# Candidate HIV-1 Tat vaccine development: from basic science to clinical trials

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## Introduction

Over the past 20 years most of the efforts in HIV vaccine development have focused on sterilizing immunity by targeting the Envelope protein (Env). However, results from preclinical and clinical trials have been largely disappointing [1–11]. Therefore, current vaccine strategies are not only aimed at preventing virus infection but also at blocking virus replication and disease onset. In particular, the control of virus replication should provide protection from disease development and reduce virus transmission, halting the HIV epidemic. This objective may be achieved by targeting virus regulatory genes, which are expressed early after infection, are essential for virus replication and pathogenesis, and are more conserved among HIV clades. This approach may be effective for both preventive and therapeutic vaccine strategies [12–68]. In this article we review the characteristics of Tat and why it was selected for use in a vaccine. We also cite the lesson learned in the development of this anti-Tat vaccine for use in human clinical trials.

## Why HIV-1 Tat?

Tat represents an optimal candidate for a vaccine controlling virus replication and blocking disease progression (Table 1).

### Role of Tat in the virus life cycle

Tat is a key viral regulatory protein produced very early after infection, even before virus integration, and is necessary for viral gene expression, cell-to-cell virus transmission and disease progression [69–85]. Furthermore, Tat is released by acutely infected cells [70,86–89] promoting HIV-1 replication [70,90,91], as well as the recruitment and activation of uninfected cells, providing new targets for HIV spread [61,70,87,90,92–95].

### Cross-sectional and longitudinal studies of Tat immune response in natural infection

The presence of anti-Tat antibodies appears to play a protective role from disease progression [96–101]. In particular, a higher prevalence of anti-Tat antibodies has been detected in asymptomatic HIV-infected individuals compared with progressed patients [98,100,102,103].

In addition, a cross-sectional assessment in 302 HIV-1-infected patients showed that anti-Tat antibodies are more frequent at an early stage (A) compared with symptomatic stages (B or C) (Table 2), whereas no differences are observed for antibodies directed against structural proteins. Furthermore, a study performed in a cohort of 252 individuals with known dates of seroconversion and a medium follow-up of 7.2 years [105] indicated a strong association of anti-Tat antibodies with slower disease progression. Moreover, none of the individuals

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**Table 1. Reasons to use the native HIV-1 Tat protein as a vaccine candidate for HIV/AIDS.**

| Biologically active Tat protein |
|---|
| Key role in the virus life cycle (early expression and release by infected cells) and in AIDS pathogenesis |
| Correlation in cross-sectional and longitudinal studies of the anti-Tat immune responses (humoral and cellular) with asymptomatic stage and non-progression to AIDS |
| Conserved immunogenic sequences among HIV-1 clades |
| Very efficiently taken up by dendritic cells inducing T helper type 1 polarization (adjuvant properties) |
| Modifies hierarchy of cytotoxic T-lymphocyte epitopes of heterologous antigens in favour of subdominant and cryptic epitopes (as a result of a modification of proteasome catalytic subunit composition) |
| Preservation of the seronegative status in vaccinees[a] |
| Use as both 'preventive' and 'therapeutic' vaccine |

See text for explanation and references.
[a]Tat-vaccinated individuals will be distinguished from infected individuals because the routine tests for HIV diagnosis detect only antibodies against structural HIV antigens and not against Tat.

who were persistently anti-Tat positive progressed to AIDS, whereas AIDS occurred in anti-Tat-negative individuals [105].

Anti-Tat cytotoxic T lymphocytes are frequently found in natural infection [24,106–109]. In particular, CD8 T-cell responses to Tat are more frequent in patients controlling viraemia [106,110], and correlate with early virus control both in humans [111,112] and monkeys [113,114].

## Tat sequence conservation among HIV clades

The immunogenic regions of Tat are conserved among the HIV-1 M group [115–118]. Cross-clade recognition of Tat B clade (BH-10) is observed with sera from Ugandan, South African and Italian patients who are infected with different subtypes [104]. In addition, the predicted Tat amino acidic sequence (1–86) is well conserved in its first 58 amino acids among the circulating virus clades and in the BH-10 Tat sequence, which derives from the first isolate of two decades ago, providing evidence that a Tat vaccine may be used in different geographical areas of the world [104].

## Immunoregulatory properties of biologically active Tat protein

Active Tat protein possesses immunomodulant and adjuvant properties that are highly advantageous in vaccine development. Native, but not oxidized, Tat protein is selectively and very efficiently taken up by monocyte-derived dendritic cells (MDDC) promoting cell maturation and T helper type 1 polarization, leading to a more efficient presentation of both allogeneic and exogenous soluble antigens [119]. Furthermore, Tat modifies the catalytic subunit composition of immunoproteasomes in B and T cells, leading to a more efficient presentation of subdominant MHC-I-binding cytotoxic T-lymphocyte epitopes of heterologous antigens both *in vitro* and *in vivo* [120,121, R. Gavioli, paper in preparation].

## Absence of seroconversion in vaccinees

Being devoid of structural HIV proteins, the Tat vaccine does not induce seroconversion, facilitating trial recruitment as well as the monitoring of vaccinees.

Taken together, these data suggest that vaccination with Tat may modify the virus–host dynamics and control HIV-1 replication both in primary infection (preventive strategy) and in infected individuals (therapeutic strategy). Therefore, the active Tat protein was chosen as a vaccine candidate against HIV/AIDS for the development of both preventive and therapeutic strategies.

## Lesson learned

Studies performed both at the level of basic and clinical research are essential to address antigen selection and to design innovative strategies for vaccine development. Dissecting the role of Tat in HIV pathogenesis, exploring its biological properties, and investigating the anti-Tat immune response in natural infection gave a twofold gain by both directing our attention to this regulatory protein and providing the necessary know-how for its development as a vaccine candidate.

**Table 2. Frequencies of IgG and IgM anti-Tat antibodies in individuals stratified according to HIV status, clinical stage and CD4 T-cell counts.**

| | IgG positive/total | Percentage | $P$[a] | IgM positive/total | Percentage | $P$[a] | IgG and/or IgM positive/total | Percentage | $P$[a] |
|---|---|---|---|---|---|---|---|---|---|
| HIV status | | | | | | | | | |
| HIV-negative | 0/132 | 0 | | 0/78 | 0 | | 0/132 | 0 | |
| HIV-positive | 40/302 | 13.2 | $<0.01$ | 21/302 | 6.9 | 0.01 | 52/302 | 17.2 | $<0.001$ |
| Clinical stage | | | | | | | | | |
| A | 37/220 | 16.8 | | 20/220 | 9.1 | | 48/220 | 21.8 | |
| B | 1/33 | 3.0 | | 1/33 | 3.0 | | 2/33 | 6.1 | |
| C | 2/49 | 4.11 | 0.01 | 0/49 | 0 | 0.03 | 2/49 | 4.1 | 0.002 |
| CD4 cell count | | | | | | | | | |
| $\geq 200$ | 37/233 | 15.9 | | 19/233 | 8.1 | | 47/233 | 20.2 | |
| $<200$ | 3/69 | 4.4 | 0.01 | 2/69 | 2.9 | 0.04 | 5/69 | 7.2 | 0.01 |

[a]Fisher's exact test. Cross-sectional assessment of serum IgG and IgM anti-Tat antibodies in 302 HIV-1-infected patients at different disease stage and 132 normal blood donors (negative controls). Anti-Tat humoral immunity was assessed by an algorithm combining two enzyme-linked immunosorbent assays as previously described [104].

## Creating the structure for HIV Tat vaccine development

The development of the Tat vaccine candidate required a complex multidisciplinary approach, accomplished by multiple milestones and regulated by national and international authorities (Fig. 1). These activities included the production of the vaccine candidate, an evaluation of its safety, immunogenicity and efficacy in preclinical models, dossier preparation, and approval for human use and clinical trials. Parallel activities consisted of: (a) studies aimed at defining the role of Tat and the Tat immune response in natural infection to identify correlates of protection and to validate tests to monitor vaccinees, and (b) capacity building to conduct advanced clinical trials in developing countries (Fig. 1). The activities undertaken for Tat vaccine development from basic research to clinical testing required the build up of 'ad hoc' structures and expertise within the Italian public sector, which represented the focus of a 10-year-long effort (Fig. 2).

## Preclinical development

### Tat vaccine production and characterization

The active substance of the Tat vaccine is the biologically active recombinant Tat protein (HTLV-IIIB strain, clone BH-10), produced in *Escherichia coli* and purified by heparin sepharose chromatography followed by high-pressure liquid chromatography [70,86,122]. This product was used for in-vitro and preclinical studies. A set of tests, which include the determination of physicochemical, immunochemical and biological properties, was selected to confirm the quality and stability of the protein (Table 3 and Fig. 3). Performing these assays is particularly relevant because Tat contains seven cysteines and is very sensitive to oxidation [70,86], which induces conformational changes, hampering its biological activity as well as recognition by conformational antibodies. For these reasons, the activity of the product was evaluated by two assays: the rescue of a Tat-defective provirus (rescue assay) and the uptake by MDDC [70,86,119]. As a result of the higher level of reproducibility and sensitivity, the uptake by MDDC has then been selected for the release of the Tat protein batches. The reliability of this test has been confirmed by comparing the results obtained by testing several lots of Tat with MDDC from a large number of normal blood donors (Fig. 4).

### Preclinical testing

Safety and immunogenicity studies were conducted in mice and monkeys with both the biologically active Tat protein or tat DNA. The results indicated that both approaches are safe because no local nor systemic toxicity was detected [17,88,123–127].

Efficacy studies in cynomolgus monkeys demonstrated that vaccination with active Tat protein can elicit a specific and broad immune response, and can control viral replication blocking disease progression after challenge with the highly pathogenic cynos-grown SHIV89.6P cy243 (Table 4) [123,124]. Of note was the fact that no residual virus hidden in resting cells was detected in the protected monkeys either in blood or lymph nodes, upon two boosts with tetanus toxoid, a stimulus known to induce virus replication [128]. Long-term protection (up to 2 years) correlated with the presence of high and stable humoral and cellular (CD4 and CD8 T-cell-mediated) responses against Tat. Vaccination with the native Tat protein thus contained viral replication in peripheral blood and tissues, preventing the development of AIDS.

Immunization with native Tat was also safe in monkeys with AIDS and no increase in viral replication nor a further decrease in CD4 T-cells was observed [129].

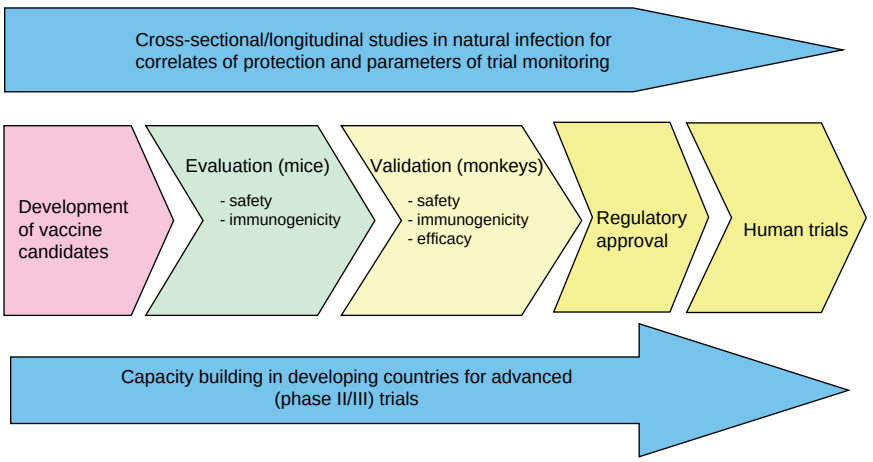


**Fig. 1. HIV Tat vaccine development.** Shown are the sequential and integrated activities for the development of the HIV-1 Tat vaccine programme from the basic research to clinical testing, including parallel activities directed at investigating the correlates of protection in natural infection and at validating laboratory testing for trial monitoring, and capacity building in developing countries for advanced clinical testing.

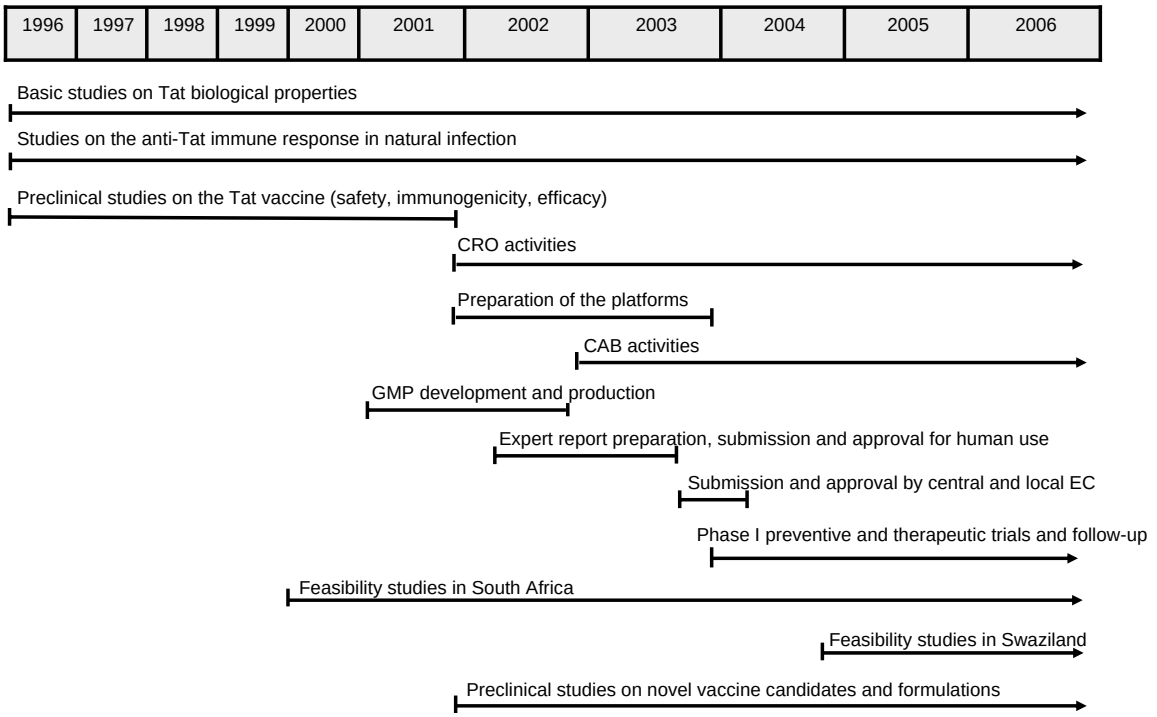


**Fig. 2. Timeline of the Tat vaccine programme.** Timeline of the activities undertaken for the development and conduct of phase I clinical trials with the Tat vaccine candidate, preparatory studies in developing countries and the development of a second/third generation of Tat-based vaccine candidates. CAB, Community advisory board; CRO, contract research organization; EC, ethical committee; GMP, good manufacturing practice.

On the basis of these data, the active Tat protein was chosen for the conduct of preventive and therapeutic phase I clinical trials (Fig. 2).

### Lesson learned

To guarantee translation to the clinical level, all preclinical activities must be conducted in compliance with regulations and procedures ensuring safety and data quality. For example, a process of production compliant with regulatory guidelines for human use should be adopted early in the developmental pipeline. Specific training programmes should be implemented to support scientists in this task.

**Table 3. Physicochemical, immunochemical and biological characterization of the Tat protein.**

| Specifications | Tests |
|---|---|
| Physicochemical properties and purity | Sodium dodecylsulphate–polyacrylamide gel electrophoresis |
| | Comassie blue staining |
| | Silver staining |
| | High-pressure liquid chromatography |
| | Endotoxin determination by LAL test |
| Immunochemical properties | Western blot |
| Biological activity | Rescue of Tat-defective provirus |
| | Uptake by dendritic cells |

LAL, Limulus amoebocyte lysate.

## Regulatory approval by the national agency within the European Union

In order to proceed to phase I clinical trials of a new vaccine in Italy, an application must be submitted to the Committee for the Evaluation of the Safety and Quality of New Drugs at Istituto Superiore di Sanità (ISS) and to the Italian Ministry of Health (Fig. 5). The process is regulated by guidelines and laws issued by European and Italian regulatory authorities (Table 5). Therefore, a dossier termed 'Expert Report' containing the required information on the quality, safety, immunogenicity and efficacy of the Tat vaccine and the clinical protocols was submitted to this Committee, which approved the use of the Tat vaccine candidate in both healthy and HIV-infected individuals (Fig. 2). After that, all the relevant documentation (clinical protocols, psychosocial protocol, investigator brochure, informed consent, clinical sites, insurance policy) (Table 6) was submitted and approved by the central (ISS) and local Ethics Committees/ Institutional Review Boards (Fig. 5). Competitive enrollment was then started in each clinical site for the conduct of both the preventive and therapeutic phase I trials (Fig. 2).

### Lesson learned

Approaching regulatory issues represents a fundamental step in building up translational research programmes, and requires a specific expertise while being extremely

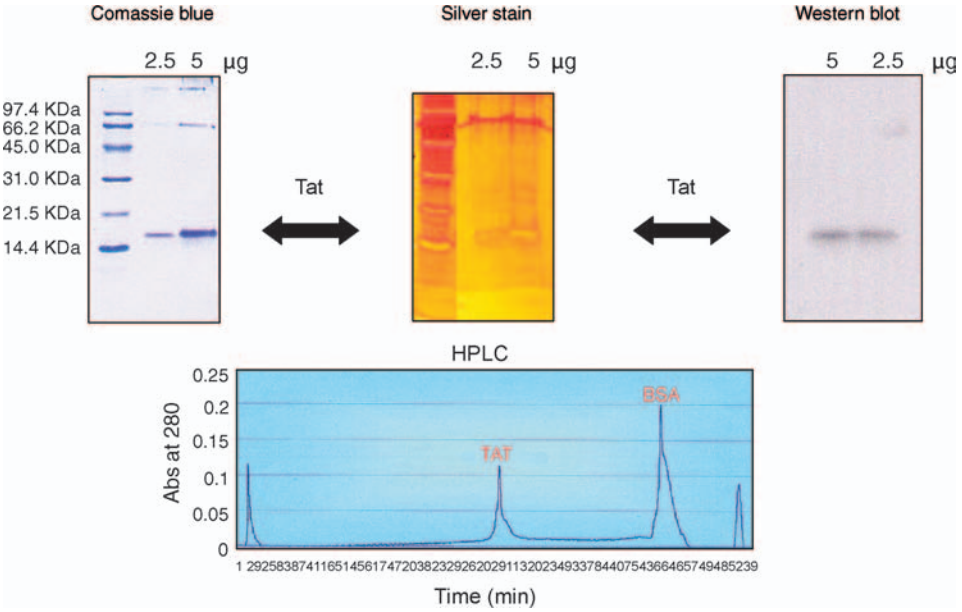


**Fig. 3. Physicochemical characterization of the Tat protein vaccine.** Shown are the Comassie blue and silver staining as well as Western blot of the Tat protein separated by sodium dodecylsulphate–polyacrylamide gel electrophoresis. The lower panel shows the results of high-pressure liquid chromatography (HPLC) of the Tat formulated with bovine serum albumin. This product was used for in-vitro and preclinical studies.

time-consuming and frustrating also because no academic training in this matter exists. Therefore, training should be implemented to support scientists in this task. The implementation of training will help in properly planning timelines and organizing human and economic resources.

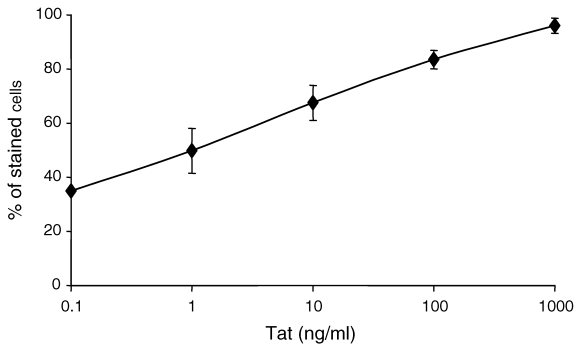


**Fig. 4. Tat uptake by monocyte-derived dendritic cells.** Monocyte-derived dendritic cells were incubated with serial concentrations (0.1–1000 ng/ml) of the native Tat protein, medium, or reconstitution buffer for 10 min. The intracytoplasmatic Tat content was evaluated by flow cytometry after staining with specific affinity purified rabbit anti-Tat polyclonal antibodies (or isotype control), followed by secondary fluorescein-isothiocyanate-conjugated anti-rabbit antibodies [119]. The percentages of positive cells (compared with isotype-stained samples) are reported. The bars indicate the standard deviation. Data are the mean of 86 experiments performed with cells from 71 different donors and eight different lots of the Tat protein.

## Good manufacturing practice Tat vaccine production for phase I studies

### Good manufacturing practice-grade process development of the Tat protein

For the good manufacturing practice (GMP) production of the Tat vaccine it was necessary to identify a validated facility adequate to sustain phase I trials, which, however, was not available in Italy. A contractor was finally identified in the United Kingdom, which produced and released the Tat vaccine according to current regulations.

The recombinant Tat protein was produced and purified by diethylaminoethyl and heparin sepharose chromatography, formulated in a suitable buffer in the presence of human serum albumin and vialed (Fig. 6). Comparability studies with the research-grade product confirmed that release specifications remained unchanged (Table 3). Amino acid terminal sequence and mass spectrometry were also performed on the GMP product. Stability tests confirmed that the vialed clinical lot retained full biological activity for up to 2 years at −80°C.

### Lesson learned

The need to find a contractor outside Italy was extremely costly and time consuming, underlining the necessity of a dedicated small-scale GMP facility in Italy. Thanks to the support of ISS and of the University of Urbino such a facility (AVITECH) has been built in Italy for Tat vaccine production following clinical trials.

**Table 4. Summary of the immunological responses and post-challenge fate of Tat-vaccinated and control monkeys.**

| | | Pre-challenge | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Group[a] | Monkey | Ab titres[b] | Tat[c] neutralization | Proliferative[d] response | CTL[e] | Tat-induced[f] TNF-α | Infection[g] after challenge | CD4[h] T-cell decline |
| Tat in | 54844 | ++ | + | + | + | + | – | – |
| RIBI (sc) | 54876 | ++ | ++ | + | ND | + | – | – |
| | 54963 | ++ | ++ | ++ | – | ND | + | + |
| Tat in | 54899 | +++ | +++ | ++ | + | + | – | – |
| Alum (sc) | 55396 | +++ | +++ | + | – | – | + | + |
| | 55240 | +++ | +++ | + | + | + | – | – |
| Tat (id) | 54222 | ± | ND | – | + | + | – | – |
| Control RIBI | 55123 | – | ND | – | – | – | + | + |
| Control alum | 55129 | – | ND | – | – | – | + | + |
| Naive | 2 | – | ND | ND | ND | ND | + | + |
| Naive | 12 | – | ND | ND | ND | ND | + | + |

ND, Not done.
[a]Monkeys were immunized subcutaneously with Tat (10 μg) and the RIBI or aluminum phosphate (alum) adjuvant. One monkey, was immunized intradermally with Tat (6 μg) in the absence of adjuvant.
[b]Antibody titres to Tat were expressed as the reciprocal of the last positive dilution: –, $<10$; ±, $\leq 100$; +, $>100 \leq 3200$; ++, $>3200 \leq 12\,800$; +++, $>12\,800$.
[c]Neutralization of Tat activity on HIV replication by the rescue assay.
[d]Lymphoproliferative response was determined by a standard $^3$H-thymidine incorporation assay. Stimulation index: –, SI $<3$; +, SI $\geq 3 <10$; ++, SI $\geq 10$.
[e]Anti-Tat cytotoxic T-lymphocyte (CTL) activity at 50 : 1 and 25 : 1 effector: target ratio at weeks 28 and 36 after immunization. Values greater than 10% were considered positive.
[f]Tumor necrosis factor alpha (TNF-α) production by peripheral blood mononuclear cells by enzyme-linked immunosorbent assay. Values below cutoff (15.6 pg/ml) were scored negative.
[g]All monkeys were challenged intravenously with 10 $MID_{50}$ of cynos-derived SHIV89.6$P_{cy243}$. Monkeys 2 and 12 were challenged with 28 and 2.8 $MID_{50}$, respectively. Infection was determined by measuring plasma viraemia and the proviral copy number.
[h]CD4-cell number was evaluated on citrated blood by a fluorescence-activated cell sorter. The decrease in the absolute number of CD4 T-cells was defined as $>50\%$. For all methodologies see Cafaro *et al*. [124] and Maggiorella *et al*. [128].

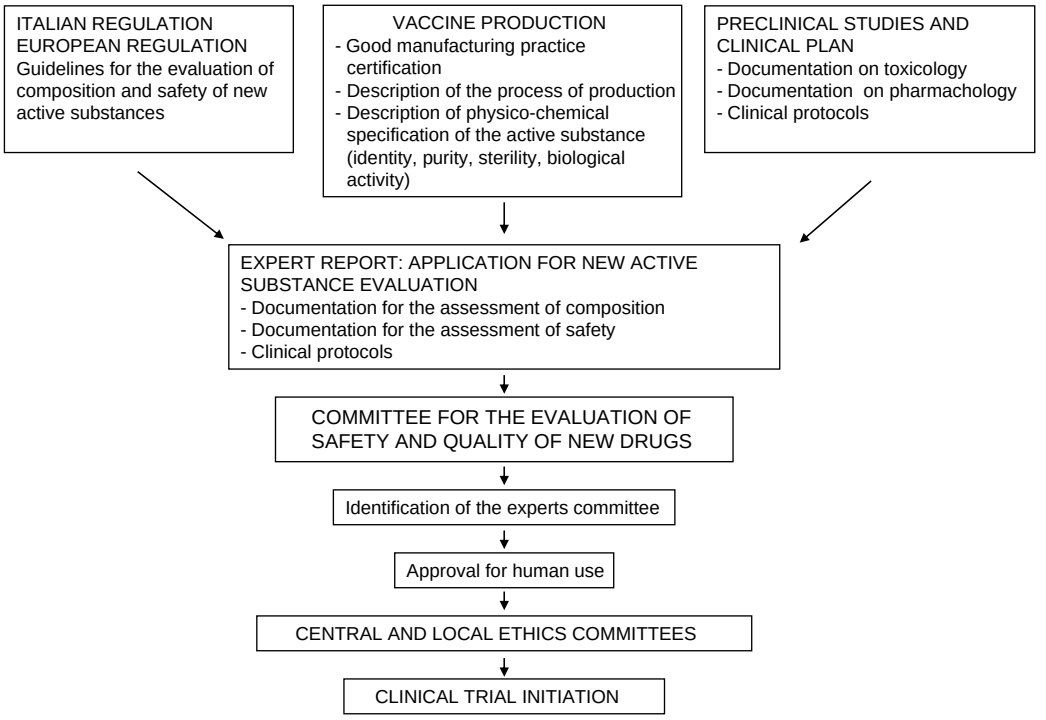


**Fig. 5. Procedures for approval of the Tat vaccine candidate for human use by regulatory agencies.** Reported are the main documentations and procedures required by the Italian regulatory authorities for approval for human use of the Tat vaccine. See text for explanation.

**Table 5. Major European guidelines for the preparation and conduct of phase I clinical trials of vaccines based on recombinant proteins.**

| Title | Emanating authority |
|---|---|
| Note for guidance on comparability of medicinal products containing biotechnology-derived proteins as active substance | CPMP/EMEA |
| Note for preclinical safety evaluation of biotechnology-derived products | CPMP/EMEA |
| Note for guidance on safety pharmacology studies for human pharmaceuticals | CPMP/EMEA |
| Note for guidance on repeated dose toxicity | CPMP/EMEA |
| Note for guidance on pharmacological and toxicological testing of vaccines | CPMP/EMEA |
| Note for guidance on good clinical practice | CPMP/ICH |
| Note for guidance on general consideration for clinical trials | CPMP/ICH |
| Note for guidance on clinical evaluation of new vaccines | CPMP/EMEA |
| Principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational products for human use, 8 October 2003 | European Parliament |
| Community procedures for the authorization and supervision of medicinal products for human and veterinarian use and establishing a European Medicine Agency, 31 March 2004 | European Parliament |
| Assessment of composition and safety of new drugs before clinical testing in humans. Law of decree, 26 April 2002 | ISS |
| Objectives and analysis of the preclinical studies required to conduct phase I clinical trials | ISS |
| Rules for simplification of the procedures for testing and controlling the new systems and experimental therapeutic protocols | |
| Law of decree, 19 December 2001 | President of the Italian Republic |

CPMP, Committee for Proprietary Medicinal Products; EMEA, European Agency for the Evaluation of Medicinal Products; ICH, International Conference on Harmonization; ISS, Istituto Superiore di Sanità.

## Establishment of clinical, laboratory and social–behavioural platforms

In order to ensure comparable read-outs for clinical trials conducted in a multicentre context, all clinical and laboratory activities, as well as psychosocial and behavioural assessments, were harmonized among the participants along common good clinical practice procedures by establishing specific and integrated platforms (Fig. 2 and Fig. 7).

### Clinical platform

Parallel preventive and therapeutic phase I clinical trials were conducted in three sites in Rome (L. Spallanzani Hospital, San Gallicano Hospital and University of Rome 'La Sapienza'), and in one site in Milan (S. Raffaele Hospital; Fig. 8). Clinical activities and responsibilities, financial support from the sponsor, property of data and biological samples and confidentiality were regulated by specific contracts between the sponsor and the clinical sites. Standard operating procedures were implemented in the clinical sites to standardize all activities encompassing prescreening, enrollment and monitoring of the volunteers (clinical evaluation, safety laboratory testing, risk

**Table 6. Major guidelines for the activity of the central ethics committee.**

| Title | Emanating authority |
|---|---|
| Declaration of Helsinki | WMA |
| Convention for the protection of human rights and dignity of the human being with regard to the application of biology and medicine: Convention on Human Rights and Biomedicine (Convention of Oviedo) | Council of Europe |
| International ethical guidelines for biomedical research involving human subjects | CIOMS/WHO |

CIOMS, Council for International Organizations of Medical Sciences; WMA, World Medical Association; WHO, World Health Organization.


**Fig. 6. Vial of the clinical lot of the Tat vaccine candidate used in the clinical trials.**

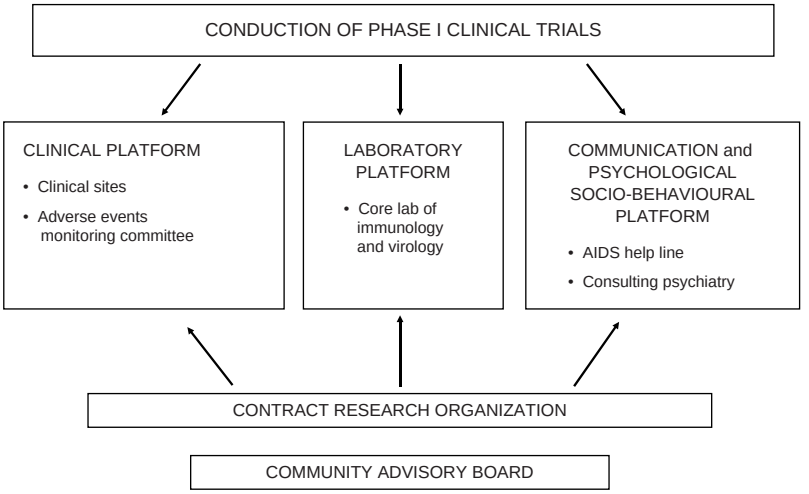


**Fig. 7. Organization of the activities for trial conduct of the Tat vaccine candidate.** Activities were organized according to specific platforms with the support of the contract research organization and the community advisory board.

assessment, and counseling on risk reduction and on avoiding pregnancy). Clinical sites were also responsible for adverse event reporting. In this regard, an independent Committee for the Evaluation of Adverse Events, composed of external clinical experts, was appointed by the sponsor. This committee held periodic meetings during the study, and submitted interim and final safety reports to the regulatory authorities.

## Laboratory platform

A dedicated Core Laboratory for Immunology and Virology was created at the San Gallicano Hospital in Rome as a joint unit with ISS (Fig. 8), and validated upon an international standard of quality (ISO 9001). Immunomonitoring was performed by a two-step strategy with a first line of testing, assessing the strength and breadth of Tat-specific B- and T-cell responses (antibody detection and mapping by enzyme-linked immunosorbent assay, Tat-specific peripheral blood mononuclear cell proliferation and γ-IFN and IL-4 production), and a second line of testing focusing at multiparametric antigen-specific profiles (proliferation coupled with an assessment of T helper types 1/2 cytokine production, multiplexed enzyme-linked immunosorbent assay for cytokines and chemokines and protein microarray), directed at validating novel methodologies for future clinical testing.

## Psychological and behavioural platform

Participation in HIV vaccine clinical trials involves intimate matters, repeated HIV testing and exposure to scientific and medical concepts that may cause anxiety,


**Fig. 8. Operative structure for the conduct of the preventive and therapeutic phase I trials.** Indicated in the figure are the main institutions and bodies involved in the operative structure for the conduct of the parallel preventive and therapeutic trials with the Tat vaccine candidate. CAB, Community advisory board; ISS, Istituto Superiore di Sanità.

stress and depression, and may also contribute to dropouts. A specific platform integrating experts from the clinical sites was therefore created (Fig. 8), and a psychosocial protocol was implemented for the assessment of psychological and sociobehavioural parameters to support volunteers throughout critical points during the study (enrollment, conclusion of the study, follow-up, screening failure or adverse events).

## Communication and enrollment

Information from the sponsor/investigators must provide a good understanding of the nature of the trial to enable potential volunteers to weigh accurately the risks and the benefits of trial participation. To this goal a specific enrollment procedure was developed. In particular, ISS announced the starting of the enrollment with a press release, which referred to the AIDS Helpline at ISS for both general information on AIDS, vaccine clinical trials and specific information on Tat vaccine trial participation (Fig. 9). The AIDS Helpline operators gave to individuals willing to participate in the trial a dedicated telephone number for each clinical site, which was chosen by the volunteers, and an alpha-numeric code needed for the first visit appointment (Fig. 9).

## Contract research organization

To guarantee the quality control and quality assurance of the clinical trials, a contract research organization was hired to provide the following services: study preparation (preparation of case report forms, submission to ethical committee, investigator qualification visits, generation and distribution of randomization codes), study initiation (study-specific monitoring visits, site initiation visits), study monitoring (routine monitoring visits, drug accountability and drug returns for destruction, resolution of queries with sites, termination visits), quality assurance (clinical site audit, database audit), data management (database design and testing, data transfer, data entry, validation and query resolution, quality control of database), analysis and reporting (statistical analysis plan design, statistical programming, statistical analysis, International Conference on Harmonization good clinical practice compliant preparation of clinical and statistical reports (Fig. 2).

## Community advisory board

A community advisory board (CAB) comprising the most representative Italian non-governmental organizations involved in all issues relating to HIV/AIDS was established to provide a communication network among communities, scientists, community care providers and the sponsor (Fig. 8). The CAB contributed to establishing the methodology for ethical information, and provided activity of counseling and communication to the volunteers. The CAB also cooperated with ISS in approaching critical situations such as confidentiality issues with trial participants.

All the activities performed by the different platforms, contract research organization and CAB were implemented and coordinated by the sponsor via numerous ad hoc meetings conducted before and during the trials.

## Lesson learned

For the conduct of preventive and therapeutic phase I studies, a network was created as a highly motivated team. Networking greatly helped the process of the harmonization of procedures and allowed an important 'exchange' of expertise among the different platforms, to the full benefit of the volunteers. In particular, the psychological platform and the CAB represented a major support to the volunteers' wellbeing.

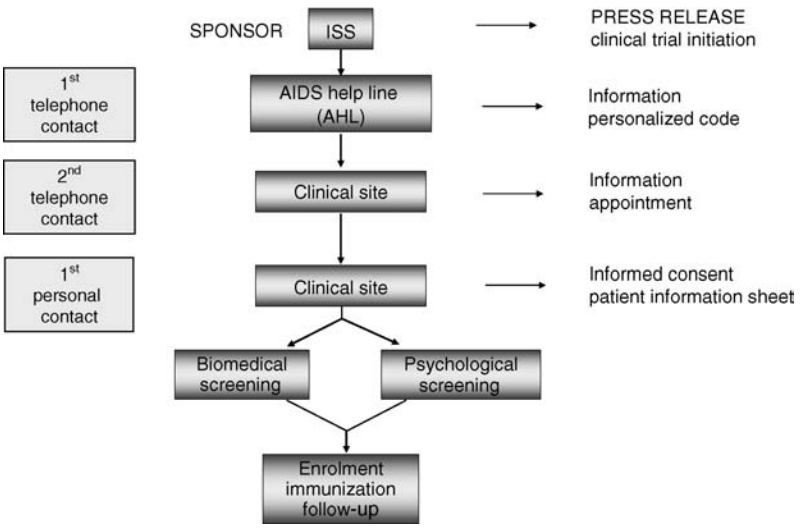


**Fig. 9. Communication and enrollment procedures established for the conduct of trials with the Tat vaccine candidate.** Reported is the algorithm developed to support the communication, recruitment and enrollment for the conduct of phase I clinical trials. See text for explanations. ISS, Istituto Superiore di Sanità.

## Parallel preventive and therapeutic phase I trial conduct

Clinical trials were conducted in healthy HIV-uninfected adults at low risk of infection (preventive protocol) and in HIV-1-infected adult asymptomatic volunteers not in therapy (i.e. CD4 T-cell counts $\geq 400$ cells/$\mu$l and viral loads $\leq 50\,000$ copies/ml; therapeutic protocol). The endpoints were to qualify the biologically active Tat protein as safe (primary endpoint) and immunogenic (secondary endpoint) in both healthy and HIV-infected individuals for its further evaluation in phase II trials (Fig. 8).

Both studies were randomized, placebo-controlled, and double-blinded. Volunteers were randomly assigned to one of two treatment arms with different routes of administration and blinded to the dosage group. In arm A, volunteers received Tat subcutaneously with alum at a dose of 7.5, 15 or 30 $\mu$g, at weeks 0, 4, 8, 12, and 16. One group of volunteers received alum plus saline solution as placebo. In arm B, volunteers received Tat intradermally without adjuvant at a dose of 7.5, 15 or 30 $\mu$g at weeks 0, 4, 8, 12, and 16; one group of volunteers received saline solution as placebo.

The study structure is described in Table 7 and all clinical, laboratory and psychological and sociobehavioural evaluations performed during the trial are shown in Table 8 and Table 9. Evaluations were conducted during the treatment phase, the 6-month follow-up and are continuing for an additional 3 years. An assessment of clinical and laboratory safety was performed at several timepoints during the study and was monitored by the Committee for the Evaluation of Adverse Events.

**Table 7. Clinical protocols of the Tat vaccine candidate: study structure.**

| Activity | Description | Schedule |
|---|---|---|
| Prescreening | 2 Visits | Weeks −4 and −1 |
| Treatment phase | 5 Immunizations | Weeks 0, 4, 8, 12, 16 |
| | 5 Visits, 24 h after each immunization | Weeks 0, 4, 8, 12, 16 |
| | 5 Visits, 7 days after each immunization | Weeks 1, 5, 9, 13, 17 |
| | 1 Visit, end of treatment phase | Week 24 |
| Follow-up | 1 Visit (ISS P-001) | Week 48 |
| | 2 Visits (ISS T-001) | Weeks 36, 48 |
| Additional follow-up | 2 Visits (ISS P-001) | Weeks 96, 144 |
| | 4 Visits (ISS T-001) | Weeks 72, 96, 120, 144 |
| Interim analysis | First database locking | Week 24 |
| Final analysis | Second database locking | Week 48 |

Reported are the key trial activities and their time schedule according to the clinical protocols designed for both the preventive (ISS P-001) and the therapeutic (ISS T-001) trials with the Tat vaccine candidate.

**Table 8. Clinical and laboratory evaluations performed in the preventive and therapeutic clinical trials of the Tat vaccine candidate.**

| | |
|---|---|
| Clinical evaluation | Physical and clinical history |
| | 12-lead electrocardiogram |
| | Psychological assessment |
| | Behavioural assessment |
| | Quality of life assessment |
| | Risk assessment |
| | Risk reduction counselling |
| | Counselling on avoiding pregnancy |
| Laboratory safety evaluation | Haematology |
| | Coagulation assessment |
| | Blood chemistry |
| | CD4 T-cell count |
| | Urine dipstick |
| | HIV-1 plasma viraemia[a] |
| Immunological evaluation | Anti-Tat IgM, IgG and IgA antibodies |
| | Lymphoproliferative response to Tat |
| | $\gamma$-IFN and IL-4 production in response to Tat (Elispot) |
| | Lymphocyte phenotyping |
| | Human leukocyte antigen typing |

[a]HIV-1 plasma viraemia was included as a safety parameter only for the therapeutic protocol.

The studies have been successfully completed. Both primary and secondary endpoints were fully achieved for both the preventive and the therapeutic trials (manuscripts in preparation), sustaining the advancement of the Tat vaccine candidate to phase IIA trials both in Italy and South Africa. On the basis of the results obtained in phase IIA, an extended 'proof-of-concept' phase IIB trial will be conducted in South Africa (preventive protocol) and in Italy (therapeutic protocol) for a preliminary evaluation of efficacy (Fig. 10).

## Lesson learned

The volunteers have established close relationships between themselves during the trial, providing an additional level of care and support. Their participation was so enthusiastic that it was proposed to the sponsor that a

**Table 9. Main objectives and activities of the psychosocial protocol.**

Support the clinical trial design and conduct:
- Contribute to the application of the inclusion/exclusion criteria indicated in the clinical protocol (identification of psychotic disorders, depression, suicidal tendency)
- Identify psychological profiles indicating the need for more accurate support during the most critical steps of the trial (enrollment, occurrence of adverse events, drop-out, conclusion of the study), to prevent drop-out and risk behaviour

Evaluate psychosocial and behavioural parameters of the volunteers:
- Assess the psychological impact of participation in the trial, with particular attention to potential anxiety symptoms
- Evaluate motivation, expectations and informational needs regarding the vaccination protocol with particular attention to the prevention of potential risk behaviour
- Evaluate the quality of life of the participants at enrollment and during the study

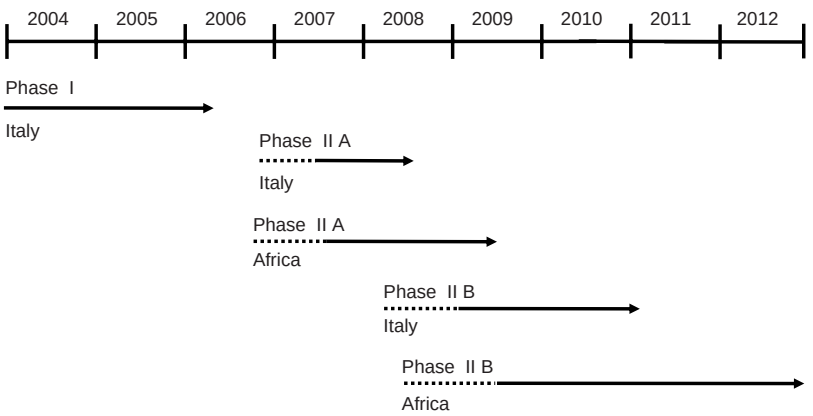


**Fig. 10. Timeline of the clinical development of the Tat vaccine candidate.** Reported is the timeline of the ongoing and future clinical testing of the Tat vaccine candidate in Italy and African countries for both the preventive and therapeutic approaches.

working group should be created to share their experience with the volunteers of the following clinical trials.

Of note is the fact that this is the first time that the same vaccine product has been tested in parallel in preventive and therapeutic trials, allowing a comparison of the safety and immunogenicity in two different populations. In particular, trials in infected subjects may give key information on the impact of vaccination on HIV infection and pathogenesis and a fast readout on vaccine efficacy, providing insights for the development of a non-sterilizing vaccine.

## Preparatory studies in Africa for the conduct of advanced clinical trials

Strengthening and building up the local clinical and laboratory capacity as well as community involvement are crucial steps that must be undertaken before starting clinical testing in African countries. Preparatory studies are also essential to estimate HIV incidence and prevalence in the populations targeted by vaccination, and to evaluate the immune cross-recognition of the vaccine antigen. To this goal, preparatory studies are ongoing in Africa (Fig. 2). In particular, cooperation with South Africa has been established with the HIV/AIDS Vaccine Division at the Perinatal HIV Research Unit at the Chris Hani Baragwanath Hospital in Soweto (Johannesburg, South Africa) within bilateral as well as European Union-funded vaccine programmes. A similar platform is being established in Swaziland.

### Lessons learned

Feasibility studies for the advanced clinical testing of a vaccine in developing countries have to be started well in advance, because a number of issues must be resolved before starting clinical trials. Priority issues are: (i) evaluating the willingness of both local political and scientific authorities, as well as key stakeholders of the community to host vaccine trials; (ii) building up laboratory and clinical capacity and identifying suitable cohorts for vaccine testing; and (iii) performing background immunological and virological field studies.

## Sponsorship of Tat vaccine clinical development

The ISS is a governmental agency with functions of the Centers for Disease Control and Prevention, National Food and Drug Administration and National Institutes of Health. As such, the ISS is strongly involved in basic and applied research in areas that represent a threat to national health, including HIV/AIDS. On the basis of the promising results from preclinical studies with the Tat vaccine, the ISS has sponsored, through the allocation of specific funds, preventive and therapeutic phase I clinical trials of the Tat vaccine. These trials represent the first public, fully government-supported phase I trials of a vaccine against HIV/AIDS in Italy. On the basis of the data obtained, the Italian government has committed to fund phase IIA and IIB preventive and therapeutic trials in Italy and South Africa.

Full sponsorship by a government agency such as the ISS represents a guarantee of the no-profit nature of the programme, while providing protection of the intellectual properties of the Tat vaccine.

### Lesson learned

Institutional sponsorship of the Italian vaccine programme was very favourably perceived by all players, including volunteers, non-governmental organizations and developing countries. At the same time, both the protection of intellectual properties as well as the advancement to phase IIB trials with public resources greatly reduce the financial risk of the private enterprises willing to develop the vaccine further.

## National and international HIV/AIDS vaccine networks

The National AIDS Centre at ISS has established networks with national and international public and private institutions focused on the development of new preventive and therapeutic vaccine strategies to curb the HIV-1 pandemic. Among them is the AIDS Vaccine Integrated Project (AVIP; http://avip-eu.org), which is a European Union-funded 5-year project involving 16 institutions from the public and private sectors from Italy, Sweden, France, Germany, Finland, United Kingdom and South Africa. The design of HIV vaccines within AVIP (Table 10) is based on two general ideas. One is a 'minimalistic' approach combining regulatory HIV proteins (Tat or Nef) with a modified (V2 deleted) Env (ΔV2 Env). The other approach aims at 'imitating' a live attenuated vaccine using as many HIV genes as necessary ('maximalistic' approach). The specific objective and activities of AVIP are described in Table 11 [130].

The Italian Concerted Action on HIV/AIDS Vaccine Development (ICAV) has been established under the National AIDS Programme coordinated by the National AIDS Centre and consists of a network of approximately 70 Italian centres. The activities of the ICAV programme are described in Table 12.

Through these and the other networks in which ISS participates, several vaccines and formulations by different participants are in the pipeline. These novel vaccine candidates also include second-generation Tat-based vaccines such as the Tat/ΔV2Env combination [AVIP, Mucosal vaccines for poverty-related diseases (MUVAPRED), very innovative AIDS vaccine (VIAV) and ISS/Novartis–Chiron agreement] and Tat alone or in combination with other HIV products delivered by micro/nanoparticles (ICAV) as well as herpesvirus vectors (ICAV) and replication-competent adenovirus vectors (Italy–USA, ISS/National Cancer Institute–National Institutes of Health) for parenteral and mucosal vaccination strategies.

**Table 10. Vaccine candidates to be tested in phase I trials within the AIDS Vaccine Integrated Project consortium.**

| Vaccine antigens | Clade |
|---|---|
| Tat ± Env (V2-deleted) | B, C |
| Nef ± Env (V2-deleted) | B, C |
| Multi-HIV antigens/epitopes[a] | A, B, C, FGH |
| HIV multigene (Nef, Rev, Tat, Gag, RT, Env) | A, B, C |

[a]Multi-HIV A, B, C, or FGH clade antigens and epitopes including full-length *Rev*, *Tat*, *Nef*, *Gag* (p17, p24) antigens and other antigens and more than 20 T-cell epitopes from *Pol*, *Protease*, *Env* antigens. Vaccine candidates are represented by four combined vaccines, which are composed of individual vaccine antigens already tested in phase I studies, or under clinical testing. Candidate vaccines will undergo trials after preclinical testing aimed at selecting the optimal formulation and immunization protocol of the antigen combination.

**Table 11. Scientific structure of the AIDS Vaccine Integrated Project.**

| Work packages |
|---|
| 1 Preclinical studies (mice, monkeys) to select the best formulation and vaccination protocol of AVIP vaccine candidates for phase I studies |
| 2 Good manufacturing practice production/toxicology, dossier preparation and regulation (approval for human use) |
| 3 Preventive phase I trials and follow-up |
| 4 Therapeutic phase I trial and follow-up |
| 5 Immunological field preparatory studies focused on cross-clade immune recognition of AVIP vaccine antigens for future phase II/III trials in developing countries |
| 6 European Vaccine against AIDS programme to support all research activities |
| 7 Coordination of science, training, business and administrative management |

Reported are the key activities of the AIDS Vaccine Integrated Project (AVIP), which are organized in work packages, each performed by a dedicated team of scientists.

## Lesson learned

The establishment of national and international networks, including private companies, public and academic institutions, is essential for vaccine development and should always include training programmes such as the AVIP International School (www.avip-eu.com), which is proving to be an optimal forum to train students, scientists

**Table 12. Scientific structure of the Italian Concerted Action on the development of a Vaccine against HIV/AIDS.**

| Work packages |
|---|
| 1 In-vitro analysis of the effects of HIV regulatory proteins and candidate vaccines on immune cells |
| 2 Natural and adaptive immune responses against candidate vaccine antigens in natural and experimental models of protection relevant to vaccine development |
| 3 Vaccine development and selection (safety and immunogenicity in animal models) |
| 4 Animal models for efficacy studies and validation of vaccine candidates |
| 5 Development and production of research grade antigens and establishment of standard operating procedures for good manufacturing practice production of candidate vaccines |
| 6 Preparatory studies (laboratory and clinical setting) for preventive and therapeutic clinical trials in adults |
| 7 Evaluation of correlates of protection in paediatric cohorts for the implementation of therapeutic and preventive HIV-1 vaccine strategies |
| 8 Epidemiological, virological and cross-clade immunological field studies for phase II/III vaccine trials in developing countries |
| 9 Behavioural and psychosocial aspects of the HIV-1 infection related to vaccine trials |
| 10 Development and standardization of techniques and diagnostic assays and preparation of standard operating procedures for vaccine testing in animal models and humans |
| 11 Coordination of science, training, business and administrative management |

Reported are the key activities of the Italian Concerted Action on the development of a Vaccine against HIV/AIDS (ICAV) programme, which are organized in work packages, each performed by a dedicated team of scientists.

**Table 13. Problems, solutions and lessons learned during the development of the Tat vaccine.**

| Problems | Solutions and lessons learned |
|---|---|
| Identification of a new vaccine target | Solution: Tat was chosen based on its role in HIV pathogenesis and on the protective role of the anti-Tat immune response in natural infection.<br>Lesson: Integrate studies of pathogenesis and immune response in natural infection in antigen selection and vaccine design. |
| Selection of the best Tat immunogen | Solution: Biologically active Tat protein was chosen for its properties (dendritic cell targeting, adjuvanticity) and easier regulatory issues.<br>Lesson: Subunit vaccines should not be dismissed. Selection depends upon the properties of the antigen. |
| Guidelines issued by regulatory authorities heavily impact production strategies | Solution: Continuous cross-talk with regulatory experts.<br>Lesson: Adopt compliant approaches as early as possible in the vaccine pipeline. Implement training programmes on regulatory issues to support clinical development. |
| Paucity of good manufacturing practice facilities for early clinical testing | Solution: A dedicated good manufacturing practice facility.<br>Lesson: Public sponsorship/contribution to create good manufacturing practice facilities dedicated to translational research. |
| Harmonization of procedures in the context of a multicentric conduct of clinical trials | Solution: Early establishment of integrated platforms harmonizing the different activities.<br>Lesson: Bring together highly motivated players acting as a team and implement training. |
| Stress in volunteers induced by trial participation | Solution: Psychological support of the volunteers from the screening phase and throughout the trial.<br>Lesson: A psychosocial–behavioural platform strongly contributes to volunteers' wellbeing and trial retention. |
| To advance vaccine clinical testing in developing countries | Solution: Early cooperation with local scientists and authorities for capacity building, feasibility studies and training.<br>Lesson: Obtain from political authorities, scientific institutions and communities an early commitment. |
| Funding of vaccine development up to clinical testing | Solution: Government sponsorship up to phase IIB – proof of concept of efficacy.<br>Lesson: Sensitize and involve key public and governmental bodies on AIDS and vaccine as the solution to stop the epidemic (show cost/benefits for public health and advancing research in general). |
| Need for scientific exchange | Solution: Creation of cooperative networks capable of providing key scientific inputs.<br>Lesson: Scientific networking is crucial to build up translational research programmes. |

and clinicians in the difficult aspects of HIV/AIDS vaccine development. Although creating these networks has been a very challenging task, particularly for management, the intellectual, scientific, and human interactions among the participants have generated true cooperative teams adding a synergistic value to research conduct.

In conclusion, the development of the Tat vaccine programme required a multidisciplinary approach, adequate economic resources, training and a great effort of managing and coordination. The programme has been fully funded and conducted by the ISS, which is the Italian health governmental agency. A great effort was, therefore, dedicated to build up a structure capable of translational research. The accomplishment of this task took 10 years and taught us important lessons (Table 13), at the same time resulting in key achievements. This structure is now ready to run the following clinical phases of the Tat vaccine, as well as new vaccine programmes. In addition, such organization offers the flexibility to update all the different areas of the programme rapidly in response to scientific needs and innovation, with no interference from private/profit interests or 'fashioned' scientific agendas, which have undermined targeting regulatory genes as well as conducting therapeutic vaccine trials that may offer new opportunities in HIV treatment. In particular, the parallel conduct of preventive and therapeutic trials with the Tat vaccine candidate has provided important insights into HIV pathogenesis and for the development of a preventive vaccine based on virus control and not on sterilizing immunity. Finally, the creation of networks for vaccine development is greatly helping in this task and provides a suitable forum for training programmes, which are greatly needed in the field.

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