Safety and efficacy of the peptide-based therapeutic vaccine for HIV-1, Vacc-4x: a phase 2 randomised, double-blind, placebo-controlled trial

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Summary

Background Present combination antiretroviral therapy (cART) alone does not cure HIV infection and requires lifelong drug treatment. The potential role of HIV therapeutic vaccines as part of an HIV cure is under consideration. Our aim was to assess the efficacy, safety, and immunogenicity of Vacc-4x, a peptide-based HIV-1 therapeutic vaccine targeting conserved domains on $p24^{Gag}$, in adults infected with HIV-1.

Methods Between July, 2008, and June, 2010, we did a multinational double-blind, randomised, phase 2 study comparing Vacc-4x with placebo. Participants were adults infected with HIV-1 who were aged 18–55 years and virologically suppressed on cART (viral load <50 copies per mL) with CD4 cell counts of 400×10^6 cells per L or greater. The trial was done at 18 sites in Germany, Italy, Spain, the UK, and the USA. Participants were randomly assigned (2:1) to Vacc-4x or placebo. Group allocation was masked from participants and investigators. Four primary immunisations, weekly for 4 weeks, containing Vacc-4x (or placebo) were given intradermally after administration of adjuvant. Booster immunisations were given at weeks 16 and 18. At week 28, cART was interrupted for up to 24 weeks. The coprimary endpoints were cART resumption and changes in CD4 counts during treatment interruption. Analyses were by modified intention to treat: all participants who received one intervention. Furthermore, safety, viral load, and immunogenicity (as measured by ELISPOT and proliferation assays) were assessed. The 52 week follow-up period was completed in June, 2011. For the coprimary endpoints the proportion of participants who met the criteria for cART resumption was analysed with a logistic regression model with the treatment effect being assessed in a model including country as a covariate. This study is registered with ClinicalTrials.gov, number NCT00659789.

Findings 174 individuals were screened; because of slow recruitment, enrolment stopped with 136 of a planned 345 participants and 93 were randomly assigned to receive Vacc-4x and 43 to receive placebo. There were no differences between the two groups for the primary efficacy endpoints in those participants who stopped cART at week 28. Of the participants who resumed cART, 30 (34%) were in the Vacc-4x group and 11 (29%) in the placebo group, and percentage changes in CD4 counts were not significant (mean treatment difference –5·71, 95% CI –13·01 to 1·59). However, a significant difference in viral load was noted for the Vacc-4x group both at week 48 (median 23 100 copies per mL Vacc-4x *vs* 71 800 copies per mL placebo; p=0·025) and week 52 (median 19 550 copies per mL *vs* 51 000 copies per mL; p=0·041). One serious adverse event, exacerbation of multiple sclerosis, was reported as possibly related to study treatment. Vacc-4x was immunogenic, inducing proliferative responses in both CD4 and CD8 T-cell populations.

Interpretation The proportion of participants resuming cART before end of study and change in CD4 counts during the treatment interruption showed no benefit of vaccination. Vacc-4x was safe, well tolerated, immunogenic, seemed to contribute to a viral-load setpoint reduction after cART interruption, and might be worth consideration in future HIV-cure investigative strategies.

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Introduction

Present combination antiretroviral therapy (cART) improves survival, enhances quality of life, and reduces the risk of onward transmission of HIV.[1,2] However, cART alone does not cure HIV infection and requires that several drugs be taken for life. Interest in therapeutic immunisation as a means to improve immune responses has greatly increased, and, more recently, there has been interest in therapeutic immunisation as a component of viral eradication or functional-cure treatment strategies.[3,4] There is now a pressing need to identify therapeutic vaccine candidates that show immunogenicity associated with virological control. Although trials of HIV vaccines in human beings have so far shown limited efficacy in this regard, a recent report assessing a dendritic-cell-based vaccine did show specific immune responses that seemed to change plasma viral-load setpoint after cART interruption in patients with chronic HIV-1 infection

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treated early in the course of the disease.[5] However, the limitations of this approach are the logistics of providing personalised dendritic-cell-based vaccines to larger populations of patients, which might ultimately restrict the use of such an approach. In the ideal scenario, a broadly applicable vaccine should induce immune responses capable of controlling viral replication in at least some patients, even after discontinuation of cART.

To this end our aim was to assess the role of Vacc-4x, a peptide-based therapeutic vaccine designed to improve and sustain immune responses to conserved domains on the HIV-1 core protein $p24^{Gag}$. This rationale is based on observations that T-cell-mediated immune responses to Gag are associated with virus control and delayed disease progression.[6] The Vacc-4x peptides are modified by aminoacid substitution to improve antigen processing and presentation on diverse HLAs. The domains of $p24^{Gag}$ represented in Vacc-4x are largely synonymous with the conserved immunologically vulnerable sector-3 regions crucial for virus assembly, and where immune escape comes at a cost to viral fitness.[7]

Vacc-4x was safe and well tolerated in previous phase 1 and 2 clinical trials in HIV-infected participants in Norway.[8,9] It was both immunogenic and induced durable immunological memory[10] and a transient reduction in viral load.[11] The present study is the first Vacc-4x placebo-controlled trial incorporating a cART interruption phase and represents one of the largest prospective, randomised, double-blind, placebo-controlled phase 2 clinical trials for a therapeutic HIV vaccine so far.

Methods

Participants

Between July, 2008, and June, 2010, we did a randomised, double-blind, placebo-controlled, phase 2 clinical trial at 18 clinical trial sites in Germany, Italy, Spain, the UK, and the USA. The study enrolled individuals aged 18–55 years, HIV-positive for at least 1 year, virologically suppressed on cART (viral load <50 copies per mL for the past 6 months), prestudy CD4 cell count of 400×10^6 cells per L or greater, and nadir (lowest ever) CD4 cell count of 200×10^6 cells per L or greater. The study was planned as a phase 2b test-of-concept study recruiting 345 participants; however, enrolment was stopped because of slow recruitment after 136 participants were randomly assigned to study groups and the study was redefined by the US Food and Drug Administration (FDA) as an exploratory phase 2 study for hypothesis-generating purposes. An independent data and safety monitoring board (DSMB) was formed to review safety data for the protection of participant safety. The DSMB reviewed safety data for the first 20 participants enrolled into the study after the fourth primary immunisation at week 4 (or had withdrawn from study before this visit). A second safety review took place when 100 participants were enrolled, progressed past treatment completion (week 18), and were followed up until week 28 (or had withdrawn from study before this visit). The DSMB could also arrange unscheduled meetings if there were any ongoing substantial safety concerns. The study continued without the DSMB raising any safety concerns. The 52 week follow-up period was completed in June, 2010, and long-term follow-up ended in June, 2011.

This study was done in accordance with the International Conference on Harmonisation Guideline for Good Clinical Practice. All participants provided written informed consent.

Randomisation and masking

The study was double-blind with respect to treatment assignment, so the participants and investigators were unaware of the identity of the study treatment received. Pharmacists, from whom treatment allocation was not masked, prepared and distributed treatment to participants. Randomisation was stratified by site, block size was three. Participants were randomly assigned (2:1) to receive either Vacc-4x or placebo. The randomisation scheme was prepared by an independent statistician and was stored securely with restricted access until any formal request to unmask study participants.

On randomisation at week 1 and subsequent entry into the treatment phase, participants were assigned a four-digit randomisation number (first two digits representing the study centre number and the final two representing an incremental number for the order participants were randomly assigned to receive study treatment at the site).

Procedures

Participants were given six immunisations with Vacc-4x (or placebo; figure 1). Participants received four primary immunisations, weekly for 4 weeks, with subsequent booster immunisations at weeks 16 and 18. After immunisation, participants remained on cART for another 10 weeks to allow time to reduce potential immune activation associated with immunisation. cART was stopped in eligible participants if their CD4 count was greater than 350×10^6 cells per L and they were virally controlled (determined at week 24) such that all eligible participants were cART free at week 28. cART was resumed if CD4 counts fell below 350×10^6 cells per L or decreased by greater than 50% compared with values at week 28, if viral load increased to greater than 300 000 copies per mL in two consecutive measurements, or if the participants developed any HIV-related or AIDS-related events (CDC clinical category B or C). The criteria for restarting cART were based on the ACTG 5197 clinical study of the Merck adenovirus vectored preventive vaccine candidate Ad5.[12] The study period ended at week 52 with a long-term follow-up until week 104. Participants who resumed cART were followed up for an additional 24 weeks to ensure they regained virus control (<50 copies per mL). During the long-term follow-up, cART resumption was in accordance with local guidelines or agreement between the participant and the investigator.

Vacc-4x was manufactured by Bachem AG (Bubendorf, Switzerland), and distributed in vials by Penn Pharmaceutical Services Ltd (Gwent, UK). Vacc-4x (or placebo) was reconstituted in water on-site by pharmacists and given intradermally superficially to the left deltoid muscle at a dose of 0·1 mL of a 12 mg/mL solution about 10 min after giving adjuvant and immediately next to the adjuvant injection site. Sargramostim (recombinant human granulocyte-macrophage colony stimulating factor), provided as a marketed product by Berlex (Seattle, WA, USA), was used as a local adjuvant and injected intradermally at a dose of 0·1 mL of a 0·60 mg/mL solution. Placebo participants received water in place of both Vacc-4x and adjuvant.

CD4 and CD8 T-cell counts, viral load (Roche Ampliprep/COBAS TaqMan HIV-1), clinical chemistry, and haematology were done by Covance Laboratory Services in the USA (Indianapolis, IN, USA) and Europe (Geneva, Switzerland).

Since only one preART value was initially collected from participants' medical records, informed consent was subsequently given to collect a second value, where available in the records, taken within 6 months of the first value. The preART viral-load setpoint was established as the mean of these two values where available. If a second value was not available, the single value was used. The viral-load setpoint (off ART) was the mean of a participant's final two viral-load values before resumption of cART or at termination of the treatment interruption period—ie, week 52.

HIV-specific T-cell responses were assessed at certified central laboratories from peripheral blood mononuclear cells prepared, cryopreserved, and shipped in liquid nitrogen from each participating site. All sites preparing the cells were accredited before the study start to ensure cells with recovery greater than 70% and viability greater than 80% on thawing.

Overlapping 15-mer peptides (offset by two aminoacids) for ELISPOT and proliferation were synthesised by Schafer-N (Copenhagen, Denmark). Interferon-γ ELISPOT analyses were done at the Division of Immunology and Allergy, Lausanne University Hospital (Lausanne, Switzerland) for all European samples and at UC Davis Immunology Specialty Laboratory (Sacramento, CA, USA) for all US samples as previously described[13] at weeks 1 (baseline), 6, 28, 44, and 52. Peripheral blood mononuclear cells were rested for 6 h after thawing and subsequently stimulated in triplicate wells in which the final amount of each antigen was 1 μg per well (15-mer peptide pools of Vacc-4x peptides, 15-mer pool for $p24^{Gag}$ corresponding to the same regions as Vacc-4x). Staphylococcal enterotoxin B (Sigma-Aldrich AG, St Louis, MO, USA) and media constituted positive and negative controls, respectively. Assays were deemed valid if the negative control had 50 spot forming units (SFU) per 10^6 cells or fewer and the positive control had greater than 500 SFU per 10^6 cells. An interferon-γ ELISPOT response was defined as positive if the triplicate test wells had a mean SFU four times or greater than the negative control and at least 55 SFU per 10^6 cells. A participant was defined as a responder if there was at least one positive response against any of the antigens and a negative response at baseline or if the response was two times the baseline value or higher.

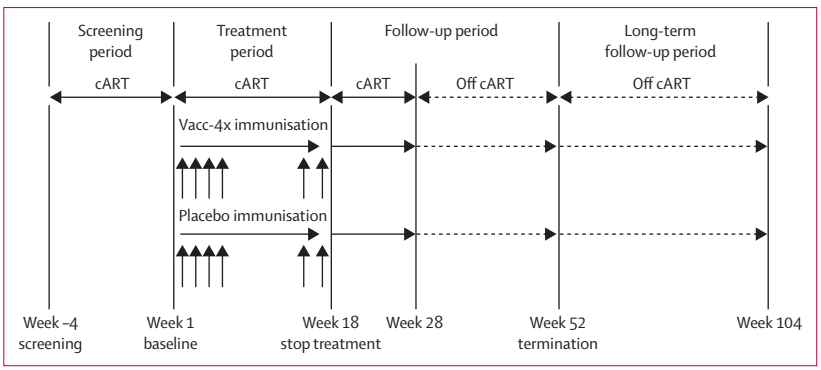


Figure 1: **Study schedule**
cART=combination antiretroviral therapy.

Ex-vivo T-cell proliferation analysis was done by use of a flow cytometry method at the Division of Immunology and Allergy, Lausanne University Hospital for all samples. After thawing and an overnight rest, peripheral blood mononuclear cells were labelled with 0·25 μmol/L 5,6-carboxy-fluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, OR, USA) as described elsewhere[13,14] and stimulated with Vacc-4x 15-mer or $p24^{Gag}$ 15-mer (1 μg/mL of each antigen). Staphylococcal enterotoxin B stimulation (100 ng/mL) served as positive control and media as negative. At day 6, cells were harvested, labelled with Aqua LIVE/DEAD stain kit (Invitrogen, Eugene, OR, USA), and then stain with CD4-ECD (Becton Dickinson, San Jose, CA, USA), CD8-PB (Becton Dickinson). Cells were fixed with CellFix (Becton Dickinson), acquired on an LSR II (Becton Dickinson), and analysed on FlowJo (version 8.8.2; Tree Star, Ashland, OR, USA). The number of lymphocyte-gated events was between 3×10^5 and 1×10^6. A positive proliferation assay response was defined as the percentage CFSE low dividing cells reactive to a specific Vacc-4x-related test antigen after baseline being three times or greater than the percentage CFSE low dividing cells in the media control.

Adverse events were recorded continuously throughout the study. Vital signs, physical examination, and clinical laboratory assessments were done at baseline and at selected timepoints throughout the study. The proportion of participants who regained virus suppression (<50 copies per mL) on cART resumption was assessed. Interleukin 6 measurements (eBioscience, San Diego, CA, USA) and detection of antibodies to recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF; USCN Life, Wuhan, China) were done by Bionor Laboratories (Skien, Norway).

Outcomes

Coprimary efficacy objectives were to establish the proportion of participants who met the criteria for resumption of cART between the interruption of cART at week 28 and the end of the study at week 52 and percentage change in CD4 counts between week 28 (interruption of cART) and the last CD4 count assessment before resumption of cART or week 52 if the participant did not resume cART in this period.

Secondary efficacy objectives were comparisons of time to restart of cART, changes in CD4 and CD8 counts, and changes in HIV RNA concentrations for Vacc-4x treated participants versus placebo. Immunogenicity was established with T-cell responses to Vacc-4x and $p24^{Gag}$ regions corresponding to Vacc-4x, in peripheral blood mononuclear cells ex vivo by use of interferon-γ ELISPOT and T-cell proliferation.

Several additional exploratory analyses were done with only those participants in the modified intention-to-treat (mITT) group (ie, all participants who received at least one intervention) who achieved a 6 month cART-free period from week 28 to study end (week 52). These were categorised as the offARTwk52 subgroup.

Statistical analysis

This study was originally designed to enrol 345 participants to have a minimum of 90% power, for a two-sided test at the 5% significance level with an assumed 15% non-evaluability rate, to detect a clinically

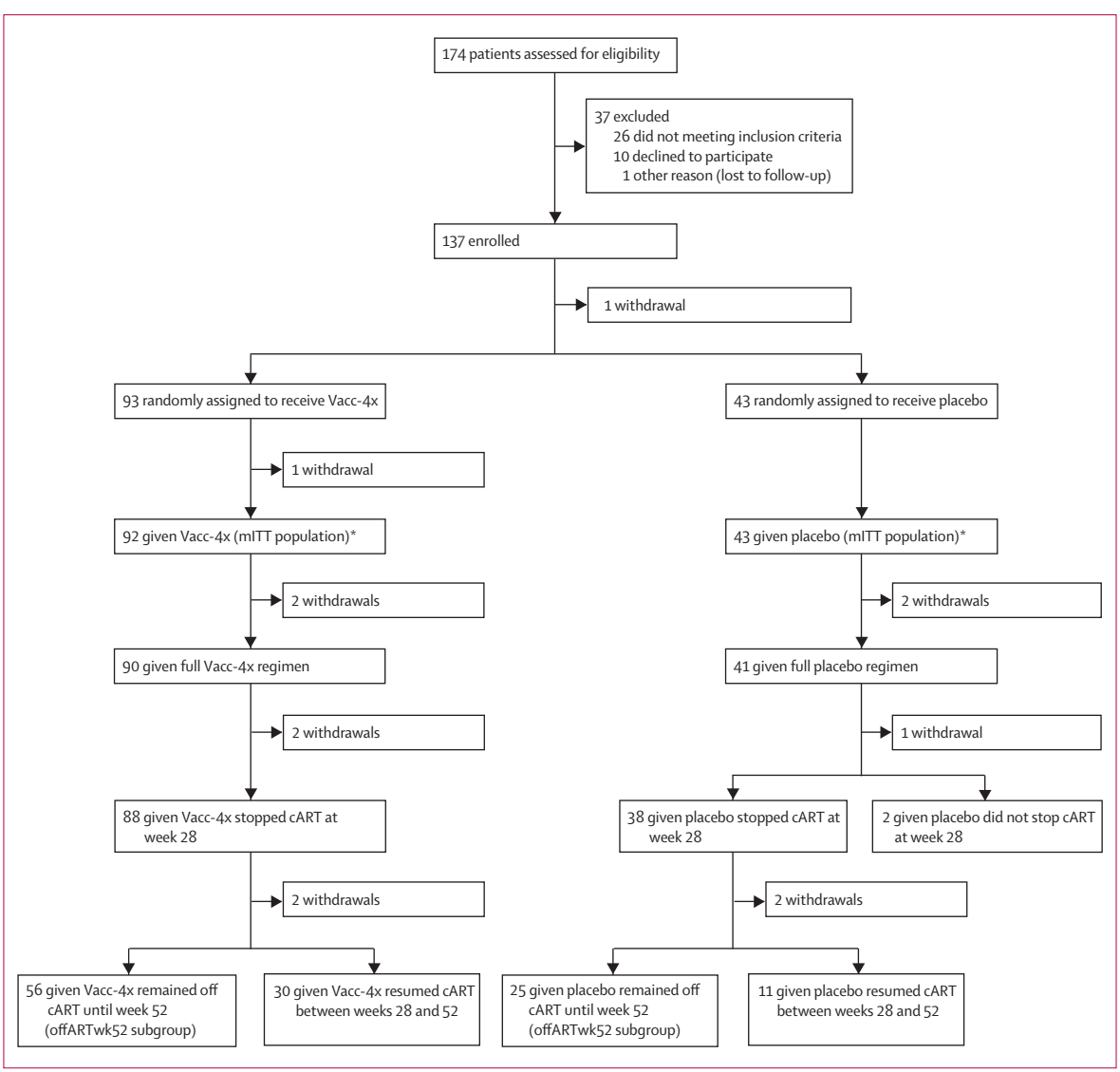


Figure 2: **Trial profile**

mITT=modified intention to treat. cART=combination antiretroviral therapy. *Safety data was based on 93 Vacc-4x participants and 42 placebo participants because one placebo participant received an injection with Vacc-4x in error.

relevant treatment difference of 20 percentage points for the primary endpoint proportion of participants who resumed cART between interruption of cART at week 28 and the end of the study at week 52 (assumed 40% Vacc-4x and 60% placebo participants needing to resume cART, which corresponds to an odds ratio [OR] of 0·44). No sample sizing on participant subgroup outcomes was envisaged at the outset of the study. However, because of slow enrolment as a result of changing attitudes to clinical studies involving cART interruption by both patients and clinicians, particularly in view of the SMART study,[15] only 137 participants were actually enrolled. 135 of these participants provided the study population pool for the exploratory analyses. Under the same original sample sizing assumptions, but with only the reduced 135 participants (92 Vacc-4x, 43 placebo) the study power to detect the same clinically relevant treatment difference is reduced to 51%.

Statistical analyses were done with standard statistical software (SAS 9.2 and SPSS 17.0). All analyses compared treatment groups (Vacc-4x *vs* placebo) unless specifically stated otherwise, and analyses were done with two-sided hypothesis tests. Because of the midtrial decision to reduce the study sample size and because, on inspection, the viral-load data tended to be non-normal, a non-parametric analysis approach was selected to analyse viral-load endpoints.

Any data from participants after cART resumption were excluded from the efficacy analyses. For the coprimary endpoints (analysis with all available participants) the proportion of participants who met the criteria for cART resumption was analysed with a logistic regression model with the treatment effect being assessed in a model including country as a covariate. Percentage change in CD4 count between week 28 (interruption of cART) and the last CD4 count assessment before cART resumption was analysed with ANOVA, including terms for treatment group and country in the model (data not shown).

For the secondary endpoint (analysis with all available participants) time to restart of cART was assessed by use of Kaplan-Meier estimation and the log-rank test (data not shown). Furthermore, viral load was analysed at each timepoint during the treatment interruption period using the Wilcoxon rank sum test.

For the exploratory analysis (offARTwk52 subgroup) the viral-load setpoint, comparing treatment groups, was analysed with the Wilcoxon rank sum test. The viral-load setpoint was compared with the preART viral-load setpoint separately for each treatment group with the Wilcoxon signed rank test.

The proportion of interferon-γ ELISPOT responders to $p24^{Gag}$ at any timepoint after baseline was compared between treatment groups with logistic regression, adjusting for the country where participants were enrolled (data not shown). Separate subgroup analyses of ELISPOT positive and negative responders at week 52 to $p24^{Gag}$ were done, analysing the viral-load setpoint, comparing treatment groups using the Wilcoxon rank sum test. Furthermore, separately within each treatment group, positive and negative responders were similarly compared. This study is registered with ClinicalTrials.gov, number NCT00659789.

Role of the funding source

The sponsor of the study was involved in study design, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

This study was done between July, 2008, and June, 2010; a 52 week follow-up period was completed in June, 2011 (figure 1). 136 participants were randomly assigned to receive study treatment (figure 2), but a withdrawal from the Vacc-4x group after randomisation left 135 in the

	Vacc-4x participants	Placebo participants
Modified intention-to-treat population*		
Sex		
Female	14 (15%)	5 (12%)
Male	78 (85%)	38 (88%)
Ethnic origin		
Black or African American	4 (4%)	5 (12%)
White	88 (96%)	37 (86%)
Asian	0	1 (2%)
Age, years	44 (28–55)	45 (20–54)
Time since diagnosis, months	175 (21–398)	203 (37–407)
Time on ART, months	95·5 (13–276)	112 (13 –197)
Nadir CD4, cells per μL	300 (200–734)	285 (200–724)
PreART CD4, cells per μL†	339 (177–1396)	370 (207–924)
Prestudy CD4, cells per μL	712 (342–1478)	619 (342–1429)
PreART viral load, copies per mL‡	80 468 (120–2 500 000)	36 055 (412–1 076 500)
offARTwk52 subgroup§		
Sex		
Female	11 (20%)	1 (4%)
Male	45 (80%)	24 (96%)
Ethnic origin		
Black or African American	3 (5%)	4 (16%)
White	53 (95%)	21 (84%)
Age, years	43·5 (28–53)	45·0 (20–54)
Time since diagnosis, months	186 (28–398)	199 (37–381)
Time on ART, months	109·5 (17–276)	121 (13–184)
Nadir CD4, cells per μL	300 (200–734)	327 (216–724)
PreART CD4, cells per μL	339 (177–1396)	385 (219–924)
Prestudy CD4, cells per μL	744 (430–1404)	599 (366–1002)
PreART viral load, copies per mL¶	60 470 (120–2 500 000)	52 731 (412–1 076 500)

Data are n (%) or median (range). *92 Vacc-4x and 43 placebo participants. †80 Vacc-4x and 39 placebo participants. ‡70 Vacc-4x and 32 placebo participants. §56 Vacc-4x and 25 placebo participants. ¶45 Vacc-4x and 18 placebo participants.

Table 1: **Baseline characteristics**

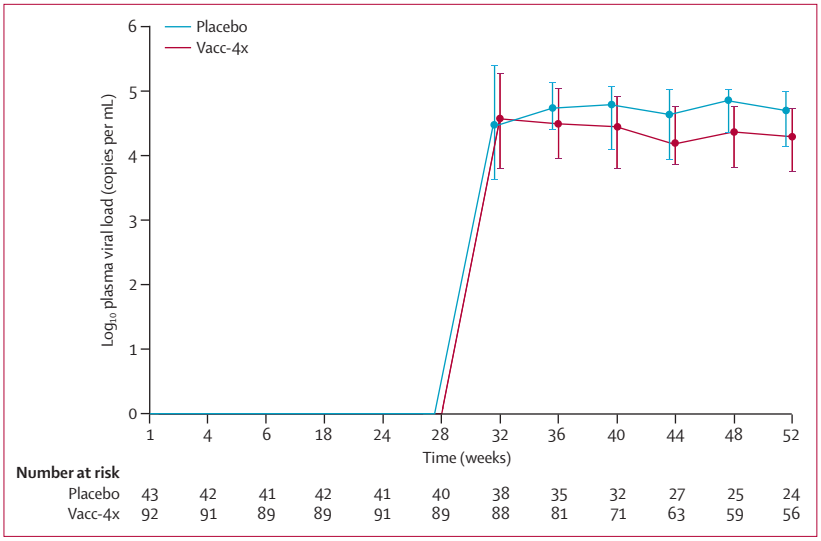


Figure 3: **Viral load over time in the intention-to-treat population**
Data are median (IQR).

	Vacc-4x	Placebo
offARTwk52 subgroup*		
Median viral-load setpoint	22 300	61 900
offARTwk52 subgroup with corresponding preART values†		
Median viral-load setpoint	24 150	50 400
Median preART viral-load setpoint	60 470	52 731

*56 Vacc-4x participants and 25 placebo participants. †45 Vacc-4x participants and 18 placebo participants.

Table 2: **Viral-load setpoint in the offARTwk52 subgroup**

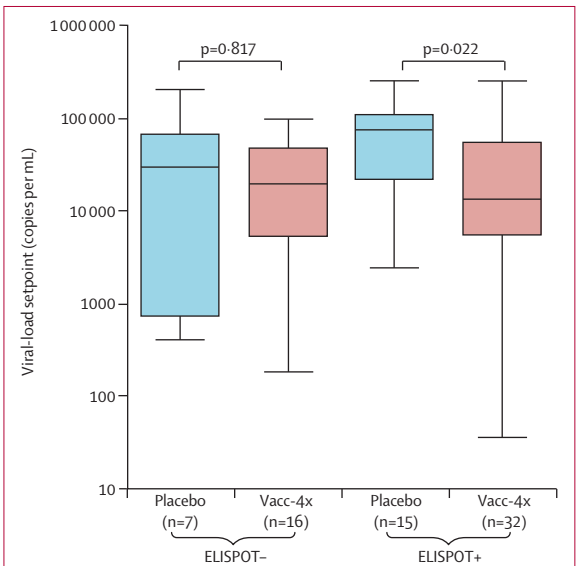


Figure 4: **ELISPOT positive and negative responders at week 52 to p24^{Gag} peptide pool in relation to viral-load setpoint for the offARTwk52 subgroup**
p values were established with Wilcoxon rank sum test. The box depicts the median and IQR, and the whiskers depict the range.

mITT population. Most participants in each group were male (>80%). There were no substantial differences between the two groups by age, sex, time of infection, and CD4 variables (table 1). The preART viral load was substantially higher in the Vacc-4x group than in the placebo groups in the ITT pobulation (table 1); however, this difference was not substantial in the offARTwk52 subgroup. Four individuals did not complete the immunisation schedule (in the Vacc-4x group one because of exacerbation of multiple sclerosis and one because the patient moved abroad; in the placebo group one because of pregnancy and one had no reason given). Five particpants did not undergo treatment interruption (in the Vacc-4x group two participants withdrew consent; in the placebo group one participant withdrew consent, one did not qualify for cART interruption, and one was lost to follow-up).

30 participants in the Vacc-4x group resumed cART between interruption of cART at week 28 and the end of the study at week 52 (34%) compared with 11 in the placebo group (29%; treatment difference OR 1·05, 95% CI 0·49–2·29; p=0·89). The 95% CI for the observed treatment difference OR crosses 1 and the lower limit is above the prespecified (within original sample-size calculation) clinically meaningful treatment difference of 0·44. Therefore, the recorded treatment difference is not significant and not clinically relevant. The reasons for restarting cART before week 52 for Vacc-4x versus placebo were because of AIDS-defining illness (one *vs* one), reduced CD4 count (15 *vs* eight), increase in viral load (two *vs* none), both CD4 and viral-load responses (two *vs* none), patient or investigator decision (nine *vs* two), and lymphadenopathy in one Vacc-4x recipient. Similarly the time to return to cART was not different (median 198 days in the Vacc-4x group *vs* 175 days in the placebo group; p=0·77). There was also no significant difference in the percentage change in CD4 cell counts between week 28 (interruption of cART) and the last CD4 cell count assessment before cART resumption (mean treatment difference –5·71, 95% CI –13·01 to 1·59; p=0·12).

For viral load, there was a statistically significant difference at weeks 48 (median 23 100 copies per mL in the Vacc-4x group *vs* 71 800 copies per mL in the placebo group; p=0·025) and 52 (median 19 550 copies per mL in the Vacc-4x group *vs* 51 000 copies per mL in the placebo group; p=0·041; figure 3).

Since not all participants achieved a 6 month cART-free period, the offARTwk52 subgroup was used to compare viral-load setpoint at the end of the study period. There was a significant reduction in median viral-load setpoint between Vacc-4x and placebo in the offARTwk52 subgroup corresponding to a 0·44 log reduction (p=0·040; table 2).

When this analysis was extended to include all participants who remained off ART for at least 12 weeks (ie, until study week 40), the results show a non-significant

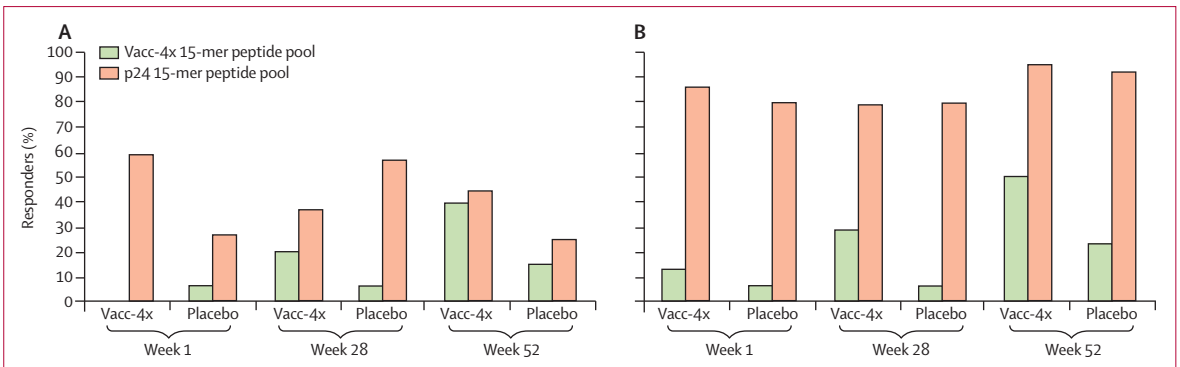
(p=0·149) difference between the study groups in viral-load setpoint also favouring the vaccine (appendix). PreART viral-load values were available for 63 participants in this subgroup. There was a significant reduction in median viral-load setpoint compared with preART values in Vacc-4x participants in the offARTwk52 subgroup (0·40 log reduction; p=0·0001). By contrast, the difference was not significant for the placebo subgroup (0·02; p=0·98) where the viral-load setpoint returned to roughly preART levels (table 2).

The proportion of interferon-γ ELISPOT responders at any timepoint to $p24^{Gag}$ (in the region spanning Vacc-4x) in Vacc-4x and placebo participants in the offARTwk52 subgroup did not differ between the two groups (data not shown). A significant difference in viral-load setpoint between interferon-γ ELISPOT responders to $p24^{Gag}$ in the Vacc-4x and placebo groups was noted in the offARTwk52 subgroup (median 13 425 copies per mL in the Vacc-4x group *vs* 76 600 copies per mL in the placebo group; p=0·022; figure 4). For the non-responders, the difference was not significant (median 19 650 copies per mL in the Vacc-4x group *vs* 30 000 copies per mL in the placebo group; p=0·82). Furthermore, there were no significant differences when comparing responders with non-responders for the Vacc-4x group (p=0·70), and separately comparing responders with non-responders for the placebo group (p=0·17).

We assessed the proliferative capacity of HIV-specific CD4 and CD8 T cells after Vacc-4x immunisation. Clear increases in assay responses to Vacc-4x antigens were noted in the Vacc-4x group (percentage of CFSE low CD4 T cells for week 1=0/30, week 28=6/30 [20%], week 52=10/25 [40%]; percentage of CFSE low CD8 T cells for week 1=4/32 [13%], week 28=9/31 [29%], week 52=13/26 [50%]) in the offARTwk52 subgroup over time, by contrast with the placebo group (percentage of CFSE low CD4 T cells for week 1=1/16 [6%], week 28=1/15 [7%], week 52=2/13 [15%]; percentage of CFSE low CD8 T cells for week 1=1/16 [6%], week 28=1/16 [6%], week 52=3/13 [23%]; figure 5). The T-cell proliferative assay responses to p24 antigens were reasonably sustained over the study period (figure 5).

See Online for appendix

Vacc-4x was safe and well tolerated (ITT population). The proportion of patients with adverse events described as "at least probably related to treatment" was greater in the Vacc-4x (81/93; 87%) than the placebo group (24/42; 57%). The difference was chiefly attributable to a higher rate of injection-site reactions (most commonly injection site erythema, induration, or pruritus); the adverse event profile was otherwise similar between the groups. A total of nine serious adverse events were recorded, five of which were in the Vacc-4x group. One serious adverse event, exacerbation of multiple sclerosis, was classified as possibly related to study treatment. One placebo participant died from myocardial infarction at week 43 while off cART. When participants resumed their original cART regimens, they responded well to reinitiation, and were well controlled (viral load <50 copies). Interleukin 6 concentrations remained within normal range, or showed no increase between baseline and week 52 (two participants displayed a four-times increase between baseline and week 52). No elevation in anti-GM-CSF antibody titre between baseline and week 6 was noted for the first 20 participants (no



	Week 1		Week 28		Week 52	
	Vacc-4x	Placebo	Vacc-4x	Placebo	Vacc-4x	Placebo
Number of participants assessed at each timepoint						
Percentage of CFSE low CD4 T cells	30	16	30	15	25	13
Vacc-4x 15-mer peptide pools	27	15	27	14	20	12
p24 15-mer peptide pools						
Percentage of CFSE low CD8 T cells	32	16	31	16	26	13
Vacc-4x 15-mer peptide pools	29	15	28	15	21	12
p24 15-mer peptide pools						

Figure 5: **Proliferative assay responses in CD4 and CD8 T-cell populations to Vacc-4x and p24 antigens (15-mer) for the offARTwk52 subgroup**
Percentage for CFSE low CD4 (A) and CD8 (B) T cells. CFSE=5,6-carboxy-fluorescein diacetate succinimidyl ester.

participants achieved a four-times increase between baseline and week 6) and was therefore not assessed for the remaining participants.

Discussion

Despite no differences between the Vacc-4x and placebo groups for the coprimary efficacy endpoints (cART resumption and changes in CD4 count over time), there was a significant reduction in viral load in the Vacc-4x group compared with placebo at the last two timepoints during cART interruption. Additional exploratory analyses were done on the subgroup of patients who successfully remained off cART for the full 24 week period. This offARTwk52 subgroup analysis showed a significant reduction in viral-load setpoint in the Vacc-4x group compared with placebo and a highly significant reduction in viral-load setpoint from historical preART values. By contrast, the viral-load setpoint for participants in the placebo group was generally the same or higher than their preART value, similar to other studies when cART is interrupted in the absence of a therapeutic intervention.[16] Of note, preART viral-load values were higher in the Vacc-4x compared with placebo groups.

GM-CSF was used as a local adjuvant in this study. It was not used in the placebo group because of a suggestion from the FDA to use a true placebo in the trial. The potential direct effects of GM-CSF in HIV infection have been investigated in several studies, results from which have varied, from favourable effects on plasma viral load to increases in plasma viral load.[17,18] Accounting for the substantially lower dose, different route of administration, and the 10 week interval from the last dose of GM-CSF to initiation of the treatment interruption, it is highly unlikely that GM-CSF has had an effect on the study outcomes.

T-cell responses were measured ex vivo using interferon-γ ELISPOT and proliferation. Since Vacc-4x aims to improve immune responses to immunologically vulnerable conserved domains on HIV-1 p24^{Gag}, responses to these conserved regions were our major focus. T-cell responses were noted to increase at weeks 44 and 52 (off ART) as a result of viral rebound. However, the observation that ELISPOT responders at week 52 in the Vacc-4x group had a lower viral-load setpoint than ELISPOT responders in the placebo group suggests a potential qualitative difference in the vaccine-induced immune response similar to that reported in earlier studies (panel).[5,37] Findings from the previous Vacc-4x phase 2 study showed very low immune escape in these conserved regions in participants who had received Vacc-4x.[38] Vacc-4x induced proliferative responses in both CD4 and CD8 T-cell subsets, while there was no corresponding increase in T-cell proliferation in the placebo group. Further analysis will be needed to establish whether a responder profile can be characterised and a surrogate marker identified for favourable outcome.

Vacc-4x was generally safe and well tolerated. Treatment interruption was well tolerated in most participants, in agreement with other therapeutic vaccine and treatment interruption trials.[5,15,19,20] However, one placebo recipient did experience a fatal myocardial infarction during cART interruption, which the investigator could not exclude as possibly related to study participation since previous studies have linked cART interruption to increased levels of inflammation and increased risk of cardiovascular events;[15] however, the measured inflammatory marker (high-sensitivity C-reactive protein) was not raised in this case.

Our study has several limitations. It is recognised that because of the smaller number of recruited participants than originally planned (137 *vs* 345) and the use of additional analyses, done on a subgroup of participants who achieved a 52 week off-ART period, these data need to be considered as exploratory. It was considered to increase the number of clinical trial sites to improve the

Panel: Research in context

Systematic review

On Aug 4, 2013, we searched PubMed for reports published at any time previously, with the term "HIV vaccine trial viral-load treatment interruption". 20 published reports of clinical trials assessing various HIV therapeutic vaccine approaches, all of which included a "structured" or "analytical" cART interruption phase, were identified.[4,5,11,12,19–35] These investigations have included various HIV-specific peptide approaches delivered either directly or through viral vectors (eg, adenovirus, canarypox, fowl pox, modified vaccinia Ankara), DNA plasmids, or even as peptide loaded dendritic cells. Many of these clinical studies have been done in small sample sizes (ie, ≤30 participants),[19,21,24,26,27,29,33,34,36] and often in a non-controlled manner,[11,21,24,33,34] making definitive conclusions difficult. Very few investigators have been able to correlate specific vaccine-induced immune responses with viral-load outcomes.[5,11] Indeed some have reported significant immunogenicity leading to higher viral rebound after cART interruption.[28]

One of the most promising approaches recently reported is that of Felipe García and colleagues,[5] who created an HIV-specific therapeutic vaccine by modifying patients' own dendritic cells. They showed that 12 weeks after cART interruption, a decrease of plasma viral-load setpoint of 1 log or greater was noted in 12 of 22 patients (55%) in the vaccinated group versus one of 11 (9%) in the control group, and, at 24 weeks interruption, in seven of 20 (35%) in the vaccinated group versus none of ten in the control group. This substantial decrease in plasma viral load was associated with increases in HIV-1-specific T-cell responses but waned over time. Also the broad applicability of a designer therapeutic vaccine approach remains to be realised.

Interpretation

We have successfully completed a large, randomised, placebo-controlled clinical trial to assess Vacc-4x. We have shown that Vacc-4x, a peptide-based therapeutic vaccine designed to improve and sustain immune responses to conserved domains on the HIV-1 core protein p24^{Gag}, is one of the few HIV therapeutic vaccines assessed so far that seems to induce proliferative responses in both CD4 and CD8 T-cell populations and contribute to a viral-load reduction after cART interruption for up to 24 weeks. Our findings suggest that Vacc-4x could be further investigated as an important component of a broader HIV cure strategy because it is now recognised that present efforts to purge the HIV viral reservoirs fail to reduce HIV-1 persistence.[4] By boosting HIV-1-specific cytotoxic T lymphocytes before reactivating latently HIV-infected cells it is thought possible to achieve reductions in HIV-1 viral reservoirs allowing functional cure of HIV infection.

recruitment rate; however, feedback from participating clinical investigators and the study sponsors made the feasibility and probable success of achieving complete recruitment within a reasonable timeframe unlikely, in view of the climate after the release of the SMART data. A protracted recruitment phase might also have resulted in study population differences, which might have affected the analysis. It was therefore agreed with regulatory agencies that redefining the study as exploratory would allow several hypothesis-generating analyses to be done.

The downsizing of the study cohort compared with the original plan made it more difficult to capture treatment-related effects. Despite this, the study remains one of the largest controlled clinical trials assessing an HIV therapeutic vaccine and the data described here provide valuable information on Vacc-4x. Even if the rate of cART resumption during the treatment interruption period was balanced between the two groups, this attrition might have introduced a selection bias favouring better outcomes that needs to be considered when interpreting the data.

In conclusion, we believe the study reported here provides initial proof of concept to support a role for therapeutic HIV vaccines, since the finding that Vacc-4x is immunogenic and capable of changing plasma viral-load setpoint after an analytical treatment interruption of cART. The mechanism behind this apparent effect remains to be elucidated, but the results accord with other studies, which have shown that immune responses to conserved regions of p24 are associated with better viral control. Therapeutic vaccines, such as Vacc-4x, might have a future role in complementing cART regimens, to improve or sustain HIV-specific immune responses and control viral load. Furthermore, there is now a growing interest in the potential contribution of therapeutic vaccination to aid functional cure through combination with histone deacetylase inhibitors, which have the potential to reactivate virus replication from the latent pool and thus generate CD4 T cells expressing HIV antigens that might serve as a target to the T-cell response enhanced by therapeutic vaccination.[3,4] With either approach, therapeutic vaccination would need to result in greater and more sustained viral-load reductions than that shown by candidate therapeutic vaccines so far. It remains to be established whether viral load can be reduced even further if Vacc-4x is combined with other interventions or after reboosting and a second treatment interruption. Such assessments are underway at present (registered with ClinicalTrials.gov, numbers NCT01704781 and NCT01712256).

Contributors

RBP was the study coordinator for the USA. JKR was the study coordinator for Europe. BP, AL, FG, DP, DMA, JvL, KA, DS, BC, WDH, RM, GM, AP, MFish, GF, MFisc, and BT were all principal investigators. GP and KE did the immunological analyses. IB was involved in protocol preparation, registration, and oversaw the study. DJ and SP did the statistical analyses. ØJ managed the serological analyses. A-OH contributed to the ELISPOT analyses. MAS liaised with the immunology central laboratories and wrote the first drafts of the report. BS initiated the study process. GP, JKR, RBP, and VW-H did the final edits.

Conflicts of interest

MAS, A-OH, and VW-H are employees of Bionor Pharma. MAS, VW-H, and BS also own shares in Bionor Pharma. RBP, JKR, BP, and GP are members of Bionor Pharma's clinical advisory board. The remaining authors declare that they have no conflicts of interest.

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References

1 Antiretroviral Therapy Cohort Collaboration. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet* 2008; **372:** 293–99.

2 Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med* 2011; **365:** 493–505.

3 Cohen J. The emerging race to cure HIV infections. *Science* 2011; **332:** 784–89.

4 Shan L, Deng K, Shroff NS, et al. Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. *Immunity* 2012; **36:** 1–11.

5 García F, Climent N, Guardo AC, et al. A dendritic cell-based vaccine elicits T cell responses associated with control of HIV-1 replication. *Sci Transl Med* 2013; **5:** 166ra2.

6 Kiepiela P, Ngumbela K, Thobakgale C, et al. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. *Nat Med* 2007; **13:** 46–53.

7 Dahirel V, Shekhar K, Pereyra F, et al. Coordinate linkage of HIV evolution reveals regions of immunological vulnerability. *Proc Natl Acad Sci USA* 2011; **108:** 11530–35.

8 Åsjö B, Stavang H, Sørensen B, Baksaas I, Nyhus J, Langeland N. Phase I trial of a therapeutic HIV type 1 vaccine, Vacc-4x, in HIV type 1-infected individuals with or without antiretroviral therapy. *AIDS Res Hum Retroviruses* 2002; **18:** 1357–65.

9 Kran AM, Sørensen B, Nyhus J, et al. HLA- and dose-dependent immunogenicity of a peptide-based HIV-1 immunotherapy candidate (Vacc-4x). *AIDS* 2004; **18:** 1875–83.

10 Lind A, Sommerfelt MA, Holmberg JO, Baksaas I, Sørensen B, Kvale D. Intradermal vaccination of HIV-infected patients with short HIV Gag p24-like peptides induces CD4 + and CD8 + T cell responses lasting more than seven years. *Scand J Infect Dis* 2012; **44:** 566–72.

11 Kran AMB, Sommerfelt MA, Sørensen B, et al. Reduced viral burden amongst high responder patients following HIV-1 p24 peptide-based therapeutic immunization. *Vaccine* 2005; **23:** 4011–15.

12 Schooley RT, Spritzler J, Wang H, et al. AIDS clinical trials group 5197: a placebo-controlled trial of immunization of HIV-1-infected persons with a replication-deficient adenovirus type 5 vaccine expressing the HIV-1 core protein. *J Infect Dis* 2010; **202:** 705–16.

13 Bart P-A, Goodall R, Barber T, et al. EV01: a phase I trial in healthy HIV negative volunteers to evaluate a clade C HIV vaccine, NYVAC-C undertaken by the EuroVacc Consortium. *Vaccine* 2008; **26:** 3153–61.

14 Cellerai C, Perreau M, Rozot V. Proliferation capacity and cytotoxic activity are mediated by functionally and phenotypically distinct virus-specific CD8 T cells defined by interleukin-7R a (CD127) and perforin expression. *J Virol* 2010; **84:** 3868–78.

15 Strategies for Management of Antiretroviral Therapy (SMART) Study Group, El-Sadr WM, Lundgren J, et al. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med* 2006; **355:** 2283–96.

16 Wit FWNM, Blackenberg DH, Brinkman K, et al. Safety of long-term interruption of successful anti-retroviral therapy: the ATHENA cohort study. *AIDS* 2005; **19:** 345–48.

17 Angel JB, High K, Rhame F, et al. Phase III study of granulocyte-macrophage colony-stimulating factor in advanced HIV disease: effect on infections, CD4 cell counts and HIV suppression. *AIDS* 2000; **14:** 387–95.

18 Jacobson JM, Lederman MM, Spritzler J, et al. Granulocyte-macrophage colony-stimulating factor induces modest increases in plasma human immunodeficiency virus (HIV) type 1 RNA levels and CD4+ lymphocyte counts in patients with uncontrolled HIV infection. *J Infect Dis* 2003; **188:** 1804–14.

19 Gudmundsdotter L, Wahren B, Haller BK, et al. Amplified antigen-specific immune responses in HIV-1 infected individuals in a double blind DNA immunization and therapy interruption trial. *Vaccine* 2011; **29:** 5558–66.

20 Maggiolo F, Airoldi M, Callegaro A, et al. CD4 cell-guided scheduled treatment interruptions in HIV-infected patients with sustained immunologic response to HAART. *AIDS* 2009; **23:** 799–807.

21 Allard SD, De Keersmaecker B, de Goede AL, et al. A phase I/IIa immunotherapy trial of HIV-1-infected patients with Tat, Rev and Nef expressing dendritic cells followed by treatment interruption. *Clin Immunol* 2012; **142:** 252–68.

22 Li JZ, Brumme ZL, Brumme CJ, et al. Factors associated with viral rebound in HIV-1-infected individuals enrolled in a therapeutic HIV-1 gag vaccine trial. *J Infect Dis* 2011; **203:** 976–83.

23 Angel JB, Routy JP, Tremblay C, et al. A randomized controlled trial of HIV therapeutic vaccination using ALVAC with or without Remune. *AIDS* 2011; **25:** 731–39.

24 Kityo C, Bousheri S, Akao J, et al. Therapeutic immunization in HIV infected Ugandans receiving stable antiretroviral treatment: a phase I safety study. *Vaccine* 2011; **29:** 1617–23.

25 Papagno L, Alter G, Assoumou L, et al. ORVACS Study Group Comprehensive analysis of virus-specific T-cells provides clues for the failure of therapeutic immunization with ALVAC-HIV vaccine. *AIDS* 2011; **25:** 27–36.

26 Rosenberg ES, Graham BS, Chan ES, et al. Safety and immunogenicity of therapeutic DNA vaccination in individuals treated with antiretroviral therapy during acute/early HIV-1 infection. *PLoS One* 2010; **5:** e10555.

27 Gandhi RT, O'Neill D, Bosch RJ, et al. A randomized therapeutic vaccine trial of canarypox-HIV-pulsed dendritic cells *vs* canarypox-HIV alone in HIV-1-infected patients on antiretroviral therapy. *Vaccine* 2009; **27:** 6088–94.

28 Autran B, Murphy RL, Costagliola D, et al. Greater viral rebound and reduced time to resume antiretroviral therapy after therapeutic immunization with the ALVAC-HIV vaccine (vCP1452). *AIDS* 2008; **22:** 1313–22.

29 Emery S, Kelleher AD, Workman C, et al. Influence of IFNγ co-expression on the safety and antiviral efficacy of recombinant fowlpox virus HIV therapeutic vaccines following interruption of antiretroviral therapy. *Hum Vaccin* 2007; **3:** 260–67.

30 Goujard C, Marcellin F, Hendel-Chavez H, et al. Interruption of antiretroviral therapy initiated during primary HIV-1 infection: impact of a therapeutic vaccination strategy combined with interleukin (IL)-2 compared with IL-2 alone in the ANRS 095 Randomized Study. *AIDS Res Hum Retroviruses* 2007; **23:** 1105–13.

31 Kilby JM, Bucy RP, Mildvan D, et al. A randomized, partially blinded phase 2 trial of antiretroviral therapy, HIV-specific immunizations, and interleukin-2 cycles to promote efficient control of viral replication (ACTG A5024). *J Infect Dis* 2006; **194:** 1672–76.

32 Jacobson JM, Pat Bucy R, Spritzler J, et al. Evidence that intermittent structured treatment interruption, but not immunization with ALVAC-HIV vCP1452, promotes host control of HIV replication: the results of AIDS Clinical Trials Group 5068. *J Infect Dis* 2006; **194:** 623–32.

33 Ide F, Nakamura T, Tomizawa M, et al. Peptide-loaded dendritic-cell vaccination followed by treatment interruption for chronic HIV-1 infection: a phase 1 trial. *J Med Virol* 2006; **78:** 711–18.

34 Harrer E, Bäuerle M, Ferstl B, et al. Therapeutic vaccination of HIV-1-infected patients on HAART with a recombinant HIV-1 nef-expressing MVA: safety, immunogenicity and influence on viral load during treatment interruption. *Antivir Ther* 2005; **10:** 285–300.

35 Kinloch-de Loes S, Hoen B, Smith DE, et al. Impact of therapeutic immunization on HIV-1 viremia after discontinuation of antiretroviral therapy initiated during acute infection. *J Infect Dis* 2005; **192:** 607–17.

36 Hejdeman B, Leandersson AC, Fredriksson EL, et al. Better preserved immune responses after immunization with rgp 160 in HIV-1 infected patients treated with highly active antiretroviral therapy than in untreated patients with similar CD4 levels during at 2 years' follow-up. *HIV Med* 2003; **4:** 101–10.

37 Plana M, Garcia F, Oxenius A, et al. Relevance of HIV- 1-specific CD4+ helper T-cell responses during structured treatment interruptions in patients with CD4+ T-cell nadir above 400/mm³. *J Acquir Immune Defic Syndr* 2004; **36:** 791–99.

38 Kran A-MB, Jonassen TØ, Sommerfelt MA, Løvgården L, Sørensen B, Kvale D. Low frequency of amino acid alterations following therapeutic immunization with HIV-1 Gag p24-like peptides. *AIDS* 2010; **24:** 2609–18.