

Parallel Conduction of the Phase I Preventive and Therapeutic Trials Based on the Tat Vaccine Candidate

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Abstract: The native HIV-1 Tat protein was chosen as vaccine candidate for phase I clinical trials in both uninfected (ClinicalTrials.gov identifier: NCT00529698) and infected volunteers (ClinicalTrials.gov identifier: NCT00505401). The rationale was based on the role of Tat in the natural infection and AIDS pathogenesis, on the association of Tat-specific immune responses with the asymptomatic stage and slow-progression rate as well as on its sequence conservation among HIV clades (<http://www.hiv1tat-vaccines.info/>).

The parallel conduction in the same clinical centers of randomized, double blind, placebo-controlled phase I studies both in healthy, immunologically competent adults and in HIV-infected, clinically asymptomatic, individuals represents a unique occasion to compare the vaccine-induced immune response in both the preventive and therapeutic setting. In both studies, the same lot of the native Tat protein was administered 5 times, every four weeks, subcutaneous (SC) with alum adjuvant or intradermic (ID), in the absence of adjuvant, at 7.5 µg, 15 µg or 30 µg doses, respectively. The primary and secondary endpoints of these studies were the safety and immunogenicity of the vaccine candidate, respectively. The study lasted 52 weeks and monitoring was conducted for an additional 3 years. The results of both studies indicated that the Tat vaccine is safe and well tolerated both locally and systemically and it is highly immunogenic at all the dosages and by both routes of administration.

Vaccination with Tat induced a balanced immune response in uninfected and infected individuals. In particular, therapeutic immunization induced functional antibodies and partially reverted the marked Th1 polarization of anti-Tat immunity seen in natural infection, and elicited a more balanced Th1/Th2 immune response. Further, the number of CD4 T cells correlated positively with anti-Tat antibody titers.

Based on these results, a phase II study is ongoing in infected drug-treated individuals (<http://www.hiv1tat-vaccines.info/>).

Keywords: HIV-Tat, vaccination, antibodies, safety, clinical trials.

INTRODUCTION

The development of a vaccine against HIV/AIDS has been mostly focused on the envelope protein (Env) of the virus with the aim of generating neutralizing antibodies and sterilizing immunity [1]. More recently, approaches have been focused at eliciting strong antiviral T cell responses against the gag, pol and nef gene products delivered by recombinant viral vectors, with the goal of preventing infection

and/or reducing virus replication and progression to disease [1,2]. However, both approaches have failed at inducing any type of protection [3,4].

Novel strategies should take into account the lesson learned by these failures as well as the evidence emerging from the natural HIV infection and be aimed at modifying the virus-host dynamic in order to prevent, or at least, to contain the establishment of a primary infection and systemic virus dissemination, possibly inducing a non-progressing disease status. Control of early virus replication, which might be achieved in the absence of sterilizing immunity, should provide protection from disease progression and reduce virus transmission to healthy individuals, thus halting the HIV epidemic. Therefore, this approach may be effective for both preventive vaccination in healthy individuals and therapeutic immunization in HIV infected subjects, respec-

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tively. Targets of such a strategy, however, should be key viral genes, which are expressed early upon infection, are essential for virus replication and pathogenesis and are conserved among the different virus clades. Among these, Tat represents an optimal candidate for a vaccine aimed at blocking disease progression [5]. Tat is a key viral regulatory protein produced very early after infection, even prior to HIV integration, and necessary for viral gene expression [6], cell-to-cell virus transmission and disease progression [7]. In fact, in the absence of Tat, no or negligible amounts of structural proteins are expressed and, therefore, no infectious virus is made [8,9]. Further, Tat is released by acutely infected T lymphocytes in the extracellular milieu [10] and enters both infected cells, in which promotes HIV-1 replication, as well as in uninfected cells in exerting multiple effects, which facilitate cell recruitment and activation, providing new cell targets for systemic virus dissemination [11,12].

Recent evidence also indicates that the native, biologically active Tat protein, possesses immunomodulatory and adjuvant properties that can be highly advantageous in vaccine development and have important implications for the immunopathogenesis of AIDS. In particular, native, but not oxidized, Tat protein is selectively and very efficiently taken up by monocyte-derived dendritic cells (MDDC), promotes cell maturation and Th1 polarization [13,14], and can redirect CTL epitopes processing and recognition [15,16].

Preclinical studies demonstrated earlier that vaccination with a biologically active Tat protein or tat DNA is safe, elicits a broad and specific immune response and, most importantly, induces a long-term protection against infection with a highly pathogenic virus (SHIV 89.6P), which rapidly causes AIDS and death in monkeys [17-19]. In addition, cross-sectional and longitudinal studies in natural infection suggested that the presence of anti-Tat humoral immune responses correlates with asymptomatic infection and with a slower disease progression [20,21], while the presence of Tat-specific CD8⁺ T cell responses correlates with early virus control both in humans [22,23] and monkeys [24,25]. Furthermore, Tat is well conserved among the circulating HIV-1 clades [26]. Homology is specifically high in the first exon-encoded portion of Tat, which contains the functional protein domains and most of the B, T-helper and CTL epitopes so far identified [26]. In addition, epitope mapping studies of sera from Italian, Ugandan and South African infected patients confirmed the cross-recognition of the BH-10 Tat, used as the vaccine candidate, providing strong formal evidence that a Tat-based vaccine may indeed represent a cross-clade vaccine approach against HIV. Finally, being devoid of structural HIV proteins, Tat vaccination does not induce seroconversion, facilitating trial recruitment as well as vaccinees' monitoring.

Therefore, the native Tat protein was chosen for the development of both preventive and therapeutic HIV/AIDS vaccine strategies and two clinical trials (ISS P-001 and ISS T-001) were sponsored by the "Istituto Superiore di Sanità" (ISS). The trials were conducted in parallel in 4 clinical centres in Italy, L. Spallanzani Hospital, San Gallicano Hospital, University of Rome "La Sapienza" (Rome) and S. Raffaele Hospital (Milan) [19,27].

Study Agent

Production of the Tat vaccine for both ISS P-001 and ISS T-001 phase I clinical trials was performed by a contractor in the United Kingdom (EXCELL BIOTECH, Livingstone, UK) according to Good Manufacturing Practices (GMP) and national and international legal requirements. The active substance of the vaccine is the biologically active recombinant Tat protein (HTLV-IIIIB strain, clone BH-10), produced in *E. coli* and purified by Diethylaminoethyl (DEAE) and Heparin Sepharose chromatography [19]. The Tat vaccine was formulated in a suitable saline buffer, in the presence 1% saccharose and 1% human serum albumin and vialled at 7.5, 15 and 30 µg dosages [19,28].

Preventive and Therapeutic Phase I Studies

The preventive and therapeutic phase I clinical trials were both randomized, double blind and placebo-controlled and conducted in 20 healthy adults volunteers (15 vaccinees and 5 placebo) without identifiable risk of HIV-1 infection [19,29] and in 27 asymptomatic HIV-infected individuals (20 vaccinees and 7 placebo), respectively [19,27,30]. Of the twenty volunteers enrolled in the preventive study, 15 (8 for Arm A and 7 for Arm B, respectively) received the scheduled vaccine injections, 5 (2 for Arm A and 3 for Arm B, respectively) received the placebo. Of the twenty-seven volunteers enrolled in the therapeutic study, 20 (11 for Arm A and 9 for Arm B, respectively) received the scheduled vaccine injections, 7 (4 for Arm A and 3 for Arm B, respectively) received the placebo (Fig. 1).

The native Tat protein was administered 5 times monthly at the doses of 7.5 µg, 15 µg or 30 µg, respectively. For each Arm (Arm A subcute or Arm B intradermal), subjects were randomized 1:1:1:1 in 4 groups to receive Tat vaccine at different dosage or placebo. The study included a 4-weeks screening period, a 16-weeks study treatment period, an 8-weeks post-immunization period and a 24-weeks follow-up period. An additional monitoring was scheduled for an additional three years (Fig. 2).

Clinical and Laboratory Platforms

All clinical and laboratory activities, as well as psychosocial and behavioral assessments, were harmonized among the clinical centers according to Good Clinical Practice (GCP) procedures and by establishing standardized and integrated platforms [19]. Clinical sites were chosen on the basis of their well-known experience as reference centers for the prevention, diagnosis and treatment of HIV infection. The activities of pre-screening, enrolment and monitoring of the volunteers (clinical evaluation, safety laboratory testing, risk assessment, and counselling on risk reduction and on avoiding pregnancy) were conducted according to study-specific Standard Operating Procedures (SOPs). The immunological and virological testing was performed by the Core Laboratory for Immunology and Virology that was established at the San Gallicano Hospital in Rome as a Joint Unit with ISS and validated upon international standard of quality (ISO 9000) [19].

A specific psychiatry and psychology platform was constituted in order to support volunteers throughout critical

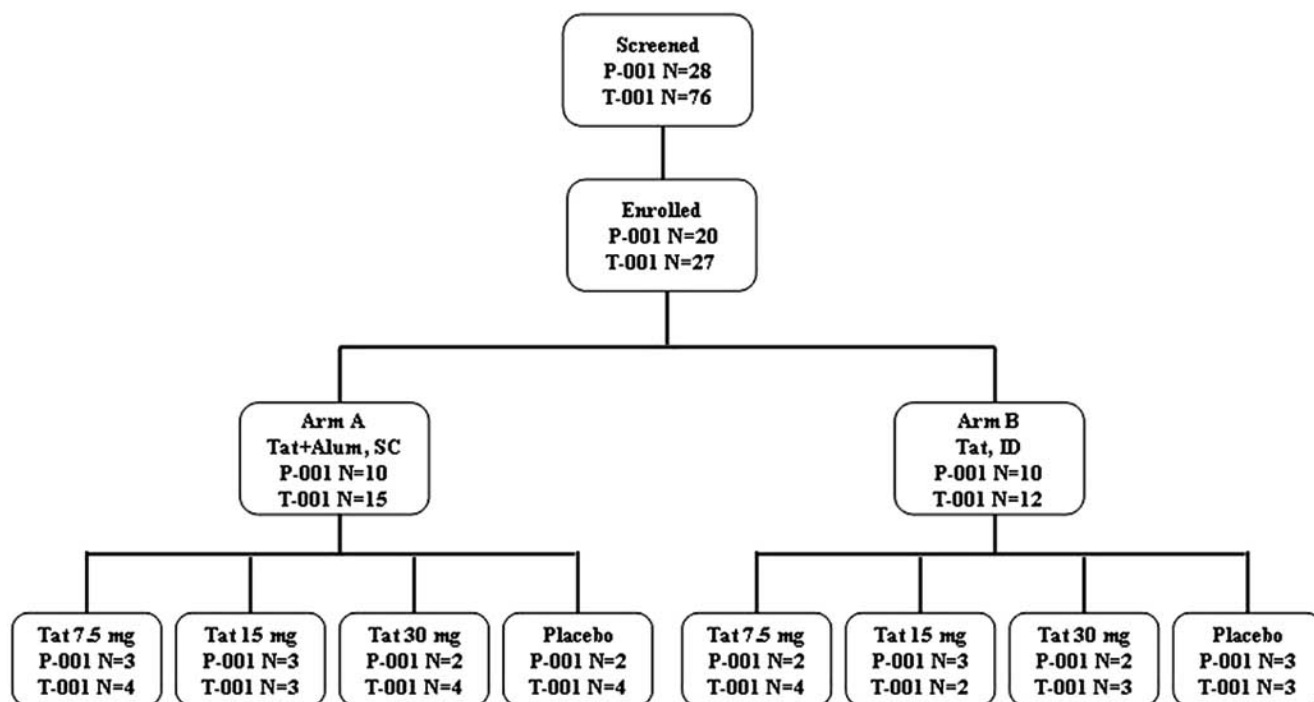


Fig. (1). Distribution of subjects by the Arm of treatment and the dosage groups for both preventive vaccination and therapeutic immunization, respectively. Subjects were stratified according to the administration Arms (subcute, Arm A or intradermic, Arm B) and randomized to enter different dosage groups to receive the vaccine (7.5, 15, 30 µg doses) or the placebo.

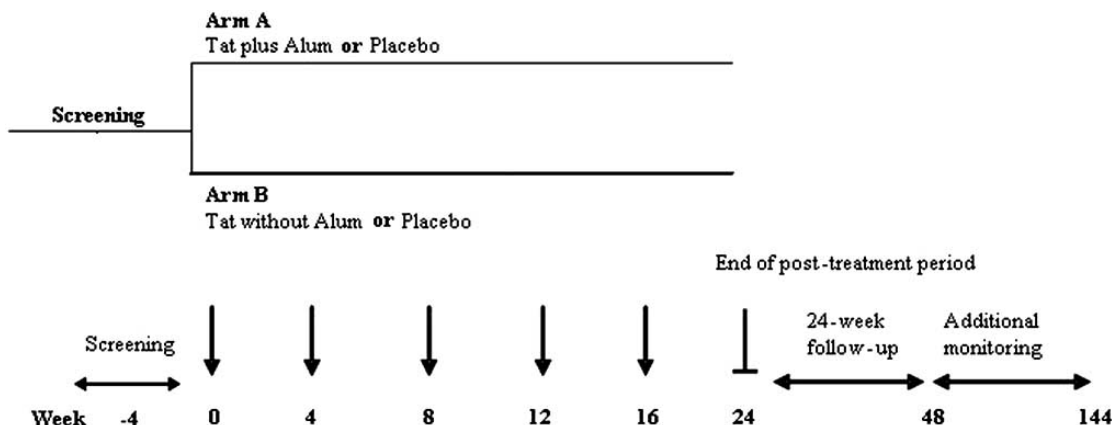


Fig. (2). Study design. The study included a 4-week screening period, a 16-weeks study treatment period, an 8-weeks post-immunization period and a 24-weeks follow-up period. An additional monitoring was scheduled for the following three years. Subjects were stratified according to the administration arms, Arm A was Tat plus Alum or placebo given subcutaneously, and Arm B was Tat without Alum or placebo given intradermally. Administration: black arrows; end-treatment: dashed arrow.

steps of the study by a structured psychological and socio-behavioural assistance [19].

Communication, Enrollment and Establishment of the Community Advisory Board

The AIDS Help-line (AHL), a call center established by ISS to provide general information on HIV/AIDS, supplied all the information on the Tat vaccine trial participation and enrolment by following SOPs in which an alpha-numeric code was provided to direct the volunteers at the first visit appointment at the clinical sites.

A Community Advisory Board (CAB) comprising the most representative Italian non-governmental organizations

(NGOs) involved in all issues relating to HIV/AIDS was established to provide a communication network among communities, scientists, community-care providers and the sponsor. The CAB contributed in establishing the methodology for ethical information and communication to the volunteers as well as counselling.

Safety

Safety was assessed during the course of the trial by monitoring the volunteers for local and systemic adverse reactions as well as of haematological (including coagulation assessment), biochemical (including liver and kidney func-

Table 1. ISS P-001 Incidence and Number of Treatment-Related AEs

System Organ Class (SOC MedDRA)	Treatment (n=15)			Severity ^c			Placebo (n=5)			Severity ^c		
	n	n/N ^a (%)	AEs ^b	1	2	3	n	n/N ^a (%)	AEs ^b	1	2	3
Local administration site disorders	15	100	149	140	9	0	3	60	8	4	4	0
Blood and lymphatic system disorders	8	53	62	58	4	0	0	0	0	0	0	0
Cardio-vascular disorders	2	13	2	2	0	0	1	20	1	1	0	0
Ear and labyrinth disorders	1	7	1	1	0	0	0	0	0	0	0	0
Eye disorders	1	7	1	1	0	0	0	0	0	0	0	0
Gastrointestinal disorders	3	20	4	3	1	0	0	0	0	0	0	0
General disorders	11	73	30	24	5	1	1	20	2	2	0	0
Kidney and urinary disorders	1	7	1	0	1	0	0	0	0	0	0	0
Nervous system disorders	7	47	14	13	1	0	1	20	1	1	0	0
Psychiatric disorders	1	7	1	1	0	0	0	0	0	0	0	0
Skeletal muscle and connective tissue disorders	4	27	11	9	2	0	1	20	5	4	1	0
Skin and subcutaneous tissue disorders	2	13	2	1	1	0	0	0	0	0	0	0

^a n/N = number of subjects reporting the events out of total evaluable subjects.

^b Number of AEs.

^c Severity grade: 1=mild, 2=moderate, 3=severe.

Table 2. ISS T-001 Incidence and Number of Treatment-Related AEs

System Organ Class (SOC MedDRA)	Treatment (n=20)			Severity ^c			Placebo (n=7)			Severity ^c		
	n	n/N ^a (%)	AEs ^b	1	2	3	n	n/N ^a (%)	AEs ^b	1	2	3
Local administration site disorders	19	95	251	237	13	1	5	71	11	9	2	0
Blood and lymphatic system disorders	9	45	29	19	10	0	3	43	4	4	0	0
Ear and labyrinth disorders	1	5	1	1	0	0	0	0	0	0	0	0
Gastrointestinal disorders	2	10	4	4	0	0	0	0	0	0	0	0
General disorders	14	70	71	53	17	1	1	14	6	6	0	0
Hepato-biliary disorders	1	5	1	0	1	0	0	0	0	0	0	0
Infection and infestation	1	5	1	0	1	0	0	0	0	0	0	0
Nervous system disorders	6	30	8	6	2	0	0	0	0	0	0	0
Psychiatric disorders	1	5	2	2	0	0	1	14	1	1	0	0
Skeletal muscle and connective tissue disorders	6	30	19	13	6	0	1	14	1	1	0	0
Skin and subcutaneous tissue disorders	1	5	2	2	0	0	1	14	1	1	0	0

^a n/N = number of subjects reporting the events out of total evaluable subjects.

^b Number of AEs.

^c Severity grade: 1=mild, 2=moderate, 3=severe.

tional parameters) and immunological parameters (including CD4+, CD8+, CD3+ T cells, NK, B cells and monocytes). Volunteers of both studies were monitored to assess immediate reactogenicity and early adverse events to the vaccine during the first 2 hours, as well as after 24 hours and 7 days after each immunization.

No clinically significant alterations were observed in the vaccinees concerning all the haematological, biochemical and immunological parameters examined.

Most of the adverse events (AEs) reported were local, transient and of mild severity and did not appear to be dose-

related [29,30]. The most frequent local adverse events were those observed at the site of injection and appeared related to the route of administration. In fact, subcutaneous administration (Arm A) was most frequently accompanied by local adverse events, possibly due to the presence of the Alum adjuvant, as previously observed [29,30]. The most common systemic adverse event was represented by transient blood disorders (mainly leucocytosis with neutrophilia and transient lymphocytopenia) (Tables 1 and 2).

Furthermore, general disorders (asthenia, chills, fever) and nervous system disorders (headache) were seldom observed in both clinical studies.

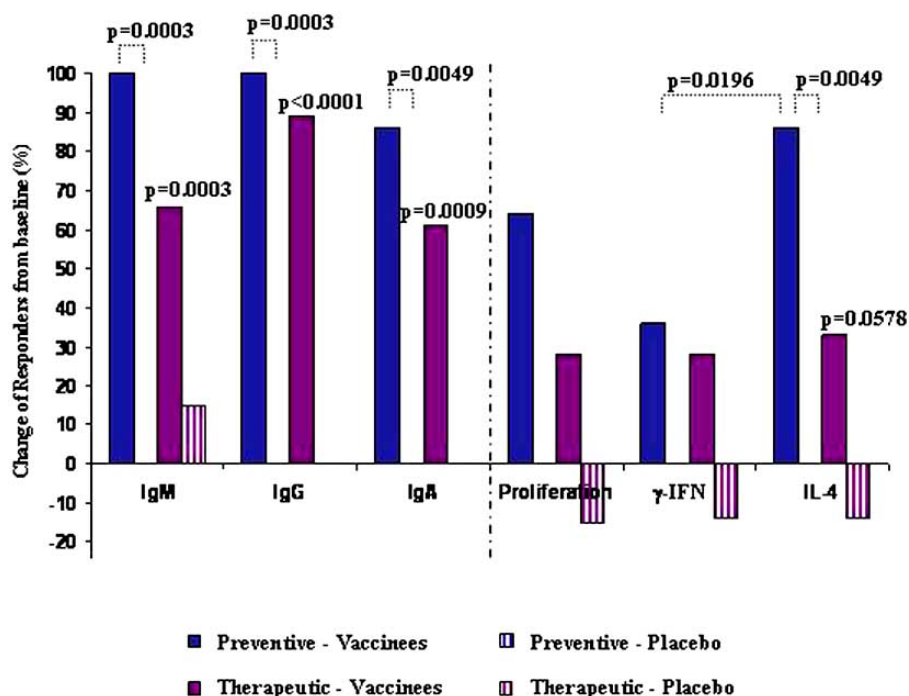


Fig. (3). Tat-specific immune responses. Pre-post immunization frequencies of responders for both humoral or cellular anti-Tat responses for the preventive and therapeutic study, respectively.

In the preventive study, a significant induction of anti-Tat humoral responses was detected in vaccinees as compared to placebo (IgM and IgG p= 0.0003; IgA p=0.0049; Fisher’s Exact Test). A statistically significant difference between treated and placebo was observed for IL-4 production (p=0.0049, Fisher’s Exact Test) and a predominance of Th2 versus Th1 T-cell response (p=0.0196, McNemar’s Test) was found in uninfected subjects vaccinated with Tat.

In the therapeutic study, vaccinated individuals showed a significant increase of the humoral response to Tat from baseline (IgM p=0.0003, IgG p<0.0001, IgA p=0.0009, McNemar’s Test) and a borderline significant increment of IL-4 production (p=0.0578, McNemar’s Test).

Peripheral blood CD4+ T cells and plasma viral load (the latter performed only in the therapeutic clinical trial) were also assessed as key safety parameters. The data on CD4+ T cell counts collected either during the treatment phase (at 24 weeks) or the subsequent follow up period (at 48-weeks) were consistent with a remarkable immunologic safety of vaccination as indicated by the preservation of pre-screening levels of circulating CD4+ T cells in the vaccinees in both studies. In particular, in the therapeutic immunization study, the placebo group experienced several fluctuations of CD4+ T cells often below the baseline levels, while the vaccinees had more stable CD4+ T cell counts [27,30]. In addition, a significant positive correlation between the levels of circulating CD4 T cells and the titers of anti-Tat antibody was found [27,30]. The determination of plasma viral load further confirmed the virological safety of the vaccination with a very limited (within the half-log) variation in the levels of plasma viremia in most cases, including vaccinees and placebo, likely as a consequence of the overall good containment of virus replication during the asymptomatic stage of infection [30].

On the basis of the results of AEs monitoring and the assessment of all the clinical and laboratory parameters, the conclusion of the safety monitoring board (independent Expert Committee on safety) was that the Tat vaccine is safe and well tolerated both locally and systemically. No further relevant events were reported during the two additional years of follow-up [27,29,30].

Immunogenicity

Immunogenicity was evaluated in 14 uninfected and 18 HIV infected volunteers that received at least three vaccine doses. This was performed by assessing the induction of anti-Tat humoral and cellular immune responses, as previously described [19,27,29,30].

Preventive Trial (ISS P-001)

Specific anti-Tat IgM and IgG were induced in all vaccinated subjects in both Arms, while anti-Tat IgA were induced in 86% subjects (100% in Arm A and 67% in Arm B, respectively) (Fig. 3). As expected, no response was observed in placebo recipients. The profile of IgM, IgG and IgA production in response to Tat indicated that the anti-Tat humoral response reached peak intensities between the third and the fourth immunization (12-16 week), decreasing thereafter during the subsequent 32-weeks (Fig. 4 and Table 3). The highest values of antibody titers were detected for IgG and IgA in Arm A and for IgM in Arm B, respectively, particularly for Tat 7.5 µg treatment group.

A significant increase of the inhibition of Tat-induced HIV replication *in vitro* (inhibition of Tat-induced HIV replication by the rescue assay) [10,26] was found in both Arms.

An anti-Tat cellular response was elicited in 93% of the individuals. Specifically, lymphoproliferation in response to

Table 3. Peak of Anti-Tat Antibody Titers in Vaccines

	ISS P-001		ISS T-001	
	GM ^a	95% C.I. ^b	GM ^a	95% C.I. ^b
IgM	156	88-279	29	18-48
IgG	4525	2584-7927	1089	631-1877
IgA	62	26-148	27	13-57

^a Geometric Mean.^b Confidence Interval.**Table 4. Tat-Specific Cellular Immune Responses**

	ISS P-001	ISS T-001
γ-IFN		
Responders ^a (%)	36	83
Peak ^b (SFC)	65 (23-112)	397 (35-1170)
IL-4		
Responders ^a (%)	86	50
Peak ^b (SFC)	24 (7-101)	16 (7-54)
Proliferation		
Responders ^a (%)	64	89
Peak ^b (SI)	6 (3-12)	9 (3-20)

^a Percent of subjects exhibiting a positive response.^b Mean (range) of peak positive responses after immunization.

Tat was found in 64% of vaccinees (62% in Arm A and 67% in Arm B respectively); γ-IFN production was detected in 36% volunteers (25% in Arm A and 50% in Arm B respectively), while IL-4 production in response to Tat was found in 86% vaccinated subjects (87% in Arm A and 83% in Arm B respectively) (Fig. 3 and Table 4).

Overall, the highest frequency of responders was observed for IL-4, in both arms, while the most elevated intensity of response (number of spots forming cells, SFC) was detected for γ-IFN. On the other hand, Arm A showed the highest mean number of positive responses for lymphoproliferation (Table 4). Concerning the Tat doses, 30 μg appeared to induce a more balanced specific cellular responses [29].

Therapeutic Immunization Trial (ISS T-001)

In the therapeutic setting, assessment of immunogenicity should take into consideration the anti-Tat response already present at enrollment. However, while vaccinees and placebo recipients had comparable humoral and cellular responses to Tat before the therapeutic immunization, the percentage of positive subjects after vaccination was significantly higher in vaccinees as compared to placebo. At baseline, 5/18 (28%) vaccinees and 3/7 (43%) placebo were positive for anti-Tat antibodies, while 15/18 (83%) and 6/7 (86%) had cellular immune response to Tat, respectively. After immunizations 18/18 (100%) vaccinees and 4/7 (57%) placebo showed specific humoral responses, while 18/18 (100%) and 4/7 (57%) were positive for anti-Tat cellular immune response, respec-

tively (Fig. 3). In particular, the frequency of vaccinees with an anti-Tat IgM response increased from 17% to 83%, while anti-Tat IgG increased from 11% to 100%, respectively. Finally, anti-Tat IgA increased from 0% at baseline to 61% of the immunized individuals. At baseline, two placebo had serum IgG or IgA, respectively, while one other subject had a transient production of anti-Tat IgM during the study. Of note, individuals already producing anti-Tat antibodies at baseline showed a different profile during the study since the former showed an increased intensity of the response in term of antibody titers, generation of additional Ig isotypes and of a larger B-cell repertoire, while the latter did not show any relevant modification of either antibody titer, Ig isotype or repertoire during the same period of observation. The determination of serum anti-Tat specific antibodies (IgM, IgG and IgA) performed up to 48-weeks after the first immunization, indicated that each vaccine dose induced a humoral response in each of the treatment Arms. However, 7.5 and 30 μg doses appeared to elicit a more balanced Tat-specific immune response [30]. The humoral immune response generated by vaccination reached peak intensities between the fourth immunization and the end of the treatment phase, decreasing thereafter during the subsequent 24-weeks of follow up (Fig. 4 and Table 3) [30]. Remarkably, the functional assessment of anti-Tat antibodies generated by vaccination showed a significant increase of the inhibition of Tat-induced HIV replication *in vitro* (HIV-rescue assay) as compared to the values gathered at baseline [30].

As previously observed in asymptomatic, antiretroviral drug-naïve individuals [22], cellular responses to Tat were found in a high proportion of the volunteers (84%) at baseline, including both vaccine and placebo recipients. Specifically, one or more cellular responses to Tat were present in 83% of the individuals randomized in the groups to be treated with the vaccine (89% in Arm A and 78% individuals in Arm B, respectively), and in 86% of the individuals randomized within the placebo. After immunization, anti-Tat cellular responses were found in 100% of the vaccinees, while a decrease was observed during the study in the placebo (from 86% to 57%) (Fig. 3 and Table 4). In particular, the frequency of lymphoproliferative responses to Tat during trial increased from 61% to 89% in the vaccinees, while they decreased in the placebo from 29% to 14%, respectively. The frequency of γ-IFN responses to Tat increased from 55% to 83% of the vaccinees, while it decreased in the placebo from 71% to 57%, respectively. IL-4 increased from 17% to 50% of the vaccinated subjects, while the only positive placebo at baseline became negative. Although both Arms of vaccine administration were effective at inducing long-term γ-IFN and lymphoproliferation responses against Tat, only vaccinees in Arm B developed a strong and persistent IL-4 response, which was very limited in individuals vaccinated by the SC route plus Alum, and was virtually absent or lost by placebo recipients. Of interest the 30 μg Tat vaccine dose administered intradermally was more effective at eliciting vaccine-specific humoral and cellular immune responses.

Comparative Assessment of Preventive and Therapeutic Immunization

A comparative analysis of the immunological data gathered in the two different clinical settings, evaluating the pre-

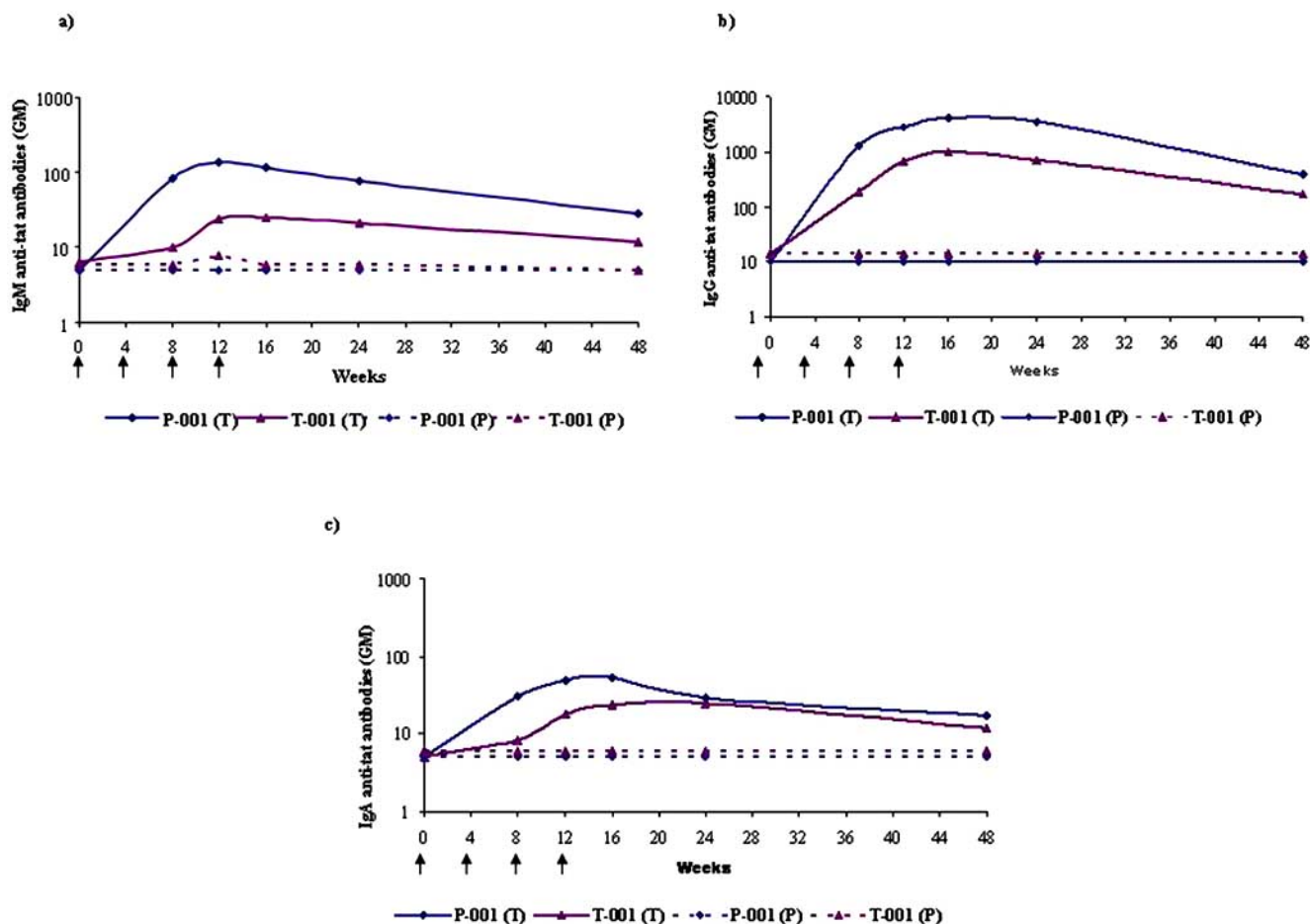


Fig. (4). Profile of anti-Tat IgM, IgG and IgA antibody production. Geometric Means of IgM (panel a), IgG (panel b) and IgA (panel c) antibody titers up to 48 weeks after the first immunization in HIV infected or uninfected subjects, respectively. Arrows indicate time of immunizations.

Preventive study (blue rhomb), therapeutic study (red triangle). The continuous and dashed lines represent vaccinated subjects and placebo, respectively.

post immunization differences, clearly showed that the immune response generated with the same vaccine lot administered in subjects at the same clinical centers differs between healthy and HIV infected individuals, even in the asymptomatic stage of infection and in the absence of any antiretroviral treatment. Remarkably, although Th1 T-cell responses appeared to be predominant in HIV-infected individuals, which had a very low frequency of humoral responses, immunization of healthy subjects elicited a prevalent Th2 B-cell response against Tat (IL-4 producing cells) (86%), and a more limited Th1 type (IFN- γ production) T-cell response (36%) ($p=0.0196$ McNemar's Test), observing also a strong lymphoproliferative response in 64% of the individuals. On the other hand, the analysis of HIV-infected individuals after therapeutic immunization showed the generation of cellular immune responses that appears still dominated by the primary Th1 type response (83%), but also show a significant induction of Th2 type responses (50%) as well as remarkable increments of lymphoproliferative responses (up to 89% of the individuals). This corresponds to a net increment of 28% for IFN- γ and proliferation and 33% for IL-4, respectively, from the baseline frequencies, suggesting that a role might

be played by the presence of memory cells (Fig. 3). Of note, the intensity of the cellular responses against Tat (SFC for IFN- γ Elispot or fold increase for lymphoproliferation) was higher in asymptomatic HIV infected subjects as compared to the healthy individuals, while the latter showed a higher frequency and intensity of IL-4 responses (Table 4).

As mentioned above, the presence of an anti-Tat humoral response was relatively rare in HIV infected individuals before immunization, as compared to the frequency of the cellular anti-Tat responses [27,30]. The reason for such a discrepancy in the setting of natural infection is unclear and may have important implications for vaccine development (i.e. targeting viral antigens, which partially or totally "escape" the host immune recognition/response). Indeed, the native Tat protein appears strongly immunogenic and capable of inducing high titers of anti-Tat antibodies in healthy subjects. In particular, at the peak intensity of anti-Tat antibody production, healthy subjects had titers superior to those observed in infected individuals (Table 3). The latter, in fact, had a slower kinetic, a lesser intensity and a more restricted breadth of the antibody repertoire as compared to healthy individuals (Fig. 4). In such therapeutic setting, the maximum

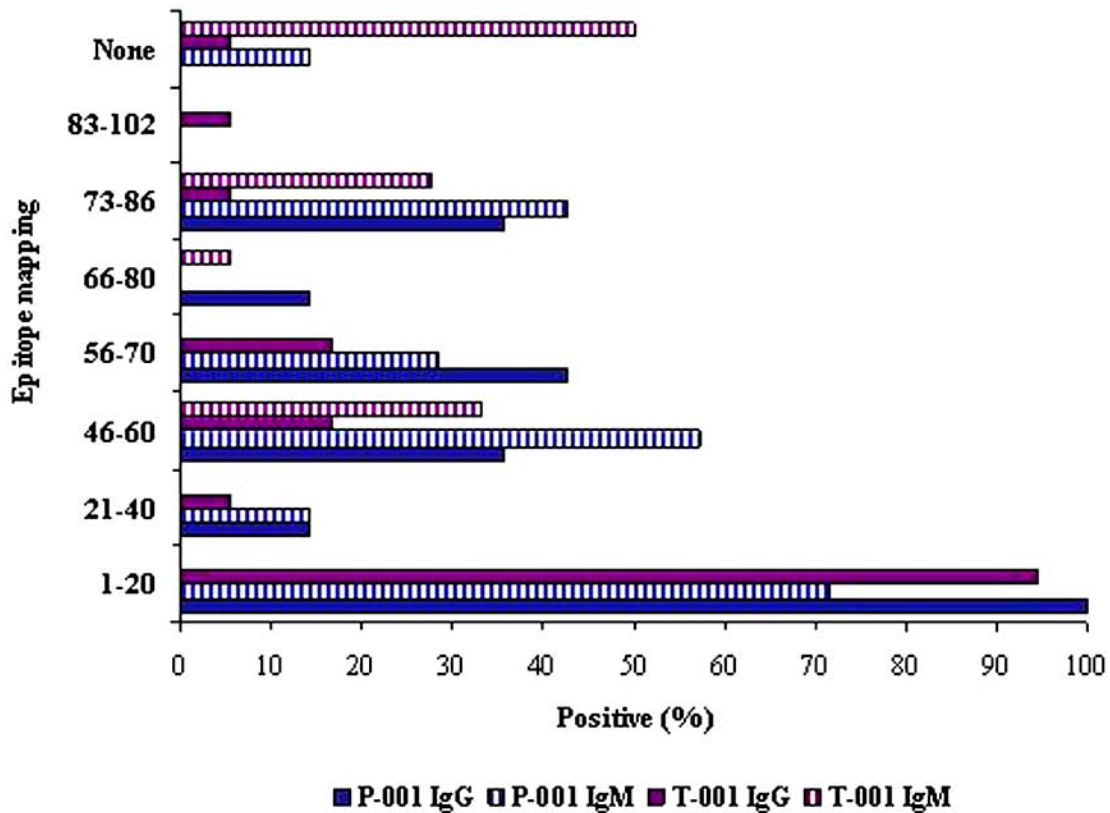


Fig. (5). Epitope-specific B-cell responses induced by Tat immunization. The repertoire of anti-Tat humoral response was determined by identifying the different epitopes recognised by IgG and IgM antibodies, respectively. The figure reports the frequencies of IgM and IgG B-cell responses against the native protein as well as against the different portions of Tat in sera from immunized HIV infected individuals (**in red**) and healthy volunteers (**in blue**).

increase of Ig titers after the immunization was observed for IgG as compared IgM and IgA, respectively (Fig. 3).

Taken together these results confirm the existence of a strong Th1 polarization of the immune response against Tat in asymptomatic HIV infected individuals, which is significantly, though partially, reverted by the therapeutic immunization with Tat that leads to a more balanced Th0 type of response, while Tat vaccination of healthy subjects generated a prevalent Th2 response.

Data from the epitope mapping studies of the anti-Tat specific B-cell response were consistent with the previous observations, since vaccination of healthy individuals induced a broader spectrum of both IgM and IgG responses capable of recognizing multiple functional epitopes of Tat, particularly against the central portion of the protein. In particular, IgM responses were mainly directed against the acidic domain of Tat [residues 1-20 (71%)], the central portion of the Tat protein [residues 46-60 (57%)] which contains the basic domain of Tat and the RGD motifs, [residues 73-86 (43%)]. IgG responses were directed mostly against the 1-20 peptide (100%) and against regions located at residues 56-70 (43%), which contains the glutamine-rich region of Tat (Fig. 5). On the other hand, HIV infected individuals had a quite restricted breadth of the B-cell response at baseline (one subject had IgG against the acidic Tat domain, at residues 1-20 peptide, while IgM responses were mainly against the basic Tat domain at residues 46-60 and toward the RGD motifs at

residues 73-86). The therapeutic immunization with Tat, however, elicited a broader array of B cell responses including antibodies directed against functional Tat domains. The most frequently recognised IgG epitope corresponded to the 1-20 peptide (94%), additional IgG responses were directed against portions located at residues 46-60 and 56-70 (17%), which contain the basic and glutamine-rich region of Tat, respectively, and at residues 73-86 and 83-102, corresponding to the C-terminal region of the protein (6%), respectively. For IgM, additional responses were observed against residues 46-60 (33%) and, to a lesser extent, against residues 73-86 (28%). Of note, none of the placebo recipients producing anti-Tat antibodies had, at any time, evidence of epitope-specific B-cell responses. Taken together these data indicate that the therapeutic immunization of asymptomatic HIV infected individuals is capable of generating and/or boosting a wider B-cell repertoire of anti-Tat antibodies, capable of recognizing multiple functional epitopes of Tat, than that seen in natural HIV infection.

CONCLUSIONS

The HIV-1 Tat is a very early regulatory protein that plays a major role in HIV replication and AIDS pathogenesis. Since it is well conserved among HIV clades, it represents an important target for the development of a vaccine against HIV/AIDS [10,27]. Vaccination with Tat is capable

of generating strong and durable humoral and cellular immune responses in both healthy individuals and in HIV-infected subjects, providing a formal evidence that this vaccine candidate is an effective immunogen for both preventive vaccination and therapeutic immunization strategies. The parallel conduction of preventive and therapeutic phase I trials also showed that the immune response to Tat vaccination differs between uninfected and HIV infected subjects, even at early, clinically asymptomatic, stages [27,30]. In HIV infection Th1 type T-cell responses to Tat (γ -IFN production) are predominant, while Th2 responses and antibody production are very limited in terms of frequency, intensity and broadness [14,27,30]. The reason for such a marked Th1 polarization of the anti-Tat immune response is unclear as yet. Recent studies, however, suggest that the interplay between native Tat protein, released during acute infection, and dendritic cells [10,31,32] leads to the transcriptional activation of TNF- α and consequent induction of Th1-associated cytokines and β -chemokines, which are capable of “diverting” the adaptive immune response toward a prevalent Th1 pathway [14]. Administration of Tat by vaccination, however, generated a balanced immune response and a broad array of Th2 type responses with production of specific antibodies in all uninfected vaccinees. This was observed also in HIV infected subjects [27,30], although, in the latter, antibodies production upon vaccination had a slower kinetic, a reduced magnitude and a limited broadness as compared to the preventive trial subjects. Nevertheless, therapeutic immunization with Tat partially reverted the marked Th1 polarization of anti-Tat immunity seen in infected individuals and effectively elicited a more balanced Th1/Th2 immune response [13].

The results of the P-001 and T-001 clinical trials confirm the full achievement of the primary and secondary endpoints of these studies, allowing to proceed to phase II clinical trials, which are ongoing in infected HAART-treated individuals in Italy (ClinicalTrials.gov identifier: NCT00751595).

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