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REVIEW

"cART intensification by the HIV-1 Tat B clade vaccine: progress to phase III efficacy studies"

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ABSTRACT

Introduction: In spite of its success at suppressing HIV replication, combination antiretroviral therapy (cART) only partially reduces immune dysregulation and loss of immune functions. These cART-unmet needs appear to be due to persistent virus replication and cell-to-cell transmission in reservoirs, and are causes of increased patients' morbidity and mortality. Up to now, therapeutic interventions aimed at cART-intensification by attacking the virus reservoir have failed.

Areas covered: We briefly review the rationale and clinical development of Tat therapeutic vaccine in cART-treated subjects in Italy and South Africa (SA). Vaccination with clade-B Tat induced cross-clade neutralizing antibodies, immune restoration, including $CD4^+$ T cell increase particularly in low immunological responders, and reduction of proviral DNA. Phase III efficacy trials in SA are planned both in adult and pediatric populations.

Expert commentary: We propose the Tat therapeutic vaccine as a pathogenesis-driven intervention that effectively intensifies cART and may lead to a functional cure and provide new perspectives for prevention and virus eradication strategies.

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1. Introduction

Although saving millions of lives since its introduction, combination antiretroviral therapy (cART) is unable to fully restore T- and B-cell number and function, incompletely silences virus replication, and does not eradicate viral reservoirs [1]. As a result, chronic inflammation and immune dysregulation persist, leading, over time, to higher risks of co-morbidities and death. In fact, despite increased access to cART, the rates of HIV morbidity/mortality are still high, due to low treatment compliance, which hampers effective virus suppression [2]. In the United States, less than one-third of HIV-infected individuals have suppressed viral loads (Centers for Disease Control and Prevention. HIV in the United States: the stages of care-CDC Fact Sheet. 2014. Available at http://www.cdc.gov/nchhstp/newsroom/docs/HIV-Stages-of-Care-Factsheet-508.pdf), mostly resulting from undiagnosed HIV infection and failure to link or retain diagnosed patients in care.

Similar problems occur in sub-Saharan countries and in particular in South Africa (SA), where the epidemic has reached vast proportions: 19.2% of the sexually active population is infected (approximately 7 million), reaching a 40% prevalence in pregnant women and in addition 240,000 children are infected [3]. About 380,000 new infections are diagnosed each year. A recent survey revealed that 46% of patients initiating cART in 2010–2014 in SA had $CD4^+$ T-cell numbers below 200 cells/μL [4]. Twenty to thirty percent of these patients will not achieve $CD4^+$ T-cell counts >500 cells/μL even after years of treatment and are at risk of disease progression [5] and comorbidities, particularly tuberculosis (TB) and cardiovascular diseases (CVD), and suffer from high mortality and morbidity rates due to AIDS and non-AIDS events [6]. Increased TB risk occurs early in infection [7], increases as $CD4^+$ count decreases, and is not reduced by cART. In the cART era, CVD have become a leading cause of morbidity and mortality [8]. Higher rates of myocardial infarction as well as a high prevalence of subclinical coronary atherosclerosis have been found in the HIV-infected population. The interplay of chronic inflammation, cART, or immune activation after initiation of cART, may accelerate and increase CVD risk.

Additionally, about two million HIV-infected children and adolescents [3] face a lifelong drug burden amidst paucity of therapy and no vaccination options. In addition to increased risks of non-AIDS-related comorbidities [9], children and adolescents are more sensitive than adults to drug-induced metabolism changes [10], which increase the long-term risk of CVD. Moreover, lifelong adherence to therapy is hampered by high pre-ART viral loads and sub-therapeutic drug concentrations due to limited pediatric drug formulations, variable pharmacokinetics, side effects, substance use, and continuous bodyweight changes [11,12]. These factors promote the emergence of HIV drug resistance mutations [13]. This and the unknown long-term toxic effects represent major concerns in these age groups [14].

Furthermore, although 48% of people living with HIV (PLWH) are already on cART, to meet the 90–90–90 goals to end the epidemic by 2030 with universal testing and

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treatment will require hundreds of thousands of people to be put on therapy (and retained in care) each year, a very demanding effort. Pre-exposure prophylaxis (PrEP) will add thousands more. Thus, to establish and implement plans toward these goals constitutes an enormous public health (PH) problem in sub-Saharan Africa, which may hamper Test and Treat programs and achievements of UNAIDS 90–90–90 goals. Indeed, SA has the largest ART program globally and now invests more than $1.5 billion annually for HIV/AIDS (SA National AIDS Council, 2015). Nevertheless, failure of $CD4^+$ T-cell reconstitution during virologically suppressive cART in people with discordant response [i.e., virological response not accompanied by recovery of $CD4^+$ T-cell counts] indicates the need for alternative treatment strategies to effectively cure the patients and eventually eradicate the infection. There is, therefore, a great need of novel HIV/AIDS treatments to offset ART shortfalls and intensify treatment outcomes.

A therapeutic HIV vaccine may represent a relevant, cost-effective contribution to increase cART effectiveness by attaining a faster/more effective response to therapy (cART intensification) and mitigating the effect of poor adherence to cART [15]. Further, it may constitute an ideal setting to safely evaluate vaccines aimed at preventing HIV-1 infection. In fact, evidence of ongoing residual virus replication or reactivation during suppressive cART somewhat recapitulates and mimics primary infection events [16]. Thus, a vaccine blocking the mechanism(s) of virus transmission in primary infection should also be able to block ongoing replication in asymptomatic and drug naive individuals as well as residual ongoing replication and/or reactivation in patients on effective cART. This may lead, respectively, to protection from infection and to a functional cure or virus eradication. Accordingly, HIV-1-infected individuals may represent a valuable and convenient model in which to initially evaluate the efficacy of preventative vaccine candidates, making the selection of promising candidates much faster and affordable than the testing in uninfected individuals.

2. The concept for developing a Tat-based vaccine

Tat, the transactivator of transcription, is a key viral product in HIV activation, replication, cell-to-cell transmission in reservoirs and disease maintenance. In particular, Tat is the first protein to be produced upon infection, even prior to integration, since it is required for virus transcription [17–19]. After integration in the host cell genome, the HIV early proteins, including Tat, Rev, and Nef, are expressed at a basal rate, which is a function of the transcriptional activity in and around the integration site [20]. This basal rate is generally low and largely determined by RNA polymerase II (RNAPII) 'pausing' after the initial synthesis of a short mRNA stretch. Tat acts by targeting the positive transcriptional elongation factor b (pTEFb) and bringing it to the paused RNAPII at the HIV long terminal repeat (LTR) promoter. This leads to RNAPII phosphorylation, which releases RNAPII from its stalled position, increasing viral transcriptional elongation by about 100 folds. Since Tat expression is driven by the same viral promoter, this establishes a strong positive transcriptional feedback loop [the Tat *circuitry*] that bypasses normal cell control of P-TEFb [21]. Studies have indicated that the Tat circuitry is characterized by transient (short-lived) stochastic bursts that, in turn, amplify stochastic basal transcriptional fluctuations at the HIV LTR promoter. This can be observed as a 'bifurcating' expression pattern in a clonal cell population, where promoter activity is high (ON state) in some cells, low (OFF state) in others [22]. These data also indicate that the OFF state is the more stable endpoint and that it generates HIV latency; thus, the Tat circuitry would serve as a 'stochastic switch' between latency and lysis. The interpretation of these studies contrasts with the notion whereby HIV latency would be deterministically determined upon the transition of infected cell from an activated state to the [non-permissive] resting state [23]. However, other studies have confirmed that HIV expression in primary cells can be persistently high even during the transition to the resting state and that the Tat circuitry can be uncoupled from cell state transitions [24]. These studies are supported by the lack of cell lysis upon treatment with non-activating 'latency reversing agents' (LRA) and by recent studies in a mouse model indicating that the Tat inhibitor didehydro-Cortistatin A can suppress HIV rebound upon cART interruption [25]. Thus, overall these studies assign a prominent role for Tat in the establishment of HIV reservoirs and in virus reactivation form latency, irrespective of immune activation stimuli, making Tat probably the single most critical protein in the virus life cycle and HIV Achille's heel [24]. However, the contribution of Tat to HIV infection goes beyond its critical role in the virus life cycle. In fact, Tat is released extracellularly and accumulates locally, since it binds through its basic region to the heparan sulfate proteoglycans present both on the membrane of neighbor cells and in the extracellular matrix, and with its Arg-Gly-Asp (RGD) region to RGD-binding integrins present on dendritic cells (DCs) and activated endothelial cells [26,27,28,29, our unpublished data]. Extracellular Tat has been shown to increase virus infectivity and to dysregulate the immune system by provoking a generalized immune activation, which hampers mounting of an effective immune responses to HIV [26–28,30–34]. In particular, extracellular Tat promotes HIV-1 replication upon entry in infected cells, while it attracts and activates uninfected cells, thus providing new targets for HIV-1 propagation [35]. In fact, extracellular Tat has been shown to bind to several chemokine receptors, including CXCR4, the HIV-1 coreceptor widely expressed on resting lymphocytes and monocytes and to chemoattract and activate them, inducing the expression of CCR5, the main HIV coreceptor [29,33,36,37].

Of note, by selectively binding Env on virus particles, Tat forms a virus entry complex and redirects the virus from the canonical receptors to integrins (α5β1, αvβ3, αvβ5), thus favoring HIV entry into DCs and other cell types of the reticular-endothelial system (i.e. long-term viral reservoirs) and transmission to T cells [28]. Moreover, by binding Env, Tat shields the virus from neutralization by anti-Env antibodies (Abs), which, in contrast, is restored and increased by anti-Tat Abs [28].

Thus, Tat represents a major target for an immune-based therapy aimed at controlling HIV replication, tissue propagation, and establishment/maintenance of virus reservoirs, factors acting together in causing chronic immune activation and

persistent immune cell dysfunctions. Further, Tat is an essential viral protein to target also because it is the strongest activator of HIV transcription and the major regulator of latency, irrespective of and independently from T-cell activation, thus representing the major obstacle to functional cure and eradication approaches [24]. Of note, cART does not prevent HIV-1 gene expression, including expression of Tat [38,39], which likely contributes to the persistence of inflammation and immune activation in virologically suppressed individuals. This constitutes the rationale to evaluate a Tat vaccine in successfully cART-treated individuals.

Epidemiological evidence confirms that an immune response to Tat may control disease progression: anti-Tat Abs have a higher prevalence in asymptomatic patients [40] and in non-progressors [41]. 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 [42]. A second study showed that asymptomatic and treatment-naïve patients with high anti-Tat Ab titers experienced a limited $CD4^+$ T-cell loss, had a low viral load, and did not meet the criterion ($CD4^+$ T cell counts <350 cells/μL) to initiate cART during the 3 years of follow-up, indicating a very slow or no progression to disease [43]. Conversely, and irrespective of anti-Env and anti-Gag Abs, anti-Tat Ab-negative patients started therapy after a mean time of 17 months. Thus, the presence of anti-Tat Abs is predictive of slower progression to AIDS.

Finally, B- and T-cell Tat epitopes are conserved among all virus subtypes [44], suggesting that Tat may represent an optimal candidate for cross-clade vaccines [35,45]. Accordingly, vaccines based on Tat are being actively pursued by us and other investigators [46,47].

3. Preclinical testing of the Tat vaccine

Based on this evidence, preclinical studies in small animals and nonhuman primates were undertaken. Immunization with the Tat protein or *tat* DNA in cynomologous macaques was found to be safe, elicited a broad and specific immune response, and, most importantly, induced a long-term protection against infection with 10 MID_{50} of the highly pathogenic SHIV89.6P, a simian immunodeficiency virus (SIV) carrying the HIV-1 *tat* gene, which rapidly causes AIDS and death in these monkeys [48–50]. Vaccinated and protected monkeys did not show signs (viral and proviral load, $CD4^+$ T-cell loss) of systemic infection throughout a 104-week follow-up, even after two boosters with tetanus toxoid, a stimulus known to activate $CD4^+$ T cells and to increase virus replication. When four of the protected monkeys were rechallenged with a fivefold higher dose (50 MID_{50}) of the same SHIV-89.6P, overt infection and $CD4^+$ T-cell loss was observed in the acute phase of infection. However, over time they regained control of infection, as indicated by the statistically significant and long-lasting reduction of viral replication and $CD4^+$ T-cell number restoration in comparison to control monkeys. This effect was associated with a strong anamnestic response to Tat, while responses to Gag and Env were nearly undetectable [51].

A retrospective analysis of 112 Mauritian cynomolgus macaques from different preclinical trials, vaccinated ($n = 67$) or not ($n = 45$) with Tat and challenged intravenously with the SHIV-89.6P, showed that vaccination induced a significant reduction of the rate of infection acquisition at 10 MID_{50} ($p < 0.0001$) and limited acute $CD4^+$ T-cell loss at 15 MID_{50} ($p = 0.0099$). Of importance, vaccination also contained $CD4^+$ T-cell depletion ($p = 0.0391$) during chronic infection, irrespective of the challenge dose [52].

4. Clinical development of the Tat vaccine

As the next step, preventative and therapeutic double-blind, placebo-controlled phase-I trials with the biologically active clade B Tat administered intradermally or subcutaneously with Aluminum phosphate (Alum) adjuvant (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 [52–55]. Subsequent trials focused on the therapeutic setting because of the several advantages it has over the preventative approach. In particular, it (i) provides a rapid first proof-of-concept of efficacy of a vaccine design and biomarkers assessment, (ii) requires a smaller sample size for efficacy trials, (iii) can be also conducted in developed countries, (iv) is much less expensive, and (v) has a broad application with key potentials in the most affected populations. Thus, therapeutic phase-II studies for cART intensification were then conducted in Italy and SA in patients on effective cART. For the subsequent trials, the intradermal route of administration was chosen since vaccination with Tat alone intradermally was more effective than the subcutaneous delivery of Tat with Alum at inducing Tat-specific cellular responses, while no significant differences between the two formulations and routes were observed in terms of tolerability and induction of humoral immune responses to the vaccine. In addition, it is easier to handle and less expensive to produce and deliver. This is a relevant advantage in the setting of large-scale vaccination campaigns in populations living in resource-limited settings.

In all the trials the vaccine administered was a subunit vaccine made of recombinant biologically active HIV-1 B Clade (BH10) Tat protein (referred herein as 'Tat') manufactured under GMP. The manufacturing process was specifically developed to prevent oxidation and to keep the protein in its native active form, which is required to induce an effective Ab response against conformational epitopes which are key for Tat neutralization of virus entry in DCs.

4.1. Therapeutic trials with the Tat vaccine

4.1.1. Phase-I trial

The randomized, double blind, placebo-controlled phase-I therapeutic trial with Tat (ISS T-001, ClinicalTrials.gov NCT00505401) was conducted in four clinical centers in Italy in 27 HIV-infected asymptomatic individuals (20 vaccinees, 7 placebo), with $CD4^+$ T cells/μL ≥400, viral load ≤50,000 copies/mL, and $CD4^+$ T-cell nadir ≥250 cells/μL, regardless of the anti-Tat serostatus at baseline. The Tat vaccine, administered five times (4 weeks apart), either

subcutaneously with Alum or intradermally without adjuvant at 7.5, 15, or 30 μg doses, was well tolerated, both locally and systemically. Notably, it did not increase plasma viremia levels, confirming former data obtained in infected macaques that bioactive Tat, at the doses used, does not promote viral replication. Conversely, the protein induced anti-Tat Abs, which persisted up to 5 years from the first immunization. Since both the primary (safety) and secondary (immunogenicity) endpoints of the study were reached [53,54,56], the Tat vaccine was advanced to phase-II studies in Italy and SA in patients on successful cART.

4.1.2. Phase-II trials

The ISS T-002 (Clinicaltrials.gov NCT00751595) was an exploratory multicenter, randomized, open label therapeutic trial conducted in Italy [57,58]. This trial enrolled 168 HIV-infected (B clade) anti-Tat Ab-negative adults on cART, who were virologically suppressed, with $CD4^+$ T-cell counts ≥200 cells/μL. Immunogenicity, safety, and immunological and virological disease biomarkers were evaluated after vaccination with 7.5 or 30 μg of Tat protein (clade B) without adjuvant, administered intradermally 3 or 5 times, one month apart. Both primary (immunogenicity) and secondary (safety) endpoints were met. Of the two vaccine doses evaluated, the 30 μg was the most effective regimen at inducing anti-Tat Abs (number of Ab responders; Ab titers, breath, and durability). A nested study conducted in 30 volunteers enrolled in the ISS T-002 trial and immunized with Tat 30 μg, 3x, or Tat 7.5 μg, 5x, also showed that Tat immunization had induced cross-clade (clades C, D, A) Abs. In fact, although none of the subjects (n = 30) were anti-Tat Ab-positive at baseline, all of them (30/30) became anti-Tat Ab-positive for clade B after immunization, and 21/30 (70%) also for other HIV Tat clades. Results also showed reduction of immune activation and durable increases of $CD4^+$ T lymphocytes, B cells, natural killer cells, and $CD4^+$ and $CD8^+$ central memory subsets [58], particularly in subjects with low $CD4^+$ T cells counts at baseline (low immunological responders). None of these changes were observed in subjects on effective cART enrolled in a parallel observational study (ISS OBS T-002, ClinicalTrials.gov NCT01024556) [57,58].

Of utmost relevance, Tat immunization induced a reduction of HIV-1 DNA load in blood, especially in volunteers receiving Tat 30 μg 3 times. 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 starting at year 3. The steep reduction of proviral DNA continued over the 8-years trial follow-up (ClinicalTrials.gov: NCT02118168). During this time anti-Tat Abs persisted in more than 50% of volunteers, as persisted $CD4^+$ T cell increases. Notably, proviral DNA progressively declined, becoming undetectable in 35% of all vaccinees, and in 48% of volunteers immunized 3 times with Tat 30 μg [58 and manuscript in preparation].

Thus, of the four vaccine regimens evaluated, the 30 μg given intradermally 3 times 4 weeks apart was the most effective at inducing Ab responses and at reducing the proviral DNA load. Accordingly, this was the regimen chosen for the confirmatory randomized, double-blind, placebo-controlled (randomization ratio 1:1), safety, and immunogenicity phase-II therapeutic trial (ISS T-003, ClinicalTrials.gov NCT01513135) that was conducted in SA in 200 HIV-infected (C clade) anti-Tat Ab-negative adults, virologically suppressed, with $CD4^+$ T-cell counts ≥200 cells/μL. Although defined as confirmatory, the ISS T-003 trial also addressed several relevant questions: 1. Would a clade B Tat vaccine be immunogenic in a population with a different genetic background, infected by different virus subtype (C clade) and treated with different drug regimens? Would a clade B Tat vaccine elicit immune responses cross-recognizing Tat clade C? Would anti-Tat Abs cross neutralize clade C Tat? Would the beneficial effects of cART intensification by the Tat clade B vaccine detected in ISS T-002 occur also in the ISS T-003 trial?

Although administered in a population with a different genetic background (black and mostly females, as opposed to the Caucasians and mostly males enrolled in the T-002 trial), infected by different (C clade) virus subtype, and treated with different drug regimens with generic drugs (Table 1), vaccination with clade B Tat was safe and induced durable, high titer anti-Tat Abs of different isotypes (Figure 1). Further, as for ISS T-002 trial, induced anti-Tat Abs were capable of cross-clade recognition (Figure 2(a)) and neutralization (Figure 3), which correlated significantly with the increase of $CD4^+$ T-cells, a key target for cART intensification [59]. Notably, T-003 volunteers had been pre-screened for Abs against Tat clade B, but not against Tat from other clades. When it was done retrospectively, it turned out that at baseline 29 of the 100 vaccinees had anti-Tat Abs directed against other HIV subtypes (Tat clade C: n = 22; 76%; D: n = 4; 14%; A: n = 12; 41%) [58]. Titers of these Abs were all significantly boosted by vaccination with Tat B clade (Figure 2(b,c)), whereas in 51 of the 68 vaccinees

Table 1. Baseline characteristics of study participants.

	n	ISS T-002	n	ISS T-003
Gender				
Male	30	78.9%	32	32.0%
Female	8	21.1%	68	68.0%
Race				
Black	0	0.0%	100	100.0%
Caucasian	38	100.0%	0	0.0%
Mixed	0	0.0%	0	0.0%
Age				
Mean ± s.d.[a]	38	43.7 ± 5.7	100	36.1 ± 5.6
Range		33.0–55.0		21.1–45.8
$CD4^+$ (cells/μL)				
Mean ± s.d.	38	617 ± 214	99	510 ± 229
Range		212–1362		137–1530
$CD4^+$ (%)				
Mean ± s.d.	38	33 ± 7	99	28 ± 8
Range		19–47		7–49
HIV RNA (copies/ml)				
<40 (cut-off assay)	36	94.7%	94	95.0%
≥ 40	2	5.3%	5	5.0%
Years from HIV diagnosis				
Mean ± s.d. [a]	38	10.5 ± 7.1	100	5.0 ± 3.0
Range		0.6–23.4		1.0–14.0
Years from ART initiation				
Mean ± s.d. [a]	37	6.7 ± 4.9	100	3.5 ± 2.0
Range		0.7–18.0		0.7–8.2
Current ART regimen				
NNRTI or NRTI-based	24	64.9%	97	97.0%
PI-based	13	35.1%	3	3.0%
Previous tuberculosis	0	0.0%	29	29.0%

n indicates the number of individuals; [a]Standard deviation.

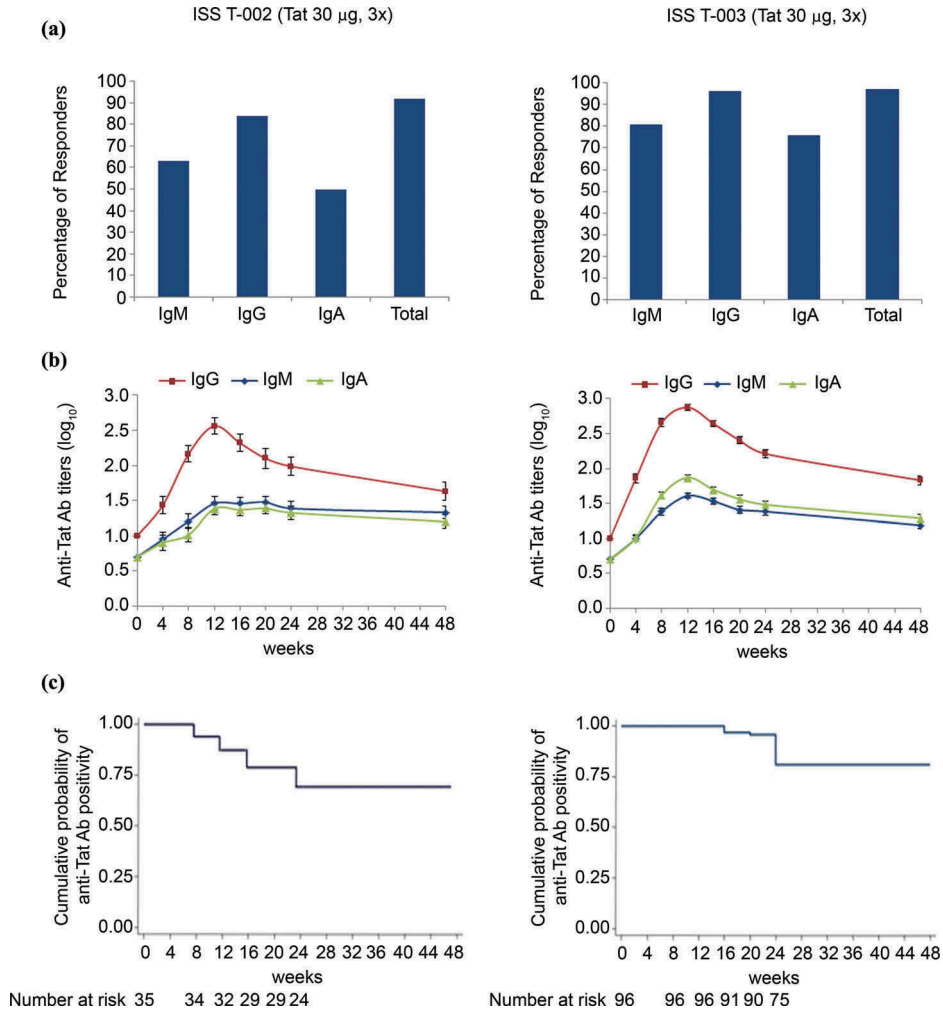

Figure 1. Anti-Tat humoral immune response in vaccinees from the ISS T-002 (left panel) and ISS T-003 (right panel) phase II trials. (A) Percentage of volunteers producing anti-Tat Abs (responders) after Tat immunization; (B) IgM, IgG and IgA *Ab* mean titers (with standard error) in responders; (C) Kaplan-Meier estimates showing the cumulative probability of anti-Tat *Ab* durability in responders after Tat vaccination. Modified with permission from [57] and [59].

with undetectable Abs against other clades at baseline who mounted anti-Tat B clade responses upon vaccination, cross-clade binding Abs were induced although to a variable extent (Table 2). The three vaccinees who did not respond to Tat vaccination were negative for cross-clade binding Abs at baseline and remained so afterwards, suggesting true unresponsiveness to Tat.

Importantly, also in this trial, the greatest $CD4^+$ T-cell increases were recorded in poor immunological responders (i.e., those with <500 $CD4^+$ T cells/µL at baseline) (Figure 4). Furthermore, vaccination limited viral load rebound and maintained $CD4^+$ T-cell counts above baseline levels in subjects non-compliant to therapy as compared to placebo, suggesting that Tat vaccine intensification of cART may counterbalance, and hopefully abrogate, the consequences for reduced treatment adherence [59]. So far, no other current treatment has been shown to achieve these effects. A follow-up study of the trial (ISS T-003 EF-UP) has been conducted to gain more insights on the extent and durability of the effect of vaccination (long-term safety, immunogenicity, efficacy).

The evaluation of the Tat vaccine efficacy data and clinical trial plans in SA was entrusted to two independent bodies: a Panel of Experts convened by National Department of Health (NDOH, 2014) and an Evaluation Workshop convened by the United Nations Industrial Development Organization (UNIDO, 2015). Both bodies advocated conduct of phase-III studies for vaccine registration in SA, and the reach-out for the needed funding to international donors. To this end a public–private partnership, the Tat Vaccine Partnership (TVP), has been established. In particular, based on the above achievements, SA and Italian stakeholders (CNAIDS/ISS, NDOH, SA Medical Research Council, SAMRC) embraced with private partners (Vaxxit, Diatheva, TCD-Global, Kiara Health), in the TVP. TVP is coordinated by the SAMRC and its mission is to contribute to achieving EDCTP2 work plan and UNAIDS goals (Sustainable Development Goals 3: Ensure healthy lives and promote well-being for all at all ages; UNAIDS 90-90-90: end the HIV/AIDS epidemics by 2030) through completion of the clinical experimentation and registration of the Tat vaccine for improved therapeutic interventions against HIV/AIDS. To this aim, the TVP reaches out to international organizations and

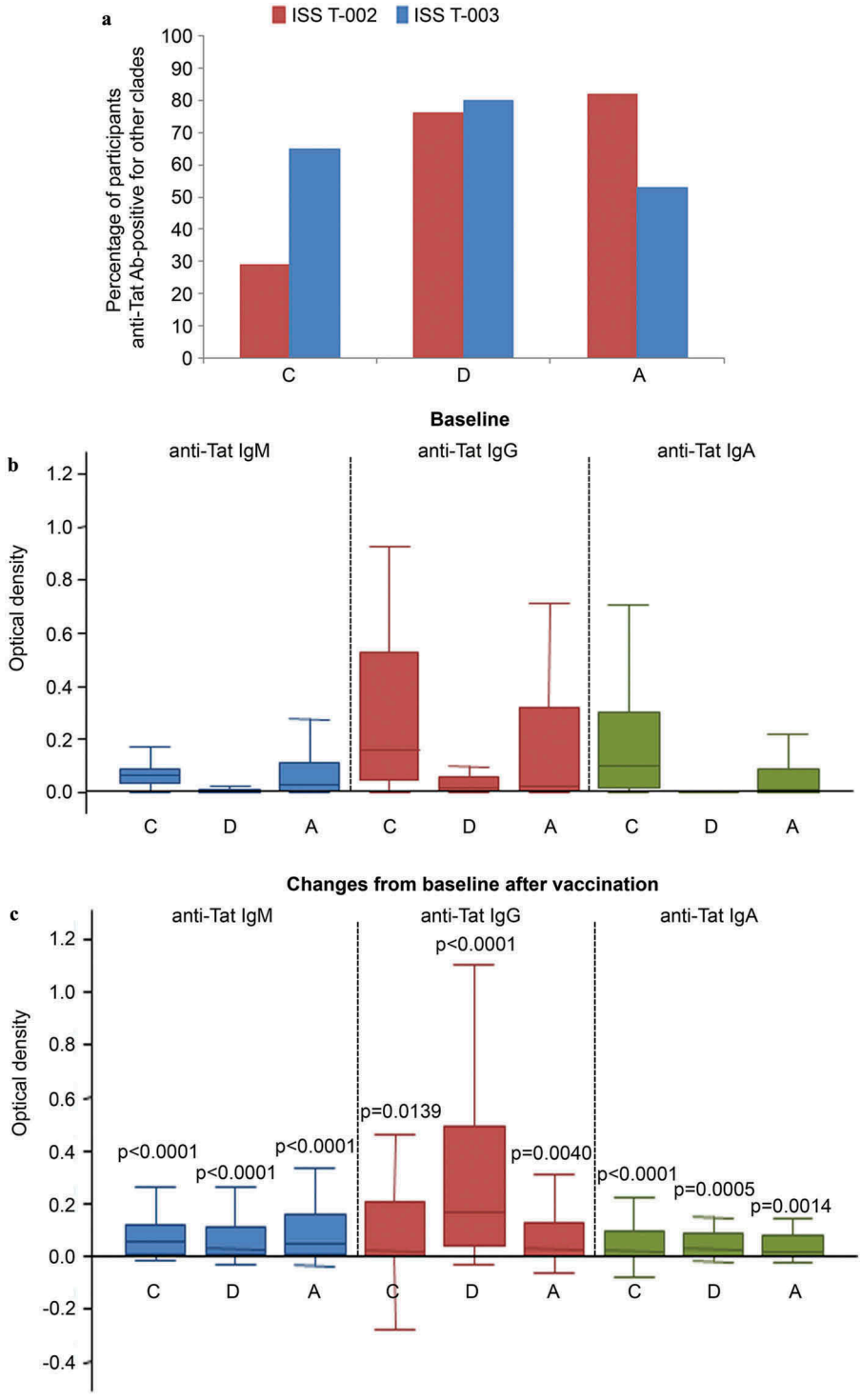

Figure 2. Induction or modulation of cross-clade anti-Tat Abs binding after immunization with Tat. (A) Percentage of vaccinees from the ISS T-002 (red bar) and ISS T-003 (blue bar) phase II trials developing after vaccination Abs recognizing Tat from other (C, D, A) clades; (B) Baseline optical density (OD) values and (C) changes from baseline OD of anti-Tat IgM, IgG and IgA against clades C, D and A in the 29 vaccinees from the ISS T-003 trial with cross-clade (C: 76%; A: 41%; D: 14%) anti-Tat Abs prior to immunization. Testing was performed at the peak of *Ab* responses (between week 12 and week 24). Statistical analysis was performed using the Wilcoxon signed-rank test. P-values assess the increase from baseline. Modified with permission from [59].

private funders for the resources needed to register the Tat vaccine for use in adults, children, and adolescents.

5. Conclusions

Results from the therapeutic trials indicate that the Tat vaccine is safe (to date over 300 people have received the vaccine, in either preventative or therapeutic trials) and immunogenic. Furthermore, the results from both phase-II trials showed a statistically significant increment of $CD4^+$ T cells (Figure 4), suggesting a return of immune functions to homeostatic levels. Moreover, in both trials $CD4^+$ T cell increased particularly in subjects with low $CD4^+$ T-cell counts at baseline (low immunological responders). Furthermore, the Tat vaccine

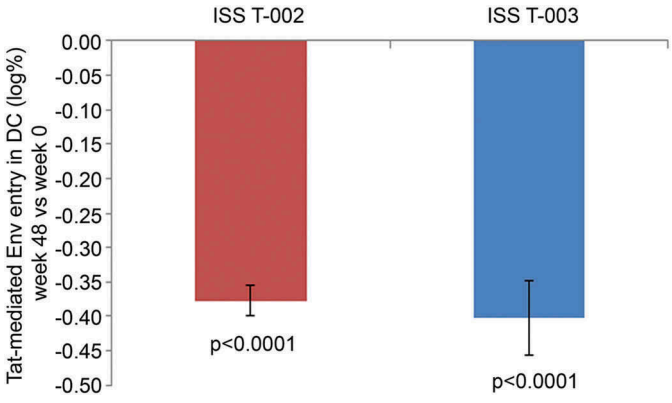

Figure 3. Neutralization of B-clade Env entry in DCs in the presence or absence of Tat (B and C clade) by sera of *Ab*-positive vaccinees, evaluated at week 48 after immunization. Data are presented as mean values with standard errors. Student's t-test for paired data was used for the analyses. Modified with permission from [59].

Table 2. Vaccinees who mounted cross-clade anti-Tat Ab responses upon vaccination with Tat B clade.

HIV clades	*n*	%
C	5	9.8
D	7	13.7
A	4	7.8
C + D	12	23.5
C + A	1	2.0
D + A	7	13.7
C + D + A	15	29.4
Total	**51**	**100.0**

promoted proviral DNA decay, thus supporting the use of Tat immunization to intensify cART [58, 59, and Ensoli et al, unpublished data]. In fact, virus reservoirs and their replenishment by cell-to-cell virus transmission are ART resistant and associated to chronic immune activation and lymphocyte dysfunction, which represent the ART unmet needs.

Taken together, these data confirm the key role of Tat in the pathogenesis of HIV/AIDS, including virus replication, transmission, immune dysregulation, disease onset and maintenance. Further, they indicate that the induction of anti-Tat immune responses is able to intensify cART efficacy and to attack cART-resistant virus reservoirs, confirming that the Tat vaccine can offset cART shortfalls while renewing perspectives for a functional cure, as suggested by promotion of proviral DNA decay [60].

Based on the above, concepts were devised for efficacy trials to be conducted in SA in adults, responding to the urgent need for cART intensification in virologically suppressed ART-treated poor-immunological responders as well as in newly diagnosed patients initiated on treatment according to the Test and Treat WHO guidelines, and for bridging trials assessing safety, immunogenicity, and efficacy of different doses of the Tat vaccine in HIV-infected patients <18 years old. In particular, the first study will be a phase-III, randomized, double-blind, placebo-controlled efficacy trial with the biologically active HIV-1 Tat protein therapeutic vaccine aimed at increasing cART efficacy by improving $CD4^+$ T-cell recovery in HIV-1-infected adult volunteers, in support of a Tat-vaccine registration application in patients with $CD4^+$ T cells ≤500/μL (cART-treated, poor immunological responders). Accordingly, vaccine efficacy will be evaluated by $CD4^+$ T-cell counts while immunogenicity will be determined by the proportion of participants who develop IgM, IgG, and IgA anti-Tat Abs or increase their titers after immunization. Exploratory laboratory testing, performed according to residual specimen availability, will evaluate immune and virological parameters, including $CD4^+$ and $CD8^+$ T-cell memory subsets, B and natural killer cells, cross-clade anti-Tat Abs, and proviral DNA, as well as biomarkers for TB reactivation and CVD risk. The second study will be a phase-IIB/III randomized, double-blind, placebo-controlled, adaptive trial directed at demonstrating the effectiveness of Tat therapeutic vaccine on $CD4^+$ T-cell recovery and viral load reduction in HIV-1-infected adult volunteers treated with cART for 3 months after diagnosis (newly diagnosed, cART-initiated). The third study will be a randomized, double-blind, placebo-controlled, age-de-escalating, dose-finding phase-I/II adaptive trial focused to evaluate the safety and immunogenicity of the HIV-1 Tat protein in HIV+ cART-treated adolescents and children (cART-treated <18 years old).

Of note, the studies conducted in Italy and SA and others planned did not account for antiretroviral treatment interruption strategies. Therefore, at present the Tat vaccine represents a way to intensify cART, and ad hoc studies are warrant to define the extent the Tat vaccine may replace or limit the need for antiretroviral therapy, which would translate in major benefit to patients and governments, including reducing the overall socio-economic burden of AIDS care. In particular, therapeutic immunization with the Tat vaccine may (i) ameliorate the effects of reduced adherence to cART, preventing selection (and transmission) of drug-resistance virus variants; (ii) allow antiretroviral treatment simplification; (iii) permit prolonged drug-free intervals; and (iv) promote reduction of virus reservoirs size.

6. Expert commentary

Overall, the results obtained with the Tat vaccine in monkeys and in humans indicate that Tat is critical in the HIV-1 life cycle, since, when targeted either during natural infection or upon vaccination, it contains viral replication with no or low progression, while in macaques vaccination appears to confine the virus at the portal of entry [28] or to confer sterilizing immunity [48,52]. Of note, data from the T-002 therapeutic trial indicate that targeting Tat in successfully cART-treated individuals reduces immune activation, as indicated by the homeostatic recovery and balancing of all lymphocytes populations and functional subsets, which we interpret as an immunological 'reset and restart', conceivably occurring upon removal of extracellular Tat from tissues. In this regard, it should be underscored that cART does not prevent HIV-1 gene expression [38] and data from many groups indicate Tat as a major dysregulator of the immune system even in the absence of HIV-1 infection. Data from the same trial also indicate that in vaccinees, but not in individuals followed in the parallel observational trial, the proviral load significantly declines, especially after 3 years from the vaccination [58]. The delayed kinetics is consistent with the evidence that the vast majority of the HIV-1 DNA does not appear to code for replication-competent virus [61], and its decay corresponds to the half-life of the

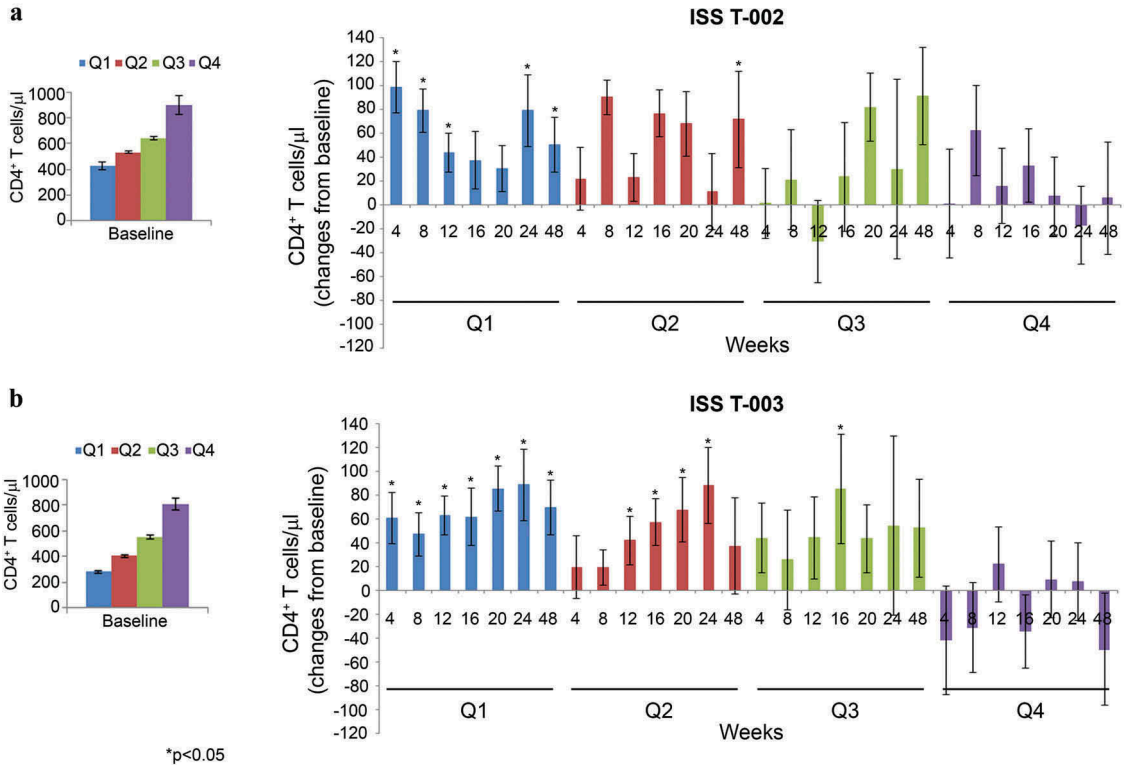

Figure 4. Changes of CD4 + T-cell counts up to week 48 in vaccinees stratified by quartiles according to baseline values. Baseline values (left panels) and changes from baseline (right panels) of CD4 + T cell counts in vaccinees from (A) ISS T-002 and (B) ISS T-003 phase II trials. Data are presented as mean values with standard errors. Longitudinal analysis for repeated measures was used. P-values assess the changes from baseline within each treatment group. Modified with permission from [59].

cell that harbors it, which in blood may be of several years [62]. Although the exact mechanism by which anti-Tat immunity promotes proviral load reduction is unknown, data indicate a correlation with the anti-Tat Ab-mediated neutralization of HIV-1 entry in DCs, suggesting blockade of replenishment of the reservoir as a possible mode of action (see Figure 1 in ref 28). In addition, killing of Tat-expressing cells, presumably carriers of replication-competent proviruses [63], by both anti-Tat Abs (through Ab-dependent cellular cytotoxicity and Ab-dependent phagocytosis) and CTLs has to be considered. However, it appears that anti-Tat Abs are a key requirement, as indicated by both the epidemiological evidence of protection from disease progression in individuals naïve to therapy [54] and the results of proviral DNA decay in subjects on cART developing anti-Tat Abs upon vaccination [58]. Conversely, the contribution of cellular anti-Tat responses alone, which are present in most infected individuals, appears lower. Accordingly, Tat-based vaccines aimed at inducing cellular responses alone failed at demonstrating any therapeutic efficacy, despite good induction of cellular responses [64]. In acute infection studies, anti-Tat cytotoxic T cells (CTL) are readily induced and escaped [65,66], indicating both that Tat is essential to the virus and must escape very rapidly from CTL control and that it may afford mutations in the linear sequence corresponding to the CTL epitope without losing its biological activities. This may also explain the lack of efficacy of a therapeutic vaccine based on a single and presumably linear universal Tat B-cell epitope [67].

In contrast, Tat is apparently unable to escape Abs indicating that structural changes affecting its conformation also impair its function. Of note, despite being reported as a very variable protein, Tat is highly conserved in the first 58 aa of exon 1 [44] and it is conceivable that most of the mutations found in the second exon do not translate into a functionally dead protein. Moreover, coevolving mutations in functionally distinct domains appear to be compensatory and to maintain Tat functions [68]. This is in substantial agreement with crystallography data indicating that, with a few constraints in the first exon, Tat is a poorly structured protein capable of withstanding mutations at several sites without losing vital functions [69,70] while maintaining the capability of interacting with an extraordinary high number of putative ligands [71].

7. Five-year view

7.1. Expected impact in clinical development of the Tat vaccine

In spite of the massive ART roll-out campaigns, SA has almost 400,000 new infections per year (>6%/year), with a 14% annual increase of drug resistance due to low or no adherence to antiretroviral treatment [2]. Late ART initiation leads to insufficient or low immunological response and low retention in care, which may jeopardize ART effectiveness and the success of Test and Treat programs. In addition, the new WHO treatment guidelines, recommending (Test & Treat, PrEP) for all PLWH, including children and adolescents (~2 million, ~82% in sub-Saharan Africa), and PrEP, for people at risk of HIV infection, pose an enormous challenge to care efforts

while widening the negative impact of ART therapy shortfalls [3,72]. In fact, in spite of therapy, patients still experience morbidities due to therapeutic deficiencies, including residual (HIV-related) immune hyper-activation, lack of immune homeostasis restoration, and persistence of virus reservoirs, placing them at higher risk of co-infections, co-morbidities, hospitalizations, and at a sevenfold increased risk of death [1,5,73,74]. PH has no interventions to fight these conditions. Moreover, in children and adolescents there is a paucity of therapy and no vaccination options [10,12,14].

Further, results from recent studies suggest reduced cART efficacy against HIV clade C, a finding that deserves further investigation and may hamper effective treatment as well as reduction of transmission of the most prevalent HIV clade worldwide [75,76].

Consequently, public HIV healthcare spending is expected to increase exponentially. In SA, access to care has steadily increased to half of the PLWH (~3.5 million) and is increasing every year to reach total coverage by 2020. Thus, the impact on PH expenditures is staggering, considering the already high price of ART delivery (>$1,000/patient/year) and effective blockade of the epidemics only partially achievable [77]. 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, AIDS, and non-AIDS co-morbidities, mitigating the negative effects of low therapy adherence, thus improving health, while reducing the overall economic burden of AIDS care (in SA, USD 200 for each hospital admission saved) [78]. The overall benefit to patients and governments will be further enhanced if therapy simplification regimens are adopted in conjunction with Tat vaccination. In fact, the outcomes promise not only to deliver a novel-intensified Tat/cART co-treatment with lower rates of treatment failure but also opens concrete perspectives for cART simplification, functional cure regimens and, possibly, administration to patients naïve to cART. If effective in counteracting the negative effects of low adherence, 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.

Both adult and pediatric clinical studies with the Tat vaccine will be conducted in SA, the country with the largest HIV epidemics in the world and at the forefront in the international fight against HIV. In fact, the HIV-1-infected pediatric population in Africa faces life-long therapy of more than 50 years on average (2.6 million in 2015, less than 1/3 receiving cART). Thus, it is extremely important to extend vaccine eligibility to these age groups. Successful completion of the pediatric trials will permit to conduct efficacy studies for registration, thus allowing provision of therapeutic vaccination coverage to virtually the whole HIV-1-infected population in SA.

Stringent regulatory review and a rigorous evaluation of the results are ensured by the MCC, sister organization of US FDA and EMA. Thus, clinical data will be used for registration also in US and Europe, respectively. Moreover, vaccine registration in SA is recognized by international organizations (WHO, UNAIDS, etc.) and, as result, will facilitate scaling of the Tat vaccine to the rest of the sub-Saharan region and Africa. The clinical studies will contribute improving clinical research capacity in the public sector and, consequently, the quality of healthcare services in the trial catchment areas, improving existing clinical sites and creating new sites for testing of other vaccines for poverty-related diseases.

The successful completion of the efficacy study in adults will lead to Marketing Authorization by the MCC for use as a novel biological co-treatment of AIDS to intensify cART efficacy. Health economics studies will be conducted to quantify the savings resulting from Tat vaccination for PH.

7.2. Expected impact of capacity building activities

Integration of healthcare, clinical research, and communities is key to improve the PH system in SA. Establishing a platform integrating clinical research units and PH facilities, diagnostic laboratories, advanced research core laboratories, and community-based organizations will raise synergies toward this goal. In particular, the clinical/laboratory network working module will allow transfer of knowledge, operational effectiveness, and expertise from the most advanced clinical research sites to the less advanced ones, with the aim of developing a public/private operational critical mass for self-sustaining capacity in clinical research.

Conducting phase-III trials in SA will also develop research capacities and will increase skills of the *in loco* personnel from the technical level to the management and directive level, contributing to mentoring and transferring HIV vaccine clinical trial expertise, therefore creating job opportunities in SA for local scientists and clinical staff at academic and non-academic levels. As result, these activities will contribute to upgrade the level of research capability.

Key issues

Combination antiretroviral therapy [cART] has several shortfalls since it does not

- eliminate chronic immune activation
- restore immune homeostasis
- eradicate HIV
- tolerate poor adherence
- prevent selection of resistant strains

cART intensification strategies are needed

The Tat vaccine intensifies cART as indicated by

- $CD4^+$ T cell gain beyond cART alone *[particularly in poor immunological responders]*
- Peripheral blood Proviral DNA decay

The Tat vaccine has the potential to be universal

- Comparable safety and immunogenicity profile in Caucasians and Blacks

- Comparable recognition of Tat from different clades [A, B, C D] and neutralization of Tat-mediated Env entry in dendritic cells

Status

- Phase II study T-002 [Italy] completed after 8 years of follow-up
- Phase II trial T-003 [SA] completed, after 3 years of follow-up

What's Next

- Conduction of Phase III efficacy trials in SA
- Registration in SA
- Conduction of Phase II/III trials in adolescents and children

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