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The therapeutic phase I trial of the recombinant native HIV-1 Tat protein

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The native HIV-1 Tat protein was chosen as a vaccine candidate based on its key role in the virus life cycle and on the correlation of Tat-specific immune responses with the asymptomatic stage and lower disease progression rates, but also due to its sequence conservation amongst the various HIV clades as well as the adjuvant effects on dendritic cells. Safety, immunogenicity and efficacy data in monkeys support the development of this vaccine concept.

Preventive and therapeutic phase I trials based on the recombinant HIV-1 Tat protein were conducted in parallel in four clinical centers in Italy (L. Spallanzani Hospital, San Gallicano Hospital, University of Rome 'La Sapienza', S. Raffaele Hospital) under the sponsorship of the Istituto Superiore di Sanità (ISS). Standard operating procedures and a centralized laboratory testing ensured harmonization and comparable read-outs [1] (http://www.hiv1tat-vaccines.info/).

The therapeutic phase I study (ISS T-001) was a randomized, double-blind, placebo-controlled trial, which was conducted in 27 HIV-infected, clinically asymptomatic individuals ($CD4^+$ T cell count $\geq 400/\mu l$, a CD4 nadir ≥ 250, and viral load $\leq 50\,000$ copies/ml) belonging to Clinical category A according to Centers for Disease Control. None of the volunteers was under antiretroviral therapy. The recombinant biologically active Tat protein [1] was administered five times, every 4 weeks, by either subcutaneous (s.c.) injection (Arm A) with Alum adjuvant (total of 15 volunteers) or intradermic (i.d.) injection (Arm B) without adjuvant (total of 12 volunteers**)**. Both arms included three different dosage groups (7.5 μg, eight individuals; 15 μg, five individuals or 30 μg, seven individuals) (total of 20 individuals) and a placebo group (total of seven individuals). The primary endpoint of the trial was safety assessment and the secondary endpoint was immunogenicity. The trial is registered in the ClinicalTrials.gov (number NCT00505401).

The data gathered during both the treatment phases (24 weeks) and the follow-up (48 weeks) indicated that the Tat vaccine is safe, well tolerated both locally and systemically, and immunogenic at all the dosages using both routes of administration. Specifically, no significant clinical or laboratory alterations of the safety parameters were found. In fact, both local and systemic adverse events, according to MedDRA dictionary coding [2], were transient, below severity grade 3, and had no association with the vaccine dosage. A safety report has been submitted to the Regulatory Agencies.

Vaccination induced or maintained Tat-specific T helper (Th)-1 cell responses, which, on the contrary, tended to be lost in the placebo group. Remarkably, vaccination elicited Th-2 responses in all participants, as well as a wide spectrum of functional anti-Tat antibodies, which are rarely seen in natural infection [1,3–6]. Overall, after vaccination by either the s.c. or i.d. route, the frequency of anti-Tat immunoglobulin M (IgM) responses increased from 17% to 83%, whereas among the placebos, one individual (Arm A) maintained the baseline IgM response and another one (Arm B) had detectable anti-Tat IgM at a single time point (week 12). The frequency of anti-Tat immunoglobulin G (IgG) and immunoglobulin A (IgA) responses increased in the vaccinees from 11% to 100% and from 0% to 61%, respectively. In contrast, the placebo group, which at baseline had one participant out of seven (14%) positive for IgG or IgA, remained unchanged. The increased frequency of seroconverters for anti-Tat IgG and IgM was statistically significant in vaccinees as compared with placebo ($P = 0.0169$ for IgM and $P < 0.0001$ for IgG, respectively; Fisher's exact test), whereas the increment of anti-Tat IgA seroconverters was borderline ($P = 0.0730$). Peak antibody titers were observed after the fourth immunization. Geometric mean values for IgM, IgG and IgA were 34, 1481, 31 (baseline 9, 15, 5) for Arm A and 22, 686, 23 (baseline 5, 13, 5) for arm B, respectively. Vaccination with Tat increased antibody titers in all participants at baseline (IgM two-fold increase, $P = 0.0958$; IgG 32-fold increase, $P = 0.015$; IgA four-fold increase, $P = 0.0845$; *t*-test for paired data) and widened the B-cell repertoire by inducing novel responses against epitopes representing functional Tat domains. On the contrary, placebo did not show any increment of humoral responses to Tat (geometric mean 6, 14, 6 at baseline and 5, 14, 6 after 48 weeks for IgM, IgG and IgA, respectively). Antibodies of all immunoglobulin classes induced by vaccination were still present after 48 weeks (Arm A 13, 252, 12 and Arm B 11, 114, 12 for IgM, IgG and IgA GM, respectively). Of note, vaccine administered by the i.d. route induced the highest titers of anti-Tat IgM with the most persistent responses.

As previously observed in the natural infection [1,3–7] (http://www.hiv1tat-vaccines.info/), the frequency of cumulative cellular responses [mostly interferon-gamma (IFNγ), and to a lesser extent, lymphoproliferation] to Tat at baseline were found in a higher proportion of the volunteers (84%, including both vaccine and placebo recipients), as compared with the cumulative frequency of humoral responses (IgM, IgG, IgA) (32% including both vaccinees and placebo). After immunization, cumulative anti-Tat cellular responses, including IFNγ, interleukin 4 (IL-4) (as assessed by Elispot) and lymphoproliferation (determined by [^{3}H]thymidine incorporation) were increased from 83% to 100% of the vaccinees, whereas a decrease was observed over time in the placebo group (from 86% to 57%). Specifically, the frequency of lymphoproliferative responses to Tat increased from 61% to 89% in the vaccinees, whereas it decreased in placebo recipients (from 29% to 14%). The frequency of IFNγ responses increased from 55% to 83% in the vaccinees, whereas it decreased in the placebo groups (from 71% to 57%). Remarkably, the frequency of IL-4 responses increased from 17% to 50% in the vaccinees, whereas the only placebo positive at baseline became negative during the study. Overall, the frequency of IL-4 and lymphoproliferation responses upon vaccination became significantly higher in vaccinees as compared with placebo recipients ($P = 0.0267$ and 0.01, respectively; Fisher's exact test). No significant differences in the intensity of these responses were found in vaccinees according to the dosage. For IFNγ, the mean peak number of spot-forming cells (SFCs) (per million of cells) was 276 and 471 (Arm A) and 201 and 383 (Arm B) at baseline and after vaccination, respectively. For IL-4, the mean SFC peak was 21 and 155 (Arm A) and 10 and 169 (Arm B) at baseline and after treatment, respectively. For lymphoproliferation, the stimulation index was 12.4 and 8.1 (Arm A) and 10 and 169 (Arm B) at baseline and after vaccination, respectively. For the placebo group, the mean SFC peak was 92 and 210 for IFNγ and 40 and 7 for IL-4 at baseline and after treatment, respectively, whereas the lymphoproliferation stimulation index was 4.3 at baseline and 7.3 after treatment, respectively. Both vaccine administration routes induced long-term IFNγ cellular responses, which lasted for the entire period of follow-up. Stronger lymphoproliferation and IL-4 responses were observed for the i.d. route, as compared with the s.c. route with Alum. These responses were virtually absent or lost in placebo.

The assessment of CD4$^+$ T cell counts and viral load in the peripheral blood was performed as a primary safety parameter in all the 27 volunteers. The data collected during the 24-week treatment phase and the follow-up (48 weeks) are consistent with a remarkable immunologic and virologic safety of the vaccination as indicated by the preservation of the levels of circulating CD4$^+$ T cells and by the absence of significant plasma viremia rebounds. The statistical analysis of the data revealed a significant positive correlation between the levels of circulating CD4$^+$ T cells and the titers of anti-Tat IgG (Arm B, $P = 0.0175$), or IgA (Arm A, $P = 0.0026$ and Arm B, $P = 0.0059$), whereas no significant correlation was found between CD4$^+$ T cells and anti-Tat IgM (Fig. 1).

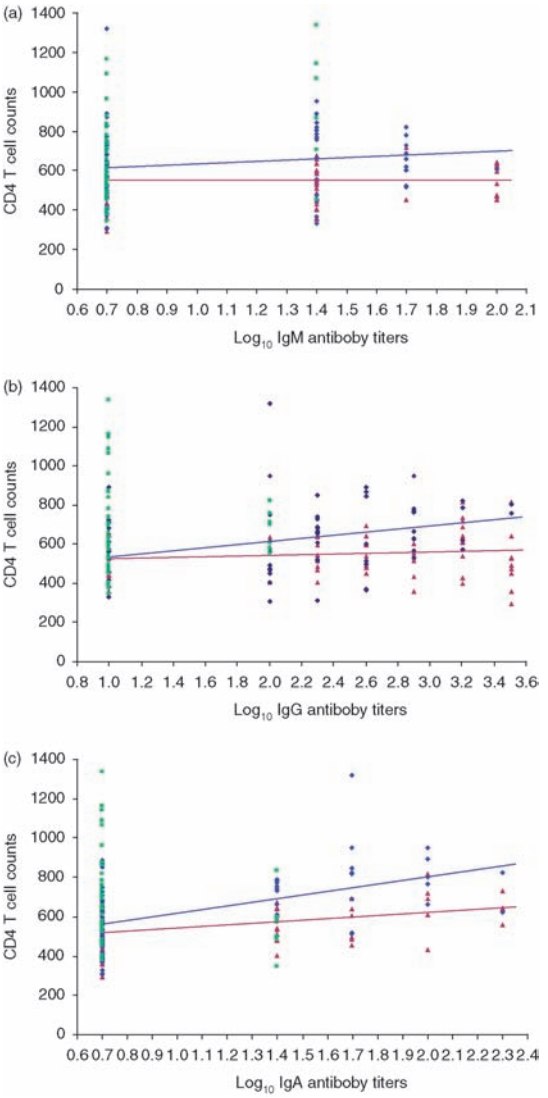

Fig. 1. Relationship between CD4$^+$ T cell counts and titers of anti-Tat IgM, IgG or IgA antibodies. The relationship between CD4$^+$ T cell counts and titers of anti-Tat IgM, IgG and IgA antibodies (a, b and c, respectively) was determined by a regression model for correlated data. The analysis included the following time points −4, 8, 12, 16, 24, 36, and 48 weeks. Arm A, s.c. (triangle in red); Arm B, i.d. (rhomb in blue); placebo (square in green). CD4$^+$ T cells vs. $\log_{10}$ IgM antibody titers: $\beta = 3$ (95% CI −69, 74) for Arm A, $\beta = 57$ (95% CI −32, 146) for Arm B; CD4$^+$ T cells vs. $\log_{10}$ IgG antibody titers: $\beta = 14$ (95% CI −40, 69) for Arm A, $\beta = 72$ (95% CI 13, 132) for Arm B; CD4$^+$ T cells vs. $\log_{10}$ IgA antibody titers: $\beta = 91$ (95% CI 32; 150) for Arm A, $\beta = 193$ (95% CI 56; 331) for Arm B. CI, confidence interval; i.d., intradermic; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; s.c., subcutaneous.

These data indicate the achievement of both the primary (safety) and the secondary (immunogenicity) endpoints of the study. On the basis of these data the Sponsor is proceeding to phase II clinical trials.

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All coauthors participated in the study and contributed to the interpretation and discussion of data, and to the development 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. M. Magnani, PhD and M.E. Laguardia, PhD developed Tat production procedures under good manufacturing practice conditions. S. Bellino, PhD and O. Longo, PhD performed all statistical analyses and contributed to the interpretation of the data. All core laboratory members, including A. Tripiciano, PhD; A. Scoglio, PhD; B. Collacchi, PhD; M.J. Ruiz Alvarez, MD; A. Arancio, PhD; V. Francavilla, PhD; and G. Paniccia, PhD, contributed to the study with the immunological and virological testing. S. Buttò, PhD contributed to the virologic and demographic analyses, A. Di Carlo, MD; G. Palamara, MD; P. Narciso, MD; A. Antinori, MD; G. D'Offizi, MD; A. Lazzarin, MD; G. Tambussi, MD; R. Visintini, MD; M. Giuliani, PhD; A. Latini, MD; M. Giulianelli; M. Carta; and C. Tassan Din, MD were involved in the conduction of the study at the corresponding institutions.

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