Effects on Immune Function of Therapeutic Immunization with HIV-1 Tat: Interim Results of a Randomized Trial

Barbara Ensoli, MD, PhD
National AIDS Center
Istituto Superiore di Sanità
Rome, Italy

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Rationale for the intensification of the Highly Active Antiretroviral therapy (HAART)

- **HAART represents a major virus-targeting intervention against HIV/AIDS, however, in spite of its success at suppressing HIV replication, HAART can only partially reduce the chronic immune activation and revert the immune dysregulation seen in successfully treated patients.**

- **In HAART-treated individuals immune dysfunction is associated with an increased risk of non-AIDS-defining illnesses, including atherosclerosis, liver and kidney diseases, tumors and accelerated aging.**

- **Tat is a key factor for disease maintenance since it is the trans-activator of HIV gene expression and replication, is persistently expressed in cell reservoirs (also under a successful HAART) and has key effects on immune activation and dysfunction.**

Due to its effects on the virus and on the immune system,
Tat represents a good candidate for HAART intensification.

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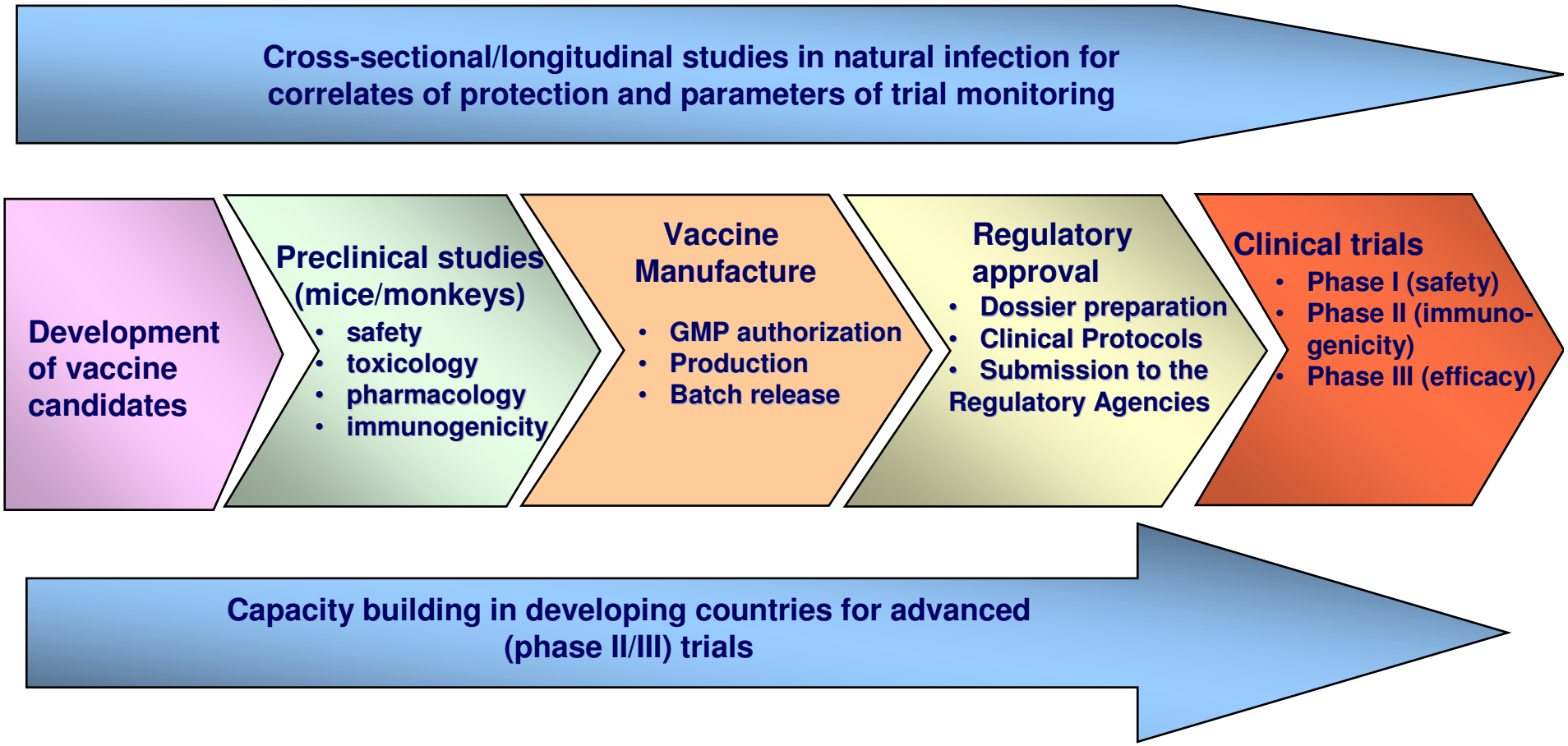
HIV/AIDS vaccine development



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ISS T-002 Phase II Trial

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

(ClinicalTrials.gov NCT00751595)

Immunization schedule: **Tat protein was administered intradermally 3 or 5 times at 2 different doses (7.5 µg or 30 µg), at week 0, 4, 8 or 0, 4, 8, 12, 16, respectively.**

Sample size: **128 subjects, 32 for each treatment group.**

Inclusion criteria: **HIV-1 infected adult subjects, anti-Tat antibody negative, of either gender, 18-55 years-old, HAART-treated with chronic suppressed infection and levels of plasma viremia <50 copies/mL in the last 6 months and without a history of virologic rebound, with CD4+ T cell counts ≥400 cells/µL and with pre-HAART CD4 nadir >250 cells/µL.**

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ISS T-002 Clinical Sites

Clinical Site, City	Principal Investigator, Co-Investigator
Policlinico Universitario, Modena	*Prof. Esposito, Dr. Mussini*
Arcispedale S. Anna, Ferrara	*Prof. Ghinelli, Dr. Sighinolfi, Dr. Segala*
IFO - San Gallicano, Roma	*Prof. Palamara, Dr. Latini*
Ospedale S. M. Annunziata , Firenze	*Prof. Mazzotta, Dr. Di Pietro*
Ospedale A. di Savoia, Torino	*Prof. Di Perri, Prof. Bonora*
Ospedale S. Raffaele, Milano	*Prof. Lazzarin, Dr. Tambussi*
Ospedale Sacco, Milano	*Prof. Galli, Dr. Rusconi*
Spedali Civili, Brescia	*Prof. Carosi, Dr. Quiros*
Ospedale S.M. Goretti, Latina	*Prof. Soscia, Dr. Mercurio, Dr. Tacconi*
Policlinico Universitario, Bari	*Prof. Pastore, Prof. Angarano, Dr. Ladisa*
Azienda Osp. San Gerardo, Monza	*Dr. Andrea Gori*

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ISS T-002 Phase II Trial

- **The primary pre-specified outcomes of the study were *Safety* and *Immunogenicity* of Tat immunization.**

- ***A second-line exploratory testing* was performed to characterize in-depth biochemical and immunological markers of disease progression, which are used to assess HAART efficacy. These include determination of cellular and biochemical markers of immune activation, regulatory T cells, cell viability and key cell subsets of the immune system (CD4 and CD8 T lymphocytes, B and NK cells, central and effector memory CD4+ and CD8+ T cells) as well as cellular responses to HIV Env and to recall antigens (Candida, Cytomegalovirus, Epstein-Barr virus, Flu virus).**

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ISS T-002 Interim Analysis

- **Due to the encouraging results, observed early during the trial, an "ad hoc" interim analysis was conducted on 87 subjects who had completed the treatment phase and were followed for up to 1 year after the first immunization.**

- **Data on 88 virologically-suppressed HAART-treated individuals, enrolled in a parallel prospective observational study at the same sites (*ISS OBS T-002, ClinicalTrials.gov NCT01024556*) were used for intergroup comparison. Of them, 32 met all the immunological and virological criteria for eligibility in the ISS T-002 clinical trial, representing, therefore, the appropriate Reference Group for a comparative assessment of the results.**

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ISS T-002 Study Results (I)

- **Immunization with Tat was safe and induced durable humoral and cellular anti-Tat immune responses, achieving the primary and secondary endpoints of the trial.**

- **The increase of regulatory T cells (T-reg) and the concomitant reduction of immune activation (CD38 expression on CD8+ T cells and biochemical markers) were associated with stable increases of the percentage and absolute numbers of both CD4+ T cells and B lymphocytes, and with the reduction of the percentage of CD8+ T cells and NK lymphocytes. As a result, the CD4/CD8 T cell ratio progressively increased.**

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ISS T-002 Study Results (II)

- **This pattern of T and B cell "repopulation" differs markedly from that reported to occur during HAART and also seen in the Reference Group and was accompanied by increases of T cell responses against Env and recall antigens (Candida, Cytomegalovirus, Epstein Barr virus, Flu virus).**

- **Of note, more immune-compromised individuals experienced greater therapeutic effects.**

These findings indicate that Tat immunization represents a promising therapeutic tool to intensify HAART efficacy and to restore the immune homeostasis.

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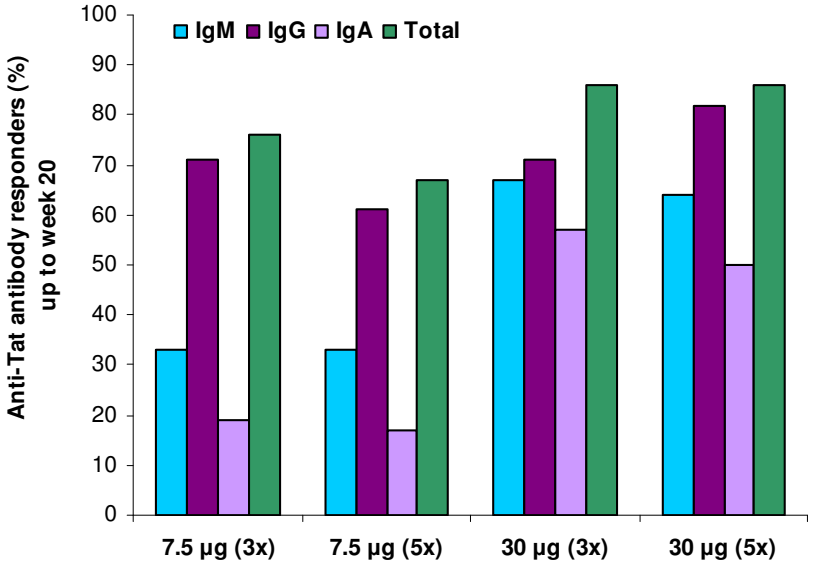
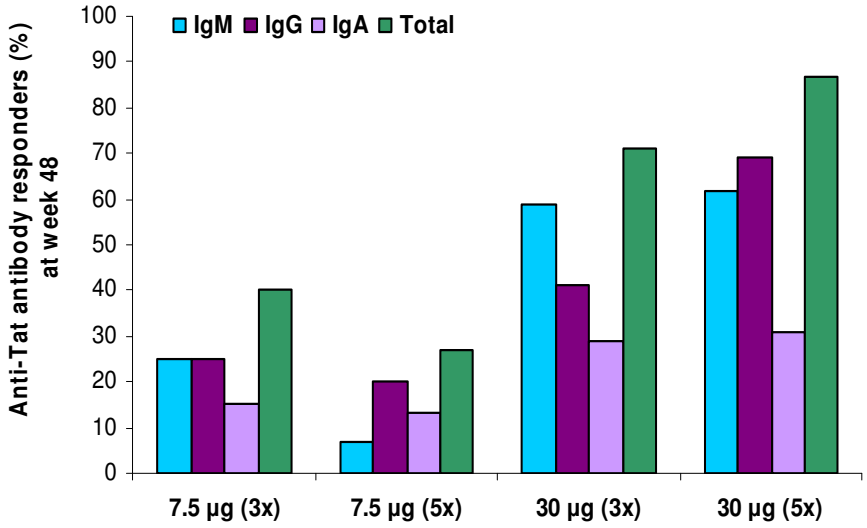
ISS T-002: Therapeutic immunization with Tat induces specific antibodies in HAART-treated individuals





The 30 µg dose of Tat was more potent than 7.5 µg at inducing anti-Tat antibodies and at maintaining long-term humoral immune responses, with little or no differences between the 3 or 5 inoculation regimens.

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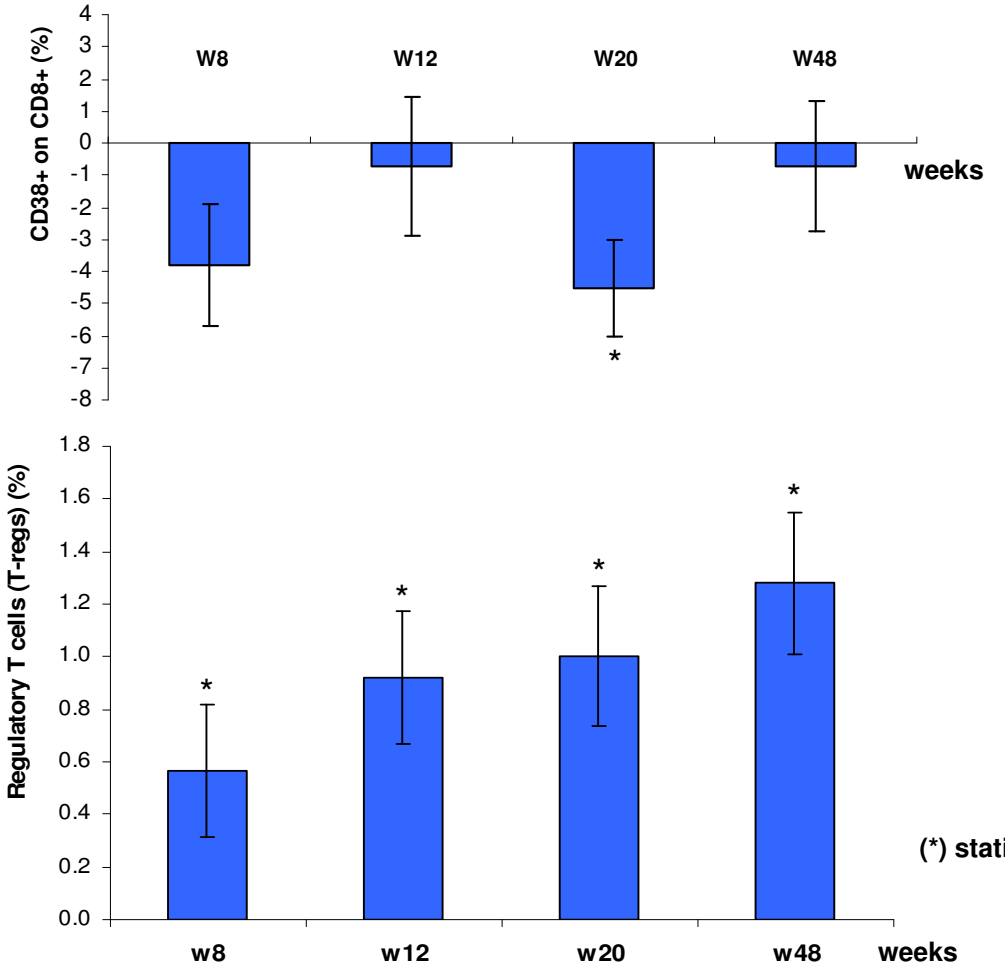
ISS T-002: Immune activation (CD38 expression) and regulatory T cells (CD25+FOXP3+CD4+)



(*) statistically significant, p<0.05

A marked downregulation of CD38 expression on CD8+ T cells and a significant and persistent increase of regulatory T cells were observed in subjects immunized with both Tat doses.

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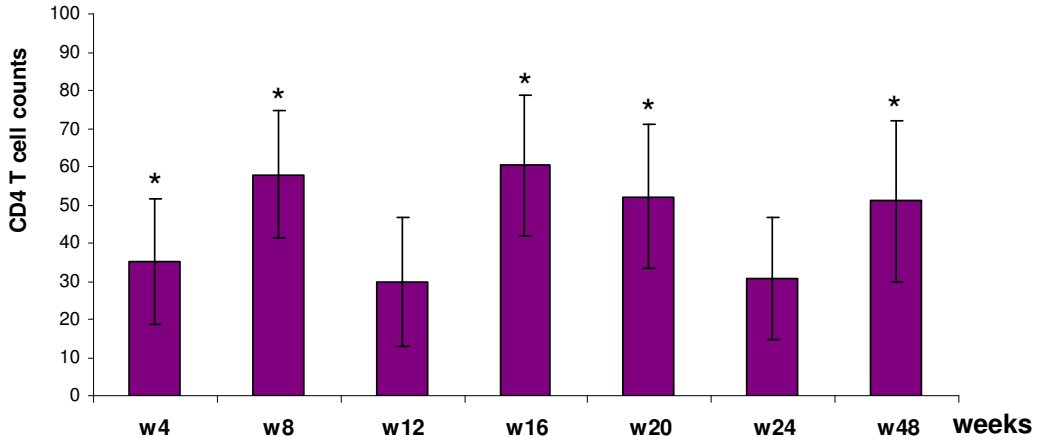
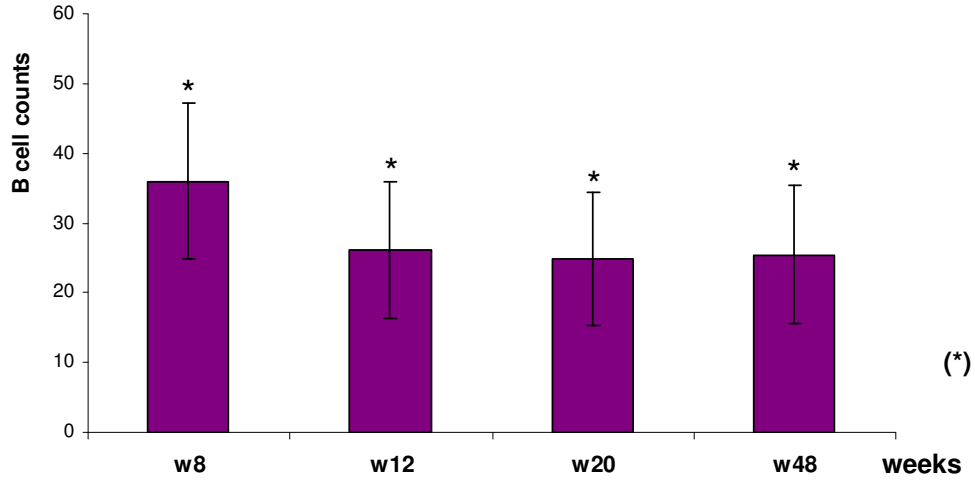
ISS T-002: Number of CD4+ T cells and B cells





(*) statistically significant, p<0.05

As compared to baseline values, the number of CD4+ T cells and B cells significantly and durably increased with both Tat doses.

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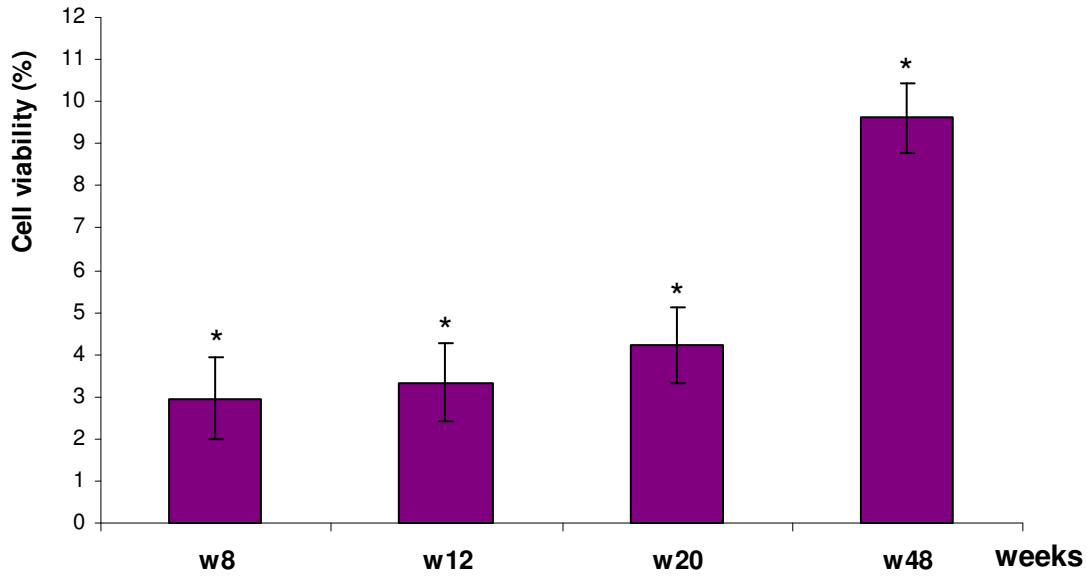
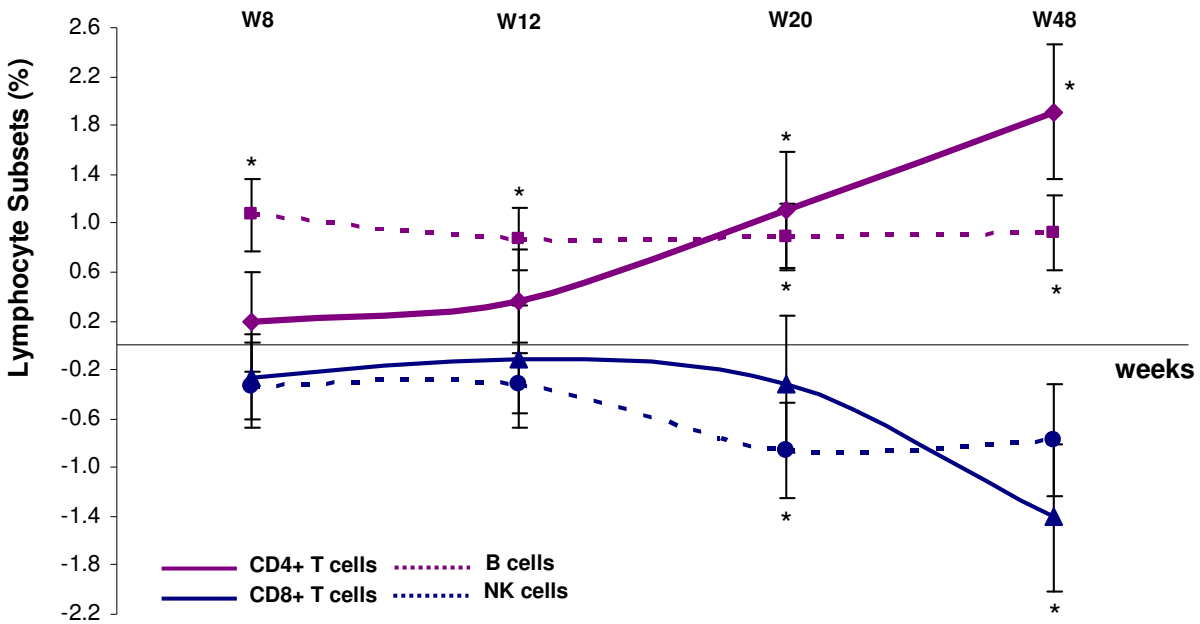
ISS T-002: Peripheral blood mononuclear cells (PBMC) viability



(*) statistically significant, p<0.05

PBMC viability was significantly and steadily increased after immunization with both Tat doses.

ISS T-002: Lymphocyte Subsets



(*) statistically significant, $p<0.05$

Immunization with Tat promoted the restoration of normal proportions of lymphocyte subsets, increasing the percentage of CD4+ T cells and B cells and reducing the percentage of CD8+ and NK cells.

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Major differences of the Observational (OBS) Study with the ISS T-002 Trial

- **In the OBS study, the decrease of CD38 expression was minor, the biochemical markers of immune activation were scarcely modified, and regulatory T cells were further decreased.**
- **No relevant changes of the CD4+ T cell number and a progressive loss of B cells were observed in the OBS study.**
- **A moderate increase of the CD4+ T cell percentage, an overall stability of B and NK subsets, a stable or further increase of the percentage of CD8+ T cells were detected in the OBS study.**

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Tat Immunization: Conclusions

- **Therapeutic immunization with Tat reduces the immune activation still present under a successful HAART and promotes the restoration of proper and effective immune responses.**

- **Results indicate that immunization with Tat acts in synergy with HAART to help restoring immune homeostasis.**

This is the first time that a therapeutic HIV/AIDS vaccine shows a targeted and selective efficacy.

These results proofs the concept of targeting Tat for a "pathogenetic therapy".

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ISS T-002 Protocol Amendment

- **In view of the urgency to improve HIV treatment and considering the results that immunization with Tat reverts biomarkers of HIV disease that persist under HAART, with higher therapeutic effects in more immune dysregulated subjects,**

a protocol amendment was approved by the Ethical Committees

- **to increase the number of patients from 128 to 160;**
- **to include more immune compromised individuals, which should most benefit from therapeutic immunization.**

Amended inclusion criteria: ***history of virologic rebound, CD4+ T cell counts ≥200 cells/μl irrespectively of pre-HAART CD4 nadir, co-infections*****.**

The trial is still continuing and it is now recruiting according to the broader inclusion criteria.

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International Advisory Board
Community Advisory Board
AIDS Help Line of Istituto Superiore di Sanità

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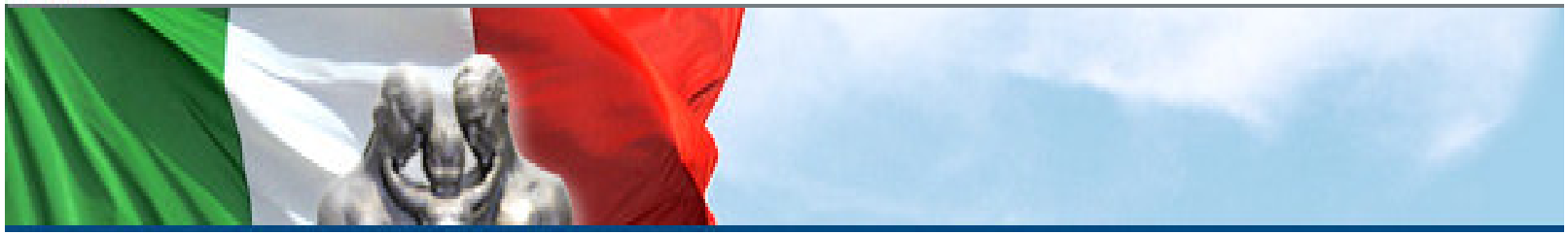
Ministero della Salute



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