MAJOR ARTICLE

Impact of Therapeutic Immunization on HIV-1 Viremia after Discontinuation of Antiretroviral Therapy Initiated during Acute Infection

Sabine Kinloch–de Loes, Bruno Hoen, Don E. Smith, Brigitte Autran, Fiona C. Lampe, Andrew N. Phillips, Li-Ean Goh, Jan Andersson, Christos Tsoukas, Anders Sonnerborg, Giuseppe Tambussi, Pierre-Marie Girard, Mark Bloch, Manuel Battegay, Nick Carter, Raphaelle El Habib, Georgia Theofan, David A. Cooper, and Luc Perrin, for the QUEST Study Group[a]

(See the editorial commentary by Gandhi and Altfeld, on pages 556–9.)

Background. Treatment strategies that would induce durable virological control of human immunodeficiency virus (HIV)–1 in the absence of continued antiretroviral therapy (ART) are highly desirable.

Methods. We assessed, in a randomized, double-blind, placebo-controlled trial, whether the addition of therapeutic vaccines (ALVAC-HIV [vCP1452] or ALVAC-HIV and Remune) to ART initiated during acute infection could increase the probability of having a plasma viral load ⩽1000 HIV-1 RNA copies/mL 24 weeks after planned discontinuation of ART.

Results. All 79 randomized subjects completed the immunization schedule, and 78 discontinued ART with no major safety concerns. After immunization, subjects in the vaccine study arms had significantly increased HIV-1–specific $CD4^+$ and $CD8^+$ T cell responses, by interferon-γ enzyme-linked immunospot assay, compared with those in the placebo study arm. Overall, 17.7% of subjects had ⩽1000 HIV-1 RNA copies/mL 24 weeks after discontinuation of ART, with no significant difference between the vaccine study arms and the placebo study arm (15.4% vs. 22.2%; difference, −6.8% [95% confidence interval, −26.8% to 10.0%]; $P = .54$).

Conclusion. Therapeutic immunization and ART, compared with ART alone, generated HIV-1–specific cellular immunity but did not lead to better virological control of HIV-1 24 weeks after discontinuation of ART. Our trial design appears to be feasible and safe for testing future immune-boosting strategies.

Antiretroviral therapy (ART) has been highly successful in reducing morbidity and mortality from HIV-1 infection [1]. However, it cannot achieve viral eradication, and an increase in HIV plasma viral load (pVL) generally occurs after discontinuation of ART. Because of problems associated with long-term ART use—such as drug toxicity, nonadherence, risk of viral resistance, and cost—a treatment strategy that could enable durable virological control of HIV-1 pVL in the absence of continued ART is highly desirable [2–4].

Received 2 December 2004; accepted 15 March 2005; electronically published 15 July 2005.

Financial support: Sanofi pasteur provided ALVAC-HIV (vCP1452) and peptides for enzyme-linked immunospot (ELISPOT) analysis and paid transport costs for samples for ELISPOT assays and regulatory costs for submission of ALVAC-HIV; GlaxoSmithKline provided study funding, Combivir, abacavir, and amprenavir; The Immune Response Corporation provided Remune and peptides for ELISPOT analysis; Roche Molecular Diagnostics provided the Amplicor Monitor kits.

[a] Author affiliations and study group members are listed after the text.

Reprints or correspondence: Dr. Sabine Kinloch–de Loes, Dept. of Medicine, Royal Free Centre for HIV Medicine, Royal Free Campus, Royal Free and University College Medical School, 23 Pond St., London NW3 2QG, United Kingdom (sabine@kinloch.u-net.com).

The Journal of Infectious Diseases 2005; 192:607–17

0022-1899/2005/19204-0008$15.00

Presented in part: 11th Conference on Retroviruses and Opportunistic Infections, 8–11 February 2004, San Francisco, California (abstract 168); AIDS Vaccine 2004 meeting, 30 August–1 September 2004, Lausanne, Switzerland.

Potential conflicts of interest: Reimbursement for attending a symposium and/or receipt of a fee for speaking, a fee for organizing education, funds for research, funds for a staff member, fees for consulting, and/or stock options apply to the following authors: S.K.–d.L., Abbott Laboratories, Bristol-Myers Squibb (BMS), Gilead, GlaxoSmithKline (GSK), and Oxxon Therapeutics; B.H., BMS, Gilead, GSK, and Hoffmann–La Roche (Roche); D.E.S., Abbott Laboratories, BMS, Boehringer Ingelheim, Gilead, GSK, Merck Sharp and Dohme, and Roche; B.A., Aventis Pasteur and GSK; F.C.L., GSK and Oxxon Therapeutics; A.N.P., Abbott Laboratories, BMS, Boehringer Ingelheim, Gilead, GSK, Oxxon Therapeutics, Pfizer, Roche, and Tibotec; A.S., Abbott Laboratories, BMS, GSK, and Roche; P.-M.G., BMS, Gilead, GSK, and Roche; M. Bloch, GSK; M. Battegay, Abbott Laboratories, BMS, Gilead, GSK, Merck Sharp and Dohme, Roche, and Trimeris; D.A.C., Avexa, BMS, Boehringer Ingelheim, Gilead, GSK, Johnson & Johnson, Merck Sharp and Dohme, Pfizer, Roche, Oxxon Therapeutics, and Virax; and L.P., Abbott Diagnostics, GSK, Merck Sharp and Dohme, Roche, and Virco. L.-E.G. is a permanent employee of GSK and owns personal shares in the company. N.C. is a permanent employee of GSK. R.E.H. is a permanent employee of Sanofi pasteur. G. Theofan is a permanent employee of The Immune Response Corporation.

HIV-1–specific T cell immunity is considered to be crucial in the control of viral replication [5, 6]. It has been argued that the initiation of ART at a very early stage of infection, rather than later, might preserve HIV-1–specific $CD4^+$ T cell responses and $CD8^+$ T cell function and limit viral diversity and spreading in lymphoid tissue [7, 8]. However, ART alone may be insufficient, because continuous ART might reduce the antigenic stimulation of the HIV-1–specific $CD8^+$ T cells and the antibodies that are necessary for maintaining functional immunity. Therefore, in patients with acute HIV-1 infection, a treatment strategy in which ART is supplemented with an immunogenic HIV-1 vaccine may represent the best chance for long-term virological control after interruption of ART [9–11]. Here, we describe the results of an international, randomized, double-blind, placebo-controlled trial in which we tested the ability of 2 immunization regimens to induce virological control of HIV-1 24 weeks after discontinuation of ART in subjects who had initiated ART at the time of primary HIV-1 infection (PHI).

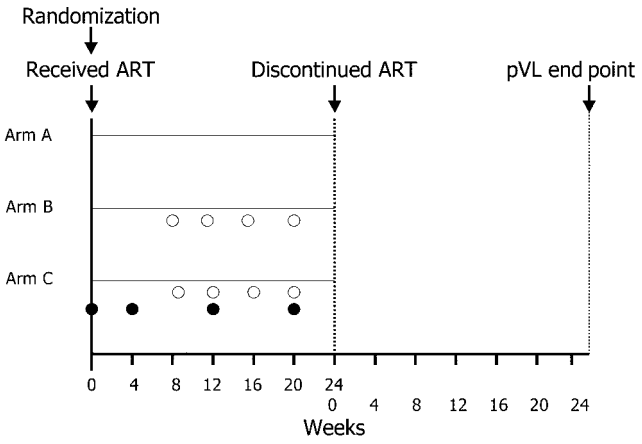

Figure 1. Study design and immunization schedule. The continuous line indicates antiretroviral therapy (ART), black circles indicate active Remune injections, and white circles indicate active ALVAC-HIV (vCP1452) injections. Arm A, ART, ALVAC-HIV placebo, and Remune placebo; arm B, ART, ALVAC-HIV, and Remune placebo; arm C, ART, ALVAC-HIV, and Remune; pVL, plasma viral load.

SUBJECTS, MATERIALS, AND METHODS

Study design. The QUEST study design has been described elsewhere (figure 1) [12]. Briefly, subjects initiated ART during PHI (≤3 bands on Western blot), continued ART for ≥72 weeks, and had pVLs <50 HIV-1 RNA copies/mL 4 weeks before randomization. The majority of subjects ($n = 69$) initiated Combivir, abacavir, and amprenavir. Ten subjects initiated ART containing 3 drugs. Subjects were divided among the following double-blind, placebo-controlled, randomized arms: arm A received ART, ALVAC-HIV (vCP1452) placebo, and Remune placebo; arm B received ART, ALVAC-HIV, and Remune placebo; and arm C received ART, ALVAC-HIV, and Remune. After a 20-week immunization period and 4 additional weeks of ART, subjects discontinued ART and were monitored for 24 weeks. Resumption of ART was advised for (1) severe clinical symptoms of acute retroviral syndrome with high pVL, (2) pVL >50,000 HIV-1 RNA copies/mL for >12 weeks, or (3) >50% $CD4^+$ T cell count decline from the count measured before discontinuation of ART.

The random allocation sequence was generated by an independent GlaxoSmithKline statistician (who used an internal system with block size 6 and stratification by country) and was concealed from investigators. Sites received numbered containers with individual code-break envelopes. Data management was coordinated by the GlaxoSmithKline Biomedical Data Sciences Group (Oakville, Ontario).

Vaccines. ALVAC-HIV (vCP1452) (Sanofi pasteur) is a modified recombinant canarypox virus [13]. It expresses HIV-1 *env* ($gp120_{MN}$) and *gag* (LAI) gene products and *nef* and *pol* gene products encompassing known human HLA-A2–restricted cytotoxic T cell (CTL) epitopes from these genes. Moreover, to enhance the overall efficiency of antigen expression, 2 vaccinia virus–derived genes, *E3L* and *K3L*, are included in the ALVAC genome. Remune is an inactivated envelope (gp120)–depleted whole virus immunogen in incomplete Freund's adjuvant (The Immune Response Corporation). Placebos were ALVAC-HIV placebo containing virus stabilizer and freeze-dried medium only and incomplete Freund's adjuvant [12]. Vaccines and/or placebos were administered intramuscularly every 4 weeks from randomization to week 20 while patients received ART (figure 1).

Outcome measures. The trial end points were (1) pVL ≤1000 HIV-1 RNA copies/mL at 24 weeks after discontinuation (and without resumption) of ART (primary end point), (2) pVL ≤400 HIV-1 RNA copies/mL throughout the 24-week period after discontinuation (and without resumption) of ART, and (3) time from discontinuation of ART to reach pVL >1000 HIV-1 RNA copies/mL. For end points 1 and 2, subjects who did not discontinue ART, resumed ART before week 24, or had missing data for pVL at week 24 were recorded as having treatment failure. For end point 3, subjects who did not discontinue ART were recorded as having treatment failure at time 0, and those who resumed ART were recorded as having treatment failure at the time ART was resumed. It was prespecified that the main analyses would compare end points between arm A and the combination of arms B and C using the intention-to-treat principle.

Statistical analysis. Sample size was limited by logistical considerations, and we aimed at enrolling 75–90 subjects, which allowed for ~80% power to detect a 30% difference between arms A and B/C for the primary end point. Proportions of successes according to end points 1 and 2 were compared between the 2 arms using the χ^2 test (or Fisher's exact test), and

95% confidence intervals (CIs) for the difference in proportions were calculated using the Wilson method (Confidence Interval Analysis version 2.0; University of Southampton). Time from discontinuation of ART to pVL >1000 HIV-1 RNA copies/mL was compared between the 2 arms using the log-rank test, and the hazard ratio (HR) and 95% CI were calculated. Values of (and changes in) virological and immunological parameters were summarized by medians and ranges or interquartile ranges (IQRs) and were compared between arms using the Mann-Whitney *U* test. Correlations between parameters were assessed using Spearman's rank correlation coefficient. In these additional analyses, subjects who withdrew from the study or resumed ART had their week 24 values imputed using the last-observation-carried-forward principle, provided that this observation was ⩾1 month after discontinuation of ART.

Laboratory methods. Testing of safety parameters, pVL measurements (Amplicor Monitor; Roche Molecular Diagnostics), and $CD4^+$ and $CD8^+$ T cell counts (standard flow cytometry) were performed at central laboratories. Cell-associated HIV-1 DNA and RNA analyses was performed at the Laboratory of Virology, Geneva University Hospital (Geneva, Switzerland), as described elsewhere [14].

Enzyme-linked immunospot (ELISPOT) assays. ELISPOT assay of HIV-1–specific interferon (IFN)–γ–producing $CD4^+$ T cells was performed on fresh cells at 5 academic laboratories using a single operating procedure established at the central laboratory (Cellular Immunology Laboratory, Pitié-Salpetrière Hospital, Paris) and derived from a method described elsewhere [15]. Briefly, cells were cultured for 40 h with HIV-1 recombinant p24 (2 μg/mL; Protein-Science) to trigger HIV-1 p24–specific $CD4^+$ T cells. Negative controls were obtained by culture with cells alone, and positive controls were obtained by culture with phytohemagglutinin (PHA; Abbott), streptokinase (Lederle), or crude extracts of cytomegalovirus (Bio-Wittaker). After washings and the addition of biotinylated second-step anti–IFN-γ monoclonal antibodies (Diaclone), spot-forming cells (sfc) were counted per 10^6 peripheral blood mononuclear cells (PBMCs) using an ELISPOT reader (Zeiss). Background values (obtained by culture with cells alone) were subtracted from positive responses. Responses that were ⩽100 sfc/10^6 PBMCs above background values were assigned a value of 0. Previous experiments confirmed that IFN-γ production after HIV-1 p24 stimulation was due primarily to $CD4^+$ T cells, because depletion of these cells resulted in an 85%–90% decrease in responses [16].

ELISPOT assay of HIV-1–specific IFN-γ–producing $CD8^+$ T cells was performed at the central laboratory, as described elsewhere [15]. Available frozen samples were shipped to the central laboratory in liquid nitrogen (in a dry shipper from Air Liquide), and IFN-γ–producing $CD8^+$ ELISPOT assays were performed in batches at the end of the study [15]. Cell samples with ⩾80% viability when assessed by trypan blue staining were tested using the same procedure as described above, except that incubation was overnight and antigens consisted of 11 pools of 15-mer synthetic peptides spanning the entire HIV-1 *gag* sequence encoded by vCP1452, overlapping by 11 aa, and composed of 9–11 peptides/pool [15]. Negative and positive controls were obtained by culture with cells alone and PHA, respectively. The sum of responses to HIV-1 Gag was calculated after background values were subtracted, and responses ⩽100 sfc/10^6 PBMCs above background values were assigned a value of 0. The origin of IFN-γ–producing cells after stimulation with this peptide pool had been confirmed using the same technique on CD8-depleted PBMCs (85% decrease in responses) [16]. IFN-γ–producing cells were also identified in 10 QUEST study samples that tested positive in response to these peptide pools in an intracellular cytokine assay [17]. These experiments confirmed that ~80% of the IFN-γ in peptide pool–stimulated PBMCs originated from $CD8^+$ T cells. The primary measures of HIV-1–specific immunity were responses to HIV-1 p24, for $CD4^+$ T cells, and to HIV-1 Gag, for $CD8^+$ T cells, at week 24 after randomization.

Ethics. This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Research Practice. All subjects provided signed, informed consent before enrollment. Independent local ethics committees and national gene therapy committees reviewed the protocol and its amendments. A data and safety monitoring board monitored the trial's progress. Clinicians, virologists, immunologists, and statisticians from Europe, Australia, and Canada formed the QUEST Core Group.

RESULTS

Subject recruitment and randomization. Of 172 subjects screened from February 1998 to November 1999, 149 initiated ART containing 4 drugs, 1 of whom was subsequently found to be HIV negative and was excluded (figure 2). Seventy-nine subjects discontinued ART, withdrew before the randomization phase, or were not eligible for randomization. From August 2000 to February 2002, the remaining 69 subjects and 10 additional subjects who entered the study under protocol amendment 10 (Europe, $n = 63$; Australia, $n = 13$; and Canada, $n = 3$) were randomized to arms A ($n = 27$), B ($n = 26$), and C ($n = 26$).

Characteristics of randomized subjects. Demographic, immunological, and virological factors at randomization were similar in arms A and B/C (table 1). Of the 79 randomized subjects, 72 (91.1%) were male, 77 (97.5%) were white, and 57 (72.2%) were homosexual. At randomization, 49 subjects (62.0%) were receiving ART containing 3 drugs, and 30 subjects (38.0%) were receiving ART containing ⩾4 drugs. In arm A, 13, 12, and 2 subjects were taking nucleoside reverse transcriptase inhibitors (NRTIs) only, NRTIs and protease inhibitors, or NRTIs and

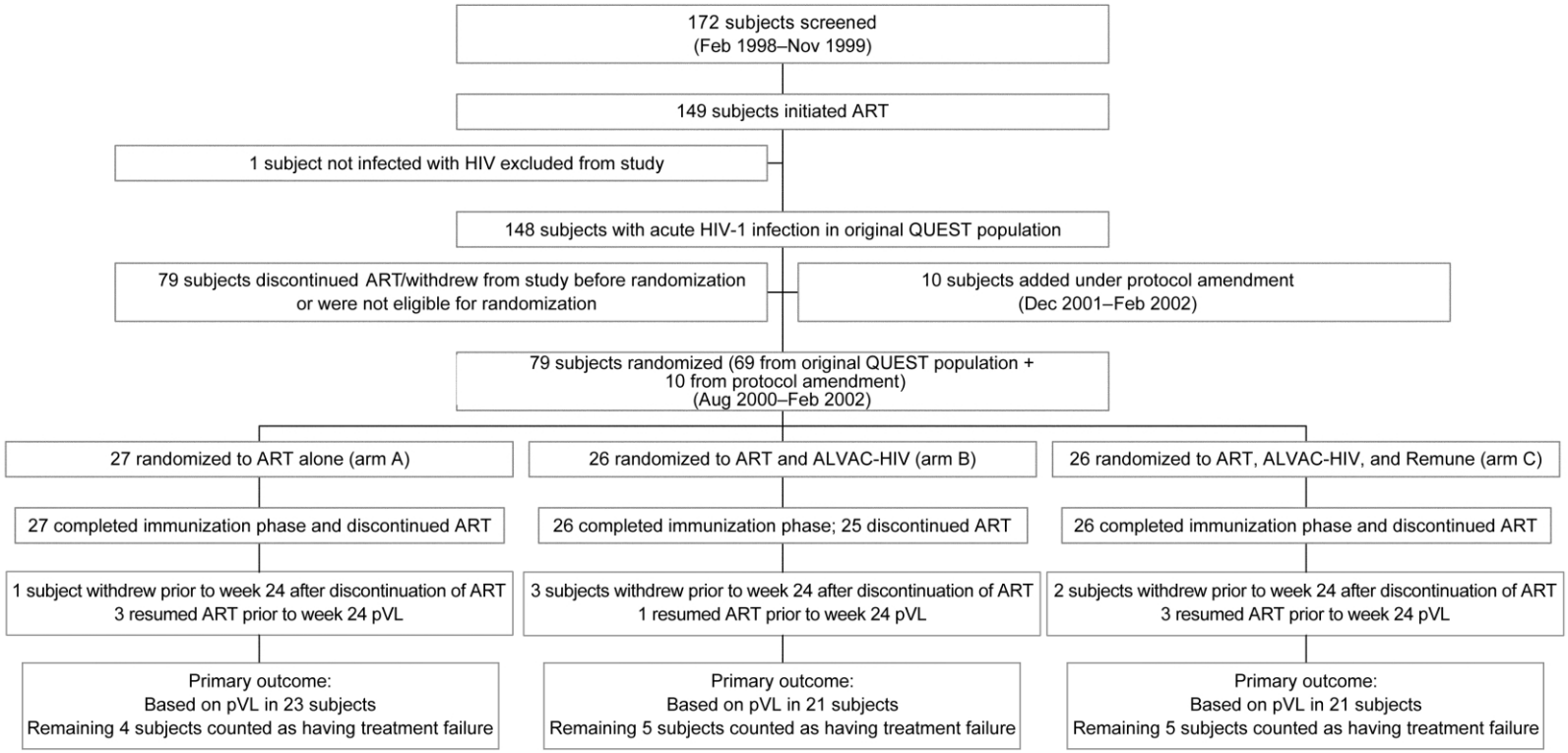

Figure 2. Flow diagram of patient recruitment and follow-up. ART, antiretroviral therapy; pVL, plasma viral load.

Table 1. Characteristics of the subjects at randomization.

	Arm A	Arm B/C	Arm B	Arm C
No.	27	52	26	26
Male sex, no. (%)	24 (88.9)	48 (92.3)	24 (92.3)	24 (92.3)
White ethnic status, no. (%)	27 (100.0)	50 (96.2)	24 (92.3)	26 (100.0)
Risk group, no. (%)				
Homosexual	20 (74.1)	37 (71.2)	18 (69.2)	19 (73.1)
Heterosexual	6 (22.2)	13 (25.0)	6 (23.1)	7 (26.9)
IDU/other	1 (3.7)	2 (3.8)	2 (7.7)	0
Age, median (range), years	37.4 (26.0–53.2)	36.6 (22.6–60.3)	36.6 (22.6–60.3)	36.2 (27.1–56.5)
ART, median (range), years	2.1 (1.5–5.2)	2.1 (1.4–5.3)	2.1 (1.4–3.8)	2.1 (1.5–5.3)
⩾4 ART drugs, no. (%)	10 (37.0)	20 (38.5)	8 (30.8)	12 (46.2)
$CD4^+$ T cell count, median (range), cells/mm^3	719 (327–1172)	795 (396–1451)	804 (451–1178)	771 (396–1451)
$CD8^+$ T cell count, median (range), cells/mm^3	605 (155–2397)	696 (240–2126)	677 (301–1647)	764 (240–2126)
Cell-associated HIV-1 RNA load, median (range), log copies/10^6 PBMCs	0.48 (0.48–2.24)	0.48 (0.48–2.31)	0.48 (0.48–1.53)	0.48 (0.48–2.31)
Cell-associated HIV-1 DNA load, median (range), log copies/10^6 PBMCs	1.38 (0.48–2.69)	1.26 (0.48–2.45)	0.95 (0.48–1.98)	1.37 (0.48–2.45)
Plasma HIV-1 RNA load ⩽50 copies/mL, no. (%)	26 (96.3)	49 (94.2)	25 (96.2)	24 (92.3)
Response to HIV-1 p24, median (range), sfc/10^6 PBMCs[a]	0 (0–260)	0 (0–617)	0 (0–617)	0 (0–437)
Response to HIV-1 Gag, median (range), sfc/10^6 PBMCs[b]	0 (0–170)	0 (0–1207)	0 (0–307)	0 (0–1207)

NOTE. Arm A, ART, ALVAC-HIV (vCP1452) placebo, and Remune placebo; arm B, ART, ALVAC-HIV, and Remune placebo; arm C, ART, ALVAC-HIV, and Remune; ART, antiretroviral therapy; IDU, injection drug user; PBMCs, peripheral blood mononuclear cells; sfc, spot-forming cells.

[a] By $CD4^+$ ELISPOT assay; arm A, $n = 17$; arm B/C, $n = 31$; arm B, $n = 16$; arm C, $n = 15$.

[b] By $CD8^+$ ELISPOT assay; arm A, $n = 15$; arm B/C, $n = 24$; arm B, $n = 11$; arm C, $n = 13$.

nonnucleoside reverse transcriptase inhibitors, respectively. In arm B/C, the respective numbers were 25, 23, and 4. The median duration of ART was 2.1 years (range, 1.4–5.3 years), and the median $CD4^+$ T cell count was 748 cells/mm^3 (range, 327–1451 cells/mm^3). The median cell-associated loads were 0.48 log HIV-1 RNA copies/10^6 PBMCs (range, 0.48–2.31 log HIV-1 RNA copies/10^6 PBMCs) and 1.30 log HIV-1 DNA copies/10^6 PBMCs (range, 0.48–2.69 log HIV-1 DNA copies/10^6 PBMCs). Four subjects had pVLs >50 HIV-1 RNA copies/mL (57, 90, 450, and 1067 HIV-1 RNA copies/mL).

Subject follow-up and adherence to protocol. All 79 subjects completed the immunization schedule, and 78 of 79 discontinued ART at a median of 25 weeks (range, 23–51 weeks) after randomization. Six subjects had no pVL measurement at week 24 after discontinuation of ART. In addition, 7 subjects resumed ART before week 24, and 3 of them fulfilled the protocol guidelines for resuming ART. Therefore, 14 subjects (4 in arm A and 10 in arm B/C) were considered to have automatic treatment failure for end points 1 and 2 (figure 2).

Immunological and virological parameters during the immunization phase. There were no significant differences between arms A and B/C in $CD4^+$ T cell count, pVL, or cell-associated HIV-1 RNA and DNA loads at week 24 after randomization or in changes in these parameters between randomization and week 24 after randomization. $CD4^+$ T cell counts tended to increase during the immunization phase: median values overall were 748 cells/mm^3 (range, 327–1451 cells/mm^3), 780 cells/mm^3 (range, 200–1350 cells/mm^3), 784 cells/mm^3 (range, 362–1559 cells/mm^3), and 778 cells/mm^3 (range, 303–1657 cells/mm^3) at 0, 8, 16, and 24 weeks after randomization, respectively. At week 24 after randomization, median $CD4^+$ T cell counts were 735 cells/mm^3 ($n = 27$) in arm A and 795 cells/mm^3 ($n = 49$) in arm B/C ($P = .36$); median changes from randomization were an increase of 59 cells/mm^3 and an increase of 57 cells/mm^3, respectively ($P = .83$). Median pVL was 0.48 log HIV-1 RNA copies/mL throughout the immunization phase. Fourteen subjects (2 [7.4%] in arm A and 12 [23.1%] in arm B/C; $P = .12$) had at least 1 measurement of pVL >50 HIV-1 RNA copies/mL during this period. Median cell-associated HIV-1 DNA load increased from 1.30 log HIV-1 DNA copies/10^6 PBMCs (range, 0.48–2.69 log HIV-1 DNA copies/10^6 PBMCs) overall to 1.56 log HIV-1 DNA copies/10^6 PBMCs (range, 0.48–2.42 log HIV-1 DNA copies/10^6 PBMCs) at week 24 after randomization (median, 1.38 log HIV-1 DNA copies/10^6 PBMCs [$n = 23$] in arm A and 1.59 log HIV-1 DNA copies/10^6 PBMCs [$n = 38$] in arm B/C; $P = .88$; changes from randomization, 0.15 and 0.19 log HIV-1 DNA copies/10^6 PBMCs, respectively; $P = .18$). Cell-associated HIV-1 RNA was undetectable (<0.48 log HIV-1 RNA copy/10^6 PBMCs) in 54 (72.0%) of 75 subjects at randomization and in 35 (57.4%) of 61 subjects at week 24 after randomization (56.5% of subjects in arm A and 57.9% of subjects in arm B/C).

HIV-1–specific responses by ELISPOT assay at week 24 after randomization. $CD4^+$ and $CD8^+$ IFN-γ ELISPOT data were available for 50 and 52 subjects, respectively. HIV-1–specific T cell responses were significantly higher in arm B/C than in arm

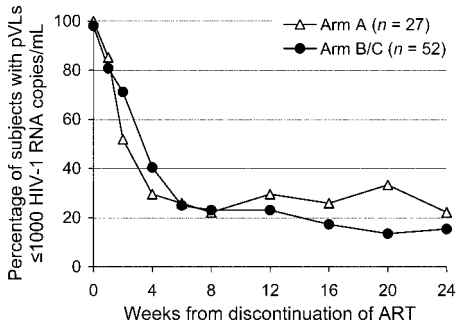

Figure 3. Percentage of subjects with plasma viral loads (pVLs) ⩽1000 HIV-1 RNA copies/mL, by week from discontinuation of antiretroviral therapy (ART). Treatment failure was recorded for subjects who failed to discontinue ART, resumed ART, or had missing data. Arm A, ART, ALVAC-HIV (vCP1452) placebo, and Remune placebo; arm B, ART, ALVAC-HIV, and Remune placebo; arm C, ART, ALVAC-HIV, and Remune.

A at week 24 after randomization for both $CD4^+$ T cells (median response to HIV-1 p24, 180 sfc/10^6 PBMCs [range, 0–2000 sfc/10^6 PBMCs] vs. 0 sfc/10^6 PBMCs [range, 0–410 sfc/10^6 PBMCs], respectively; $P = .006$) and $CD8^+$ T cells (median response to HIV-1 Gag, 275 sfc/10^6 PBMCs [range, 0–4255 sfc/10^6 PBMCs] vs. 0 sfc/10^6 PBMCs [range, 0–230 sfc/10^6 PBMCs]; $P = .002$). There was no significant difference between arm B and C in responses by $CD4^+$ T cells (median responses to HIV-1 p24, 100 sfc/10^6 PBMCs [range, 0–690 sfc/10^6 PBMCs] vs. 180 sfc/10^6 PBMCs [range, 0–2000 sfc/10^6 PBMCs], respectively; $P = .11$) or $CD8^+$ T cells (median responses to HIV-1 Gag, 208 sfc/10^6 PBMCs [range, 0–1630 sfc/10^6 PBMCs] vs. 357 sfc/10^6 PBMCs [range, 0–4255 sfc/10^6 PBMCs], respectively; $P = .24$).

Comparison of groups for end points after discontinuation of ART. Overall, 14 (17.7%) of 79 subjects were classified as having success for the primary end point (end point 1), and there was no significant difference between arms A and B/C (6/27 [22.2%] and 8/52 [15.4%] subjects, respectively; difference in percentages, −6.8 [95% CI, −26.8 to 10.0]; $P = .54$). Only 3 (3.8%) of 79 subjects fulfilled the success criterion for end point 2 (2/27 [7.4%] subjects in arm A and 1/52 [1.9%] subjects in arm B/C; difference in percentages, −5.5 [95% CI, −21.5 to 4.3]; $P = .27$). Median time to pVL >1000 HIV-1 RNA copies/mL (end point 3) was 29 days overall, and this was similar in arms A and B/C (28 and 29 days, respectively; HR 1.00 [95% CI, 0.61–1.64]; $P = .99$).

Other virological and immunological parameters after discontinuation of ART. The proportion of subjects with pVLs ⩽1000 HIV-1 RNA copies/mL fell rapidly during the first 6 weeks after discontinuation of ART and remained relatively stable thereafter (figure 3). Table 2 shows that arms A and B/C were similar with respect to pVL distribution at week 24 after discontinuation of ART. There were no significant differences between arms A and B/C in pVL, $CD4^+$ T cell count, $CD8^+$ T cell count, or cell-associated HIV-1 RNA and DNA loads at week 24 after discontinuation of ART or in changes in values before discontinuation to week 24 after discontinuation of ART (table 3). During this period, $CD4^+$ T cell count declined (median change, −139 cells/mm^3 [range, −1009 to 383 cells/mm^3]), whereas pVL (median change, 3.3 log HIV-1 RNA copies/mL [range, 0 to 5.6 log HIV-1 RNA copies/mL]), $CD8^+$ T cell count (median change, 310 cells/mm^3 [range, −304 to 1082 cells/mm^3]), and cell-associated HIV-1 RNA (median change, 1.19 log HIV-1 RNA copies/10^6 PBMCs [range, −1.37 to 2.74 log HIV-1 RNA copies/10^6 PBMCs]) and HIV-1 DNA (median change, 0.57 log HIV-1 DNA copies/10^6 PBMCs [range, −1.10 to 2.38 log HIV-1 DNA copies/10^6 PBMCs]) loads increased.

Correlates of pVL at week 24 after discontinuation of ART. Although HIV-1–specific T cell responses, as measured by IFN-γ ELISPOT assay, were significantly higher in arm B/C than arm A after immunization, there was no significant correlation between these responses at week 24 after randomization and pVL at week 24 after discontinuation of ART for either $CD4^+$ T cell response to HIV-1 p24 ($n = 47$; $r = -0.03$; $P = .82$) or $CD8^+$ T cell response to HIV-1 Gag ($n = 49$; $r = -0.04$; $P = .81$) (figure 4). In addition, there was no significant correlation between pVL at week 24 after discontinuation of ART and $CD4^+$ T cell count ($n = 76$; $r = 0.12$; $P = .30$), $CD8^+$ T cell count ($n = 76$; $r = 0.04$; $P = .76$), or cell-associated HIV-1 RNA load ($n = 74$; $r = 0.07$; $P = .56$) before discontinuation of ART, but a weak positive correlation was found with cell-associated HIV-1 DNA load before discontinuation of ART ($n = 74$; $r = 0.26$; $P = .027$).

Adverse events during the immunization phase and after discontinuation of ART. In total, 9 (33%), 14 (54%), and 18 (69%) subjects in arms A, B, and C, respectively, had treatment-related adverse events (AEs). The most common AEs were reactions at the injection site (8, 7, and 12 subjects in arms A, B, and C, respectively), musculoskeletal pain (2, 3, and 2 sub-

Table 2. Plasma viral load (pVL) distribution at week 24 after discontinuation of antiretroviral therapy (ART).

pVL	Arm A ($n = 27$)	Arm B/C ($n = 52$)	Arm B ($n = 26$)	Arm C ($n = 26$)
⩽50 copies/mL	3 (11.1)	1 (1.9)	1 (3.8)	0 (0.0)
51–1000 copies/mL	3 (11.1)	7 (13.5)	1 (3.8)	6 (23.1)
1001–10,000 copies/mL	7 (25.9)	15 (28.8)	8 (30.8)	7 (26.9)
10,001–100,000 copies/mL	8 (29.6)	14 (26.9)	8 (30.8)	6 (23.1)
>100,000 copies/mL	2 (7.4)	5 (9.6)	3 (11.5)	2 (7.7)
Treatment failure	4 (14.8)	10 (19.2)	5 (19.2)	5 (19.2)

NOTE. Data are no. (%) of subjects. Treatment failure was recorded for any subject who failed to discontinue ART, resumed ART before week 24 after discontinuation of ART, or had missing pVL data at week 24. Arm A, ART, ALVAC-HIV (vCP1452) placebo, and Remune placebo; arm B, ART, ALVAC-HIV, and Remune placebo; arm C, ART, ALVAC-HIV, and Remune.

Table 3. Virological and immunological parameters after discontinuation of antiretroviral therapy (ART).

Parameter	Arm A		Arm B/C			Arm B		Arm C	
	No. of subjects	Median (IQR)	No. of subjects	Median (IQR)	*P*, arm A vs. arm B/C[a]	No. of subjects	Median (IQR)	No. of subjects	Median (IQR)
Plasma HIV-1 RNA[b]									
Week 24 after discontinuation of ART	27	3.9 (3.0–4.9)	49	4.2 (3.4–4.8)	0.61	24	4.4 (3.5–4.8)	25	3.9 (3.1–4.9)
Change from before discontinuation of ART	27	3.5 (2.3–4.1)	49	3.2 (2.4–4.1)	0.97	24	3.4 (3.0–4.3)	25	2.8 (2.2–3.8)
$CD4^+$ T cell count[c]									
Week 24 after discontinuation of ART	26	656 (520–739)	49	621 (495–773)	0.65	24	565 (486–777)	25	625 (514–773)
Change from before discontinuation of ART	26	−150 (−194 to 1)	49	−139 (−349 to −51)	0.25	24	−121 (−368 to −25)	25	−199 (−336 to −86)
$CD8^+$ T cell count[c]									
Week 24 after discontinuation of ART	26	1002 (826–1292)	49	1068 (736–1350)	0.96	24	1063 (696–1274)	25	1174 (838–1587)
Change from before discontinuation of ART	26	435 (142–646)	49	233 (137–439)	0.070	24	257 (100–439)	25	233 (137–485)
Cell-associated HIV-1 RNA load[d]									
Week 24 after discontinuation of ART	23	2.00 (1.51–2.74)	42	1.91 (1.45–2.64)	0.80	21	2.02 (1.34–2.50)	21	1.79 (1.53–2.88)
Change from before discontinuation of ART	23	1.01 (0–2.21)	42	1.26 (0.73–1.54)	0.76	21	1.35 (0.87–1.54)	21	1.23 (0.52–1.52)
Cell-associated HIV-1 DNA load[d]									
Week 24 after discontinuation of ART	23	1.87 (1.30–2.52)	42	2.05 (1.58–2.47)	0.60	21	1.93 (1.58–2.47)	21	2.09 (1.86–2.44)
Change from before discontinuation of ART	23	0.57 (0–0.85)	42	0.57 (0.17–1.02)	0.63	21	0.57 (0.22–1.11)	21	0.56 (0.04–0.89)

NOTE. The last-observation-carried-forward principle was used (if the observation was ⩾1 month after discontinuation of ART) for subjects with missing week 24 values and those who resumed ART. Arm A, ART, ALVAC-HIV (vCP1452) placebo, and Remune placebo; arm B, ART, ALVAC-HIV, and Remune placebo; arm C, ART, ALVAC-HIV, and Remune.

[a] Mann-Whitney *U* test.
[b] ln log copies/mL.
[c] ln cells/mm^3.
[d] ln log copies/10^6 peripheral blood mononuclear cells.

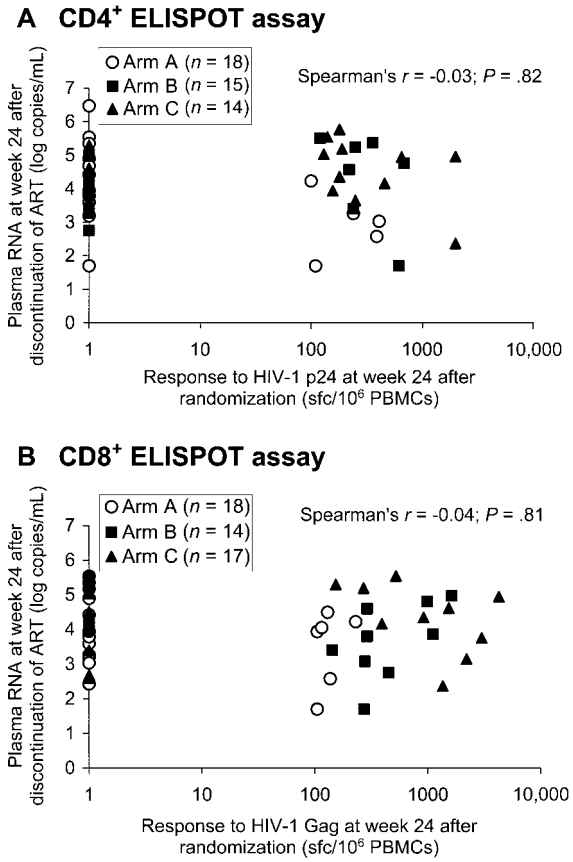

Figure 4. Association between HIV-1–specific responses at week 24 after randomization and plasma viral load at week 24 after discontinuation of antiretroviral therapy (ART) in subjects with available samples (47 subjects for $CD4^+$ T cell data and 49 subjects for $CD8^+$ T cell data), using the last-observation-carried-forward principle (if $\geq$1 month after discontinuation of ART) for subjects with missing week 24 values and those who resumed ART. Arm A, ART, ALVAC-HIV (vCP1452) placebo, and Remune placebo; arm B, ART, ALVAC-HIV, and Remune placebo; arm C, ART, ALVAC-HIV, and Remune; ELISPOT, enzyme-linked immunospot; sfc, spot-forming cells.

jects), and fatigue (1, 3, and 3 subjects). There were no serious treatment-related AEs. No AE led to premature discontinuation of the vaccines or withdrawal from the study. No cases of severe acute retroviral syndrome or AIDS or a $CD4^+$ T cell count <200 cells/mm^3 were noted during follow-up.

DISCUSSION

We report here the results of the first international, randomized, double-blind, placebo-controlled trial of therapeutic immunization in subjects who had initiated ART at the time of PHI. Adherence to the immunization regimen and planned discontinuation of ART was excellent. Overall, 17.7% of subjects had pVLs $\leq$1000 HIV-1 RNA copies/mL 24 weeks after discontinuation of ART. This proportion did not differ between the vaccine arms and the placebo arm. The upper bound of the 95% CI for the difference in proportions (10%) shows that the trial could rule out any substantial benefit of this immunization strategy for this end point in this clinical setting. There was no difference between the vaccine arms and the placebo arm in the time from discontinuation of ART to pVL >1000 HIV-1 RNA copies/mL or in median pVL at week 24 after discontinuation of ART.

The use of a planned discontinuation of ART provided a means of assessing the potential for virological control by measuring the level of pVL after discontinuation of ART. We considered a 6-month period after discontinuation of ART to be sufficient to allow for the establishment of a stable viral set point. The safety of this strategy in our population was demonstrated by the absence of severe acute retroviral syndrome or progression to AIDS and high median $CD4^+$ T cell counts. Similar strategies involving a planned interruption in ART have been selected for therapeutic immunization studies of chronically infected subjects, and time to resumption of ART was used as the primary end point [18, 19].

The choice of vaccine candidates for this study was dictated by their safety records in HIV-1–infected subjects, immunogenicity, and availability [20–22]. Remune was used preferentially to increase $CD4^+$ T helper cells, and ALVAC-HIV was used to increase CTLs [22–25]. The combination of vaccines in a single treatment arm was selected for its potential to enhance both components of T cell immunity. Our results demonstrate that these vaccines were immunogenic, in that they increased $CD4^+$ and $CD8^+$ T cell responses, as measured by IFN-γ ELISPOT assay, compared with the results with the placebo vaccines. However, the level of immunogenicity before discontinuation of ART was not correlated with pVL 6 months after discontinuation of ART. Our study was, therefore, not able to demonstrate a link between levels of HIV-1–specific T cell responses, as measured by IFN-γ ELISPOT assay, and viremia. This is in contrast to findings from studies of animals, long-term nonprogressors (LTNPs) [16], and subjects with acute and chronic HIV-1 infection [18, 19, 26–30]. A possible explanation for our results is that the immune responses induced by immunization were not of sufficient magnitude to affect viral replication after discontinuation of ART [5]. However, in a study of structured treatment interruptions during PHI, $CD4^+$ and $CD8^+$ T cell responses of similar intensity were found to be associated with virological control [31].

When our results are compared with responses by LTNPs measured by the same ELISPOT assay, median levels of IFN-γ production by $CD8^+$ T cells to HIV-1 Gag in our study were 10-fold below those observed in LTNPs, whereas $CD4^+$ T cell responses to HIV-1 p24 did not differ [16]. Responses in LTNPs were further correlated with the pVL, the cell-associated HIV-1 DNA load, and an absence of disease progression [16]. Further emphasis may be needed not only on the number of IFN-γ–producing cells but also on the type of immune function elicited

for protection—such as the simultaneous production of interleukin-2 and IFN-γ [16, 32, 33], which may not have been elicited by the immunization procedure used in our study. Studies to characterize the kinetics of expansion, diversity, and differentiation of HIV-1–specific T cells in order to further investigate immunological correlates of virological control in our population are in progress. Ongoing follow-up of the QUEST cohort will assess the virological, immunological, and clinical effects of the immunization strategy over a longer period.

Because we aimed to enable virological control without ART, we chose what was considered to be the best clinical setting (i.e., PHI) and stringent virological end points. An association between pVL at set point and clinical prognosis has been demonstrated in previous studies [34]. However, our results should not preclude the use of other populations and end points in other assessments of this type of strategy. Our results may not be automatically applicable to chronically infected subjects. It remains unclear which of the 2 clinical settings is best suited for testing the efficacy of candidate immunogens.

In our study, 17.7% of all randomized subjects had pVLs ≤1000 HIV-1 RNA copies/mL at 6 months after discontinuation of ART. This raises the question of whether initiation of ART during PHI was beneficial in itself. This question cannot be directly evaluated by our study, because we did not include an ART-free control arm. There are no results from randomized, placebo-controlled trials of potent combination ART during PHI. Observational comparisons, although subject to bias, provide the only means available to address this issue. A comparison between the proportion of subjects with low pVLs after discontinuation of ART in our study population and the prevalence of low pVLs at a comparable time point after HIV seroconversion in untreated cohorts will be the subject of another study. Ultimately, results from randomized controlled trials are needed to provide definitive evidence of the effect that ART has during PHI [35].

In conclusion, an overall 17.7% of our subjects who had initiated ART at the time of PHI fulfilled our primary end point of pVL ≤1000 HIV-1 copies/mL 24 weeks after discontinuation of ART. However, although the therapeutic immunization strategy we used was safe and immunogenic, we found no evidence that it increased the likelihood of low pVL after discontinuation of ART. Trials with designs and subject populations such as ours may be an appropriate means to evaluate immune-boosting agents and may contribute to the development of alternative strategies to long-term ART.

AUTHOR AFFILIATIONS

Departments of Medicine (S.K.–d.L.) and Primary Care and Population Sciences (F.C.L. and A.N.P.), Royal Free Centre for HIV Medicine, Royal Free and University College Medical School, London, and GlaxoSmithKline Research and Development, Greenford, United Kingdom (L.-E.G. and N.C.); Cellular and Tissue Immunology Laboratory, INSERM U543, Pitié-Salpetriere Hospital (B.A.), and Department of Infectious and Tropical Diseases, Rothschild Hospital, Assistance Publique–Hôpitaux de Paris, Paris (P.-M.G.), Department of Infectious Diseases, University Medical Center, Besançon (B.H.), and Sanofi pasteur, Lyon, France (R.E.H.); National Centre in HIV Epidemiology and Clinical Research, University of New South Wales and St. Vincent's Hospital, Sydney (D.E.S. and D.A.C.), and Holdsworth House General Practice, Darlinghurst, New South Wales, Australia (M. Bloch); Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden (J.A. and A.S.); Immune Deficiency Treatment Centre, Montreal General Hospital, Montreal, Quebec, Canada (C.T.); Clinic of Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy (G. Tambussi); The Immune Response Corporation, Carlsbad, California (G. Theofan); Division of Infectious Diseases, Basel University Hospital, Basel (M. Battegay), and Laboratory of Virology, Division of Infectious Diseases, Geneva University Hospital, Geneva, Switzerland (L.P.)

QUEST STUDY GROUP

Recruiting centers. Australia: M. Bloch (Holdsworth House General Practice, Darlinghurst, New South Wales), D. Baker (Doctors Clinic, Surry Hills, Sydney), R. Finlayson (Taylor Square Private Clinic, Surry Hills, Sydney), P. Grey, D. E. Smith, D. A. Cooper (National Centre in HIV Epidemiology and Clinical Research and St. Vincent's Hospital, Sydney); Belgium: P. Hermanns, K. Kabeya, N. Clumeck (Department of Infectious Diseases, St. Pierre Hospital, Brussels); Canada: M. Harris, J. Montaner (John Ruedy Immune Deficiency Clinic, St. Paul's Hospital, University of British Columbia, Vancouver), C. Tsoukas (Immune Deficiency Treatment Centre, Montreal General Hospital, Montreal, Quebec); France: B. Hoen (Department of Infectious Diseases, University Medical Center, Besançon), P.-M. Girard (Department of Infectious and Tropical Diseases, Rothschild Hospital, Paris), J. Modai (Department of Infectious Disease, St. Louis Hospital, Paris), P. Canton, T. May (Department of Infectious Diseases, Centre Hospitalier Universitaire Nancy, Vandoeuvres-les-Nancy), D. Sereni (Department of Internal Medicine, St. Louis Hospital, Paris), C. Katlama (Department of Infectious Diseases, Pitié-Salpetrière Hospital, Paris); Germany: S. Staszewski (Klinikum der JW Goethe Universität, Zentrum der Inneren Medizin, Frankfurt), H. J. Stellbrink (Medizinische Poliklinik, Universitätsklinikum Eppendorf, Hamburg); Italy: G. Tambussi, A. Lazzarin (Clinic of Infectious Diseases, San Raffaele Scientific Institute, Milan); Sweden: H. Gaines, S. Lindback, A. Blaxhult (Department of Infectious Diseases, Karolinska University Hospital, Stockholm); Switzerland: M. C. Bernard, B. Hirschel, L. Perrin (Division of Infectious Diseases, University Hospital, Geneva), K. Wolf, M.

Battegay (Division of Infectious Diseases, Basel University Hospital, Basel), P. Vernazza (Department für Innere Medizin, Kantonspital, St. Gallen), R. Weber (Department für Innere Medizin, Kantonspital, Zurich); United Kingdom: S. Kinloch–de Loes, T. Drinkwater, Z. Cuthbertson, P. Byrne, M. Youle, M. Tyrer, S. Bhagani, M. A. Johnson (Royal Free Centre for HIV Medicine and Ian Charleson Day Centre, Royal Free Hospital, London), C. Higgs, D. Hawkins, B. Gazzard (Kobler Centre, Chelsea & Westminster Hospital, London), A. Friedman (University Hospital of Wales, Cardiff), M. Fisher (Claude Nicol Centre Research Department, Royal Sussex Country Hospital, Brighton).

GlaxoSmithKline QUEST Team (Greenford, UK). V. Mallet, S. Turkish, S. Fortes, H. Maseruka, H. Steel, D. Thorborn, H. McDade, L.-E. Goh. GlaxoSmithKline monitors: M. Haberl, J. Young (Australia); D. Luyts, I. van Steenberg (Belgium); S. Pratt, T. Russell (Canada); L. Beauvais, J. M. Vauthier (France); M. Sikora (Germany); C. Gussetti, C. M. Anghileri, V. Piva, D. Fendt (Italy); G. Larsson (Sweden); C. Python, I. Schauwecker, E. Gremlich (Switzerland); K. Studdard, P. Humphreys, U. Loughrey (United Kingdom).

The Immune Response Corporation (Carlsbad, California). R. Moss, G. Theofan.

Sanofi pasteur (Lyon, France). D. Blanc, C. di Vita, V. Mazarin, B. Mouterde, R. El Habib.

Data Safety Monitoring Board. M. Schechter (Canada), I. Weller (United Kingdom), R. Luethy (Switzerland), J. M. Molina (France).

Core Group. S. Kinloch–de Loes, D. A. Cooper, L. Perrin, B. Hoen, A. Sonnerborg, C. Tsoukas, J. Andersson, F. C. Lampe, A. N. Phillips, B. Autran.

Statistical analysis. F. C. Lampe, N. Carter, A. N. Phillips.

Roche Molecular Systems (Alameda, California). B. Dale, A. Capt.

Laboratory support. L. Wegmann, S. Yerly, L. Perrin (Laboratory of Virology, Geneva University Hospital, Geneva, Switzerland); A. Samri, B. Autran (Cellular and Tissue Immunology Laboratory, INSERM U543, Pitié-Salpetrière Hospital, Paris, France); J. Zaunders, P. Cunningham, A. Kelleher (Centre for Immunology, St. Vincent's Hospital, National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia); S. Martins, G. Janossy (HIV Immunology Laboratory, Department of Immunology and Molecular Pathology, Royal Free and University College Medical School, London); A. L. Spetz, J. Andersson (Karolinska University Hospital, Stockholm, Sweden); G. Tambussi, A. Lazzarin, A. Galli (Clinic of Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy).

Acknowledgments

We thank the scientists, for advice; the physicians and nurses, for patient referral and clinical care; and the subjects, for participation in the study.

References

1. Mocroft A, Vella S, Benfield TL, et al. Changing patterns of mortality across Europe in patients infected with HIV-1. Lancet **1998**; 352: 1725–30.
2. Allen MA, Kelleher AD, Zaunders J, Walker BD. STI and beyond: the prospects of boosting anti-HIV immune responses. Trends Immunol **2002**; 23:456–60.
3. Fagard C, Oxenius A, Gunthard H, et al. A prospective trial of structured treatment interruptions in human immunodeficiency virus infection. Arch Intern Med **2003**; 163:1220–6.
4. Kovacs A, Connors M. HIV-1 and immune control: can we change the course of HIV-1? Lancet **2004**; 363:833–4.
5. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1 specific CD4+ T cell responses associated with control of viremia. Science **1997**; 278:1447–50.
6. Oxenius A, Price DA, Easterbrook PJ, et al. Early highly active antiretroviral therapy for acute HIV-1 infection preserves immune function of CD8+ and CD4+ T lymphocytes. Proc Natl Acad Sci USA **2000**; 97:3382–7.
7. Altfeld M, Rosenberg ES, Shankarappa R, et al. Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. J Exp Med **2001**; 193:169–80.
8. Tenner-Racz K, Perrin L, Stellbrink HJ, et al. Low lymph node viral load despite high viremia in seroconverters supports early HAART intervention [abstract 758]. In: Program and abstracts of the 8th Conference on Retroviruses and Opportunistic Infections (Chicago). Alexandria, VA: Foundation for Retrovirology and Human Health, **2001**: 274.
9. Hoff R, McNamara J. Therapeutic vaccines for preventing AIDS: their use with HAART. Lancet **1999**; 353:1723–4.
10. Ogg GS, Walker BD. Levels of human immunodeficiency virus type 1-specific cytotoxic T-lymphocyte effector and memory responses decline after suppression of viremia with highly active antiretroviral therapy. J Virol **1999**; 73:6721–8.
11. Autran B, Carcelain G. Boosting immunity to HIV—can the virus help? Science **2000**; 290:946–9.
12. Goh LE, Perrin L, Hoen B, et al. Study protocol for the evaluation of the potential for durable viral suppression after quadruple HAART with or without HIV vaccination: the QUEST study. HIV Clin Trials **2001**; 2: 438–41.
13. Clinical Investigator's Brochure. ALVAC(2) 120 (B,MN)GNP (vCP1452). Swiftwater, PA: Aventis Pasteur, **1999**.
14. Yerly S, Perneger TV, Vora S, Hirschel B, Perrin L. Decay of cell-associated HIV-1 DNA correlates with residual replication in patients treated during acute HIV-1 infection. AIDS **2000**; 14:2805–12.
15. Alatrakchi N, Duvivier C, Costagliola D, et al. Persistent low viral load on antiretroviral therapy is associated with T cell-mediated control of HIV replication. AIDS **2005**; 19:25–33.
16. Martinez V, Costagliola D, Bonduelle O, et al. Combination of HIV-1–specific CD4 Th1 responses and IgG2 antibodies is the best predictor for persistence of long-term nonprogression. J Infect Dis **2005**; 191: 2053–63.
17. Sun Y, Iglesias E, Samri A, et al. A systematic comparison of methods to measure HIV-1 specific CD8 T cells. J Immunol Methods **2003**; 272: 23–34.
18. Levy Y, Gahery-Segard H, Duriere C, et al. Immunological and virological efficacy of a therapeutic immunization combined with interleukin-2 in chronically HIV-1 infected patients. AIDS **2005**; 19:279–86.
19. Tubiana R, Carcelain G, Vray M, et al. Therapeutic vaccination with ALVAC HIV vCP 1433: a recombinant canarypox vaccine in chronically HIV-1 infected patients treated with HAART: VACCITER (ANRS 094) [abstract 61]. In: Program and abstracts of the 10th Conference on Retroviruses and Opportunistic Infections (Boston). Alexandria, VA: Foundation for Retrovirology and Human Health, **2003**:78.
20. Trauger RJ, Daigle AE, Giermakowska W, Moss RB, Jensen F, Carlo DJ. Safety and immunogenicity of a gp120 depleted, inactivated HIV-1 im-

munogen: results of a double blind, adjuvant controlled trial. J Acquir Immune Defic Syndr Hum Retrovirol **1995**; 10(Suppl 2):S74–82.
21. Trauger RJ, Ferre F, Daigle AE, et al. Effect of immunization with inactivated gp120-depleted human immunodeficiency virus type 1 (HIV-1) immunogen on HIV-1 immunity, viral DNA, and percentage of CD4 cells. J Infect Dis **1994**; 169:1256–64.
22. Ferrari G, Berend C, Ottinger R, et al. Replication-defective canarypox (ALVAC) vectors effectively activate anti-human immunodeficiency virus-cytotoxic T lymphocytes presented in infected patients: implications for antigen-specific immunotherapy. Blood **1997**; 90:2406–16.
23. Robbins GK, Addo MM, Troung H, et al. Augmentation of HIV-1 specific T helper cell responses in chronic HIV-1 infection by therapeutic immunization. AIDS **2003**; 17:1121–6.
24. Maino VC, Suni MA, Wormsley SB, Carlo DJ, Wallace MR, Moss RB. Enhancement of HIV-1 antigen specific CD4+ T cell memory in subjects with chronic HIV-1 infection receiving HIV-1 immunogen. AIDS Res Hum Retroviruses **2000**; 16:539–47.
25. Markowitz M, Jin X, Hurley A, et al. Discontinuation of antiretroviral therapy commenced early during the course of human immunodeficiency virus type 1 infection with or without adjunctive vaccination. J Infect Dis **2002**; 186:634–43.
26. Hel Z, Venzon D, Poudyaal M, et al. Viremia control following antiretroviral treatment and therapeutic immunization during primary SIV_{251} infection of macaques. Nat Med **2000**; 6:1140–6.
27. Tryniscewska E, Nacsa J, Lewis MG, et al. Vaccination of macaques with long-standing SIVmac251 infection lowers viral set-point after cessation of therapy. J Immunol **2002**; 169:5347–57.
28. McMichael AJ, Hanke T. HIV vaccines 1983–2003. Nat Med **2003**; 9: 874–80.
29. Lisziewicz J, Bakare N, Lori F. Therapeutic vaccination for future management of HIV/AIDS. Vaccine **2003**; 21:620–3.
30. Lu W, Wu X, Lu Y, Weishong G, Andrieu JM. Therapeutic dendritic cell vaccine for simian AIDS. Nat Med **2003**; 9:27–32.
31. Rosenberg ES, Altfeld M, Poon SH, et al. Immune control of HIV-1 after early treatment of acute infection. Nature **2000**; 407:523–6.
32. Harari A, Petitpierre S, Vallelian F, Pantaleo G. Skewed representation of functionally distinct populations of virus-specific CD4 T cells in HIV-1 infected subjects with progressive disease: changes after antiretroviral therapy. Blood **2004**; 103:966–72.
33. Younes SA, Yassine-Diab B, Dumont AR, et al. HIV-1 viremia prevents the establishment of interleukin 2-producing HIV-specific memory CD4+ T cells endowed with proliferative capacity. J Exp Med **2003**; 198:1909–22.
34. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med **1997**; 126:946–54.
35. Smith DE, Walker B, Cooper DA, Rosenberg ES, Kaldor JM. Is antiretroviral treatment of primary HIV infection clinically justified on the basis of current evidence? AIDS **2004**; 18:709–18.