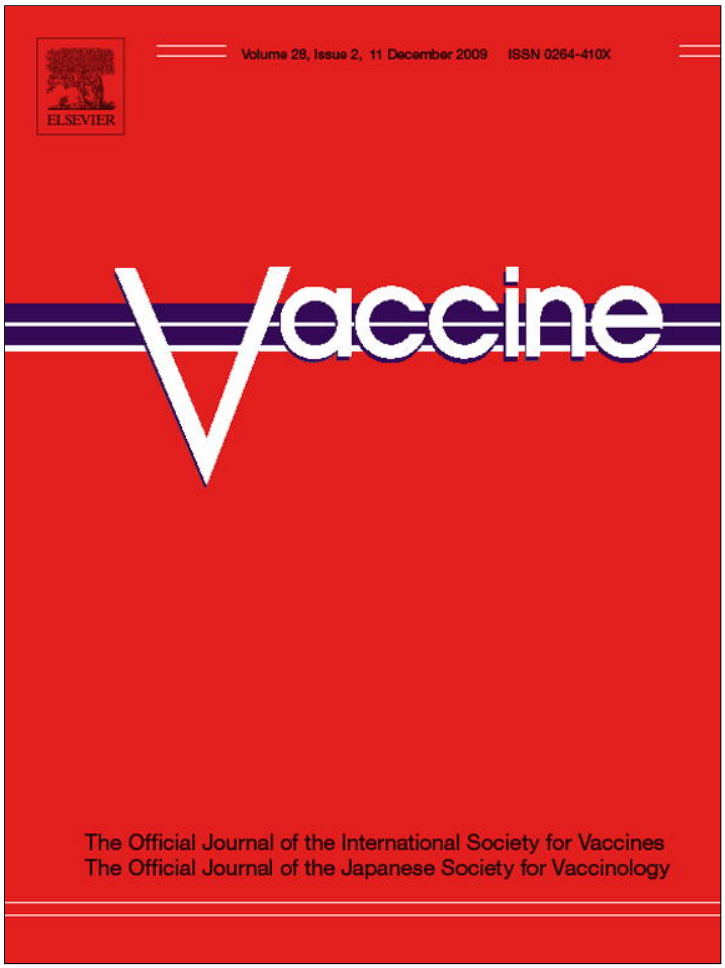

Vaccine 28 (2010) 371–378

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

The preventive phase I trial with the HIV-1 Tat-based vaccine

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ARTICLE INFO

Article history:
Received 20 April 2009
Received in revised form 1 October 2009
Accepted 12 October 2009
Available online 29 October 2009

Keywords:
Clinical trials
HIV-1 Tat
Vaccination

ABSTRACT

The native HIV-1 Tat protein was chosen as vaccine candidate for phase I clinical trials based on its role in the natural infection and AIDS pathogenesis, on the association of Tat-specific immune response with the asymptomatic stage as well as on its sequence conservation among HIV clades.

A randomized, double blind, placebo-controlled phase I study (ISS P-001) was conducted in healthy adult volunteers without identifiable risk of HIV infection. Tat was administered 5 times monthly, subcute in alum or intradermic alone at 7.5 μg, 15 μg or 30 μg, respectively (ClinicalTrials.gov identifier: NCT00529698). Vaccination with Tat resulted to be safe and well tolerated (primary endpoint) both locally and systemically. In addition, Tat induced both Th1 and Th2 type specific immune responses in all subjects (secondary endpoint) with a wide spectrum of functional antibodies that are rarely seen in natural infection, providing key information for further clinical development of the Tat vaccine candidate.

1. 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. 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 protection [3,4].

Novel vaccine 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 virus dissemination, and 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, halting the HIV epidemic. Thus, this approach may be effective for both preventive and therapeutic vaccination. Targets of such 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. Further, Tat is released by the infected T lymphocytes in the extracellular milieu [8] and enters both infected

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doi:10.1016/j.vaccine.2009.10.038

cells, in which promotes HIV-1 replication, while exerting multiple effects on uninfected cells, which facilitate, directly or indirectly, cell recruitment and activation, providing new cell targets for systemic virus dissemination [9,10].

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 and promotes cell maturation and Th1 polarization [11]. Finally, Tat modifies the hierarchy of cytotoxic T lymphocytes epitopes of heterologous antigens favouring the presentation of subdominant and cryptic epitopes [12,13].

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 of cynomologous monkeys against infection with a highly pathogenic virus (simian human immunodeficiency virus 89.6P), which rapidly causes AIDS and death in this model [14]. On the other hand, cross-sectional and longitudinal studies in natural infection indicated that the presence of an anti-Tat humoral immune response correlates with asymptomatic infection and with a slower disease progression [15,16], while the presence of CD8+ T-cell responses to Tat correlates with early virus control both in humans [17,18] and monkeys [19,20]. Furthermore, Tat is well conserved among the circulating HIV-1 clades [21]. 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 cytotoxic T lymphocytes epitopes so far identified [21]. 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, the Tat vaccine candidate does not induce any apparent seroconversion, facilitating trial recruitment as well as vaccinees monitoring.

Therefore, the native Tat protein was chosen for the development of both preventive and therapeutic [22–24] HIV/AIDS vaccine strategies and on this basis two clinical trials were sponsored by "Istituto Superiore di Sanità" (ISS). The Preventive study was conducted in four clinical centers in Italy, L. Spallanzani Hospital, San Gallicano Hospital, University of Rome "La Sapienza" (Rome) and S. Raffaele Hospital (Milan) [22–24].

2. Materials and methods

2.1. Study agent

Production and release of the Tat vaccine 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 Tat vaccine is the biologically active recombinant Tat protein (human T-cell lymphotrophic virus-IIIB strain, clone BH-10), produced in *Escherichia coli* and purified by DEAE and Heparin Sepharose chromatography. The Tat vaccine was formulated in a suitable saline buffer, in the presence 1% saccarose and 1% human serum albumin and vialed. Regulatory approval was obtained after completion of the evaluation of all the required documentation by the "Committee for evaluation of safety and quality of new drugs" according to the DPR No. 439/2001 of the Italian Ministry of Health, as well as by the central (Istituto Superiore di Sanità – ISS) and local Ethics Committees (Institutional Review Boards – IRB) following the guidelines and specific requirements issued by the European and Italian regulatory authorities [22].

Table 1
Demographic characteristics.

Subjects ($n=20$)	Arm A (SC) Vaccination ($n=8$)	Arm B (ID) Vaccination ($n=7$)	Placebo ($n=5$)
Sex			
Male	7 (87.5%)	6 (85.7%)	4 (80.0%)
Female	1 (12.5%)	1 (14.3%)	1 (20.0%)
Race			
Caucasian	8 (100.0%)	7 (100.0%)	5 (100.0%)
Age (years)	41.6 ± 5.6	33.9 ± 11.6	36.0 ± 9.5
Weight (kg)	83.6 ± 17.1	68.1 ± 11.1	63.6 ± 11.3
Height (cm)	178.2 ± 7.0	171.8 ± 7.4	169.4 ± 4.5
BMI	26.1 ± 3.8	23.1 ± 3.8	22.0 ± 2.8

Mean ± SD.

2.2. Study design

The preventive phase I study was a randomized, double blind, placebo-controlled clinical trial (ISS P-001) conducted in healthy adult volunteers without identifiable risk of HIV-1 infection (Table 1). As summarized in Fig. 1, the Tat vaccine was administered monthly for 5 times (weeks 0, 4, 8, 12, 16) either subcute (SC) in alum (Arm A) or intradermic (ID) without adjuvant (Arm B) at 7.5 μg, 15 μg or 30 μg, respectively. Twenty volunteers were enrolled, 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. The study included a 4-week screening period, a 16-week study treatment period, a 8-week post-immunization period and a 24-week follow-up period. An additional monitoring was scheduled for the following 2 years.

2.3. Clinical and laboratory platforms

All clinical and laboratory activities, as well as psychosocial and behavioural assessments, were harmonized among the clinical centers according to Good Clinical Practice (GCP) procedures and by establishing standardized and integrated platforms [22]. 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 of Immunology and Virology at the San Gallicano Hospital in Rome as a Joint Unit with ISS and validated upon international standard of quality (ISO 9000).

A specific platform constituted by psychiatrics and psychologists was implemented to develop a psychosocial protocol for the assessment of psychological and socio-behavioural issues in order to support volunteers throughout critical steps of the study.

2.4. Communication, enrolment and establishment of the Community Advisory Board (CAB)

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 a standard operating procedure in which an alpha-numeric code was provided to direct the volunteers at the first visit appointment at the clinical sites [22].

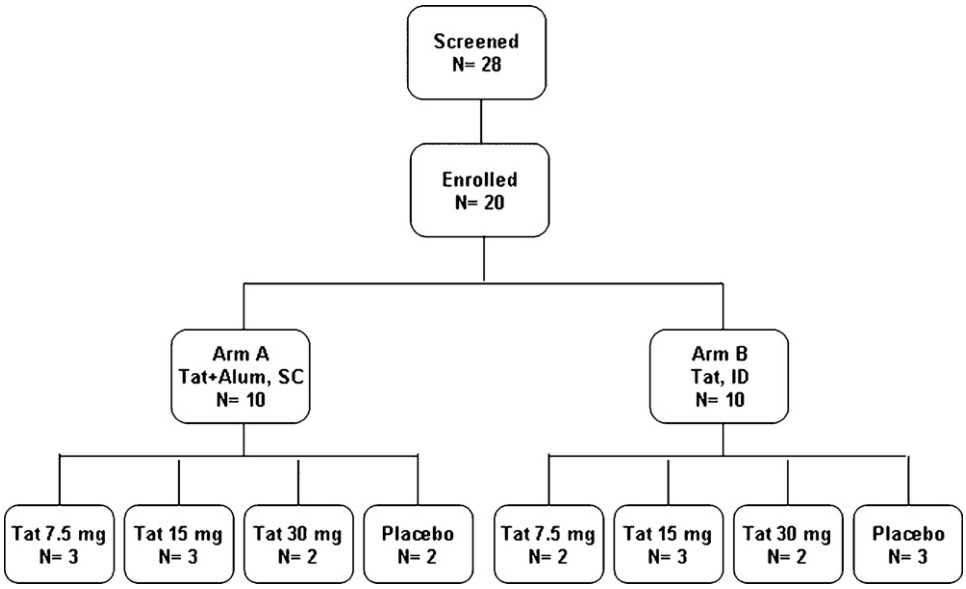

Fig. 1. Subjects by Arm of treatment and dosage groups. Subjects were stratified by administration Arms (SC, Arm A or ID, Arm B) in different dosage groups to receive the vaccine (7.5 μg, 15 μg, 30 μg doses) or placebo.

A 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 and counselling at the clinical sites [22].

2.5. Safety and laboratory assessments

Evaluation of safety included physical examination and clinical laboratory assessments (haematology, serum chemistry and urinalysis) in addition to the close monitoring of local and systemic adverse events. Evaluation of CD4+ T-cell counts and lymphocyte phenotype were also performed in order to assess the immunological safety of the vaccine candidate. These determinations were performed at the clinical sites by standard Flow Cytometry, applying common SOPs and the same lot of reagents, which were provided by the sponsor.

2.6. Measurement of serum antibody against the Tat protein and epitope mapping

Anti-Tat antibody titers were assessed by ELISA as previously described [21]. Serum samples obtained at the indicated time points were tested at serial dilutions and titers were determined as the reciprocal of the last positive sample dilution. A value higher than 100 for IgG and 25 for IgM and IgA, respectively, was considered as positive.

Epitope mapping of anti-Tat IgM/IgG was performed by ELISA using purified peptides designed to partially overlap the primary Tat sequence (aa 1–20; aa 21–40; aa 36–50; aa 46–60; aa 56–70; aa 66–80; aa 73–86; aa 83–102), as previously described [21]. Sera were considered positive when the mean *A* value of wells coated with anti-Tat peptide was higher than 0.35, and the Δ value was higher than 0.15.

2.7. Serum neutralization of extracellular Tat activity by the Tat-induced HIV-1 rescue assay

HLM1 cells (1.0×10^5), a HeLa-CD4+ cell line containing an integrated copy of an HIV-1 *tat*-defective provirus whose replication is rescued by the addition of exogenous Tat [8,25], were seeded in 24-well plates in 500 μl of Dulbecco's modified essential medium containing 10% of fetal bovine serum (FBS) (GIBCO-BRL, Grand Island, NY). After 24 h of culture, medium was replaced with 300 μl of fresh medium containing 2.5 μg/ml of recombinant Tat protein pre-incubated alone or with diluted human sera overnight at 4 °C with gentle shaking. Forty-eight hours later, the rescue of HIV-1 replication was monitored in the culture supernatants by an antigen capture assay (EIA) for the quantification of HIV p24 core antigen (INNOGENETICS N.V.). Values were expressed as the percentage of inhibition of virus rescue considering 100% of rescue the value obtained with Tat alone or with Tat in the presence of control sera which was assumed as 100% of rescue. Each serum was tested in duplicate. Pre-immunization sera were the controls. Results were evaluated by comparing the relative intensity of the neutralizing activity found before treatment with those obtained after vaccine administration, both normalized on the maximal Tat-induced HIV replication obtained in the experimental system.

2.8. T-cell proliferation assay

The proliferative response to Tat and to recall antigen (Candida), was assessed by the [^{3}H]Thymidine (Amersham Life Science, Buckingamshire, UK) incorporation assay on Ficoll-purified PBMCs (2×10^5 per well) cultured either alone or in the presence of the Tat protein (1–5 μg/ml) or the pool of Tat peptides (5 μg/ml) or recall antigen.

After 6 days of incubation at 37 °C in 5% CO_2, cell cultures were pulsed with 1 μCi/well of [^{3}H]Thymidine. The incorporated radioactivity was measured by a β-Counter (Wallac, 1205 Betaplate, Turku, Finland). Proliferative responses were considered positive only when the stimulation index (SI, ratio of stimulated to unstimulated cells) was ≥3.

2.9. Interferon-γ (γ-IFN) and interleukin-4 (IL-4) EliSpot assay

γ-IFN and IL-4 EliSpot assays were performed using commercial plates and kits (R&D Systems, Europe Ltd.). Briefly, after Ficoll separation, 3×10^5 peripheral blood mononuclear cells (PBMC)/well were plated in RPMI 1640 containing 10% FBS in the presence of

three distinct pools of 15mer-Tat peptides (5 μg/ml each) overlapping by 10 amino acids and spanning the entire Tat sequence (aa 1–102) (UFP Service, University of Ferrara, Italy). Phytohemoagglutinin (2 μg/ml) and anti-CD3 antibody (αCD3 1 μg/ml, R&D Systems, Europe Ltd.) were used as positive controls and medium alone as negative control, respectively. After incubation at 37 °C in a humidified 5% CO_2 chamber (24 h for γ-IFN and 48 h for IL-4) the antibody for the detection was added into each well and plates were incubated at 4 °C overnight. Plates were then revealed by a combined treatment with Streptavidin-AP and BCIP/NBT Chromogen and, after drying, were processed by an EliSpot Reader (AID ELISPOT Reader System). The tests were considered valid only when the positive controls (phytohemoagglutinin and αCD3) showed a number of spots forming cells (SFC)/well ≥100 and 50, respectively. γ-IFN EliSpot was considered positive only when the number of SFC/well was ≥9 and fold increase over control was ≥3. The IL-4 EliSpot was considered positive only when fold increase was ≥3.

2.10. Statistical methods

Being a phase I trial, this study was designed to recruit a limited number of participants. At the end of the study, 20 subjects have been treated, which provided a reasonable level of statistical confidence for the safety and for a primary evaluation of the immunogenicity of the vaccine candidate.

Safety was assessed in terms of adverse events and laboratory measurements. The mean change from baseline of lymphocyte phenotype (CD4+, CD8+, natural killer and B cells) was evaluated.

All adverse events (AEs), reported according to the MedDRA Dictionary, were classified by body system, preferred term, severity and relationship with the vaccination; the incidence of adverse events "possibly", "likely" or "clearly" related to vaccination were computed.

Frequency of anti-Tat humoral and cellular responses were evaluated and compared between the two treatment groups by the Fisher's Exact test. The induction of anti-Tat antibody titers (IgM, IgG and IgA) was analyzed by the geometric means over the 48 weeks of the study; anti-Tat inhibition antibody titers at 50% was evaluated after the third immunization (week 12) for the two vaccine administration routes using the Student's *t*-test for paired data. The intensity of cellular immune response to Tat was analyzed by the mean peak of positive response for each Arm, in terms of spots $\times 10^6$ cells for γ-IFN/IL-4 production and stimulation index for lymphoproliferation.

All statistical tests have been performed at two-sided with a 5% significance level.

Statistical analyses and data processing have been performed using SAS® software (SAS Institute, Cary, NC, USA).

3. Results

A total of 28 subjects were screened and 20 HIV-1 uninfected adult volunteers at low risk of infection were enrolled and randomized in different groups to receive the vaccine or placebo, according to the administration routes and the different dosages. The demographic characteristics of subjects among the treatment groups were well balanced regarding age and sex as well as the body mass index (BMI) (Table 1). The study included a 4-week screening period, a 16-week study treatment period, an 8-week post-immunization period and a 24-week follow-up period. An additional monitoring was scheduled for the following 2 years.

Of the twenty volunteers enrolled, 15 (8 for Arm A and 7 for Arm B, respectively) received the scheduled vaccine injections, 5 of them (2 for Arm A and 3 for Arm B, respectively) received the placebo. Since two volunteers interrupted the treatment after the first and the second immunization, respectively, the immunogenicity population is represented by 18 subjects that received at least 3 immunizations. Of these two volunteers, one (Arm B, 15 μg Tat dosage) decided to discontinue the study, while the second (Arm A, placebo) interrupted the treatment as a consequence of a creatine phosphokinase (CPK) increase and hypochondriac polarization.

3.1. Safety

The evaluation of safety parameters was performed considering all the volunteers (20 subjects) that received at least one dose of the study medication (safety population).

Safety was assessed by monitoring the volunteers for local and systemic adverse reactions during the first 2 h, after 24 h and 7 days after each immunization.

Clinical evaluation of safety included monitoring of haematological (including coagulation assessment), biochemical (with liver and kidney functional parameters) and immunological parameters (including CD3+, CD4+, CD8+ T cells, natural killer, B cells and monocytes).

As already described for the therapeutic trial [23,26], no clinically significant alterations of haematological, biochemical and immunological parameters were observed. Most of the adverse events were local, mild and transient, as commonly observed for vaccines in commercial use. The most common systemic adverse event was represented by transient blood disorders (mainly leucocytosis with neutrophilia and transient lymphocytopenia). Incidence and number of local/non-local AEs "possibly", "likely" or "clearly" related to the immunization (including the grading of severity) by each vaccination Arm and placebo group are detailed in Table 2.

No events with a grade of severity >2 were reported. Overall, subjects immunized with Tat + alum showed a higher number of local and non-local adverse events as compared to the other groups, suggesting an effect of the alum adjuvant, as previously observed [23,24,26]. One serious adverse event (ophthalmic neuritis), defined by the investigator as "unlikely" related to the vaccination, was reported in the placebo group after the V immunization, consequently the subject discontinued the study. The *Committee for the Evaluation of Adverse Events* concluded 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.

3.2. Immunogenicity

Vaccine immunogenicity was evaluated in the 18 volunteers that received at least three vaccine doses. None had any humoral or cellular anti-Tat response at the time of enrolment.

3.3. Anti-Tat humoral response

The anti-Tat humoral immune response was assessed at the pre-immunization, during the treatment phase at week 8 (4 weeks after the II immunization), at week 12 (4 weeks after the III immunization), at week 16 (4 weeks after the IV immunization), at week 24 (8 weeks after the V immunization) and at week 48 (32 weeks after the V immunization).

After vaccination, anti-Tat IgM and IgG were found in all vaccinated subjects [14/14 (100%)], while anti-Tat IgA were induced in 86% (12/14) of the subjects [8/8 (100%) in Arm A and 4/6 (67%) in

Table 2
Incidence and number of treatment-related AEs.

System Organ Class (SOC)	Arm A ($n=8$)			Severity[a]			Arm B ($n=7$)			Severity[a]			Placebo ($n=5$)			Severity[a]		
	n	n/N[b] (%)	AEs[c]	1	2	3	n	n/N[b] (%)	AEs[c]	1	2	3	n	n/N[b] (%)	AEs[c]	1	2	3
Local administration site disorders	8	100	77	71	6	0	7	100	72	69	3	0	3	60	8	4	4	0
Blood and lymphatic system disorders	5	63	48	45	3	0	3	43	14	13	1	0	0	0	0	0	0	0
Cardio-vascular disorders	0	0	0	0	0	0	2	29	2	2	0	0	1	20	1	1	0	0
Ear and labyrinth disorders	1	13	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eye disorders	1	13	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gastrointestinal disorders	2	25	3	3	0	0	1	14	1	0	1	0	0	0	0	0	0	0
General disorders	7	88	24	19	4	1	4	57	6	5	1	0	1	20	2	2	0	0
Kidney and urinary disorders	1	13	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Nervous system disorders	4	50	8	7	1	0	3	43	6	6	0	0	1	20	1	1	0	0
Psychiatric disorders	1	13	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skeletal muscle and connective tissue disorders	3	38	6	5	1	0	1	14	5	4	1	0	1	20	5	4	1	0
Skin and subcutaneous tissue disorders	1	13	1	0	1	0	1	14	1	1	0	0	0	0	0	0	0	0

[a] Severity grade: 1 = mild, 2 = moderate and 3 = severe.
[b] n/N = number of subjects reporting the events out of total evaluable subjects.
[c] Number of AEs.

Arm B, respectively]. No responses were observed in the placebo groups. The comparison between vaccinated and placebo groups was statistically significant ($p < 0.0001$, Fisher's Exact test). IgM, IgG and IgA production reached peak intensities between the third and the fourth immunization (12–16 weeks), decreasing during the subsequent 32 weeks (Fig. 2A–C). In particular, SC administration induced peak IgG and IgA titers while the ID route gave the highest IgM titers.

Concerning the vaccine dosages, Tat 7.5 μg gave the highest antibody titers. The induction of specific antibodies was statistically significant ($p < 0.01$, Student's t-test) in both Arms.

3.4. Analysis of epitope-specific B-cell responses against Tat

Epitope mapping of anti-Tat IgM and IgG, performed during the 48 weeks study period (Fig. 3), showed a broad spectrum of B-cell responses. IgM were mainly directed against the amino terminal of Tat at residues 1–20 (10/14, 71%), the basic domain at residues 46–60 (7/14, 50%), and the RGD motif, at residues 73–86 (8/14, 57%), respectively. IgG were mostly directed against the 1–20 peptide (14/14, 100%) and against residues 56–70 (6/14, 42%), which contain the glutamine-rich region of Tat.

3.5. Tat-specific neutralization by sera from vaccinated individuals

Titers of anti-Tat neutralizing IgG and IgM antibody were determined after the third immunization (week 12) by the HIV rescue assay, which is based on the measurement of the inhibition of Tat-induced HIV replication in vitro [21]. The results showed a significant production of anti-Tat neutralizing antibody in both treatment Arms (Arm A $p = 0.0042$ and Arm B $p < 0.0001$, Student's t-test) (Fig. 4).

3.6. Cellular immunity

The development of a cellular immune response against Tat was monitored at the pre-immunization and during the treatment phase at week 5 (1 week after the II immunization), at week 13 (1 week after the IV immunization), at week 17 (1 week after the V

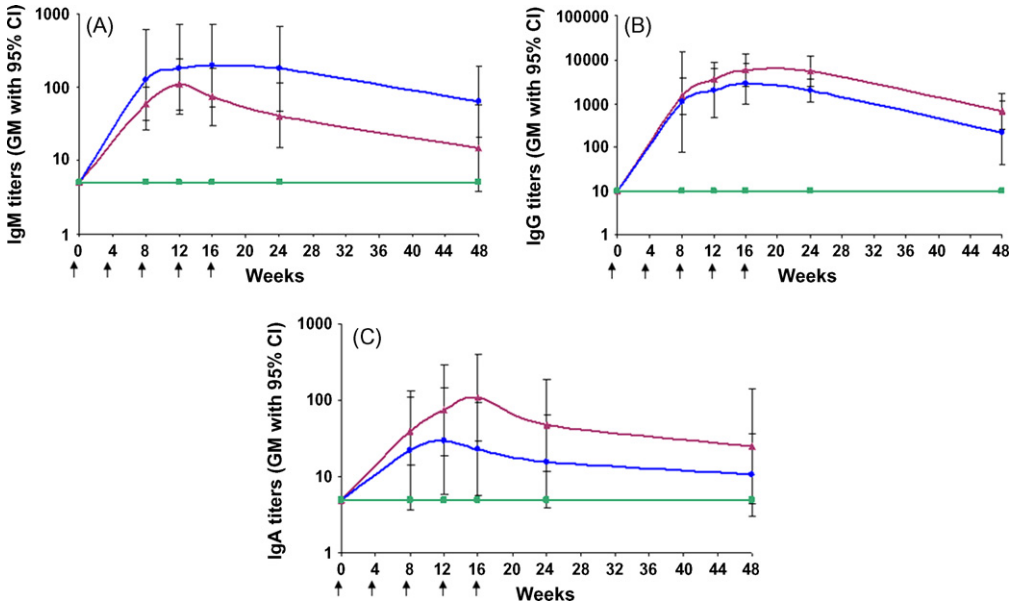

Fig. 2. Anti-Tat IgM, IgG and IgA geometric mean titers by route of administration. Geometric Means (GM) with 95% confidence intervals (CI) of IgM (panel A), IgG (panel B) and IgA (panel C) antibody titers, respectively, are represented on a logarithmic scale. Immunizations are indicated by the arrows (weeks 0, 4, 8, 12, 16). Arm A, SC (red triangle); Arm B, ID (blue circle); placebo (green square). The induction of specific anti-Tat antibody was statistically significant ($p < 0.01$) in both Arms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

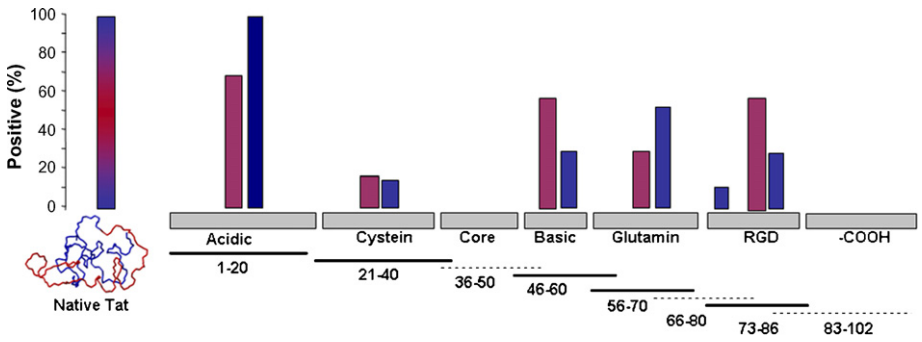

Fig. 3. Epitope-specific B-cell responses induced by Tat vaccination. Epitope mapping of antibody generated by vaccination. The histograms indicate the frequencies of IgM (in red) and IgG (in blue) specific B-cell responses, respectively, against the native Tat and toward distinct Tat domains in the vaccinated individuals. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

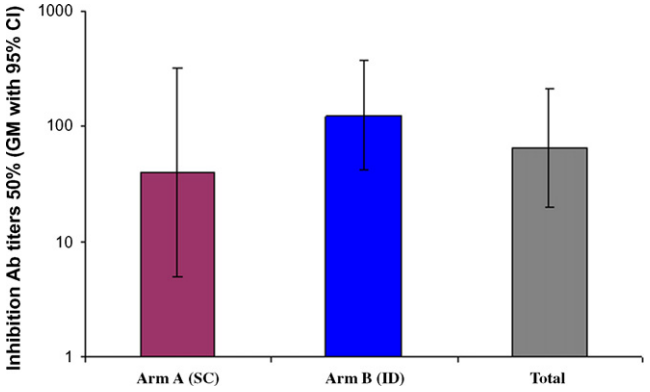

Fig. 4. Neutralization of Tat-induced HIV replication by sera (rescue assay). Geometric mean (GM) of Tat neutralizing antibody titers, corresponding to 50% of inhibition of virus replication (by the Tat-induced HIV rescue assay), are represented on a logarithmic scale with 95% CI for both Arms, either by separate or cumulative data, respectively. A significant production of anti-Tat neutralizing antibody was found in both treatment Arms. Arm A (in red), $p = 0.0042$; Arm B (in blue), $p < 0.0001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

immunization) and at week 48 (32 weeks after the V immunization).

After immunization, anti-Tat cellular responses were generated in 13/14 (93%) of the vaccinees (Table 3). Specifically, lymphoproliferation was found in 9/14 (64%) of vaccinees [5/8 (62%) in Arm A and 4/6 (67%) in Arm B, respectively] γ-IFN production in 5/14 (36%) [2/8 (25%) in Arm A and 3/6 (50%) in Arm B] and IL-4 production in 12/14 (86%) [7/8 (87%) in Arm A and 5/6 (83%) in Arm B, respectively]. The highest intensity (number of SFC/million) was detected for γ-IFN (Arm A, 82 SFC and Arm B 53 SFC, respectively), while IL-4 peak intensities were 31 SFC and 14 SFC, for Arm A and Arm B, respectively.

Table 3
Tat-specific cellular immune responses.

	Arm A (SC)	Arm B (ID)	Placebo
EliSpot γ-IFN			
Responders[a] (%)	25	50	0
N. positive responses[b]	1.5	1.0	
Peak[c] (SFC)	82 (53–112)	53 (23–109)	
EliSpot IL-4			
Responders[a] (%)	87	83	0
N. positive responses[b]	1.8	1.2	
Peak[c] (SFC)	31 (7–101)	14 (11–20)	
Proliferation			
Responders[a] (%)	62	67	0
N. positive responses[b]	2.6	1.5	
Peak[c] (SI)	7 (3–12)	5 (4–6)	

[a] Percent of subjects exhibiting a positive response, weeks 5–48.
[b] Mean of positive responses for each subject over the 4 time points evaluated.
[c] Mean peak (range) of positive responses (SFC/10^6 cells), weeks 5–48.

Overall in both Arms the highest frequency of responders was observed for IL-4, while the most elevated intensity of the response (number of SFC) was detected for γ-IFN. On the other hand, Arm A showed the highest mean number of positive responses for lymphoproliferation (Table 3).

4. Discussion

These results indicate that the administration of the Tat vaccine to uninfected subjects is safe, well tolerated and immunogenic, as already observed in the phase I therapeutic trial [23,24] and in preclinical studies [19,20,25]. Tat vaccination induced strong and durable humoral and cellular immune responses in all vaccinees. Remarkably, vaccination elicited a wide repertoire of B-cell responses directed at multiple key domains of Tat, with an effective, statistically significant induction of Tat-specific neutralizing antibody. These responses are rarely seen in the natural infection [11,23,27,28] and correlate significantly with the asymptomatic stage and long-term non-progression to disease [15, and Poli et al., personal communication]. Of note, both administration Arms gave comparable frequencies of IL-4 and lymphoproliferation while ID vaccination (Arm B) was the most effective at inducing γ-IFN responses.

The parallel conduction of preventative and therapeutic phase I trials with the same vaccine lot also showed that the immune response to Tat vaccination differs between uninfected and HIV infected subjects, even at early, clinically asymptomatic, stages [23]. 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 [23]. 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 the native Tat protein, released during acute infection, and dendritic cells [8,11,27] 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 [28–32]. Administration of Tat by vaccination, however, generated a balanced immune response and a broad array of Th2 type responses with production of specific antibody in all uninfected vaccinees. This was observed also in HIV infected subjects [23], although, in the latter, antibody production upon vaccination had a slower kinetic, a reduced magnitude and a limited broadness as compared to the preventative

trial subjects. Nevertheless in infected individuals a statistically significant positive correlation between the number of CD4 T cells and anti-Tat antibody titers was observed [15,23]. In fact, Tat vaccination partially reverted the marked Th1 polarization of anti-Tat immunity and effectively elicited a more balanced Th1/Th2 immune response [22–24]. The results of the ISS P-001 clinical trial confirm the full achievement of the primary and secondary endpoints of the study, allowing to proceed to further development of an anti-HIV/AIDS vaccine based on Tat protein alone or in combination with other structural HIV antigens (http://www.hiv1tat-vaccines.info/).

Acknowledgements

Conflict of interest statement: None declared. *Financial support:* This work was funded by "ISS special funding for Tat vaccine trials", the Italian Concerted Action on HIV-AIDS Vaccine Development (ICAV, National AIDS Program) and the Italian Ministry of Health.

The authors wish to give a special thank to all volunteers who participated in the trial.

The authors thank all the collaborators at the clinical centers involved with the conduction of the study, the Committee for the Monitoring of AEs, Community Advisory Board, AIDS Help-line members for their valuable contribution.

The authors wish to thank Mrs. P. Sergiampietri for the editorial assistance.

Appendix A.

All co-authors contributed to the conduction of the study and critical revision of the manuscript.

B. Ensoli MD PhD, V. Fiorelli PhD, F. Ensoli MD PhD, P. Monini PhD and E. Garaci MD, conceived, designed, and supervised the conduct of the study. A. Lazzarin MD, R. Visintini MD, P. Narciso MD and A. Di Carlo MD contributed to the conduction of the study. M. Magnani PhD developed Tat production procedures under GMP conditions.

The Clinical Trial Group: S. Bellino PhD and O. Longo PhD (National AIDS Center, Istituto Superiore di Sanità), contributed to the analysis and interpretation of the data and to manuscript preparation. A. Tripiciano PhD, A. Scoglio PhD, B. Collacchi PhD, M.J. Ruiz Alvarez MD, A. Arancio PhD, V. Francavilla PhD and G. Paniccia PhD (National AIDS Center, Istituto Superiore di Sanità and San Gallicano Hospital, Rome) performed all the immunological and virological testing. M.E. Laguardia (University of Urbino, Urbino, Italy), cooperated to GMP Tat production.

The Clinical Sites Team: Co-investigators at the clinical sites include G. Tambussi MD PhD and C. Tassan Din MD, San Raffaele Hospital, Milan; G. Palamara MD and A. Latini MD, San Gallicano Hospital, Rome; A. Antinori MD and G. D'Offizi MD, National Institute for Infectious Diseases "L. Spallanzani", Rome. Psychologists at the clinical sites supported the volunteers during the study. M. Giuliani PhD, San Gallicano Hospital, Rome; M. Giulianelli PhD, National Institute for Infectious Diseases "L. Spallanzani", Rome; M. Carta PhD, University "La Sapienza", Rome.

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