SYNOPSIS

NAME OF SPONSOR: National HIV/AIDS Research Centre Istituto Superiore di Sanità (ISS) **NAME OF FINISHED PRODUCT:** Not applicable **NAME OF ACTIVE INGREDIENT** Biologically active recombinant Tat protein		

TITLE OF STUDY

A Phase II randomized, open label, immunogenicity and safety trial of the vaccine based on the recombinant biologically active HIV-1 Tat protein in anti-Tat (antibody) negative HIV-1 infected HAART-treated adult subjects.

STUDY SITES

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PUBLICATION (reference)
Ensoli B et al. PLoS ONE 2010; Monini P. et al. PLoS ONE 2012; Ensoli F et al. Retrovirology 2015; Sgadari C et al., Frontiers in Immunol., 2019

STUDY PERIOD
First subject enrolled: September 09th, 2008
Last subject completed (48 weeks of study): May 05th , 2012

PHASE OF DEVELOPMENT
Therapeutic exploratory (Phase II)

STUDY OBJECTIVES
To demonstrate that the Tat vaccine is immunogenic in HIV-1 infected, anti-Tat antibody (Ab) negative subjects treated with antiretroviral therapy

METHODOLOGY
Randomized, open label study

NUMBER OF SUBJECTS
Number of planned subjects: n. 160 evaluable subjects
Number of subjects included in the study: n. 168 subjects (138 male, 30 female subjects)

Treatment Group	Subject enrolled	Subject completed treatment
Group I – Arm A	44	39
Group II – Arm A	41	35
Group I – Arm B	43	41
Group II – Arm B	40	38
TOTAL	168	153

MAIN CRITERIA FOR INCLUSION

1. Age 18-55 years;
2. Anti-Tat Ab negative subjects;
3. Human Immunodeficiency Virus 1 (HIV-1) infected subjects under successful Highly Active Anti-Retroviral Therapy (HAART) with HIV plasma viremia < 50 copies/mL in the last 6 months prior to screening;
4. Subjects with any pre-HAART CD4 nadir;
5. $CD4^+$ T cell counts ≥ 200 cells/μl at enrolment;
6. Availability for the planned study duration;
7. Negative pregnancy test for women of childbearing age (to be performed during the screening phase and just before the immunizations) and use of an acceptable mean of contraception (condom, hormonal or mechanical methods) for one month prior to immunization and at least until 48 weeks form the first immunization;
8. Signed Informed Consent.

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MAIN CRITERIA FOR EXCLUSION

1. Concomitant AIDS-related opportunistic diseases;
2. Concomitant neoplastic diseases;
3. History of malignant neoplastic diseases [*NOTE: Subjects with history of non malignant neoplastic diseases completely resolved according to the fulfilment of all the specific recovery criteria, in agreement with the current guidelines in medical oncology are eligible*];
4. History of encephalopathy, neuropathy or unstable Central Nervous System (CNS) pathology, immunodeficiency, autoimmune disease, angina or cardiac arrhythmias, or any other clinically significant medical problems;
5. Any evidence, as judged by the Investigator, of unstable cardio-vascular disease (e.g. unstable hypertensive disease needing modification or introduction of an anti-hypertensive treatment);
6. Chest radiography showing evidence of active or acute cardiac or pulmonary disease within 6 months prior to study screening visit;
7. History of anaphylaxis or serious adverse reactions to vaccines as well as serum IgE levels exceeding 1000 U.I./mL;
8. History of serious allergic reaction to any substance, requiring hospitalization or emergency medical care (e.g. Steven-Johnson syndrome, bronchospasm, or hypotension);
9. Active tuberculosis documented Purified Protein Derivative (PPD) skin test within one year [*NOTE: if the PPD skin test is positive, then a chest x-ray will be done and if no findings consistent with active pulmonary tuberculosis and no indications exist for prophylaxis or treatment, the subject is eligible for participation in this trial*];
10. Medical or psychiatric condition which preclude subject compliance with the protocol. Specifically, persons with psychotic disorders, major affective disorders, suicidal ideation are to be excluded;
11. Current use of psychotropic drugs prescribed for major psychotic disorders;
12. Concomitant participation in any experimental study;
13. Current or prior therapy with immunomodulators or immunosuppressive drugs and anticoagulant drugs within 30 days prior to study medication administration;
14. Live attenuated vaccines within 60 days of study inclusion [*NOTE: Medically indicated sub-unit or killed vaccines (e.g., influenza, pneumococcal, hepatitis A and B) are not exclusionary, but should be given at least 4 weeks away from HIV immunizations*];
15. Administration of blood products or immunoglobulin (Ig) in the past year;
16. Previous participation in an HIV-1 vaccine trial (subjects who participated as placebo in an HIV-1 vaccine trial and have never been effectively administered with an HIV-1 vaccine are eligible);
17. Drug and/or alcohol abuse;
18. Use in the last 6 months or concomitant use of Chemokine Receptor-5 (CCR-5) inhibitors and/or HIV integrase inhibitors and/or HIV fusion inhibitors;
19. Pregnant or lactating women.

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TEST PRODUCT, REFERENCE PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Test Product:

Biologically active recombinant Tat protein

Tat (7.5 µg or 30 µg) was administered intradermally (id) according to the following schedule:

- Arm A - Group I: Tat (7.5 µg) administered 5 times (5x) at weeks 0, 4, 8, 12, 16;
- Arm A - Group II: Tat (30 µg) administered 5x at weeks 0, 4, 8, 12, 16;
- Arm B - Group I: Tat (7.5 µg) administered 3x at weeks 0, 4, 8;
- Arm B - Group II: Tat (30 µg) administered 3x at weeks 0, 4, 8.

DURATION OF TREATMENT AND FOLLOW-UP

Sixteen weeks for subjects randomized to arm A and 8 weeks for subjects randomized to arm B. Total study duration was 144 weeks, however the trial was amended to close the study when the last enrolled subject reached the 48 weeks from the first immunization in order to allow the initiation of an “ad hoc” observational study of follow-up until week 180 from the first immunization (see below ISS T-002 EF-UP results).

CRITERIA FOR EVALUATION OF SAFETY

The safety evaluations included any adverse events, and any significant change in haematological/biochemical and coagulation laboratory parameters.

For all laboratory safety parameters, results at each visit were compared to the results at baseline, and the clinical relevance of significant differences was determined.

STATISTICAL METHODS

The study was a randomized, open label, Phase II clinical trial directed to evaluate the immunogenicity and the safety of the HIV-1 Tat protein-based vaccine.

Two populations were considered for the statistical analyses: the immunogenicity population (155 subjects), representing all randomized individuals who received at least 3 immunizations (excluding also two subjects with poor ARV compliance), and the safety population (168 subjects), representing all randomized subjects who received at least one administration of the vaccine.

The trial was closed at week 48 after the first immunization with a subgroup of patients that was followed up to 3 years (n = 76 up to 96 weeks and n = 45 up to 144 weeks).

Kaplan-Meier method was used to assess the cumulative probability of anti-Tat Ab persistence, by treatment groups and compared by the Log-Rank test. Peak intensity of Abs and cellular responses was compared between Tat doses by Wilcoxon rank-sum test. McNemar’s and Wilcoxon signed-rank tests were used to compare pre-post immunization frequencies and intensity of anti-Tat cellular responses. Logistic regression model was used to evaluate the risk factors to be responders to Tat.

Longitudinal analysis for repeated measurements, multivariate analysis of variance for repeated measures, longitudinal regression analysis using a random-effects regression model, generalized estimating equation with adjustment for repeated measures were used in order to evaluate virological and immunological parameters over time and their relationship.

RESULTS OF IMMUNOGENICITY AND EXPLORATORY TESTINGS

Immunogenicity

Tat immunization induced anti-Tat Abs in most vaccinees (79%), with the highest frequency in the Tat 30 µg groups (89%), and particularly after 3 administrations (92%), as compared to the 7.5 µg arms (70%).
The anti-Tat Ab response was detected since week 4 from the first immunization, and the frequency of responders continued to increase reaching peak values at week 12 and week 20 for the 3X and the 5X immunizations schedule, respectively, with a significant dose-response relationship. Subjects immunized 3X with Tat had the peak of anti-Tat Ab titers at week 12, while for subjects immunized 5X the peak of anti-Tat Ab titers was at week 20.
Statistically significant odds ratios (OR, risk to be responder) were detected for Tat 30 µg 3x or 5x as compared to the reference (Tat 7.5 µg 3x), whereas no differences were observed between 7.5 µg 5x and the reference (Ensoli F, 2015).
In the Tat 30 µg groups Abs persisted significantly longer, as compared to the Tat 7.5 µg groups. The 30 µg doses were also more effective at inducing anti-Tat Abs of different isotypes, and peak IgG titers. In addition, Tat immunization increased the percentage of responders and intensity of anti-Tat cellular responses, including IFN-γ, IL-4, IL-2 production and CD4+-, CD8+-T cell proliferation, as compared to baseline, without significant differences among the treatment groups (Ensoli F, 2015).

Exploratory Analyses

Immune restoration

A significant increase of CD4+ T cell number was observed early after Tat immunization, peaking at year 2 (up to about 100 cells/µl increase, $p<0.0001$) and persisting at year 3. At the same time, Tat immunization maintained stable levels of CD8+ T cells. An early and significant increase of B cells was also induced by Tat immunization that reached the highest value at year 3. Natural killer (NK) cell numbers were also significantly increased in vaccinees at year 2 and 3. Changes of CD4+ and CD8+ T cells, B and NK cells were comparable with both drug regimens (NNRTI/NRTI- or PI-based) (Ensoli F, 2015).
Of note, the increase of CD4+ T cells after vaccination was independent from the CD4+ T cell nadir and at year 3 subjects with nadir ≤250 cells/µl had increases greater, although not significantly different, than those with a nadir >250 cells/µl (increase of 132 cells/µl $p=0.0012$ vs 85/µl cells $p=0.0003$, respectively). The increase of CD4+ T cells and the maintenance of CD8+ T cells after vaccination were associated with a statistically significant increase of CD4+/CD8+ ratio overtime, due to the increase of CD4+ T cells.
Changes in CD4+ and CD8+ T cells were accompanied by an early, durable and statistically significant restoration of functional subsets. In particular, early (year 1) and durable (up to year 3) increases of CD4+ central memory T cells (Tcm) occurred in vaccinees. This was associated with decreases of naïve and effector memory T cells (Tem), which, however, in most cases did not reach statistical significance. Changes in the CD8+ T cell compartment followed those seen in the CD4+ T cell subsets. In particular, statistical significant increases of CD8+ naïve and Tcm cells with a concomitant decrease of the Tem subset were observed at year 2 and/or year 3. Similar results were observed in vaccinees with both drug regimens (NNRTI/NRTI- or PI-based) for the CD4+ T cell subsets, whereas for the CD8+ T cell subsets NNRTI were associated with greater and significant decreases of CD8+ Tem and increases of CD8+ Tcm as compared to PI-based regimens (Ensoli F, 2015).
Finally, the 30 µg, 3x group was the only one showing significant increases of NK cells and CD38+HLA-DR+/CD8+ T cells, a phenotype associated with increased killing activity in elite controllers (Ensoli F, 2015).

HIV proviral DNA

HIV-1 DNA was evaluated longitudinally up to 3 years (144 weeks): a reduction of HIV-1 DNA was seen in vaccinees, particularly under PI-based regimens. In particular, the percentage of proviral reduction in all vaccinees under PI-based regimens ranged from 26% (week 48) to 60% (week 132), and in the Tat 30 µg, 3x under PI-based regimens ranged from 53% (week 72) to 85%, (week 144), followed by Tat 7.5 µg, 5x (range from 53% to 67%, at week 84 and 108, respectively) and Tat 30 µg, 5x (range from 31% to 47%, at week 96 and 72, respectively). The estimate for HIV-1 DNA decay in the Tat 30 µg, 3x group under PI-based regimens reached over 70% reduction after 3 years, with a half-life of 88 weeks. (Ensoli F, 2015)
The greatest and most significant HIV-1 DNA reduction occurred in the presence of both anti-Tat IgM and IgG Abs at year 2 and, particularly, at year 3 from vaccination, whereas in the presence of a single anti-Tat Ab isotype, it was observed only in year 3. Anti-Tat Ab negative vaccinees had a reduction of borderline

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significance at year 3, possibly explained by the fact that most of these subjects (76%) had Tat-specific cellular responses (Ensoli F, 2015).

Neutralization of Tat-mediated Env entry in dendritic cells (DC)

Neutralization of the entry of oligomeric Env into DC in the presence or absence of Tat was assessed in the Tat 30 µg, 3x regimen group, the most effective regimen ad reducing HIV DNA, by analyzing sera at week 0 (baseline) and at week 12, 20 and 48 after immunization. Sera blocking Tat-mediated Env entry in DC by ≥50% versus baseline were indicated as neutralizing. A significant inverse relationship was observed between Tat-mediated Env entry in DC and anti-Tat Ab binding titers of all isotypes, particularly at week 48. At this time, neutralization was observed in 90% of the subjects with anti-Tat Abs and correlated with both anti-Tat IgM and IgG titers. Of note, the presence of anti-Tat Abs and neutralizing activity at week 48 predicted a significant reduction of HIV-1 DNA, which was consistently detected at year 2 and particularly at year 3 (Ensoli F, 2015)

SAFETY RESULTS

The adverse events (AE) reported during the study indicated that the Tat vaccine may induce mild injection site reactions, and systemic effects, which were generally well tolerated. A total of 103 patients experienced at least one AE during the 48 weeks of the study and the mean number of AE reported per patient was 3.63 (range 1-23). These AE were mainly mild and unrelated to the study treatment. General disorders and administration site reactions were the most frequently reported AE with a possible, probable or definite relationship to study treatment. Serious AE were recorded in 10 vaccinees. For 8 of them the events were judged “unrelated (n. 6) or unlikely related (n. 2)” to the study drug. The remaining 2 patients with serious AE manifested in one case “possibly related” peripheral neurological symptoms (dysarthria, motor aphasia, facial weakness, tongue paresthesia associated with retrosternal constriction) which resolved after 10 days; in the other case a “probably related” right Bell’s paralysis was diagnosed which resolved within 3 months. These events were evaluated by the Data Safety Monitoring Board (DSMB) overseeing the study and judged as manifestations occurring more frequently in HIV-infected patients and also relatively common after the administration of different commercial vaccines. After the 48 weeks of the study, no relevant AE were recorded. Three subjects experienced serious AE judged as “unrelated” to the study treatment that included 2 subjects with hepatic enzymes increase and S. aureus infection and abdominal pain, respectively, which were completely resolved within 4 months, and a third subject with ischemic hemianopsia which was resolved with sequelae.

Based on the overall safety data the DSMB judged the Tat vaccine as safe and well tolerated.

CONCLUSIONS

The results of the ISS T-002 study not only confirm previous findings of safety and immunogenicity of the Tat vaccine, but also show that immunization with Tat in HAART-treated, virologically-suppressed subjects increases T, B and NK cell numbers and promotes restoration of CD4+ and CD8+ functional T cell subsets toward homeostasis. Immune restoration started early after vaccination, increased upon time and was durable (up to 3 years). In particular, a mean increase of CD4+ T cells up to values of about 100 cells/μl was observed in vaccinees at year 3, which appears highly meaningful in subjects with a mean of 6 years of therapy. Further, in vaccinees CD4 T cell number increased progressively independently from the nadir. This is of clinical relevance since it is known that subjects with a nadir ≤250 cells/μl do not respond as well to therapy. Taken together these immunological changes appear consistent with a general restoration of immune functions, well beyond what is achieved upon HAART. In this regard the significant increase of central memory CD4+ T cells and the significant reduction of effector memory CD8+ T cells in association with the expansion of central memory CD8+ T cells appear of particular relevance. Overall, all treatment groups experienced, although to a different extent, immune restoration with the 7.5 μg, 3x performing the least.

Immune restoration was followed by a significant and progressive HIV-1 DNA decay in blood, particularly with Tat 30 μg, 3x under PI-based regimens, which was associated with the presence of both anti-Tat IgM and IgG Abs and Env neutralization, which predicted HIV-1 DNA decay.

The results indicate that the Tat vaccine candidate is safe and immunogenic. In addition, Tat-induced immune responses appear to be necessary to restore immune homeostasis and effective antiviral activities, and to block the replenishment and reduce the size of the viral reservoir. Thus, Tat immunization represents a promising pathogenesis-driven intervention to intensify HAART efficacy.

FURTHER EXTENSION OF ISS T-002 TRIAL FOLLOW-UP

To further extend the ISS T-002 trial follow-up, an observational study was conducted to evaluate the persistence of anti-Tat immune response and to monitor immunological and virological parameters (including CD4+ T-cell counts and HIV proviral DNA load) up to 132 weeks in vaccinees who had received at least 3 immunizations (ISS T-002 EF-UP).

Ninety-three volunteers enrolled in the ISS T-002 trial agreed to enter the extended follow-up study, which started on September 2013 and ended on July 2016, when the participants had a median follow-up of 120 weeks (range 72-132). One patient was excluded from all the analyses because persistently not compliant to HAART. Five patients dropped out of the study: 1 after 24 weeks (discontinued HAART), 1 after 36 weeks (relocated), 1 after 48 weeks (not compliant to study visits), 1 after 48 weeks (death for lung tumor) and 1 after 84 weeks (lost to follow-up) (Sgadari, 2019).

The results of the ISS T-002 EF-UP indicate that the Tat therapeutic vaccine promotes long-lasting specific humoral responses, restoration of the immune system and effective antiviral activities capable of reducing HIV reservoirs up to 90% after 8 years from the 1st immunization.

In particular, the Tat vaccine induced anti-Tat Abs persisting for the entire follow-up period in a high proportion of participants, particularly in the Tat 30 μg regimens, in which anti-Tat Abs were still detectable after 8 years in about 50% of the vaccinees (Sgadari, 2019).

Immune restoration started early after vaccination (14, 38), increased upon time and persisted up to the end of the 8 years follow-up. In particular, CD4+ T-cells increased over time up to year 5 to plateau thereafter with a net average gain of about 100 cells/μL. Of note, CD4+ T-cell count restoration occurred even in subjects with a CD4+ T-cell nadir ≤250 cells/μL, which is associated with a poor immunological recovery upon HAART initiation and long-term negative outcomes. Similarly, significant CD4+ T-cell increases were recorded in poor-immunological-responders (i.e. <500 CD4+ T-cells/μL at baseline), who are known to experience progression and comorbidities. Overall, improvements of CD4+ T-cell counts were observed in all treatment groups, although to a different extent, with the 30 μg, 3x group performing the best and the 7.5 μg, 3x group performing the least. The CD4+ T-cell increase was paralleled by the increase of the CD4+/CD8+ T-cell ratio, an important marker of immune reconstitution in virologically suppressed HAART-treated patients (Sgadari, 2019).

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The increase of the CD4+/CD8+ ratio above HAART levels upon Tat vaccination was paralleled by a continued, long-lasting decay of HIV-1 proviral DNA throughout the 8 years of follow-up. Notably, proviral decay was more pronounced in vaccinees experiencing CD4+/CD8+ T-cell ratio or CD4+ T-cell increases, while it was less pronounced in vaccinees with increasing CD8+ T-cell counts or with residual viremia and/or viral blips. Further, although CD4+ T-cell increases plateaued after year 5, proviral DNA continued to decay. In their whole, these data indicate a key role for immune homeostasis restoration by the Tat vaccine and suggest that immunological recovery achieved with Tat immunization was sufficient for a continued, long-term control of the virus reservoirs. (Sgadari, 2019)

Overall, our data suggest that immunization with Tat may accelerate HIV-1 proviral DNA decay through multiple actions of anti-Tat Abs including i) blocking of Tat-induced CD4+ T-cell transitioning through a functional cell state primed for latent HIV infection, ii) relieving Tat-mediated inhibition of CTL responses, and iii) blocking Tat-dependent enhancement of HIV infection in low virus-producing compartments (i.e., conceivably, lymphoid tissues with suboptimal drug penetration). This conclusion is also suggested by the presence in vaccinees of anti-Tat Abs predicting HIV DNA decay

In conclusion, the findings reported herein indicate that anti-Tat immune responses are needed to offset HAART shortfalls and to promote return to immune homeostasis, which likely contributes to restoration of effective anti-viral responses that, together with anti-Tat immunity, are capable of attacking HAART-resistant virus reservoirs. Thus, Tat immunization represents a promising pathogenesis-driven intervention to intensify HAART efficacy while renewing perspectives for a functional cure.