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Long-term HIV-specific responses and delayed resumption of antiretroviral therapy after peptide immunization targeting dendritic cells

Anne-Marte B. Kran^a, Birger Sørensen^b, Maja A. Sommerfelt^b, Jørgen Nyhus^b, Ingebjørg Baksaas^c and Dag Kvale^a

Long-term HIV-specific immune responses and clinical outcomes were evaluated in HIV-infected patients previously immunized with p24-like peptides (Vacc-4x) targeting dendritic cells (DC). Vacc-4x-induced cellular immune responses were unchanged 1.5 years after completing immunization, and 62% were still off combined antiretroviral treatment (CART). The magnitude of early Vacc-4x responses determined whether the resumption of CART was clinically indicated 2 years after enrolment. These observations encourage further exploration of both Vacc-4x and other HIV peptide-based immunization regimens targeting DC.

Combination antiretroviral treatment (CART) inhibits the reproduction of HIV particles and reverses disease progression [1], but several factors call for alternative treatment strategies: high cost, persistent low-grade viral replication, incomplete normalization of T-cell functions, drug-related side-effects, and emerging primary HIV infections with multidrug-resistant virus.

Therapeutic immunization aims to attenuate disease progression by modulating HIV-specific immune responses, but the correlates of effective immunity remain to be defined [1–4]. So far, most immune response-inducing candidates have neither been linked with clinical benefit nor with the sustained control of viral replication [2,4,5]. However, a recent study by Lévy *et al.* [6] suggested that immunization in combination with CART and IL-2 improved HIV-specific immunity and lowered the viral set-point after stopping CART. Furthermore, Lu *et al.* [7] showed that autologous monocyte-derived dendritic cells (DC), pulsed *ex vivo* with inactivated autologous virus, induced a prolonged reduction of HIV RNA in a substantial number of patients, highlighting the potential of immunotherapy strategies targeting DC.

We have recently targeted DC *in vivo* with four modified HIV p24-like peptides (Vacc-4x) using the DC-stimulating granulocyte macrophage-colony stimulating factor as a local adjuvant in an open-label, randomized, dose-finding prospective phase II clinical trial with 40 patients on CART. Cellular immune responses were enhanced in 90% of the patients in a dose-related manner [8]. Immunizations were followed by a 4-week treatment interruption to rule out side-effects and allow exposure to autologous virus, and CART was resumed for 8 weeks until week 38. Patients then interrupted CART for 14 more weeks, completing the original trial at week 52. The magnitude of the preceding Vacc-4x responses,

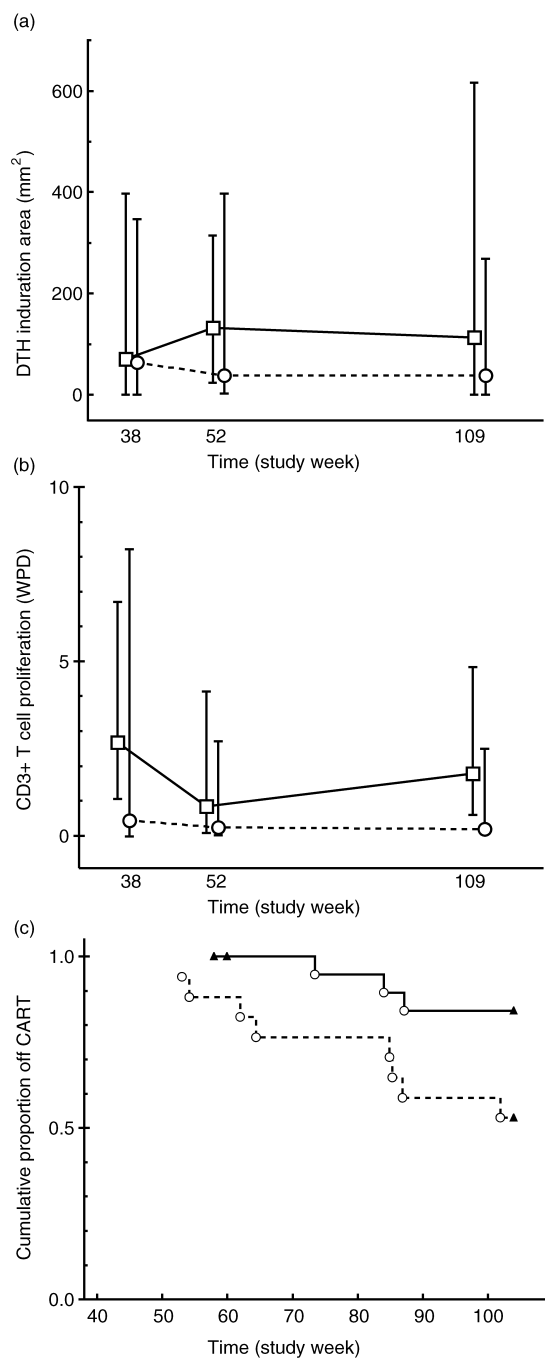


Fig. 1. Longitudinal observations in Vacc-4x patients after interruption of combined antiretroviral treatment. Higher Vacc-4x-specific T-cell responses of patients (a,b) within the high dosage arm (solid line, $n = 17$) compared with the low dosage-arm (dashed line, $n = 15$) at study weeks 38 [combined antiretroviral treatment (CART) stop], 52 (end of study) and 109 (follow up), in terms of (a) delayed-type hypersensitivity (DTH) induration area, and (b) T-cell proliferation ($P < 0.04$). Medians and interquartile ranges are indicated. DTH was evaluated 48 h after the intradermal application of Vacc-4x peptides and was expressed as skin infiltrate areas; positive DTH was defined above 10 mm² according to placebo [8]. No adverse events to DTH were

irrespective of the Vacc-4x dose, was related to lower HIV-RNA set-points and lower CD4 T-cell declines at week 52 without any other confounding factors [9]. Although these data need to be confirmed, a therapeutic HIV vaccine should fulfil at least two demands that we wanted to address in this follow-up study, namely the longevity of vaccine-related cellular immune responses and clinical efficacy.

All of the 37 eligible Vacc-4x patients were evaluated clinically with measurements of HIV RNA and CD4 T lymphocyte counts 109 (108–114) weeks (median, interquartile range) after enrolment in the Vacc-4x trial, i.e. 70 weeks after CART was stopped. Vacc-4x and HIV-specific T-cell responses *in vitro* and *in vivo* [delayed-type hypersensitivity (DTH) tests] were performed in the 32 patients who were able to participate within the assay period. One patient did not receive a DTH test because of a previous allergic reaction [9]. The study was conducted with informed patient consent and was approved by the Norwegian Medicines Control Authority and the Regional Ethics Committee.

After completion of the original study at week 52, a resumption of CART was clinically indicated in two of the patients (5%). All participants were subsequently followed post-study every third month by their regular consultants at our polyclinic. CART was resumed without knowledge of the Vacc-4x responses, either according to medical advice from the patient's physician based on current treatment guidelines [10,11] ($n = 10$), or on the patient's personal initiative to ensure better protection for partners ($n = 2$). At follow-up, 23 of the patients (62%) were still without CART with CD4 cell counts at 410 cells/ μ l (300–460) and HIV RNA at 82 000 copies/ml (31 000–200 000), whereas 14 (38%) had

Fig. 1. (continued)

observed. Vacc-4x-specific T-cell proliferation was evaluated *in vitro* after 7 days in antigen-stimulated peripheral blood mononuclear cells and expressed as the weighed percentage divided [8]. (c) Kaplan-Meier time analysis of the proportion of immunized Vacc-4x patients who remained off CART 2 years after enrolment. Patients were stratified on the magnitude of Vacc-4x DTH induration areas obtained after immunization at study week 38, being either above (solid line) or below (dashed line) the median DTH at this timepoint. Kaplan-Meier product limit estimates showed a lower risk of well-immunized patients resuming CART ($P = 0.01$, Cox F-test). Almost identical data were obtained when stratification was based on DTH responses immediately after immunization at study week 26 ($P = 0.03$). Censored cases, indicated by ▲, included timepoints for those two patients who started CART for personal rather than medical reasons. Overall, CD4 T-cell counts were 400 cells/ μ l (320–470) with a CD4 cell loss from week 38 to follow-up of 230 cells/ μ l (100–340). CART-free patients ($n = 23$, 62%) had been without medication for 71 weeks (48–75).

restarted effective CART after 41 weeks (22–49), with CD4 cell counts of 245 cells/ μ l (208–343) increasing to 375 cells/ μ l (325–515) at follow-up. One Vacc-4x low-responder (DTH < 25th percentile) died from cryptococcal meningitis after a diagnostic delay, approximately 11 months after CART was stopped, and death was judged to have no relation to Vacc-4x.

One of the aims of HIV immunotherapy is to alleviate the drug burden; trial follow-ups will therefore include CART-free periods. However, several studies have reported a drop in HIV-specific T-cell clones from blood during longer interruptions of CART [12,13]. We have recently questioned whether blood is the right test compartment in this particular situation, and pointed out the complexity of evaluating the durability of stimulated HIV-specific responses [14]. In this follow-up study, we found that the T cell-specific responses to Vacc-4x persisted both quantitatively and qualitatively: 26 of the retested patients (84%) still had positive DTH reactions ($n = 31$), and 25 out of 32 (78%) had detectable Vacc-4x-specific T-cell proliferative responses in the peripheral blood mononuclear cells, no different from after completed immunization (90 and 80%, respectively) [8]. Quantitatively, the Vacc-4x-specific T-cell proliferation and DTH reactions correlated ($R = 0.65$, $P < 0.0001$), and were unchanged in magnitude compared with study weeks 38 (CART stop) and 52 (end of study) (Wilcoxon paired tests, Fig. 1). This was true, independent of the Vacc-4x immunization dosages and whether patients had resumed CART or not (data not shown). However, the previously described Vacc-4x high dose advantage in terms of Vacc-4x-specific immune responses [8,9] persisted throughout ($P < 0.04$, Mann–Whitney; Fig. 1).

As a result of the paucity of HIV-related complications, we [9] and others [1] have used pseudomarkers for clinical progression in HIV as efficacy parameters, such as CD4 lymphocyte counts and HIV RNA. However, an analysis of these pseudomarkers was difficult at follow-up because a fraction of patients in every analytical subgroup had resumed CART for various reasons and at different timepoints. Clinical outcomes in relation to Vacc-4x immune responses were therefore evaluated by the time-dependent resumption of CART based on standard clinical care. When patients were stratified according to their post-immunization DTH responses at study week 38 before the last CART-stop, analogous to our previous study [9], DTH high-responders having DTH greater than the median remained CART-free longer than 2 years after enrolment, in a Kaplan–Meier analysis ($P = 0.01$, Cox's F-test; Fig. 1).

In conclusion, Vacc-4x peptides induced qualitatively and quantitatively durable cellular immune responses, even in the presence of HIV viremia. The magnitude of early Vacc-4x responses was the only discriminating factor as to whether the resumption of CART was indicated in

clinical practice, although a placebo-controlled trial is required to prove a causative relationship. The fact that most of the patients were still CART-free approximately 1.5 years after completing immunization confirmed the safety of Vacc-4x. These observations altogether encourage further exploration of both Vacc-4x and other HIV peptide-based immunization regimens targeting DC.

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^aUllevål University Hospital, University of Oslo, Department of Infectious Diseases, NO-0407 Oslo, Norway; ^bBionor Immuno, NO-3703 Skien, Norway; and ^cMericon, NO-3703 Skien, Norway.

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