
viral proteins or particles may be addressed by improve-  
ments in ART or the antiviral immune response. Recent  
reports have demonstrated the recovery of replication-  
competent HIV immediately post-mortem from follicular  
dendritic cells in patients on ART.<sup>18</sup> and suggested that

hematopoietic stem cells are a source of persistent HIV.<sup>19</sup>  
However, these observations are controversial.<sup>20–22</sup>

One of the most daunting tasks facing researchers as  
they attempt to cure HIV is to ensure that any curative  
therapy reaches sanctuary anatomical sites where virus  
infection may persist. For instance, the central nervous  
system (CNS) could potentially serve as a reservoir of  
persistent infection as the blood brain barrier may limit  
access of anti-retroviral drugs and immune surveillance,  
and so this may present a challenge to clearance of HIV  
in CNS cells such as astrocytes and microglia.<sup>23,24</sup> A cure  
for HIV cannot be achieved without tackling the potential that  
these sites may be a source of virus that can rebound in  
the absence of anti-retroviral therapy.

### Future perspectives

The only case of curative therapy to date involves Timothy  
Brown, known as “the Berlin patient.” Mr Brown received  
a stem cell transplant containing a deletion in the CCR5  
gene (CCR5 delta 32) as part of a gene therapy procedure  
for leukemia. CCR5 is a receptor found on the surface  
of T-cells, macrophages and other immune cells and is  
required for the entry of HIV into cells. Patients who have  
a mutation in this gene are naturally immune to HIV infec-  
tion.<sup>25</sup> During the gene therapy procedure, Mr. Brown's  
own immune cells were first destroyed and then replaced  
with stem cells containing a deletion in the CCR5 gene  
(CCR5 delta 32 variant).<sup>26</sup> His immune system was then  
replenished with immune cells that have a deletion in  
their CCR5 genes, thus rendering his cells resistant to HIV  
infection.

Given this precedent, researchers have recently engi-  
neered a series of enzymes called zinc finger nucleases  
(ZFNs) to excise CCR5 from CD4+ T-cells *ex-vivo*. These  
cells are then infused back into the patient with the hope  
of replacing the patient's own CD4 T-cells with clones  
that are CCR5 negative. Clinical trials using this curative  
approach are ongoing.<sup>27,28</sup>

As researchers move forward with the quest to eradi-  
cate HIV infection, it is likely that we will learn more about  
the persistence of HIV and our directions and opinions  
may change. Total eradication of infection (“sterilizing  
cure”) may be possible using traditional small-molecule  
chemical drugs, with or without the help of immunothera-  
peutic approaches such as vaccines or antibody thera-  
pies. A “functional cure” might be achieved in which there  
remains residual virus genetic material, but ART is no  
longer required to halt disease progression and prevent  
infectiousness. Given the limited access to ART for mil-  
lions across the world, these approaches will have to be  
developed with a global scale of implementation in mind  
as the need exists everywhere and is more acute where  
resources are less plentiful. Such welcomed solutions to  
the HIV pandemic can only be achieved if we mount a  
sustained and dedicated effort.

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