

# Developments in HIV-1 immunotherapy and therapeutic vaccination

Peter Lawrence Smith\*, Helen Tanner and Angus Dalglish

Address: St George's, University of London, Institute for Infection and Immunity, 1 Cranmer Terrace, London SW17 0RE, UK

\* Corresponding author: Peter Lawrence Smith (plsmith@sgul.ac.uk)

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## Abstract

Since the human immunodeficiency virus (HIV-1) pandemic began, few prophylactic vaccines have reached phase III trials. Only one has shown partial efficacy in preventing HIV-1 infection. The introduction of antiretroviral therapy (ART) has had considerable success in controlling infection and reducing transmission but in so doing has changed the nature of HIV-1 infection for those with access to ART. Access, compliance, and toxicity alongside the emergence of serious non-AIDS morbidity and the sometimes poor immune reconstitution in ART-treated patients have emphasized the need for additional therapies. Such therapy is intended to contribute to control of HIV-1 infection, permit structured treatment interruptions, or even establish a functional cure of permanently suppressed and controlled infection. Both immunotherapy and therapeutic vaccination have the potential to reach these goals. In this review, the latest developments in immunotherapy and therapeutic vaccination are discussed.

## Introduction

An effective prophylactic vaccine for HIV-1 remains elusive [1]. Few vaccines have entered phase III trials and only one has shown partial protection against HIV-1 infection [2]. Although insight gained from these trials is informing future vaccine research, the current failures have prompted the development of strategies aimed at eradicating the virus in infected individuals. Such efforts to induce a "sterilizing cure" include gene therapy and stem cell therapy. The recent report of an HIV-1-infected patient who after transplantation with chemokine receptor 5 (CCR5) $\Delta$ 32/ $\Delta$ 32 stem cells was absent of detectable viral replication despite the cessation of ART provides encouragement that, in principle, eradication is an achievable goal [3]. However, it remains to be seen whether any eradication approaches are effective or practical on a large scale, and thus ART remains the best means of controlling both infection of individuals and the HIV-1 epidemic.

ART has been very successful in reducing the rate of HIV-1 infection and progression to AIDS. Treatment of

ART early in infection has been shown to reduce the transmission of HIV-1 from person to person [4], whilst examples of early, intensive ART administration have demonstrated an ability to clear detectable infection in a new-born child [5]. The use of ART as a pre-exposure prophylactic treatment in high-risk groups has resulted in a reduction in HIV-1 infection, providing evidence that ART can be used to effectively reduce the incidence of HIV-1 infection in addition to controlling existing infection [6].

## Problems with antiretroviral therapy

Despite the success of ART, there are problems associated with its use and this emphasizes the need for additional therapies, particularly those permitting breaks in ART medication. ART is ineffective against latent viral reservoirs [7] and does not completely stop expression of some HIV-1 genes. This failure necessitates continued ART for life, which is logistically difficult and expensive, particularly in low- to middle-income countries in which the majority of HIV-1-infected individuals reside. The generation of ART-resistant strains of HIV-1 continues to

be a problem [8], and the need for continued ART creates issues in both access and compliance along with complications associated with ART use. Such complications include metabolic syndrome [9], increased cardiovascular disease [10], quality-of-life changes [11], and organ damage [12]. Not surprisingly, side effects associated with ART are linked with non-compliance [13]. Whilst ART has reduced the incidence of HIV-1-associated neurodegenerative disorders (HANDs), many HIV-1 patients with successful ART therapy still exhibit HANDs. Penetration of ART across the blood-brain barrier is problematic and may not reach central nervous system compartments of HIV-1 infection [14]. Conversely, some antiretroviral drugs are associated with neurological side effects [15,16]. Finally, initiation of ART in patients with HIV-1 and a co-infection such as tuberculosis risks the onset of immune reconstitution inflammatory syndrome [17,18]. Thus, although ART is vital in treating HIV-1 infection, reliance on ART creates additional problems to be addressed.

The success of antiretroviral therapy as a lifelong treatment has transformed the nature of HIV-1 infection. In successfully treated individuals, HIV-1 infection is now a chronic immunological disease [19]. Delaying the initiation of ART or planned interruption is associated with increases in morbidity and mortality, and yet continued treatment of ART over a number of decades causes toxicity. Evidence suggests that even antiretroviral-naïve patients with normal CD4<sup>+</sup> T-cell counts are at a greater risk of suffering from serious HIV-1 associated non-AIDS diseases [20], highlighting the potential use of ART in patients with good CD4<sup>+</sup> T cell counts and yet patients successfully treated with ART often fail to adequately reconstitute healthy immune responses [21].

The mechanism of this immune dysfunction is thought to be the immune activation induced by HIV-1. This immune activation involves innate inflammatory responses such as monocyte activation and production of pro-inflammatory cytokines, including interleukin (IL)-6. Skewed T-cell ratios, generalized T-cell activation, ineffective T-cell effector functions, and the selective depletion of T-cell subsets, particularly T helper 17 (Th17) cells and effector memory CD4<sup>+</sup> T cells, are also observed in HIV-1-dependent immune activation.

### **HIV-1-dependent immune activation**

The causes of immune activation and dysfunction are not completely understood. Lipopolysaccharide translocation across damaged mucosal barriers contributes to immune activation by creating an inflammatory environment within the mucosa-associated lymphoid tissue [22].

Depletion of CD4<sup>+</sup> T-cells, particularly Th17 cells, in the gut leading to compromised mucosal barrier function is thought to be important in this process, but it is unclear how the Th17 cells are being depleted when only a fraction of T cells are actually infected [23-25]. Other factors influencing immune activation include sensitivity to type I interferon and co-infection. It is likely that multiple factors relating to viral replication synergize to promote the activation and subsequent dysregulation of the immune system, but it is currently unclear which factors are principal causes rather than consequences of HIV-1-induced immune activation. It is also unknown why some level of immune failure continues to persist after HIV-1 replication has been effectively controlled by ART. Prophylactic or therapeutic vaccination will likely need to address immune activation in order to succeed. A large number of immunotherapy approaches have been studied for their ability to improve anti-HIV-1 immune responses and control infection. These include both non-specific immunotherapy and antigen-specific therapeutic vaccination. Both approaches will be described here with a focus on some of the most notable recent studies in immunotherapy and therapeutic vaccination.

### **Immunotherapy**

Immunotherapy, aimed at reducing inflammation, preventing immune activation by HIV-1, or promoting effective immune responses, is currently being investigated (Table 1). Diverse approaches are being studied, and a number of trials are completed or under way.

### **Antibody therapy**

Antibodies used to either enhance immune responses or inhibit negative regulatory pathways are being investigated. Increases in programmed cell death (PD)-1 are associated with immune dysfunction in HIV-1-positive patients [26], and *in vitro* antibody blockade of PD-ligand (L)-1 and IL10R $\alpha$  results in increases in cytokine expression, including interferon-gamma (IFN- $\gamma$ ) in HIV-1-infected patients [27]. Memory CD4<sup>+</sup> T cells expressing high levels of PD-1 contain more proviral DNA than PD-1-low cells, and blocking PD-1 may activate HIV-1 replication and thus facilitate the clearance of these infected cells.

Anti-cytotoxic T-lymphocyte antigen 4 (CTLA4) has been studied for its ability to enhance immune responses to tumors [28]. T regulatory cells express high levels of CTLA4 and are implicated in inhibiting immune responses during HIV-1 infection. *In vitro* experiments using cells from healthy and HIV-1-infected individuals showed that CTLA4 is upregulated in the CD4<sup>+</sup> T cells

**Table 1. Selected recent immunotherapy for HIV-1 infection**

Immunotherapy	Treatment	Responses	Reference
Promoting immune responses			
Cytokines	IL-2 during ART treatment interruptions	No evidence of improved HIV-1-specific immunity	[35]
	IL-7 dose escalation trial in ART-treated patients	Increases in CD4 <sup>+</sup> T-cell numbers	[37]
Drugs	IL-21 administered to SIV-infected Rhesus macaques	Increases in natural killer-cell and CD8 <sup>+</sup> T-cell function and Th17 cells and a reduction in bacterial translocation and immune activation	[38,39]
	Immunomodulatory drugs	Enhanced T-cell responses to dendritic cells electroporated with GAG or NEF mRNA	[46]
Correcting immune activation and dysfunction			
Drugs	Aspirin	Reductions in CD38, HLA-DR, and sCD14	[40]
	COX-2 inhibitor celecoxib	Celecoxib reduced the expression CD38 and inflammatory markers	[41]
Antibodies	Anti-PD-1	Blocking PD-1 results in increased cytokine expression in HIV-1 patients	[27]
	Anti-CTLA4 treatment of SIV-infected macaques	Decreased TGF- $\beta$ , IDO, and viral RNA in SIV-infected macaques	[30]
	Anti-CCR5 Maraviroc	Reduction in CD38 HLA-DR-expressing CD4 <sup>+</sup> T cells and normalized CD8 <sup>+</sup> T-cell skewing	[32]
Probiotics	Symbiotic plus dietary fiber	No alteration in bacterial translocation	[48]
	Prebiotic and probiotic	Reductions in bacterial DNA, CD4 <sup>+</sup> T-cell counts, and IL-6	[49]

ART, antiretroviral therapy; COX-2, cyclooxygenase type 2; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; SIV, simian immunodeficiency virus; TGF- $\beta$ , transforming growth factor-beta.

from infected patients and inversely correlated with CD4 counts and anti-HIV-1 T-cell responses whilst positively correlating with the percentage of activated CD4<sup>+</sup> T cells [29]. Using the anti-CTLA4 antibody MDX-010 in simian immunodeficiency virus (SIV)-infected macaques treated with ART, decreases in transforming growth factor-beta (TGF- $\beta$ ), indoleamine 2,3-dioxygenase (IDO), and viral RNA expression were observed alongside increased SIV-specific T-cell effector function [30].

The CCR5 antagonist Maraviroc is used for HIV-1 entry blockade in combination with ART [31] but also acts as an immune modulator. In a recent study on the impact of Maraviroc intensification, Maraviroc normalized HIV-1-associated CD8<sup>+</sup> T-cell skewing and reduced the number of CD38- and human leukocyte antigen (HLA)-DR-expressing CD4<sup>+</sup> T cells [32].

### Cytokines

Reductions in IL-2 are associated with HIV-1-induced immune dysfunction. For this reason, trials were conducted in ART-treated subjects, resulting in improved CD4<sup>+</sup> T-cell counts [33]. A trial involving intermittent IL-2 therapy of ART-naïve patients was also undertaken and demonstrated sustaining increases in CD4<sup>+</sup> T-cell numbers that could allow for deference of ART [34]. However, the recent SILCAAT (Subcutaneous Recombinant, Human Interleukin-2 in HIV-Infected Patients with Low CD4<sup>+</sup> Counts under Active Antiretroviral Therapy) and ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial) trials intended to assess the effect of IL-2 in HIV-1-infected individuals with

ART-induced viral suppression revealed no evidence for improved HIV-1 immunity [35], whereas a later trial intended to assess the effect of IL-2 in ART-naïve patients demonstrated an increase in the number of CD4<sup>+</sup> T cells without effecting viral load [36]. These modest effects, along with toxicity associated with IL-2 therapy, have prompted research on immune modulatory properties of other cytokines during HIV-1 infection.

Alternative cytokines for improving T-cell homeostasis include IL-7 and IL-21. A dose escalation trial of recombinant human IL-7 (rhIL-7) in ART-treated HIV-infected persons was performed. Doses of rhIL-7 up to 20  $\mu$ g/kg were tolerated and showed increases in naïve and central memory CD4<sup>+</sup> T cells. In individuals with low T-cell receptor (TCR) diversity, IL-7 therapy could improve the available TCR repertoire [37].

IL-21 has recently been used in a trial of chronically untreated SIV-infected macaques in which both natural killer (NK)-cell and T-cell cytotoxicity was improved along with increased antibody production and no increases in viral load [38]. In another trial of IL-21-treated SIV-infected macaques, higher levels of intestinal Th17 cells were observed and were associated with reduced levels of intestinal T-cell proliferation, microbial translocation, and systemic activation/inflammation in the chronic infection [39]. These trials indicate that IL-21 may affect beneficial immune responses while ameliorating intestinal immune activation, making treatment with IL-21 a promising novel approach.

### Anti-inflammatory and immunomodulatory drugs

Aspirin has broad anti-inflammatory properties. HIV-1-infected individuals with ART-controlled infection were administered low-dose aspirin for 1 week. After therapy, decreases in CD38 and HLA-DR expression on T cells, soluble CD14, and platelet activation were observed [40].

Cyclo-oxygenase type 2 (COX-2) inhibits T-cell activation and is upregulated in HIV-1 disease. This has prompted the investigation of COX-2 inhibitors in HIV-1 infection to determine whether they are capable of correcting immune activation. The COX-2 inhibitor celecoxib was studied in a trial of HIV-1 patients without ART. Celecoxib reduced CD38 expression of CD8<sup>+</sup> T cells, inflammatory markers, and the expression of PD-1 and improved vaccine responses [41].

The immunomodulatory drugs (iMiDs) thalidomide, lenalidomide, and pomalidomide have demonstrated the ability to improve immune responses [42-45]. An *in vitro* study of iMiDs alongside GAG or NEF mRNA-electroporated dendritic cells (DCs) resulted in higher numbers of cytokine-secreting HIV-specific CD8<sup>+</sup> T cells, including polyfunctional HIV-specific CD8<sup>+</sup> T cells with an enhanced lytic capacity and an ability to promote the activation of CD8<sup>+</sup> T cells at a lower concentration of antigen [46]. Immunomodulatory drugs such as iMiDs warrant further studies alongside immunotherapeutic vaccination.

### Probiotics

ART does not correct HIV-1-induced damage to the intestinal mucosa and only partially controls T-cell exhaustion and activation [47]. Probiotic therapy is being investigated to ameliorate this damage. In a trial in HIV-infected subjects on antiretroviral therapy, a synbiotic formulation, containing probiotic bacteria and dietary fiber, was provided each day, versus a fiber-only placebo formulation. Bacterial translocation was not altered between the probiotic bacteria and placebo groups, and markers of immune activation were unchanged [48]. Another symbiotic, a combination of pre- and probiotics, was used in a study of ART-naïve HIV-1-infected subjects [49]. Subjects received the symbiotic, just the pre- or probiotic, or placebo. Reductions in bacterial DNA, increases in CD4<sup>+</sup> T-cell counts, and significant reductions in IL-6 were observed in the symbiotic group whilst the probiotic group exhibited significant increases in beneficial bacteria and reductions in harmful bacteria such as *Clostridium*.

A number of diverse immunotherapy approaches are being investigated in order to promote effective immune

responses. Some of these have demonstrated promising results in terms of reducing markers of immune activation or positively effecting CD4<sup>+</sup> T-cell homeostasis. However, generalized immune modulation does not specifically target HIV-1 or directly enhance anti-HIV-1-specific immune responses. Given that HIV-1 patients will already exhibit some measure of immune dysfunction, even when successfully treated with ART, therapeutic vaccine approaches will be vital in the generation of therapy capable of either providing a functional cure or allowing structured treatment interruptions.

### Therapeutic vaccines

T-cell immunity has emerged as a vital component of protective immune responses in HIV-1 disease [50]. Genetic polymorphisms associated with protection reside only in genes encoding HLA class I molecules [51], and effective polyfunctional T-cell immune responses to conserved HIV-1 epitopes are present in long-term non-progressor patients who control HIV-1 infection and elite controllers [52-53]. In light of this, a number of vaccines aimed at inducing effective therapeutic immune responses are in development and most of them are intended to promote anti-HIV-1 T-cell immunity (Table 2). It is hoped that the therapeutic generation of HIV-1-specific immunity can control infection and limit transmission. Some vaccines assessed for an immunotherapeutic effect were initially designed to induce prophylactic immunity, whereas others are intended to specifically activate therapeutic T-cell immune responses.

### Viral vector vaccines

Viral vectors, particularly adenovirus and pox virus, have generated considerable interest because of the low cost and ease of manufacture, along with their ability to induce gene expression within the cytosol and the large quantity of vaccine DNA that can be incorporated to induce humoral and cellular immunity after a single vaccination [54]. Although viral vector vaccines have been intended for prophylactic immunity, they also induce T-cell immune responses that may have therapeutic effects.

A phase III trial of 16,402 healthy volunteers with low risk of HIV-1 infection in Thailand measured the efficacy of a prime-boost regimen using four doses of a recombinant canarypoxvirus (ALVAC) expressing HIV *gag*, *pro*, and *env* genes (vCP1521) along with gp120 and Alum in the last two doses. Volunteers in the vaccine group acquired 31.2% fewer HIV-1 infections than those in the placebo group after 3 years of follow-up. Neither broadly neutralizing anti-serum nor broadly reactive T-cell responses were induced. Anti-gp120 antibodies were present in 90% of vaccinated patients after 20 weeks. Antibody-dependent cellular cytotoxicity

**Table 2. Selected recent anti-HIV-1 therapeutic vaccine studies in HIV-1-positive and healthy individuals**

Vaccine type	Vaccine	Immune responses	Viral load	Reference
Viral vector ALVAC	Recombinant canary pox viral vector genetically engineered to express HIV-1 Gag and Pro (subtype B LAI strain) and CRF01_AE (subtype E) HIV-1 gp120, administered to uninfected individuals	Weak evidence of increased CD4 <sup>+</sup> T-cell count upon HIV-1 infection	No overall statistically significant reduction in pre-HAART viral load upon HIV-1 infection	[2,55]
MVA-B	A recombinant MVA-B-expressing monomeric gp120 and the fused Gag-Pol-Nef (GPN) polyprotein of clade B	Polyfunctional CD8 <sup>+</sup> and CD4 <sup>+</sup> T-cell responses along with Env-specific antibody responses in 95% of volunteers	Uninfected volunteers	[58]
NYVAC	Phase I trial of NYVAC, expressing Gag, Pol, Nef, and Env from an HIV clade B isolate injected intramuscularly into 10 HIV-infected patients successfully treated with antiretroviral therapy	Increased HIV-specific T-cell responses in virtually all vaccines, mostly GAG-specific pre-existing and new detected responses	Not studied	[59]
Ad35-GRIN	Andovirus vector containing GAG, reverse transcriptase, integrase, Nef, and Env	Polyfunctional HIV-specific cellular immune responses and humoral responses	Uninfected volunteers	[61]
AVX101	Alphavirus replicon containing GAG	Limited immune responses	Uninfected volunteers	[62]
DNA PENNVAX DNA	Three plasmids expressing ENV, GAG and Pol, plasmid containing IL-12	Developed CD4 <sup>+</sup> or CD8 <sup>+</sup> T-cell responses after repeat vaccination	Uninfected volunteers	[63]
DermaVir	Dose escalation study of plasmid DNA containing 13 complete and 2 non-functional HIV-1 genes/proteins that self-assemble	Induction of significantly increased GAG-specific T-cell responses	Not studied	[66]
GTU-multi-HIVB	Phase II trial of fusion gene expressing Rev, Nef, TAT, GAG + CTL epitopes clusters from Pol and Env. Intradermal and Intramuscular vaccination of in HIV-1-infected ART-naïve patients	The increase in HIV-1-specific CD4 T-cells was observed predominantly in the TNF- $\alpha$ -secreting CD4 T-cell population while both HIV-1-specific TNF- $\alpha$ and IFN- $\gamma$ CD8 T-cell populations increased following immunization.	IM group with a decrease in log p HIV-RNA 0.47 log units (95% CI from -0.75 to -0.19) compared with placebo ( $P = 0.001$ )	[68,69]
Dendritic cell DCV2/MANON07-ORVACS	Autologous DC pulsed with whole inactivated HIV-1	Increased HIV-1-specific T-cell responses	Decrease of plasma viral load setpoint $\geq 1$ log was observed in vaccinated groups and was associated with a consistent increase in HIV-1-specific T-cell responses	[71]
mRNA-electroporated DC	mRNA vaccine encoding Tat, Rev, and Nef	Induced and/or enhanced HIV-1-specific CD4 <sup>+</sup> and CD8 <sup>+</sup> T-cell but did not correlate with the number of weeks off cART	Viral load in plasma unchanged from historical control data	[72,73]
Sub unit Vacc4X	Four modified peptides from the GAG protein containing MHC class I and II restricted epitopes	Delayed-type hypersensitivity (DTH) reactions. T-cell responses in 80% to 90% of patients allowing for structured treatment interruption	Significantly improved viral load ratios for DTH <sup>hi</sup> (compared with DTH <sup>lo</sup> ) groups: 0.58 (0.27-1.33) and 1.26 (0.90-2.02). Correlate with stable CD4 <sup>+</sup> T-cell counts	[74-78]
Subdominant HIV-1 peptides and CAF01	Multiple subdominant epitopes restricted to HLA supertypes	Induction of CD4 <sup>+</sup> and CD8 <sup>+</sup> T-cell responses	No significant changes in viral load	[79]
TAT	Whole TAT protein	Modified pattern of CD4 <sup>+</sup> and CD8 <sup>+</sup> T-cell activation and decreased markers of immune activation	Subjects on ART during study	[80]

ALVAC, canarypoxvirus; ART, antiretroviral therapy; cART, combination antiretroviral therapy; CI, confidence interval; DC, dendritic cell; HAART, Highly Active Antiretroviral Therapy; IFN- $\gamma$ , interferon-gamma; IL, interleukin; IM, intramuscular; MHC, major histocompatibility complex; MVA-B, modified vaccinia virus Ankara vector expressing Env, Gag, Pol, and Nef proteins of HIV-1 subtype B; NYVAC, New York Vaccinia virus; TNF- $\alpha$ , tumor necrosis factor-alpha.



with gp120-coated targets was detected in 75% of vaccines for clade B gp120 but reduced by 20 weeks. CD4<sup>+</sup> T-cell proliferation was the most substantial response detected in vaccinated individuals [2].

An extended evaluation of ALVAC in trial volunteers who subsequently acquired HIV-1 was carried out to determine whether the vaccine had therapeutic efficacy. CD4<sup>+</sup> T-cell counts and viral load were measured; however, the vaccine did not affect the clinical course of HIV disease after infection [55].

New York Vaccinia virus (NYVAC) and Modified vaccinia virus Ankara (MVA) are replication-deficient poxvirus vectors. They are stable and safe and can deliver a large amount of integrated DNA into cytosolic locations within an infected cell. Both vectors have been used in the development of HIV-1 vaccines. Over 30 clinical trials using MVA-based HIV-1 vaccines have been conducted [54]. Some of these trials have demonstrated impressive immunogenicity, such as the phase I trial of an MVA vector expressing Env, Gag, Pol, and Nef proteins of HIV-1 subtype B (MVA-B). Three doses of MVA-B in healthy volunteers induced broad and polyfunctional CD8<sup>+</sup> and CD4<sup>+</sup> T-cell responses along with Env-specific antibody responses in 95% of volunteers [56,57].

NYVAC is highly attenuated due to the deletion of 18 open reading frames in the viral genome. NYVAC gene expression results in antigen-specific immune responses but is unable to produce infectious virus in humans. Clinical trials have been conducted by using NYVAC-based HIV-1 vaccines [54]. Prime-boost vaccine protocols using DNA and NYVAC containing Env, Gag, Pol, and Nef antigens have proven to be particularly immunogenic, inducing T-cell responses in 90% of individuals, including durable and polyfunctional responses along with anti-gp140 antibody responses [58,59].

Although poxvirus-based vaccines are intended primarily for prophylactic vaccination, their ability to induce broad and polyfunctional CD8 and CD4<sup>+</sup> T-cell responses indicates that they also have promise as therapeutic vaccines, particularly after deletion of viral immunomodulatory genes and if vaccination strategies include immunomodulatory agents.

A phase I trial of recombinant replication-defective adenovirus serotype 35 (Ad35) vectors containing gag, reverse transcriptase, and integrase and nef (Ad35-GRIN) or env (Ad35-ENV), both derived from HIV-1 subtype A isolates, was performed. Broad and polyfunctional HIV-specific cellular immune responses and humoral responses were seen in the majority of volunteers

immunized with either vaccine and increased after subsequent second vaccination [60].

AVX101, an alphavirus replicon vaccine containing HIV-1 subtype C gag, was studied in a clinical trial in healthy HIV-1-uninfected adults. AVX101 was well tolerated, however; in contrast to the preclinical data, immune responses in humans were limited. Only low levels of binding antibodies and T-cell responses were seen at the highest doses [61].

In summary, viral vectors have demonstrated the ability to induce functional immune responses and are undergoing further modification to enhance their immunogenicity. Further trials are planned.

### DNA vaccines

DNA vaccination is safe and has demonstrated promising immunogenicity in animal models. These results have prompted the development of DNA-based HIV-1 vaccines. PENNVAX DNA vaccine is a mixture of three expression plasmids encoding HIV-1 Clade B Env, Gag, and Pol in addition to an IL-12 DNA plasmid. These were administered in three intramuscular vaccinations followed by electroporation. Most subjects vaccinated with PENNVAX plus IL-12 plasmid with electroporation developed either CD4<sup>+</sup> or CD8<sup>+</sup> T-cell responses after repeat vaccination [62].

DermaVir is a single plasmid DNA immunogen including 13 complete and two non-functional HIV-1 proteins which self-assemble into virus-like particles [63]. In chronically infected macaques, DermaVir administered with antiretroviral drugs suppressed viral load rebound after treatment interruption and improved survival [64]. In a dose escalation study, DermaVir was found to be safe and immunogenic in HIV-1-infected individuals. HIV-specific effector CD4<sup>+</sup> and CD8<sup>+</sup> T cells expressing IFN- $\gamma$  and IL-2 were detected against several antigens in every subject. DermaVir administration was associated with a trend toward greater HIV-specific, predominantly central memory T-cell responses [65], and the use of IL-7 and IL-15 as adjuvants with DermaVir significantly enhanced the number of GAG-specific central memory T cells from mice [66].

The DNA vaccine GTU-Multi-HIVB consists of a fusion gene expressing HIV-1 proteins Rev, Nef, and Tat as well as Gag p17/p24 and a stretch of 11 cytotoxic T lymphocyte (CTL) epitope clusters from Pol and Env [67]. The induction of CD8<sup>+</sup> T-cell and antibody responses to Rev, Nef, Tat, Env, and Gag were observed in BALB/c mice. In a phase II trial of untreated, otherwise-healthy, HIV-1-infected adults, significant

declines in log proviral RNA and increases in CD4<sup>+</sup> T-cell counts were observed in the vaccine group compared with placebo which were maintained in patients with some HLA haplotypes [68]. Early DNA-based vaccines suffered from poor immunogenicity, particularly compared with viral vectors; however, improvement in plasmid vectors, adjuvant, and delivery means that DNA vaccinations such as those detailed above warrant further study [69].

### Dendritic cell vaccines

DC vaccines are attractive due to the ability to ensure that vaccine antigen reaches the cells most able to activate protective immune responses; however, they are relatively time-consuming and expensive.

In the DCV2/MANON07-ORVACS Study, autologous monocyte-derived dendritic cells (MDDCs) pulsed with heat-inactivated whole HIV have been used in a phase I trial which demonstrated immunogenicity associated with a control of viral replication. Patients on ART with CD4<sup>+</sup> more than 450 cells/mm<sup>3</sup> received three immunizations with MDDCs or with non-pulsed MDDCs. Vaccination that resulted in significant decreases in viral load during ART interruption was associated with an increase in HIV-1-specific T-cell responses [70].

In a phase I/IIa clinical trial, HIV-1-infected ART-treated patients received four vaccinations with autologous DCs electroporated with an mRNA vaccine encoding Tat, Rev, and Nef and linked to the HLA class II-targeting sequence of DC-LAMP. ART was interrupted and after 96 weeks 6 out of 17 patients remained off therapy. Enhanced CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses specific for the immunogens were observed in most of the patients, but there was no correlation with the number of weeks off ART, and CD4<sup>+</sup> T-cell counts and plasma viral load did not differ from historical control data [71,72].

### Subunit vaccines

Vacc-4x consists of peptides derived from conserved domains within HIV-1 p24 Gag and induced T-cell responses in 90% of patients which were associated with reduced viral loads [73]. One and a half years after immunization, Vacc-4x responses were unchanged and 62% were still on ART treatment interruption [74]. Seven years after immunization, CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses were observed in 95% and 68% of subjects, respectively, and 55% of subjects demonstrated CD107a and IFN- $\gamma$  CD8<sup>+</sup> T-cell responses. Immunized patients spent a median of 2.2 years free of ART prior to successfully resuming treatment [75]. Subsequent boosting with Vacc-4x 7 years after initial immunization resulted in an increase in CD8<sup>+</sup> T-cell proliferation in 80% of subjects. Responders demonstrated increased

CD8<sup>+</sup> T-cell degranulation and CD4<sup>+</sup> T-cell IFN- $\gamma$  expression. Increases in tumor necrosis factor-alpha (TNF- $\alpha$ ) and IL-1 $\alpha$ , IL-1 $\beta$ , and IL-10 were associated with non-responders, whereas IL-10- and TGF- $\beta$ -mediated down-regulation of Vacc-4x-specific CD8<sup>+</sup> T-cell proliferation increased only in non-responders, indicating that boosting with Vacc-4x peptides can enhance T-cell responses but is also capable of promoting regulatory immune responses, highlighting the need to address immune regulation in therapeutic vaccination [76]. Taken together, these studies provided evidence of long-lasting T-cell memory responses to a peptide-based immunotherapeutic candidate for HIV-infected patients [77]. Further studies of Vacc-4x include vaccination with the iMiD Revlimid and with an additional vaccine aimed at inducing the production of antibodies to the C5 region of gp120. The C5 region is thought to influence immune activation, and anti-C5 antibodies are correlated with long-term non-progressor status and HIV-1-specific T-cell responses (unpublished data).

Subdominant peptide epitopes from HIV-1 restricted to common HLA supertypes were used with the adjuvant CAF01 to vaccinate treatment-naïve HIV-1-infected individuals. All 10 patients induced new CD4 and CD8 T-cell responses specific for one or more vaccine epitopes; however, there were no significant changes in HIV-1 viral load or CD4 T-cell counts [78]. These studies on peptide vaccination highlight the importance of selecting optimal peptide combinations for vaccination.

A phase II clinical trial of virologically suppressed ART-treated HIV-1-infected individuals receiving therapeutic immunization with Tat was conducted. Immunization with TAT was safe and induced immune responses, including modifying the pattern of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell activation, a reduction in markers of immune activation, and increases in regulatory T cells (Tregs). Increases in central and effector memory and reduction in terminally differentiated CD4<sup>+</sup> and CD8<sup>+</sup> T cells were accompanied by increased responses against Env and recall antigens. Of note, more immune-compromised individuals experienced greater therapeutic effects [79].

Protein and peptide vaccines are among the safest, cheapest, and most stable. They benefit from a relatively simple design allowing for thorough characterization of induced immune responses and avoidance of complications that have been associated with complex vaccine preparations. Peptide vaccines show promise as an immunotherapeutic vaccine strategy alongside restorative immunotherapy.

### Latently infected cells and histone deacetylase inhibitors

The ability of vaccination or ART treatment to cure HIV-1 infection is limited by the phenomenon of HIV-1 latency. Latency is established, particularly in CD4<sup>+</sup> T cells, early in infection by unknown mechanisms and is thought to be responsible for viral rebound after ART cessation. Recently, drugs capable of activating HIV-1 replication have been studied with the aim of removing latent viral reservoirs with ART or vaccination [80]. Among these, histone deacetylase (HDAC) inhibitors show promise in reactivating HIV-1 reservoirs and are currently in phase I and II clinical trials. Acetylation of lysine residues found on histone molecules is associated with gene transcription by increasing the accessibility of transcription factors. HDACs deacetylate lysine residues in histone molecules and in so doing prevent transcription. Valproic acid inhibits class I and II HDACs and, in a clinical study of four patients undergoing intensified ART, significantly reduced latent infection in three patients [81].

Vorinostat is a Food and Drug Administration-approved HDAC inhibitor that suppresses HDACs capable of binding to the HIV-1 promoter. In a trial of 11 ART-treated patients who had no measurable viral plasma RNA but who demonstrated increased *ex vivo* HIV-1 RNA production in response to Vorinostat, subsequent treatment of these patients resulted in an increase in HIV RNA expression in resting CD4<sup>+</sup> T cells [82].

Other HDAC inhibitors, targeting different HDACs and first developed as anti-cancer chemotherapy, are being tested for putative *in vitro* effects on HIV-1 latency [83-85]. Inhibitors targeting class I HDACs are particularly promising since these HDACs are linked to HIV-1 latency [86].

The promising early data using HDAC require further study to determine to what extent they reduce the HIV-1 reservoir and what proportion of latently infected cells are susceptible. A recent study indicated that T cells with reactivated HIV-1 survive and that antigen-specific cytotoxic immune responses are required to kill these infected cells. This indicates that combinations of HDAC inhibitors with immunotherapeutic vaccination may be required to successfully clear latent viral reservoirs [87].

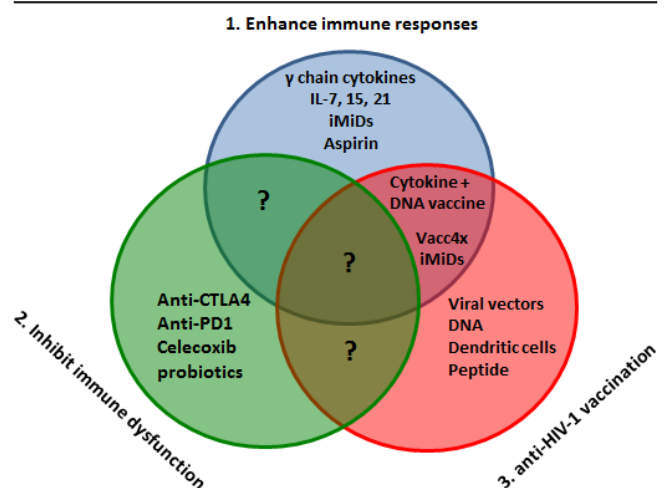
### Conclusions

The limitations of ART and poor efficacy demonstrated by prophylactic vaccine candidates are well documented. This creates an urgent need for additional therapies, and numerous immunotherapeutic approaches are being investigated, some with encouraging results. The study of therapeutic vaccine formulations capable of activating

effective and sustained anti-HIV-1 immune responses warrants continued emphasis. Although non-antigen-specific immunotherapy may prove insufficient to replace ART or allow structured treatment interruptions, it will likely be a necessary prerequisite for effective anti-HIV-1 therapeutic vaccination (Figure 1). Studies involving combinations of immunotherapy and therapeutic vaccination are currently lacking but have the potential to correct immune dysfunction and enhance the capacity of the immune system to respond to therapeutic vaccination. The ability to combine immunotherapy, vaccination, and agents capable of reactivating latent viral reservoirs, such as HDAC inhibitors, represents an exciting strategy to develop a functional cure of HIV-1 infection. Many of these approaches are in early clinical development, and the results from current and future trials will provide insight regarding which approaches might best combine to control infection.

At present, both immunotherapy and therapeutic vaccination suffer from a lack of knowledge regarding which immune activation markers represent causative rather than just associated agents of HIV-1 disease progression. The presence of immune activation predicts disease progression independently of viral load and CD4<sup>+</sup> T-cell number, and this creates a difficulty in interpreting the significance of clinical trial data. Many trials do not detail vaccine-specific immune responses and measures of viral

Figure 1. Immunotherapy and therapeutic vaccination for HIV-1



Immunotherapy to enhance immune responses (1) or inhibit immune dysfunction (2) should be studied alongside therapeutic vaccination (3) in order to develop a functional cure consisting of controlled immune responses and repressed viral replication. Abbreviations: CTLA-4, cytotoxic T-lymphocyte antigen 4; IL, interleukin; iMiD, immunomodulatory drug; PD-1, programmed cell death-1.



replication alongside markers of immune activation. It may be necessary to observe how all three of these correlate change over time during structured treatment interruptions in order to assess the effectiveness of novel therapy. The success of immunotherapy and therapeutic vaccination will depend upon a greater understanding of the causative factors responsible for immune activation and of the immune responses that provide protection in elite controller patients.






## Abbreviations

Ad35, adenovirus serotype 35; ALVAC, recombinant canarypoxvirus; ART, antiretroviral therapy; CCR5, chemokine receptor 5; COX-2, cyclooxygenase type 2; CTLA4, Cytotoxic T-lymphocyte antigen 4; DC, dendritic cell; HAND, HIV-1-associated neurodegenerative disorder; HDAC, histone deacetylase; HIV-1, human immunodeficiency virus-1; HLA, human leukocyte antigen; IFN- $\gamma$ , interferon-gamma; IL, interleukin; iMiD, immunomodulatory drug; MDDC, monocyte-derived dendritic cell; MVA, Modified vaccinia virus Ankara; NYVAC, New York Vaccinia virus; PD-1, programmed cell death-1; rhIL-7, recombinant human IL-7; SIV, simian immunodeficiency virus; TCR, T-cell receptor; TGF- $\beta$ , transforming growth factor-beta; Th17, T helper 17.

## Disclosures

The authors declare that they have no disclosures.

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