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REVIEW

HIV therapeutic vaccines aimed at intensifying combination antiretroviral therapy

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ABSTRACT

Introduction: Although successful at suppressing HIV replication, combination antiretroviral therapy (cART) only partially restores immune functions and fails to reduce the latent HIV reservoir, thus requiring novel interventions for its intensification.

Areas covered: Here are reviewed therapeutic vaccine candidates that are being developed to this goal. Among them, the Tat vaccine has been shown to promote immune restoration, including CD4 + T-cell recovery in low immunological responders, and to reduce the virus reservoirs well beyond what achieved with long-term suppressive cART.

Expert opinion: The authors propose the Tat vaccine as a promising vaccine candidate for cART intensification toward HIV reservoirs depletion, functional cure, and eradication strategies, suggesting that targeting a key protein in the virus life cycle is pivotal to success.

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1. Introduction

The HIV epidemics has reached vast proportions and represents one of the major global health challenges, with important social and economic implications for the public health systems. UNAIDS estimates that approximately 37 million people are living with HIV, with a total of 940,000 AIDS-related deaths and 1,8 million new infections in 2017 [www.unaids.org/en/resources/fact-sheet; 1]. Combination antiretroviral therapy (cART) has saved millions of lives since its introduction in the early 90s and has shown efficacy at blocking virus replication and increasing CD4+ T-cell counts, preventing progression to AIDS. However, only 59% of people living with HIV have access to treatment [1]. Moreover, cART is unable to fully restore T- and B-cell number and function, incompletely silences virus gene expression and does not block replenishment of viral reservoirs, where the virus hides and reactivates, and hence low level chronic inflammation and immune dysregulation persist, leading over time to an increased risk of comorbidities, co-infections and death [2]. Thus, the rates of HIV morbidity/mortality are still higher, as compared to the general population, a situation worsened by the increasing occurrence of HIV drug resistance due to insufficient compliance to treatment [3], often caused by drug-related toxicity.

Although the initiation of cART generally leads to a rapid reduction in HIV-1 RNA plasma levels and to an increase in peripheral CD4+ T-cell counts, some patients experience a 'discordant response', whereby the HIV-1 RNA plasma level is below the limit of detection but the restoration of CD4 + T-cell number is blunted. Approximately 25% of patients who start cART with a CD4+ T-cell count below 200 cells/μL do not achieve a CD4+ T-cell count >500 cells/μL even after 7–10 years of treatment or longer, and may have an elevated risk of non–AIDS-related morbidity and mortality [4]. Further, there are individuals who never achieve normal CD4+ T-cell counts even after years of therapy [4]. Depending on the mechanism for these suboptimal immunological outcomes, novel immune-based therapeutic approaches may be necessary to restore immune competence in these individuals. Moreover, the majority of patients in resource-limited settings often start cART with a CD4+ T cell count <200 cells/μL [5]. Thus, in addition to implement access and retention to therapy, treatment intensification strategies to reduce comorbidities and mortality are also needed.

To date, however, attempts to intensify cART have failed to show benefit in patients with late-stage HIV infection and/or inadequate response to antiretroviral therapy [6,7]. Therefore, novel intensification strategies are needed. In this context, an effective therapeutic HIV vaccine in conjunction with antiretroviral therapy may represent a relevant, cost-effective approach to increase the effectiveness of cART by: **i**) attaining a faster/more effective response to therapy (cART intensification), **ii**) mitigating the effect of poor adherence to cART, **iii**) delaying viral rebound when off cART, thus allowing for cART-free periods, and **iv**) attacking the cART resistant virus reservoir through vaccine-induced HIV-specific immune responses. Therefore, a multi-pronged approach will be needed to achieve the goal of curing HIV infection.

2. Therapeutic vaccines

Historically, therapeutic immunological strategies to fight HIV infection had three major objectives: **i**) to restore immune function; **ii**) to enhance the efficacy of cART in suppressing

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Article highlights

- cART has several shortcomings since it does not: **i)** completely silence virus gene expression, **ii)** block replenishment of viral reservoirs, **iii)** eliminate low level chronic inflammation, **iv)** fully restore T- and B-cell number and function, **v)** reverse immune dysregulation, and **vi)** prevent selection of resistant strains.
- Therapeutic HIV vaccine in conjunction with antiretroviral therapy may represent a relevant, cost-effective approach to increase the effectiveness of cART by: **i)** accelerating time-to-response to therapy, **ii)** blocking or reducing virus transmission, **iii)** solving unmet needs of ART (immune activation, immune defects, proviral DNA), **iv)** allowing drug simplification or virus control despite low adherence to therapy.
- The Tat vaccine intensifies cART as indicated by: **i)** CD4+ T-cell increase beyond cART alone (particularly in poor immunological responders), **ii)** increased peripheral blood proviral DNA decay, **iii)** reduction of immune activation/dysregulation, **iv)** increased number and function of CD4+ and CD8+ T cells, B, NK cells and memory subsets (increase of central memory and decrease of effector memory T cells)
- The Tat vaccine has the potential to intensify cART efficacy by: **i)** reducing the rate of treatment failure, **ii)** reducing the prevalence of AIDS and non-AIDS co-morbidities, **iii)** restoring the immune response that may lead to greater efficacy of routine immunizations and containment of co-infections, and **iv)** allowing periodic drug-free time.

virus replication, particularly in the early days of highly active antiretroviral therapy (mid 1990s), when few drugs were available and not sufficiently potent to effectively suppress viral replication; and **iii**) to replace cART, thus providing a functional cure.

Although a number of therapeutic HIV/AIDS vaccine strategies have been developed over the years, as reviewed elsewhere [8], here, we specifically review the rationale and development of therapeutic vaccines currently under clinical investigation for cART intensification.

2.1. Objectives of therapeutic vaccination for cART intensification

The goal of therapeutic vaccines is to provide effective immunity against HIV by inducing anti-viral CD8+ T cells (CTLs), helper CD4+ T cells, and/or neutralizing antibodies (NAbs) [9]. Each arm of these adaptive immune responses is considered important in contributing to the control of virus replication. With regard to cellular immune responses, in addition to increasing their magnitude, the generation of poly-functional T cells capable of producing multiple cytokines and performing effector functions is considered very important, as HIV specific T-cell polyfunctionality has been shown to be associated with long-term non-progression [10]. Also, induced cellular responses have to be broad, as HIV mutates very rapidly and escapes immune pressure [11]. In addition, recent studies have identified T follicular helper cells (Tfh) as a significant source of virus production and a major component of the total viral reservoir [12]. Since these cells reside in B-cell follicles/germinal centers, it may be critical to generate CXCR5+ CD8+ T cells that can home to B-cell follicles and suppress or clear HIV-infected Tfh. HIV-specific CD4+ T-cell responses are critical to the development and maintenance of functional CD8+ T-cell and B-cell responses. Further, CD4+ T cells with cytolytic function have been reported to be associated with enhanced viral control, although it is unclear whether these responses can be primed by vaccination [13]. As chronic HIV infection is associated with impaired dendritic cell (DC) function and DCs are critical for generating protective cellular and humoral immune responses, therapeutic vaccines strategies aimed at enhancing or replacing DC function are also needed [14].

Although cART intensification by a therapeutic vaccine will benefit all individuals on antiretroviral therapy, to induce or augment immune responses against HIV antigens is particularly important in poor immunological responders to therapy, supporting a more effective immune restoration and virological control. Further, therapeutic vaccination may be of particular value in resource-limited countries, where access to cART and retention in care are difficult and represent a major obstacle to achieve control of the epidemics. In fact, the rate of new infections often exceeds the rate of antiretroviral roll-out, further fueling the HIV epidemics [15]. An effective therapeutic vaccination could, therefore, also help to control the epidemics.

In this scenario, combining ART with a therapeutic vaccine could elicit or boost anti-viral CD8+ CTL responses, increase CD4+ T-cell counts and functionality, as well as induce NAbs. This may lead to control of viral replication and purging of the HIV reservoir, hopefully reaching compartments, such as the central nervous system and, possibly, lymphoid tissues and cell types, such as monocyte/macrophages, that current antiretroviral drugs fail to adequately reach and/or exert their effects on [16].

Several therapeutic vaccines are being evaluated in phase I/II clinical trials, in naïve or cART-treated patients. To date, despite the efforts made, only slight clinical benefits have been reported [17,18]. Similarly, no significant reduction of the viral reservoir was observed in the few therapeutic vaccine trials that measured it [19], with the notable exception of cART intensification by the Tat therapeutic vaccine (see below). It is conceivable that the reduced immunocompetence observed in HIV-1 infected individuals limits the capability of therapeutic vaccines to elicit powerful protective immune responses. Alternatively, ineffective viral targets were utilized. However, cART intensification by therapeutic vaccination early in the course of infection, when the immune system functions are not severely deteriorated and virus reservoirs are not fully established, may be required to ensure strong virus control and to effectively attack the reservoirs, thus leading to a functional cure and, possibly, virus eradication.

2.2. Therapeutic vaccine clinical trials for cART intensification

Strategies and outcome of therapeutic vaccine clinical trials performed in the last years have been extensively reviewed elsewhere [14,20–23]. Here we focus on the therapeutic vaccines in clinical trials used as a cure adjunct to intensify cART, not to replace it. The most representative therapeutic clinical trials for cART intensification performed in the last 5 years and the novel approaches undertaken are summarized in Tables 1

Table 1. Therapeutic vaccines for cART intensification recently evaluated in humans.

Vaccine type	Trial Name	Registration number	Phase of clinical testing	Description	Immune responses	Virological responses	References
Peptide-based	IPROTECT1	NCT02041247	2	3S is a highly conserved motif of HIV-gp41 protein coupled with CRM197 carrier in aluminum salt adjuvant	Increase in CD4 counts observed in patients with higher anti-3S response	No effect on HIV-DNA levels	24
Peptide-based	Vacc-4x + lenalidomide	NCT01704781	1-2	Consists of four, slightly modified HIV Gag p24 consensus peptides	CD4 counts increase observed in the Vacc4x/ Lenalidomide group	Not assessed	25
Multiclade HIV plasmid DNA vaccine	VRCHIVDNA016-00-VP	NCT00270465	1	Plasmid DNA encoding for clade B Gag, Pol, and Nef and clade A, B, and C Env	HIV-specific T-cell responses against Gag, Pol, and Env with increased polyfunctionality	No reduction in HIV RNA and in the frequency of latently infected resting CD4 T cells	26
pDNA nanomedicine vaccine	LC002 DermaVir	NCT00270205	1-2	15 HIV antigens in a synthetic pDNA nanomedicine formulation for intradermal delivery	HIV specific, predominantly central memory T-cell responses	Not assessed	28
ChAdV63. HIVconsv and MVA.HIVconsv vaccines	ChAd-MVA. HIVconsv-BCN01	NCT01712425	1	One chimeric protein constructed by assembling the 14 most conserved regions of the HIV-1 proteome and inserted into 2 non-replicating vectors: an attenuated chimpanzee adenovirus serotype 63 (ChAdV63) and a modified vaccinia virus Ankara (MVA), to be delivered sequentially in a heterologous prime-boost regimen	Induction of broad and potentially novel T-cell responses against the HIVconsv epitopes	No effect on the size of the latent viral reservoir	30
mRNA-based dendritic cell vaccine	iHIVARNA-01	NCT02888756	2	mRNA encoding a mixture of antigen presenting cell (APC) activation molecules (CD40L, a constitutively active variant of TLR4 and CD70, referred as TriMix) and the HIV target antigens contained in HIVACAT	Increased frequencies of HIV-1-specific T-cell responses	No effect on HIV-DNA levels, HIV-RNA expression and ultrasensitive viral load (VL) transiently increased	33
ART intensification + DNA/Ad5 vaccine	EraMune 02	NCT00976404	2	Maraviroc + raltegravir intensification plus HIV DNA prime vaccine (VRC-HIVDNA016-00-VP), followed by HIV rAd5 boost vaccine (VRC-HIVADV014-00-VP)	Increase of immune responses to Gag, Pol, and Env after the rAd5 booster	No effects on HIV-DNA levels and HIV-RNA expression	34
DCs-based vaccine + LRA	VORVAX	NCT02707900	1	AGS-004 (autologous dendritic cells electroporated with RNA encoding CD40L and the HIV antigens Gag, Vpr, Rev, and Nef) + vorinostat	Not available	Not available	Not available
Tat protein	ISS T-002	NCT00751595 and NCT02118168 (extended follow-up study)	2	Biologically active clade B Tat protein	Increase in CD4, B and NK counts. Restoration of functional CD4 and CD8 T cell subsets. Increased T-cell responses against Env and recall antigens. Reduction of immune activation	HIV-1 DNA reduction 3 years after vaccination, which continued to decay in the 8 years of follow-up	80-82
Tat protein	ISS T-003	NCT01513135 and NCT02712489 (extended follow-up study)	2	Biologically active clade B Tat protein	Increase in CD4 counts. Cross-clade neutralizing antibody activity	In patients with detectable VL at week 48, lower geometric mean levels in vaccinees	88

and 2. The Tat therapeutic vaccine will be described separately since, at present, it represents the most advanced strategy in clinical development, showing efficacy in immune restoration and proviral DNA decay.

2.2.1. Therapeutic vaccines for cART intensification recently evaluated in humans

Therapeutic vaccine candidates and strategies that completed at least Phase I trials in the last 5 years are listed in Table 1 and are briefly discussed below. Although the strategies undertaken differ, they are all aimed at inducing mostly CD8+ T cell-mediated responses, with a special regard to memory responses needed for ensuring long-lasting immunity.

2.2.1.1. Peptide-based vaccines. VAC-3S is a therapeutic vaccine aimed at inducing a humoral immune response against a highly conserved region of the envelope protein gp41 of HIV-1 known as 3S, developed by InnaVirVax (a spin-off of INSERM, Evry, France). The gp41 3S motif has been shown to induce the expression of the natural killer (NK) ligand NKp44L on uninfected CD4+ T cells, rendering them susceptible to lysis by NKp44C activated NK cells. IPROTECT1 was a clinical trial assessing the therapeutic properties of VAC-3S when added to long-term standard antiretroviral therapy in the course of HIV-1 Infection (ClinicalTrials.gov Identifier: NCT02041247). The study demonstrated that VAC-3S is safe and induced a significant antibody (Ab) response, higher in patients with higher CD4+/CD8+ T-cell ratio at baseline. An increase in CD4+ T-cell count was observed in patients with higher Ab titers [24].

Vacc-4x, is a therapeutic vaccine consisting of four synthetic peptides of the HIV-1 p24Gag protein, which was evaluated in combination with the immune modulator Lenalidomide to determine whether the association could improve CD4+ T-cell counts in persons with low pre-ART CD4+ nadir, which often experience incomplete immune reconstitution despite effective and continuous ART (ClinicalTrials.gov Identifier: NCT01704781). A significant mean CD4+ T-cell increase was observed in the Vacc-4x/Lenalidomide group, compared to baseline, and higher than the corresponding mean CD4+ T-cell increase in the Vacc-4x/placebo group, although the difference between the two groups was not significant [25].

2.2.1.2. DNA-based vaccines. A prime-boost strategy with DNA/adenovirus 5 was tested in a double-blind study in 17 HIV-infected individuals on suppressive antiretroviral therapy (the VRC 101 study; ClinicalTrials.gov Identifier: NCT00270465) [26]. The vaccine was able to induce significantly stronger HIV-specific T cell responses against Gag, Pol, and Env, with increased polyfunctionality and a broadened epitope-specific CTL repertoire. However, DNA prime and rAd5 boost did not reduce HIV RNA by standard or single copy assays and did not reduce the frequency of latently infected resting CD4+ T cells measured by the viral outgrowth assay.

DermaVir is a topically administered pDNA nanomedicine therapeutic vaccine expressing HIV (Clade B) virus-like particles consisting of 15 antigens, developed by Genetic Immunity (Budapest, Hungary), which induces predominantly central memory T-cell responses. DermaVir is applied topically on the skin of the upper back and upper ventral thighs using a specific medical device (DermaPrep) after an exfoliation procedure [27].

DermaVir was tested in the A5176 phase 1 trial in 25 chronically HIV-infected subjects on suppressive cART (ClinicalTrials.gov Identifier: NCT00270205), and its administration was associated with a trend toward greater and predominantly central memory HIV specific T-cell responses [28]. This short-term study was not designed to assess efficacy, as neither clinical nor virological endpoints were included in the study design [28].

The BCN 01 trial was a phase I, open-label, non-randomized, multicenter prime/boost therapeutic vaccination study in acute HIV-1-infected individuals to evaluate the safety and immunogenicity of a heterologous prime-boost vaccine regimen in which the Chimpanzee adenovirus serotype 63 (ChAdV63) and Modified Vaccinia virus Ankara (MVA) were used to deliver the HIVconsv vaccine. The HIVconsv is a chimeric protein constructed by assembling together 14 regions that are highly conserved among the four major HIV-1 clades A, B, C and D and aimed at eliciting T-cell responses [29].

Vaccination led to a marked and highly selective expansion of preexisting as well as induction of new T-cell responses to HIVconsv, but failed to impact the size of the latent viral reservoir. Of note, T-cell responses were more robust after a prolonged (24 weeks) time interval between the ChAdV63.HIVconsv priming and the MVA.HIVconsv boosting as compared to an 8-week interval, (ClinicalTrials.gov Identifier: NCT01712425) [30], consistent with the prolonged in vivo persistence of the ChAdV63.HIVconsv vector and subsequent superior T-cell response maturation.

2.2.1.3. mRNA-based dendritic cell (DC) vaccines. DCs are considered the best professional antigen presenting cells for eliciting adaptive immune responses [31], and therapeutic vaccination strategies aimed at targeting DCs have been proposed over the years [14]. Recently, a novel strategy has been developed, consisting in the delivery of mRNAs encoding a mixture of activation molecules that are functional in DCs (i.e. CD40L + CD70 + a constitutively active variant of Toll like receptor 4, referred as TriMix) combined with a novel HIV immunogen, termed HIVACAT T-cell Immunogen (HTI). HTI sequence design was driven by functional immune data from close to 1,000 individuals from four different cohorts in three continents [32]. It is represented by a single mRNA sequence constituted by 16 joined fragments encoding conserved and highly immunogenic HIV-1 target epitopes of 10–70 amino acids each, in Gag, Pol, Vif and Nef. The novel vaccine combining Trimix and HTI, termed iHIVARNA was evaluated in 21 chronic HIV-1 infected patients under stable ART (ClinicalTrials.gov Identifier: NCT02888756). Intranodal (inguinal lymph nodes) vaccination had no impact on HIV-DNA levels, whereas HIV-RNA expression and (ultrasensitive-measured) viral load (VL) were transiently increased at weeks 5 and 6 at the highest dose of iHIVARNA, and these changes were positively correlated with HIV-1-specific-induced immune responses [33].

2.2.1.4. Therapeutic vaccine candidates combined with latency reversing agents. In a further approach therapeutic vaccine candidates have been combined with latency reversing agents (LRA), mobilizers of the reservoir, to reactivate latent HIV, which then become target of the vaccine induced HIV-specific immune response, leading to progressive reduction of the virus reservoir in patients on continuous ART, and eventually to eradication of the infection.

The EraMune 02 study was a clinical trial of therapy intensification by using raltegravir–maraviroc therapy (to reduce any residual cell-to-cell spread of HIV), followed by HIV DNA prime and rAd5 boost vaccination, as it was postulated that this vaccine would reactivate latent virus, induce replication, and improve the ability of the host immune system to clear virus producing cells [34] (ClinicalTrials.gov Identifier: NCT00976404). This strategy did not significantly increase HIV gene expression or reduced significantly the total cell-associated HIV DNA in either peripheral blood or rectal tissue in chronically infected patients with long-term HIV RNA suppression [34]. However, immune responses to Gag, Pol, and Env were boosted after administration of the rAd5 vaccine.

The VORVAX trial was a phase I/IIa study aimed at evaluating the association of serial AGS-004 vaccinations in combination with serial doses of vorinostat, a LRA, on the reactivation of persistent proviral HIV, as well as on HIV-specific immune responses, and on the frequency of resting CD4+ infected T-cells (ClinicalTrials.gov Identifier NCT02707900). AGS-004 is a therapeutic HIV vaccine, developed by Argos Therapeutics, and produced from autologous monocyte-derived DCs (MDDCs) that are electroporated with RNA that encodes CD40L (which is required for DC maturation) and the HIV antigens Gag, Vpr, Rev, and Nef, which are derived from an individual's pre-ART plasma, and reinjected into the patient intradermally [35]. No results of the VORVAX trial were published as the study was terminated because Argos could no longer provide the AGS-004 HIV vaccine.

2.2.2. Novel therapeutic vaccine candidates for cART intensification currently under investigation

New approaches and therapeutic candidates will be tested in clinical trials for cART intensification in the next few years. All the novel ongoing approaches here described aim to induce specific T cell responses, and are expected to be more effective. They include: **i)** the HTI vaccine; **ii)** the bivalent HIV-1 mosaic antigens vaccine; **iii)** DNA-based vaccines; **iv)** the tHIVconsvX vaccine; v) dendritic cell-based vaccines. These are briefly described in Table 2 and below.

2.2.2.1. The HTI vaccine. AELIX Therapeutics in collaboration with IrsiCaixa will evaluate in the AELIX-002 phase I study the safety and immunogenicity of the previously described HTI vaccine candidate delivered this time in a heterologous DNA prime (DNA.HTI) – viral vector boost (MVA.HTI and ChAdOx1.HTI) vaccination regimen in HIV-1 positive patients who started cART within the first 6 months since HIV-1 acquisition (ClinicalTrials.gov Identifier: NCT03204617). This combined administration is expected to strongly enhance immune responses to HIV, and the enrollment of early diagnosed, early treated HIV-infected individuals in the trial should favor the expansion of vaccine-associated immune responses, as the immune system deterioration is limited. Safety and immunogenicity are the endpoints of the trial, expected to be completed by January 2020.

2.2.2.2. The bivalent HIV-1 mosaic antigens vaccine. To address the challenge of global HIV-1 diversity, bioinformatically optimized bivalent global 'mosaic' antigens were developed to expand immunologic coverage of HIV-1 M group viruses. To express mosaic Env and Gag-Pol immunogens, adenovirus serotype 26 (Ad26) vectors were used, which differ substantially from Ad5 vectors in cellular receptor usage, tropism, innate inflammatory responses, adaptive immune phenotypes, as indicated by enhanced HIV Gag-specific CD8+ T-cells and memory T-cell responses generated by Ad26 [36]. Further, baseline NAb titers against adenoviruses in human populations are much lower for Ad26 as compared to Ad5, reducing the risk of unwanted responses to the vaccine due to preexisting immunity to the vector [36]. Phase I clinical trials in healthy HIV-uninfected adults with prototype Ad26 vector expressing a single HIV-1 Env insert have demonstrated the induction of robust Env-specific immune responses in both peripheral blood and colorectal mucosa. Vaccine regimens included priming with Ad26 vectors expressing mosaic Env/Gag/Pol antigens and boosting with Ad26 or MVA vectors expressing the same antigens with or without aluminum adjuvanted clade C Env gp140 protein [37]. A Phase I study of 2 different regimens of tetravalent Ad26.Mos4.HIV prime followed by boost with modified vaccinia Ankara (MVA)-mosaic or Ad26.Mos4.HIV plus a combination of mosaic and clade C gp140 protein will be evaluated in HIV-1 infected adults on suppressive antiretroviral treatment (ClinicalTrials.gov Identifier: NCT03307915). The trial, whose primary purpose is to assess safety and tolerability, will be completed by February 2022 and will be sponsored by Janssen Vaccines & Prevention B.V.

2.2.2.3. DNA-based vaccines. New approaches under investigation are aimed at inducing efficient CTL responses targeting conserved elements of Gag, generated by selecting regions characterized by stringent conservation, association with control of viremia in HIV-infected patients, and broad human leukocyte antigen coverage independently of alleles associated with virus control [38]. This vaccine will be evaluated for safety, immunogenicity, and preliminary assessment of efficacy in HIV-1 infected persons on ART. A phase I/II trial will be completed by October 2020 (ClinicalTrials.gov Identifier: NCT03560258).

Another innovative therapeutic DNA-based vaccine is a multi-clade gag/pol/env DNA vaccine administered together with an IL-12 DNA plasmid (PENNVAX, Inovio) that has been also studied for HIV prevention and it is known to be safe and highly immunogenic. PENNVAX®-GP consists of expression plasmids that encode synthetic HIV-1 multiclade consensus Gag, Pol and Env proteins administered with the IL-12 DNA as adjuvant (INO-9012), by electroporation. A Phase I/II trial will compare the immunogenicity and anti-reservoir activities of gag/pol DNA versus gag/pol/env DNA (both administered with IL-12), and will determine whether the presence of Env in

Table 2. Novel therapeutic vaccine candidates for cART intensification currently under investigation.

Vaccine type	Trial name	Registration number	Phase of clinical testing	Status	Description
HIVACAT-T immunogen (HTI)	AELIX-002	NCT03204617	1	Recruiting	HTI sequence is represented by a single mRNA sequence constituted by 16 segments (11 to 78 amino acids (aa) long) linked to each other by single, dual or triple alanine amino acid linkers. The final polypeptide sequence included regions from p17, p24, p2p7p1p6, Protease, RT, Integrase, Vif, Nef. The HTI vaccine candidate is delivered in an heterologous DNA prime (DNA.HTI) – viral vector boost (MVA.HTI and ChAdOx1.HTI) vaccination regimen
Bivalent HIV-1 mosaic antigens	Adenovirus 26 MVA + Clade C gp140 + Mosaic gp140	NCT03307915	1	Recruiting	Polyvalent 'mosaic' proteins assembled from natural clade B and C Gag, Pol, and Env sequences by in silico recombination and optimized to provide maximal coverage of potential T-cell epitopes. Heterologous prime-boost regimen
DNA-based vaccine	HIV-1-Gag Conserved-Element DNA Vaccine	NCT03560258	1-2	Recruiting	Sequences of at least 8 aa in length, in which all aa are conserved in at least 98% of all sequences and correlate epitope recognition with VL. Two plasmids, each with 7 conserved segments of 12–24 aa in length
DNA-based vaccine	HIV DNA Vaccine Encoding Gag, Pol and Env Proteins With IL-12 Plasmid	NCT03606213	1-2	Recruiting	HIV DNA vaccine encoding Gag/Pol (PENNVAX-GP) versus Gag/Pol/Env (INO-6145), both with IL-12 plasmids (INO-9102), administered by electroporation
T-cell vaccine vectored by DNA	HIVconsv	NCT03844386	1	Recruiting	14 most conserved HIV-1 subprotein domains, irrespective of known CD8+ T-cell epitopes
DC-based vaccine	DC-HIV04	NCT03758625	1	Recruiting	DC-HIV vaccine composed of autologous DCs matured with an optimized cocktail (a1DC) or with a standard prostaglandin E2 cocktail (pgDC) and loaded with autologous -inactivated HIV or conserved HIV Gag and Pol peptide pool. **Control vaccine will be** composed of autologous DCs matured with a1DC or with a standard prostaglandin E2 cocktail (pgDC), but not loaded with antigens

a DNA vaccine blunts T-cell responses to more conserved Gag-specific and Pol-specific epitopes, and may have a measurable effect on reservoir (ClinicalTrials.gov Identifier: NCT03606213). The trial is estimated to end by December 2020.

2.2.2.4. The tHIVconsvX vaccine. T cell vaccines targeting the most conserved regions of the HIV-1 proteome are expected to induce more effective immune responses. To this aim, a T-cell vaccine expressing novel immunogens, designated tHIVconsvX, assembling the 14 most conserved regions of the HIV-1 proteome into one chimeric protein, vectored by DNA, simian (chimpanzee) adenovirus and poxvirus MVA, has been developed. The tHIVconsvX immunogens combine the three leading strategies for elicitation of effective CD8+ T-cell responses: **i)** use of regions of HIV-1 proteins functionally conserved across all M group viruses (to make HIV-1 escape costly on viral fitness), **ii)** inclusion of bivalent complementary mosaic immunogens (to maximize global epitope matching and breadth of responses, and block common escape paths), and **iii)** inclusion of epitopes known to be associated with low VL in infected untreated people (to induce field-proven protective responses) [39]. A Phase I trial is being conducted in HIV-infected participants with durable viral suppression who will be randomly assigned to receive vaccination with MVA. tHIVconsv or placebo (ClinicalTrials.gov Identifier: NCT03844386), in order to assess safety and immunogenicity and to evaluate their immune system's ability to kill HIV. The study will end in August 2020.

2.2.2.5. Dendritic cell-based vaccines. This vaccine is composed of autologous DCs matured with an optimized cytokine cocktail (a1DC) or with a standard prostaglandin E2 cocktail (pgDC) and loaded with autologous inactivated HIV or with a conserved HIV Gag and Pol peptide pool. The vaccine will be tested in the DC-HIV04 trial, a Phase I study to evaluate safety and immunogenicity in ART-treated HIV-infected adults (ClinicalTrials.gov Identifier NCT03758625). This study will also evaluate four different methods of making the vaccine to verify which method may result in better immune responses. The study will end in May 2022.

3. The tat-based therapeutic vaccine

3.1. The concept for developing a therapeutic Tat vaccine for cART intensification

To increase the chances of success, the National HIV/AIDS Research Center (CNAIDS) at the Istituto Superiore di Sanità (ISS) in Rome, Italy, has adopted a 'pathogenetic approach' aimed at targeting key viral products responsible for reactivation of latent HIV, HIV reservoir replenishment and disease persistence under cART. Among them, Tat is a key HIV regulatory protein produced very early after infection, prior to virus integration, and necessary for viral gene expression, cell-to-cell virus transmission and disease progression [40–42]. A large part of Tat is released extracellularly and accumulates in tissues where it increases virus infectivity and activates immune cells while disabling the immune response to HIV [42,43].

Extracellular Tat mimics chemokines [44], induces HIV co-receptor expression [45,46] and can enter both infected and uninfected cells, particularly through the RGD-binding integrin receptors (α5β1, αvβ3, αvβ5) that are highly expressed on DCs, macrophages and activated endothelial cells. As a result, it facilitates R5-tropic viruses transmission to neighbor cells

[47] even in the absence of cell activation [48], sustains active virus replication, reactivates latent HIV [42,43] and enhances virus infectivity [49].

Of note, the Tat protein has been detected in highly purified virions [50], further supporting its key role in virus transmission and establishment of infection. In fact, Tat interacts with trimeric Env of different clades forming a virus entry-complex that targets DCs through the integrin receptors recognized by Tat, while diverting HIV from the Env canonical C-type lectin receptors (CLRs) [51]. This is because the cysteine-rich region of Tat engages the CCR5-binding regions of Env, including the V3 loop and a region of Env partially overlapping the other Env CCR5-binding site, whereas the Tat RGD sequence remains free and directs the virus to the RGD-binding integrins present on the DC surface [51]. These results were confirmed by cryoelectron microscopy [52]. Of note, by binding to Tat, Env appears to be shielded against HIV neutralizing Abs, which in the presence of Tat are much less effective. Consequently, Tat renders ineffective anti-Env Abs capable of blocking HIV entry through the CLRs pathway, whereas anti-Tat Abs become crucial for preventing HIV entry in DCs [51]. As a result, HIV acquisition by relatively poor susceptible cell targets, but important viral reservoirs such as DCs and macrophages and other cells of the reticular-endothelial system, is greatly increased [51]; through these actions, Tat favors and enhances HIV transmission to T cells [51].

Finally, Tat also markedly affects the immune system. It promotes DC maturation toward a T helper (Th)-1 polarizing phenotype, augmenting allogeneic and Ag-specific presentation by DCs and increasing Th1 responses against recall Ags [53]. In particular, it was reported that native Tat protein is very efficiently taken up by DCs, and, upon cellular entry, promotes their maturation and activation, leading to more efficient presentation of both allogeneic and exogenous soluble antigens and resulting in increased T cell responses against heterologous antigens [53]. In addition, the Tat protein modulates the CTL epitope hierarchy of heterologous antigens in vitro by modifying the catalytic subunit composition of the immunoproteasome, leading to broadened antigen processing and presentation, and to increased CTL recognition of subdominant epitopes [54]. Moreover, Tat modulates programming and secretory capacity of CD8+ T cells, suggesting that it may be involved in the development of CD8+ T lymphocytes with an effector profile, promoting the induction of short-living CD8+ effectors T cells, which undergo apoptosis upon encountering antigens in the presence of Tat [53–57].

Consistently with the role of Tat in HIV pathogenesis, epidemiological studies have shown that asymptomatic patients [58] and non-progressors (individuals who control viral load levels sufficiently to remain symptom-free with a CD4+ cell count above 500 cells/mm^3 for several years, and avoid or delay progression to AIDS) [59] have a higher prevalence of anti-Tat Abs as compared to progressors, and that a Tat-specific Ab response correlates with reduced risk of progression to AIDS.

In a 14-year longitudinal study of 252 individuals with known seroconversion dates, the risk of developing AIDS was 60% lower for anti-Tat Ab-positive as compared to anti-Tat Ab-negative individuals [60]. A second study showed that asymptomatic and treatment-naïve patients with high anti-Tat Ab titers contained CD4+ T-cell loss and VL, and did not initiate cART during the 3 years of follow-up, irrespective of anti-Env and anti-Gag Abs, indicating a very slow or no progression to disease [61]. Conversely, anti-Tat Ab-negative patients started therapy after a mean time of 17 months [61]. Thus, the presence of anti-Tat Abs is predictive of slower progression to AIDS.

Of importance, Tat is quite conserved among all subtypes in its immunogenic domains. A previous study, in fact, has shown an effective cross-recognition of a B-clade laboratory strain–derived Tat protein isolated more than 30 years ago by sera from Italian, South African and Ugandan individuals infected with different local viruses, owing to the high similarity of Tat epitopes among the different HIV clades [62]. Notably, the major degree of conservation was identified in the 1–58 amino acid region, which contains most of the immunogenic B- and T-cell epitopes. These data suggested that a Tat-based vaccine is capable of inducing a broad immune response against different virus clades [62].

Further, because of the intrinsic immunomodulatory properties, biologically active Tat does not require adjuvants, a relevant advantage in terms of clinical development, production and delivery costs [63].

As none of the available antiretroviral agents attack the latent reservoirs and are all ineffective at completely suppressing HIV expression and/or replication in low drug-penetration compartments, expression of HIV proteins, including Tat, persists [64], contributing, together with the ensuing residual viremia, to the persistent low-grade inflammation and immune activation, which, in turn, further fuel virus reactivation/replication [65] and HIV reservoirs replenishment. These abnormalities underlay and sustain residual disease in patients on effective cART, and conceivably represent the major obstacle to a functional cure or virus eradication. Thus, being the strongest activator of HIV transcription and the major regulator of latency [66], Tat arguably represents the major obstacle to functional cure and eradication approaches and, conversely, an important pathogenetic target for therapeutic vaccine approaches.

3.2. Therapeutic trials with the Tat vaccine

Results of preclinical studies of immunization with the Tat protein in monkeys indicated that the vaccine was safe, immunogenic and effective at inducing long-term protection against a highly pathogenic virus (SHIV 89.6P) [67–73]. As confirmed by others [74,75], protection correlated with anti-Tat Ab and anti-Tat cellular responses [67,73]. Thus, Tat-specific immunity appears to be key to prevent HIV acquisition and spreading.

Subsequent preventative and therapeutic, double blind, placebo-controlled phase I trials with the biologically active Tat (ISS P-001, ClinicalTrials.gov NCT00529698; ISS T-001, ClinicalTrials.gov NCT00505401) were successfully completed in Italy, meeting both primary (safety) and secondary (immunogenicity) endpoints [76–79]. As vaccination per se may trigger HIV replication and the Tat protein used to vaccinate

volunteers is biologically active, special attention was paid with regard to safety, both in the preclinical studies and phase I clinical trials, which also included volunteers not on cART. Monitoring for local and systemic adverse reactions as well as for hematological, biochemical, coagulation and immunological parameters demonstrated that the Tat vaccine was safe and did not induce virus replication, as indicated by the preservation of the levels of circulating CD4+ T cells and by the absence of significant plasma viremia rebounds [76,77,79]. As reported below, safety was confirmed in two much larger phase II trials.

Moving forwards, the therapeutic use was prioritized over the preventive as a shorter and cost-effective route to proof-of-efficacy [15]. Therapeutic phase II studies for cART intensification were conducted in Italy and South Africa (SA) in patients on successful ART.

The Italian phase II study (ClinicalTrials.gov NCT00751595) was an exploratory phase II open label therapeutic trial, randomized on the different regimens utilized. It enrolled 168 HIV-infected (B clade) anti-Tat Ab-negative adults on long-term cART (more than 6 years of therapy), virologically-suppressed, with CD4+ T-cell counts ≥200 cells/mmc, and evaluated immunogenicity, safety and immunological and virological disease biomarkers after vaccination with 7.5 or 30 µg of the Tat protein (clade B) without adjuvant, administered intradermally 3 or 5 times, one month apart. Both primary (immunogenicity) and secondary (safety) endpoints were met. Results also showed reduction of immune activation, and durable increases of CD4+ T cells, B cells, NK cells and CD4+ and CD8+ central memory T-cell subsets with reduction of effector memory cells, indicating a shift of the immune response toward restoration of immune homeostasis [80] (Table 3). None of these changes were observed in subjects on effective cART, negative for anti-Tat Ab and not immunized with the Tat vaccine, who were enrolled in a parallel observational study conducted at the same clinical Centers (ISS OBS T-002] (ClinicalTrials.gov NCT01024556] [80,81] and represented the external reference group for disease biomarkers according to ICH guidelines [European Medicines Agency. Note for Guidance on Choice of Control Group in Clinical Trials (CPMP/ICH/364/96). January 2001] [80–82]. In fact, the potential bias of an external nonrandomized control group was limited according to recommendations of the regulatory guidelines by enrolling subjects in the same clinical centers, with the same inclusion criteria of the trial, by using the same procedures, and by simultaneous evaluations by the same core laboratory.

Table 3. Results of the T-002 Trial.

(A) **Endpoints met: both primary (*Immunogenicity*) and secondary (*safety*)**

(B) **Immune Restoration**

- Increase of CD4+ T-cell number
- Restoration of functional CD4+ and CD8 + T-cell subsets
- Increase of B cell and NK cell number
- Increased cellular responses to Env and recall antigens *(Candida, Flu, CEF)*
- Reduction of immune activation

(C) **Reduction of HIV proviral DNA**

- Persistent and steep HIV-1 DNA reduction after 3 years from vaccination (*highest with 30 µg 3x in the PI-based drug regimens*)
- Continued decay of HIV-1 DNA after 8 years of follow-up from vaccination
- HIV-1 DNA reduction predicted by neutralization of Tat-mediated Env entry

(D) **Induction of cross-clade neutralizing anti-Tat Abs**

Ensoli B, PLoS ONE 2010; Ensoli F, Retrovirology 2015; Sgadari C, Front Immunol 2019

The CD4+ T-cell increases observed in vaccinees vs. controls (mean of about 100 cells/mmc) were statistically significant, and quartile analysis showed that CD4+ T-cell increases were particularly high in patients with low CD4+ T-cells at baseline (≤500/mmc), indicative of potential clinical benefit for low immunological responders.

Of note, Tat immunization induced a reduction of HIV-1 DNA load in blood, especially in volunteers receiving Tat 30 µg, given 3 times, that continued throughout the 8-years trial follow-up (ClinicalTrials.gov: NCT02118168) (see below). HIV-1 DNA decay was associated with anti-Tat Abs and neutralization of Tat-mediated Env entry in DCs, which predicted at 48 weeks the significant HIV-1 DNA reduction observed since year 3 after immunization. Tat-specific cellular responses also contributed to HIV proviral DNA reduction. Further, the induction, upon vaccination, of CD38+ HLA-DR+ CD8+ T cells and NK cells endowed with killer activity against virus-infected cells may have also contributed to reduction of the virus reservoir [81].

During the 8-year follow-up, anti-Tat Abs persisted in more than 50% of volunteers, CD4+ T cells continued to increase and HIV proviral DNA continued to decrease, becoming undetectable in blood in 34% of all vaccinees, and in 48% of volunteers in the Tat 30 µg 3 times group [82]. These results indicate that the induction of anti-Tat immune responses is necessary to intensify cART efficacy and to attack the cART-resistant virus reservoir [82] (Figure 1). A striking finding of this study is represented by the continued HIV DNA decay that, in vaccinees of the 30 µg 3× regimen, was reduced up to 10% of its pre-vaccination size (Figure 2). This, indeed, conflicts with the known modest, and very slow decelerating kinetics decay of proviral DNA after about 4–5.5 years of cART into a residual, steady reservoir core [83–85], as also observed by us in the reference control group of our trial [81]. Notably, the kinetics rate of HIV DNA decay in Tat vaccinees appear to be generally much faster as compared to patients treated for comparable period of times. In fact, in two recent studies evaluating DNA decay kinetics in patients on long-term cART, the HIV DNA half-life was quantitated in 7–19 years [86,87], as compared to only 3 years for Tat vaccinees. Finally, the kinetics of DNA decay appear remarkably and consistently faster for Tat vaccinees even when compared to long-term cART patients with comparable levels of intermittent viremia, which is a factor known to be associated with slower rates of decay [82] (Table 4). These data suggest that Tat immunization accelerates latent HIV reservoir decay in cART-treated patients by several potential mechanisms: **i**) blocking Tat-dependent enhancement of HIV infection in low virus-producing tissue compartments; **ii**) blocking Tat-induced CD4+ T-cell transitioning through a functional

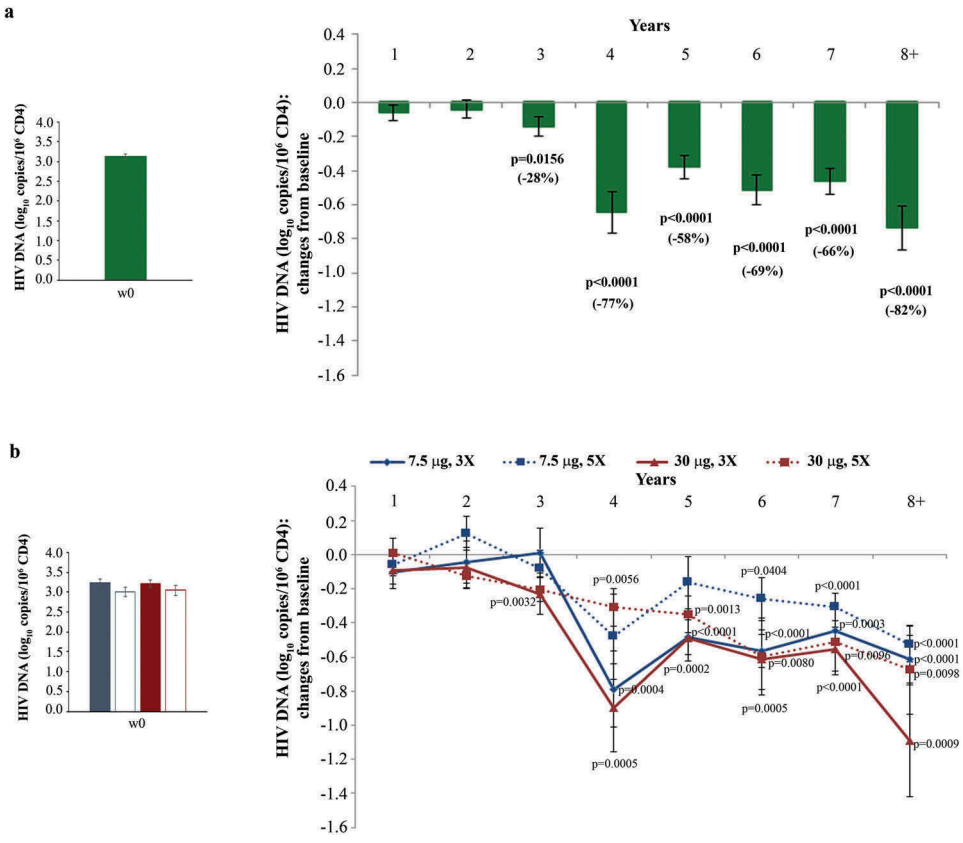
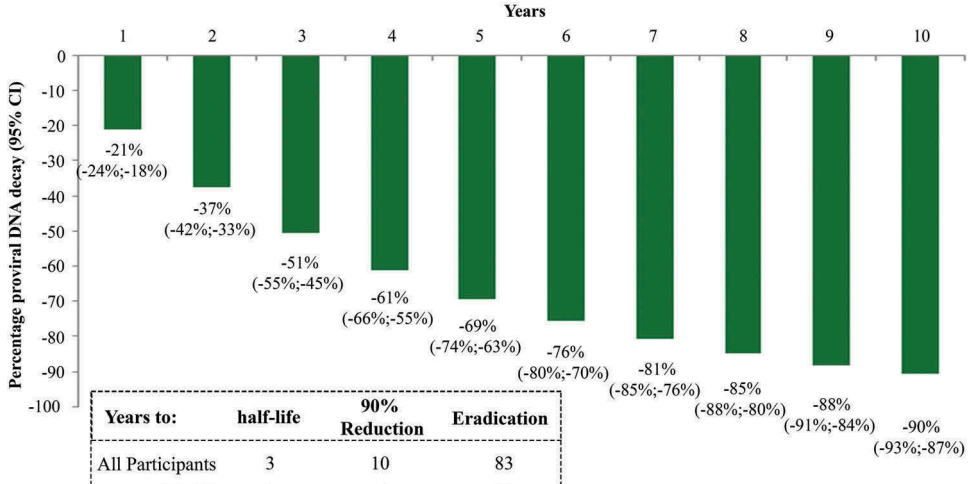


Figure 1. Changes of blood HIV DNA load over baseline in all vaccinees (A) and stratified by treatment groups (B) during follow-up. Baseline values (left panels) and annual changes (right panels) of HIV DNA levels (expressed as log10 copies/10^6 CD4+ T cells) from ISS T-002 study entry in all vaccinees (A), and stratified by Tat vaccine treatment groups (B) are shown. The number of participants tested are as follows: year 1 n = 89, year 2 n = 59, year 3 n = 42, year 4 n = 36, year 5 n = 51, year 6 n = 75, year 7 n = 58, year 8+ n = 37. Data are presented as mean values with standard error. A longitudinal analysis for repeated measurements was applied. p-values assess the values at year 1–8 after immunization vs. baseline values. Modified with permission from [82].



Years to:	half-life	90% Reduction	Eradication
All Participants	3	10	83
7.5, 3X	3	10	77
7.5, 5X	4	15	122
30, 3X	2	8	66
30, 5X	3	10	83

Figure 2. Kinetics parameter estimates of proviral DNA decay in vaccinees stratified by Tat vaccine regimens. Estimates of HIV-1 DNA annual decay in all vaccinees expressed as the percentage of HIV-1 DNA decay with 95% confidence interval (upper panel) or years to 50% reduction [half-life (t1/2)], to 90% reduction and to eradication for all vaccinees and by Tat vaccine regimens (lower panel) are shown. Modified with permission from [82].

Table 4. Kinetics of proviral DNA decay for the Tat vaccinees as compared to similar cohorts of virologically suppressed patients.

Reference study	Sgadari et al, Front Immunol 2019	Golob et al, AIDS 2018	Jaafoura et al, Nat Comm 2014
HIV reservoir measured	Total HIV DNA	Total HIV DNA	Integrated DNA
cART duration before enrolment	6 years (mean)	≥5 years	≥2 years*
Vaccination	Tat vaccine	None	None
cART continuation throughout the study	Yes	Yes	Yes
Proviral DNA decay kinetics parameter	**Half-life**	**Half-life**	**Half-life**
All patients	2-3 years	12 years	NA
Persistent virological suppression (VL = 0)	1 year	7 years	>7 years
Residual viremia (≥1 ≤40 copies/mL)	3 years	12 years	NA
Viremic blips (≥40 copies/mL)	4 years	22 years	NA
Proviral DNA decay kinetics parameter	**Eradication^**	**Eradication^**	**Eradication****
Persistent virological suppression (VL = 0)	31 years	NA	>200 years

*Only patients with no detectable viremic blips were considered in the study; ^Total body reservoir; **Total Blood reservoir*

cell state (effector memory CD4+ T cells) primed for latent HIV infection; **iii**) blocking Tat-mediated cell survival, thus increasing the turn-over (replacement) of latently-infected memory CD4+ T cells with uninfected cells; **iv**) relieving Tat-mediated inhibition of CTL responses; and **v**) increasing cell mediated immunity to Tat and to other HIV antigens (e.g., HIV Env) (Table 5).

A confirmatory randomized, double-blind, placebo-controlled (randomization ratio 1:1), safety and immunogenicity phase II therapeutic trial (ISS T-003, ClinicalTrials.gov NCT01513135) was then conducted in SA in 200 HIV-infected (C clade) anti-Tat Ab-negative adults, virologically suppressed, with CD4+ T-cell counts ≥200 cells/mmc. The clade B Tat vaccine (30 μg) was administered intradermally 3 times at monthly intervals [88]. The vaccine was safe and induced durable, high titers anti-Tat Abs that were capable of cross-clade recognition and neutralization, which correlated with the increase of CD4+ T-cell increase, a key target for cART intensification [88]. Of note, vaccination contained the VL rebound and maintained CD4+ T-cell counts above baseline levels in subjects non-compliant to therapy as compared to (non-compliant) placebo, suggesting that Tat vaccine intensification of cART may counterbalance incomplete adherence to treatment. A follow-up study of this trial (ISS T-003 EF-UP) has been completed and is under analysis.

Overall, the Tat vaccine proves for the first time that cART may be intensified by therapeutic immunization and that proviral DNA load may be progressively lowered.

Table 5. Potential mechanisms of HIV DNA decay by vaccine induced anti-Tat immunity.

Tat immunotherapy may accelerate HIV-1 proviral DNA decay in cART-treated patients through one or more of the following anti-Tat Abs and cell mediated mechanisms
• Block of Tat-dependent enhancement of HIV infection in low virus-producing tissue compartments
• Block of Tat-induced CD4+ T-cell transitioning through a functional cell state (effector memory) primed for latent HIV infection
• Block of Tat-mediated survival of latently-infected memory CD4+ T cells, thus increasing the turn-over (replacement) with uninfected cells
• Relieve of the Tat-mediated inhibition of CTL responses
• Increase of HIV-specific immunity

Ensoli F, Retrovirology 2015; Sgadari C, Front Immunol 2019

4. Conclusion

Therapeutic vaccines based on structural HIV gene products and immunotherapies have demonstrated their effectiveness in inducing immune responses, but, to date, have failed to obtain a permanent suppression of viral replication or a significant decay of the HIV-1 reservoirs, alone or even when added to long-term cART. There is, therefore, a growing interest in assessing new candidates or strategies to improve the magnitude and the quality of immune and virological responses. In this context, cART intensification by the therapeutic Tat vaccine has proved effective at promoting immune system restoration by improving CD4+ T-cell recovery and immune system functions, while reducing virus reservoirs and immune activation/dysregulation (Table 3). These combined effects may reduce the negative effects of non-adherence to therapy on virus transmission, hence, global community VL, new infections, and drug resistance. Moreover, an intervention that restores immune responses may allow periodic drug-free time. At this regard, reduction of ART would be a major advancement in HIV care, given the drugs toxicity, opening concrete perspectives for implementation of novel HIV/AIDS treatments and guidelines, including ART simplification and functional cure regimens, issues particularly important in the pediatric population.

5. Expert opinion

To date, no therapeutic vaccine or immunotherapy alone has obtained a prolonged remission of VL in patients [17–19]. Nevertheless, there is a growing interest in assessing new candidates or strategies capable of improving the magnitude and the quality of the immune responses with the aim of controlling viral replication and containing or reducing virus reservoirs. Many important immuno-virologic features of HIV infection are formidable obstacles fighting the success of current strategies, including poor vaccine antigen processing and presentation by DCs, vaccine inducing responses that target epitope escape, CTLs unable to migrate to sanctuaries (i.e. B-cell follicle), an adverse host immune environment (inflammation, immune activation, tolerance), the persistence of a latent reservoir virtually invisible to patrolling HIV-specific T cells.

Stem cell therapy, gene therapy, cell therapy, immune-based therapies (therapeutic vaccines, bNAbs, checkpoint

inhibitors) and activation of HIV-1 from latent reservoir are different strategies to improve the immune system or to target HIV-1 reservoirs that will be taken into account in the near future to achieve an HIV cure. It is conceivable that a combination of these strategies (i.e. innovative therapeutic vaccine candidates, bNAbs, checkpoint inhibitors, LRA, and cell therapy) will be required to succeed. Moreover, although these approaches holds great promises, further insights into the mechanism of action and potency are still needed for their full development, to improve their stability and delivery efficiency and to decrease the dose. Based on the results of the above described studies, a better understanding of the mechanism of action of therapeutic vaccines, the identification and development of new delivery systems, and the improvement of vaccine design will be attained. Additionally, future improvements should increase antigen-specific immune responses and the magnitude of memory B and T cell responses.

In the next 5 years the safety profile and the feasibility of implementation of some very complex approaches (stem cell therapy, gene therapy, cell therapy) will be determined. In addition, progresses have been made in redirecting immune responses to more vulnerable and conserved sites of the virus (HIVconsv, Mos.HIV, iHIVARNA), in developing new targeting strategies (mRNA iHIVARNA, nanoparticles), adjuvants (IL-15, anti-PD-1), LRA (romidepsin, vorinostat, TLR7/9), or a combination of these strategies to achieve the functional cure of HIV-1 infection [89].

In this regard, Tat could be an excellent vaccine candidate due to its capacity to accelerate HIV-1 proviral DNA decay in cART-treated patients, to promote the restoration of memory T-cell homeostasis and to reduce the immune dysregulation [80,82]. Moreover, Tat enters efficiently MDDCs, enhancing their Ag-presenting function, and drives Th1-specific immune responses, that may favor its own presentation and the induction of specific immune responses, but it may also adjuvate T-cell responses to other Ag [53,55]. In addition, by modifying the immune proteasome composition and function, Tat alters the Ag processing and presentation, leading to increased CD8 + T-cell responses to subdominant epitopes, thus, modulating the CTL epitopes hierarchy and breath of the response [54]. This may have an impact on both the control of HIV-1 infection and the use of Tat for vaccination strategies based on other HIV antigens as well as against other pathogens. For example, in the context of a live attenuated Herpes simplex (HSV) vaccine, the presence of Tat in an attenuated HSV vector increased memory CD8+ T-cell responses against epitopes called 'asymptomatic', because present in subjects without recurrences, induced anti-HSV IgG2a, an indicator in the mouse model of a Th1-driven B-cell response, and protected mice from death after a lethal challenge with a wild type HSV as compared to mice immunized with the HSV vector without Tat [90,91].

The different therapeutic uses of Tat vaccination in HIV infection are to be confirmed in dedicated trials to verify whether administration of Tat in patients receiving long-term (late) cART can provide prolonged post-treatment control (extended drug-free period of time with low or undetectable VL) in an analytic treatment interruption trial, opening new perspectives for functional cure and eradication strategies. Further, the Tat vaccine should be evaluated in low immunological responders to verify whether it ameliorates response to cART, at therapy initiation with the aim of reducing time to virological and immunological response. Of particular relevance it will be also to evaluate the Tat vaccine in HIV-infected cART-treated adolescents and children who face the longest time on antiretroviral treatment and therefore are the ones most needing approaches which may lead to maintenance of virus control despite poor adherence, to the adoption of therapy simplification regimens, or to prolonged time off-therapy (ATI) (Table 6). Further, Tat could be used as co-treatment to improve PrEP efficacy. Needless to say that it will be very important to evaluate in these context the effects of the Tat vaccine on HIV-DNA in lymphoid tissues and deeper compartments to determine the impact of vaccination on solid tissue HIV reservoirs and residual disease.

Public HIV healthcare spending is expected to increase exponentially. Thus, the impact on Public Health (PH) expenditures is staggering, considering the already high price of ART delivery (>$1,000/patient/year) [92]. In fact, the progressively higher cost of the medication (the current calculated yearly cost of treating all HIV-infected patients in Western and Central Europe is approximately 7 billion €) remains an important issue and will increase with the introduction of costly therapies such as those based on HIV bNAbs.

In this scenario, the development of new HIV/AIDS treatments based on the use of the Tat vaccine as co-treatment of ART will allow the PH system to better target resources toward more advanced policies of HIV care. Vaccination with Tat is expected to intensify cART efficacy by reducing the rate of treatment failure, and the prevalence of AIDS and non-AIDS co-morbidities, and by restoring the immune response that may lead to greater efficacy of routine immunizations, containment of co-infections, and allow periodic drug-free time, particularly in infants, children and adolescents facing lifelong cART and its severe side-effects causing low therapy-adherence. Moreover, the increased decay of cART-resistant latent HIV reservoirs by Tat vaccination [82] promises to blunt HIV rebound upon low adherence, one of the main mechanisms for development of drug resistance and virus transmission. Notably, vaccination with Tat in 'very early' treatment of acute infection could stall the expansion of HIV reservoirs, opening new perspectives for a functional cure for HIV infection.

Table 6. Clinical studies plans with the therapeutic Tat vaccine.

• **In Italy**
Analytical Therapy Interruption (ATI) Proof of Concept (PoC) trial to evaluate post-treatment viremic control in individuals on effective cART treated with rTat (volunteers from the T-002 phase II trial)
• **In South Africa**
A multi-center, randomized, double blind, placebo-controlled, efficacy trial to evaluate cART intensification after vaccination with the HIV-1 Tat protein in low immunological responders HIV-infected adult volunteers
A multi-center phase IIB/III randomized, placebo-controlled, double blind adaptive trial to evaluate cART intensification after vaccination with the HIV-1 Tat protein in HIV-infected adult volunteers at therapy initiation
A multi-center randomized, double blind, placebo-controlled, age-de-escalating, dose-finding phase I/II adaptive trial to evaluate the safety and immunogenicity of the HIV-1 Tat protein vaccine in HIV-infected cART-treated adolescents and children

Taking all the above into account, Tat vaccination may lead to new treatment guidelines capable of reducing second-line treatments and allow use of the saved resources to provide a wider access to therapy at the regional level. Therefore, intensified Tat/cART co-treatment will contribute to improved disease management and reduction of the HIV economic burden on PH.

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Declaration of interest

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