

Research Paper

Influence of IFN γ Co-Expression on the Safety and Antiviral Efficacy of Recombinant Fowlpox Virus HIV Therapeutic Vaccines Following Interruption of Antiretroviral Therapy

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Original manuscript submitted: 05/05/07

Manuscript accepted: 06/25/07

Previously published online as a *Human Vaccines* E-publication:
<http://www.landesbioscience.com/journals/vaccines/article/4627>

KEY WORDS

therapeutic vaccine, HIV, pox virus vector vaccine

ACKNOWLEDGEMENTS

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ABSTRACT

Therapeutic immunization to stimulate host immune responses and control human immunodeficiency virus (HIV-1) replication is being investigated as a supplementary treatment for the management of HIV infection.

On completion of an earlier study involving three vaccinations while taking combination antiretroviral therapy (CART), twenty-five subjects with plasma viral load (pVL) <50 copies/mL received a booster vaccination with either placebo (n = 7); fowl pox vaccine (rFPV) expressing HIV-1 Gag/Pol; [partial construct - PC (n = 8)] or rFPV coexpressing HIV-1 Gag/Pol and human interferon- γ [full construct - FC (n = 10)]. One week after the booster vaccination, participants stopped ART and were monitored for safety, pVL and immunological parameters for \leq 20 weeks.

The time weighted mean change (SD) from baseline plasma HIV RNA was 1.80 (0.72), 1.78 (0.91) and 0.96 (0.91) log₁₀ copies/mL for placebo, PC and FC recipients respectively (p = 0.06; mean differences between placebo and FC). Laboratory evaluations did not reveal differences in anti-HIV specific immune responses between study arms. No difference between treatment arms for host genetic factors known to affect pVL was demonstrated.

In conclusion, vaccination with FC was associated with a trend toward lower rates of HIV replication following cessation of ART relative to placebo or PC. The promising antiretrovirological effect supports further study of FC in a larger trial with a broader population of patients with HIV disease.

INTRODUCTION

Combination antiretroviral therapy (CART) has resulted in marked reductions in morbidity and mortality for patients infected with HIV.^{1,2} However, it has limitations,^{3,4} including the inability to eradicate HIV, the association with acute and chronic toxicity and the potential emergence of drug resistant virus.^{3,5} Furthermore, while CART is associated with increased memory and naïve CD4+ T-cell numbers it does not reconstitute the HIV specific T-cell responses often observed in long term non-progressors.⁶ Given these limitations, other treatment approaches such as therapeutic vaccines designed to generate an HIV specific immune response that controls HIV viraemia are being evaluated.^{7,8} To date, therapeutic vaccination has shown limited impact upon clinical outcomes of HIV disease (for review see refs. 7 and 9).

The use of recombinant live viral vectors to deliver antigens in a fashion mimicking natural HIV-1 infection to patients receiving CART is a promising approach currently being tested with mixed results.¹⁰⁻¹² Here we have examined the effect of vaccination with recombinant fowlpox virus vector vaccines expressing HIV-1 Gag/Pol with and without the Th1 cytokine human interferon- γ (IFN γ) gene¹³ on viral control and immune responses during an analytical treatment interruption (ATI) in patients who commenced stable CART soon after primary infection. This study extends a previous safety and immunogenicity trial where the vaccines were shown to be safe and well tolerated without generating significant anti-HIV immune responses despite both constructs being immunogenic in macaques.^{13,14} The cytokine coexpressing construct was specifically designed to stimulate Th1 T-cell-mediated anti-HIV immune responses so as to overcome the Th2 bias associated with HIV infection.¹⁵⁻¹⁸

METHODS

Study design. This study was open to patients who had enrolled in an earlier randomized, placebo-controlled, safety and immunogenicity study of these vaccines.¹⁴ Subjects were required to have completed 52 weeks of follow-up, have received the three study vaccinations in that trial, to have continued CART throughout, and maintained plasma viral load (pVL) <50 copies/mL, and also provided written informed consent.

Participants received a 1.0 mL intramuscular booster vaccination with either placebo, 5×10^7 plaque-forming units (pfu)/mL of a fowl pox vaccine expressing HIV-1 *Gag/Pol*; (partial construct, PC) or a fowl pox vaccine coexpressing HIV-1 *Gag/Pol* and human interferon- γ (full construct, FC). The vaccine booster was administered in keeping with the randomisation schedule applied during the initial phase of study. The double-blind concealment was retained on all patients (including those not consenting to participate in this protocol) throughout the 20 week duration of this extension study and until closure of the database and completion of initial analysis plan.

One week after vaccination participants interrupted CART (analytical treatment interruption) and were closely followed clinically, with weekly CD4+ T cell counts and pVL for ≤ 20 weeks. A diagrammatic representation of the study design and immunization schedule in the previous and current trials is presented in Figure 1A.

The protocol provided guidelines for re-initiation of CART as follows; pVL >100,000 copies/mL during the first six weeks following cessation of CART and/or a CD4+ count <200 cells/ μ L and/or, symptoms of HIV primary infection requiring treatment and/or a pVL set point (after a pVL spike) >20,000 copies/mL. These criteria were not obligatory and clinicians were asked to exercise good clinical judgement in making decisions about reinitiating CART.

Additional long term follow-up data comprising CART status, pVL and CD4+ T-cell counts were collected to July 7, 2006 (up to 198 weeks after booster vaccination) through an ongoing observational cohort study.

Outcome measures. Safety was assessed using clinical and laboratory assessments. Serious adverse events and adverse events were collected for all subjects regardless of causality.

Efficacy was assessed using changes from baseline pVL (<50 copies/ml). The protocol-defined primary endpoint was time-weighted mean area under the curve change from baseline log pVL until reintroduction of CART. This measure was to be compared a priori between placebo recipients and the combined results from PC plus FC recipients. Secondary endpoints were pVL after cessation of CART (post-vaccination pVL set-point), as well as differences between treatment groups for kinetics and rate of pVL recrudescence and median time to re-initiation of CART. A series of post hoc analyses compared endpoints between FC recipients and placebo or PC recipients. In addition, we conducted post hoc analysis of time to pVL above a number of threshold levels.

Cell-mediated immunogenicity was assessed via T-cell proliferation and IFN γ ELISpot in blinded batched analysis of cryopreserved peripheral blood mononuclear cells (PBMC) collected at baseline and 2, 4, 9, 16 and 20 weeks after booster vaccination. Mean changes in cell-mediated immunogenicity at week 2 were formally compared between placebo and PC recipients and the FC groups.

Statistical analysis. This study was exploratory and no power calculations were determined. Formal statistical comparisons of quantitative variables were based on ANOVA methods or equivalent non-parametric tests. Binary variables were analyzed using Fisher's exact or chi-squared tests. Time to event endpoints were summarized using Kaplan-Meier plots and treatment groups compared using

Cox regression. All p-values and confidence intervals were two-tailed. There was no adjustment of p-values for multiple comparisons. All formal comparisons were performed without adjustment for baseline characteristics. Analyses were also performed and adjusted for covariates that were imbalanced at baseline.

Covariate analyses were performed to identify covariates associated with good virological outcomes. Analyses were performed separately for the time weighted average log pVL, time to detectable pVL and time to re-initiation of CART. Covariates assessed included age, duration of anti-retroviral treatment, estimated time from HIV exposure to starting CART, seroconversion symptoms and CD4+ and CD8+ cell counts at the extension study baseline. Safety data were summarized by treatment received with no formal statistical comparisons.

Enzyme-linked immunospot (ELISpot) assays. Assays were performed as previously described.¹⁹ A positive result was defined as greater than twice background level and greater than 50 sfu/ 10^6 cells after subtraction of background (media alone). Staphylococcal enterotoxin B 5 μ g/mL (Sigma Pharmaceuticals Ltd.) and whole CMV lysate 1/2000 (Biowhittaker, Maryland, USA) mixed with CEF peptides were used as mitogen and antigen positive controls respectively. Vaccine specific responses were measured using a single HXB Gag and two HXB Pol peptide pools and whole AT2 inactivated HIV (Gift from J. Lifson, NCI, USA). A HXB Nef peptide pool was used to monitor HIV specific responses that would not be directly modulated by vaccine administration. All peptide pools were composed of 15 mers over-lapping by 10 used at a final concentration of 2 μ g/ml. All peptide sets were obtained from the NIH AIDS Research and Reference Reagent Program.

Proliferation assays. Assays were performed as previously described.²⁰ Results were expressed as stimulation index with responses with a stimulation index >2 reported as a positive response.

PHA 1 μ g/mL (Murex Diagnostics, Dartford, UK) plus IL-2 10 U/mL was used as the mitogen positive control. Antigens tested were the same as those as used in the ELISpot, except that whole recombinant p24 protein 1 μ g/mL (Baculovirus derived, Protein Sciences, Connecticut, USA) was substituted for the whole inactivated HIV.

Detection of IFN α , IRF-1 and CIITA mRNA. IRF-1 (interferon regulatory factor-1) and CIITA (Class II Transactivator) mRNA levels were measured as IFN γ induces their up-regulation. The endogenous expression of IFN α mRNA was studied as it was previously shown to correlate with vaccine responsiveness in macaques.²¹ Total RNA isolated from PBMC using the Qiagen RNeasy kit and cDNA generated using random hexamer primers and Taqman Reverse Transcriptase Reagents (ABI). Amplification of IFN α was performed using previously published primers and probe.²² Real time PCR was performed to amplify IRF-1 and CIITA from PBMC cDNA. Primers and probe utilised to detect a 68 bp IRF-1 fragment were: 5'-TCCAG/CACTGTCGCCATGT-3', 5'-GCACAACCTCCACTGGGATGT-3, and 5'-CTGTCAGCAGCACTCTCCCCGACTG-3' respectively (/ indicates exon-exon boundary) and to detect a 93 bp CIITA fragment were 5'-AGCAGGCTGTTG TGTGACATG-3', 5'-GGAGTCCTGGAAGACATACTGGTC-3' and 5'-AGGGAGGCTTATGCCAATATCGCGG-3' respectively. β -actin gene was amplified using Taqman β -actin Detection Reagents (ABI).

Determination of host factors via chemokine and chemokine receptor polymorphisms and class I HLA typing. Delta 32 CCR5, CCR2 and SDF-1 polymorphisms and CCR5 polymorphisms were performed, according to the published methodology.²³ Class I HLA typing was performed by standard sequencing techniques.

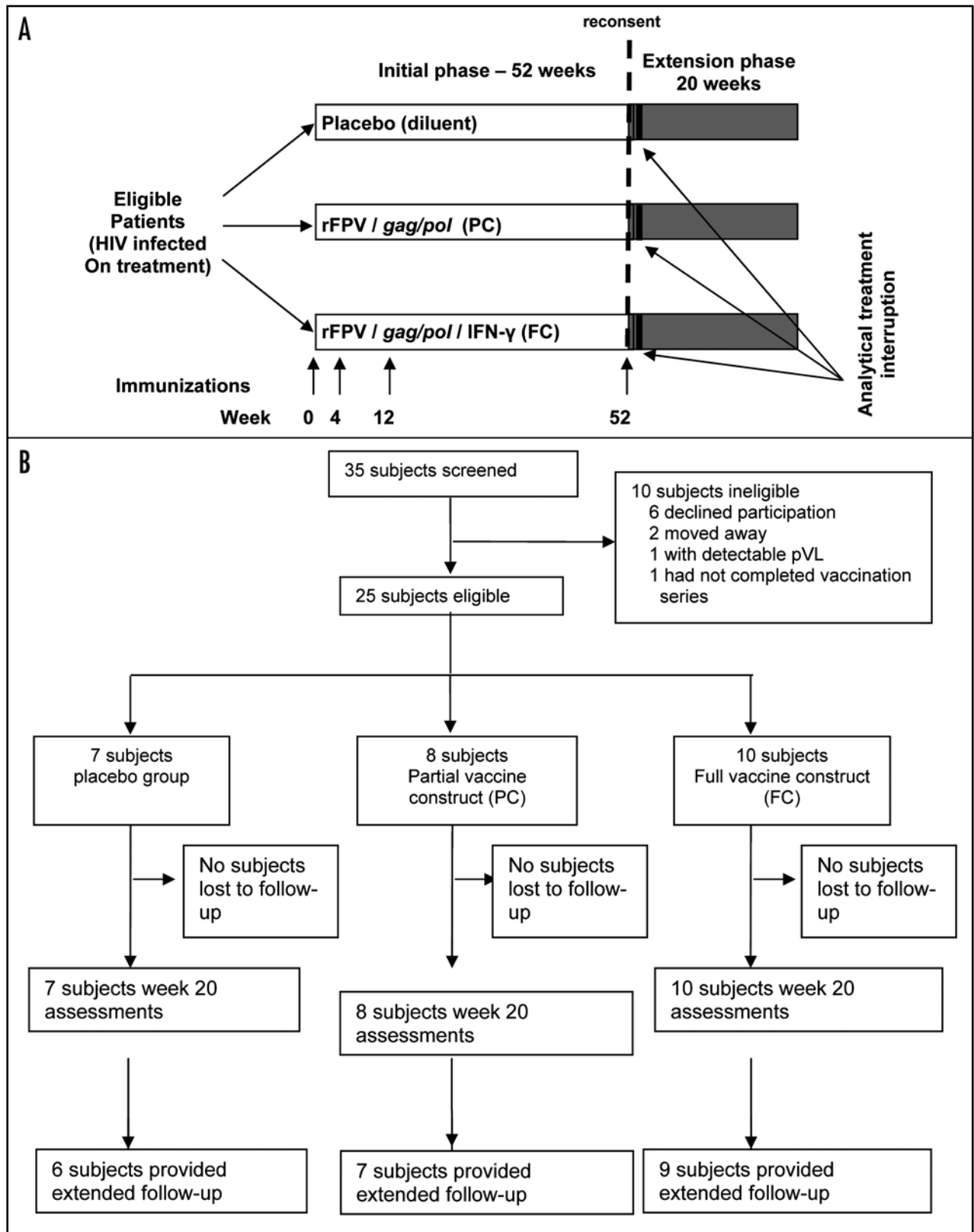


Figure 1. Study design and patient disposition. (A) Flow diagram of study design and immunization schedule. Thirty-five HIV infected subjects undergoing CART in trial VIR-NCHR-01 (12 Placebo, 11 PC, 12 FC) were vaccinated at Weeks 0, 4, 8 and 12 and followed for 52 weeks. At week 52 those subjects consenting to participate in the rollover study VIR-NCHR-02 were boosted with the respective vaccine (Placebo, PC or FC) prior to undertaking an analytical treatment interruption one week later. Subjects were followed for 20 weeks following the booster vaccination. (B) Flow Diagram of study design and patient disposition.

Table 1 **Demography and baseline characteristics**

	Placebo	Partial Construct	Full Construct
N	7	8	10
Male (%)	100	100	100
Caucasian (%)	100	100	80
Age, mean ± SD years	43.3 ± 3.4	38.0 ± 7.8	39.2 ± 4.6
CD4+ cell count, mean ± SD cells/μL	699 ± 241	814 ± 217	827 ± 139
Patients diagnosed with seroconversion illness (%)	71	88	90
Estimated time from HIV infection to commencing ART, mean ± SD months	2.9 ± 3.6	2.8 ± 3.5	1.1 ± 1.5
Total duration of ART, mean ± SD months	60.6 ± 18.3	64.9 ± 8.8	52.0 ± 23.5
Antiretroviral therapy (history)			
NRTI (%)	100	100	100
NNRTI (%)	42.9	62.5	30
PI (%)	85.7	100	100
Antiretroviral therapy (current)			
NRTI (%)	85.7	75	90
NNRTI (%)	42.9	50	10
PI (%)	57.1	50	90
Host Genetic Factors: Chemokine/chemokine receptor polymorphisms			
CCR5 -d32 heterozygotes*	1	0	0
CCR2-64I heterozygotes*	0	0	0
SDF1-31A/ 31A homozygotes	0	1	0

*No homozygotes were detected.

RESULTS

Patient disposition and baseline characteristics. Twenty-five subjects (from 35 in the original trial) participated in this extension study. The reasons for non-participation are summarized in Figure 1B. All 25 enrolled patients (seven placebo recipients, eight PC recipients and ten FC recipients) completed this extension protocol to week 20. One FC recipient was found to have a plasma VL at baseline of 1,310 copies/mL. The pVL one week later was below the limit of assay detection (<50 copies/mL) so all data from this patient were included in the analyses. Selected demographic and baseline clinical characteristics are shown in Table 1. The vaccine groups were well balanced with the exception of mean CD4+ cell count that was approximately 100 cells/μL lower in the placebo group than either of the PC or FC groups. As a consequence we undertook duplicate analyses of study outcomes that adjusted for baseline CD4+ cell count. The mean time interval between original diagnosis of HIV infection and commencement of antiretroviral therapy was somewhat lower in recipients of FC relative to either the PC group or placebo recipients (1.1 months vs 2.8 months and 2.9 months respectively).

A total of 22 patients (six placebo, seven PC and nine FC) contributed long term data (for up to 178 weeks) following completion of the initial 20 week follow-up.

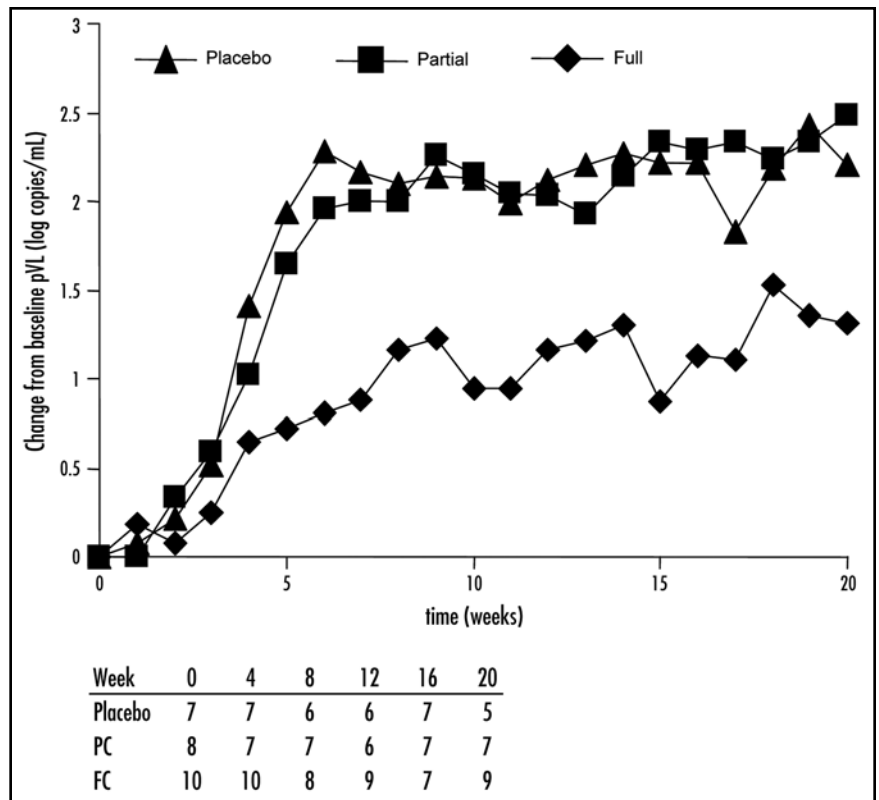


Figure 2. Change in mean HIV RNA plasma virus load from baseline (Log scale) in the ATI for each treatment group upon discontinuation of combination antiretroviral therapy. The number of patients in each group continuing in the ATI as a function of the study week is summarized in the lower panel.

Table 2 Immunogenicity data

Assay System	Placebo	Partial Construct (PC)	Full Construct (FC)
<u>Elispot (sfu/10⁶ PBMC)</u>			
Gag Antigen			
Mean base line \pm SD (week 0)	55 \pm 91	73 \pm 86	31 \pm 49
Mean change from baseline \pm SD (week 2)	81 \pm 101	25 \pm 118	21 \pm 35
Mean change from baseline \pm SD (week 20)	832 \pm 1011	431 \pm 516	270 \pm 482
Time weighted change from baseline \pm SD	426 \pm 465	244 \pm 353	107 \pm 205
Nef Antigen			
Mean base line \pm SD (week 0)	122 \pm 173	108 \pm 211	41 \pm 74
Mean change from baseline \pm SD (week 2)	-14 \pm 102	19 \pm 118	11 \pm 53
Mean change from baseline \pm SD (week 20)	357 \pm 398	211 \pm 271	183 \pm 440
Time weighted change from baseline \pm SD	169 \pm 273	319 \pm 350	91 \pm 232
CMV Antigen			
Mean base line \pm SD (week 0)	972 \pm 695	797 \pm 620	971 \pm 761
Mean change from baseline \pm SD (week 2)	87 \pm 824	197 \pm 715	105 \pm 739
Mean change from baseline \pm SD (week 20)	326 \pm 730	133 \pm 639	220 \pm 975
Time weighted change from baseline \pm SD	39 \pm 609	4 \pm 560	73 \pm 563
<u>Lymphoproliferation (Stimulation Index)</u>			
Gag Antigen			
Mean base line \pm SD (week 0)	4.1 \pm 7.1	7.7 \pm 7.6	5.6 \pm 4.8
Mean change from baseline \pm SD (week 2)	-1.9 \pm 4.1	-0.7 \pm 5.6	2.2 \pm 7.8
Mean change from baseline \pm SD (week 20)	-1.8 \pm 8.5	-5.8 \pm 7.1	-1.5 \pm 6.8
Time weighted change from baseline \pm SD	-2.5 \pm 5.9	-5.3 \pm 5.4	-0.4 \pm 6.6
Nef Antigen			
Mean base line \pm SD (week 0)	0 \pm 0	0.5 \pm 1.5	0 \pm 0
Mean change from baseline \pm SD (week 2)	0 \pm 0	0 \pm 0	0.8 \pm 1.8
Mean change from baseline \pm SD (week 20)	0.7 \pm 1.65	-0.5 \pm 1.5	0 \pm 0
Time weighted change from baseline \pm SD	0.3 \pm 05	-0.5 \pm 1.4	0.7 \pm 08
CMV Antigen			
Mean base line \pm SD (week 0)	24.8 \pm 36	11.2 \pm 13.8	21.2 \pm 22.8
Mean change from baseline \pm SD (week 2)	1.1 \pm 2.4	2.0 \pm 12.1	-1.9 \pm 15.9
Mean change from baseline \pm SD (week 20)	-7.0 \pm 27.2	-1.7 \pm 5.5	11.7 \pm 44.4
Time weighted change from baseline \pm SD	-10.0 \pm 30.0	-1.9 \pm 6.0	1.6 \pm 15.0

Virologic measures. Figure 2 demonstrates that the time-weighted mean (sd) change from baseline pVL over 20 weeks was 1.80 (0.72), 1.78 (0.91) and 0.96 (0.91) log₁₀ copies/mL for placebo, PC and FC respectively ($p = 0.253$, comparing FC and PC recipients with placebo; $p = 0.077$ comparing PC with FC recipients). Post-hoc analysis comparing FC with placebo recipients (mean difference 0.84 log₁₀ copies/mL, 95% CI -0.04, 1.72, $p = 0.060$) approached a significant difference. Similar conclusions were reached in analyses that controlled for baseline CD4+ cell count (data not shown).

The majority of patients experienced some form of viral rebound during the trial. Individual patient viral load plots are shown in Figure 3A–C and summary data are shown in Figure 2. The individual patient plots for placebo and the PC recipients are somewhat similar. The majority of patients experienced a significant increase within 5–7 weeks after cessation of antiretroviral therapy with only 1 of the 15 patients (7%) showing evidence of longer term control of pVL. In contrast, the pattern of recrudescing pVL in FC recipients was somewhat dissimilar. Only two patients had abrupt increases in pVL during the first 5–7 weeks, three patients continued to suppress HIV replication to low levels for the duration of follow-up and the remaining patients experienced later and blunted increases in HIV replication. An analysis comparing the rates of change in

pVL per day between vaccine groups did not reveal any significant differences (data not shown). All recipients of placebo, PC and FC had detectable pVL at weeks 9, 12 and 16, respectively. There was no significant difference in the time to detectable pVL in the combined FC and PC groups, compared with placebo (hazard ratio 1.21, 95% CI 0.40–2.97, $p = 0.682$). We performed a post-hoc analysis of time to pVL >10,000 copies/mL (illustrated in Fig. 4). Estimates of median time to pVL \geq 10,000 copies/mL suggest approximately 42, 38 and >145 days respectively for placebo, PC and FC recipients. For the comparison of FC versus PC, this difference is statistically significant (hazard ratio = 3.52, 95% CI-1.01, 12.32, $p = 0.049$).

A total of seven patients (three placebo, three PC and one FC) recommenced CART during the 20 weeks of the Analytical Treatment Interruption (ATI) although re-initiation was not entirely consistent with the protocol guidelines. Two PC recipients recommenced CART in advance of the specified criteria and two patients (one placebo and one FC) recommenced later than recommended. There were no significant differences in the time to re-initiation of CART between vaccine groups (data not shown). At day 140 (20 weeks), the estimated probability of a patient remaining off ART was 58%, 62% and 90%, for placebo, PC and FC recipients respectively.

In the longer term follow-up of patients five FC, three PC and one placebo recipient remained off CART. The total time off therapy for these patients is between 1171–1386 days. There was however no significant difference between vaccine groups for time to reinitiation of ART (hazard ratio = 1.98 95% CI 0.64, 6.11; $p = 0.235$ comparing FC and PC and hazard ratio = 2.70 95% CI 0.7, 10.3; $p = 0.145$ comparing FC with placebo).

In a further series of post-hoc analyses we examined covariates associated with virological outcomes. In a multivariate model, the only covariates significantly associated with differential pVL outcomes were the vaccine group and age. Relative to recipients of FC, PC recipients had significantly greater time-weighted mean change in pVL from baseline (0.84 log greater pVL, $p = 0.029$). Relative to recipients of FC, placebo recipients had different time-weighted mean change from baseline pVL, but this was not significant (0.72 log greater pVL, $p = 0.088$). In addition, subjects aged 36–44 years had higher risk for greater pVL than younger patients (0.85 log greater pVL, $p = 0.042$).

Immunologic measures. There were no statistically significant differences in the total number or proportions of CD4+ or CD8+ T lymphocytes between treatment groups during follow-up (data not shown). Neither were there significant changes from baseline in these measures within any of the vaccine groups (data not shown).

ELIspot responses. IFN γ ELIspot data are summarized in Table 2. No significant increase in response was observed upon comparison of anti-Gag responses of FC or PC with placebo at week two suggesting vaccination did not impact on the ability of T-Cells to produce IFN γ . There was no significant difference in mean difference of the time weighted change in Gag response from week 0 when FC was compared to PC and placebo [mean = -222.3 (95% CI -524.5, 79.9; $p = 0.141$)] or upon comparison of FC with placebo [mean = -319.2 (95% CI -688.3, 49.9; $p = 0.0848$)]. Similar results were observed in respect to Nef antigen, when either Nef responses for FC were compared to PC and placebo [mean = -104.6 (95% CI -350.9, 217.9; $p = 0.388$)] or upon comparison of FC with placebo [mean = -78.1 (95% CI -348.7, 192.5; $p = 0.5459$)]. For both Gag and Nef antigens there was an increase in response with time during the ATI suggesting this was due to viral rebound. Furthermore when time weighted change in ELIspot responses from baseline to Gag was compared to time weighted log pVL change from baseline there was a weak correlation of borderline significance ($R = 0.4$, $p = 0.053$).

Lymphoproliferative response. Lymphoproliferative data are summarized in Table 2. Neither Gag nor Pol responses were significantly increased at week 2 following booster vaccination. There was no measured increase in proliferative responses during the treatment interruptions. There were no significant differences observed upon comparison of mean time weighted change from

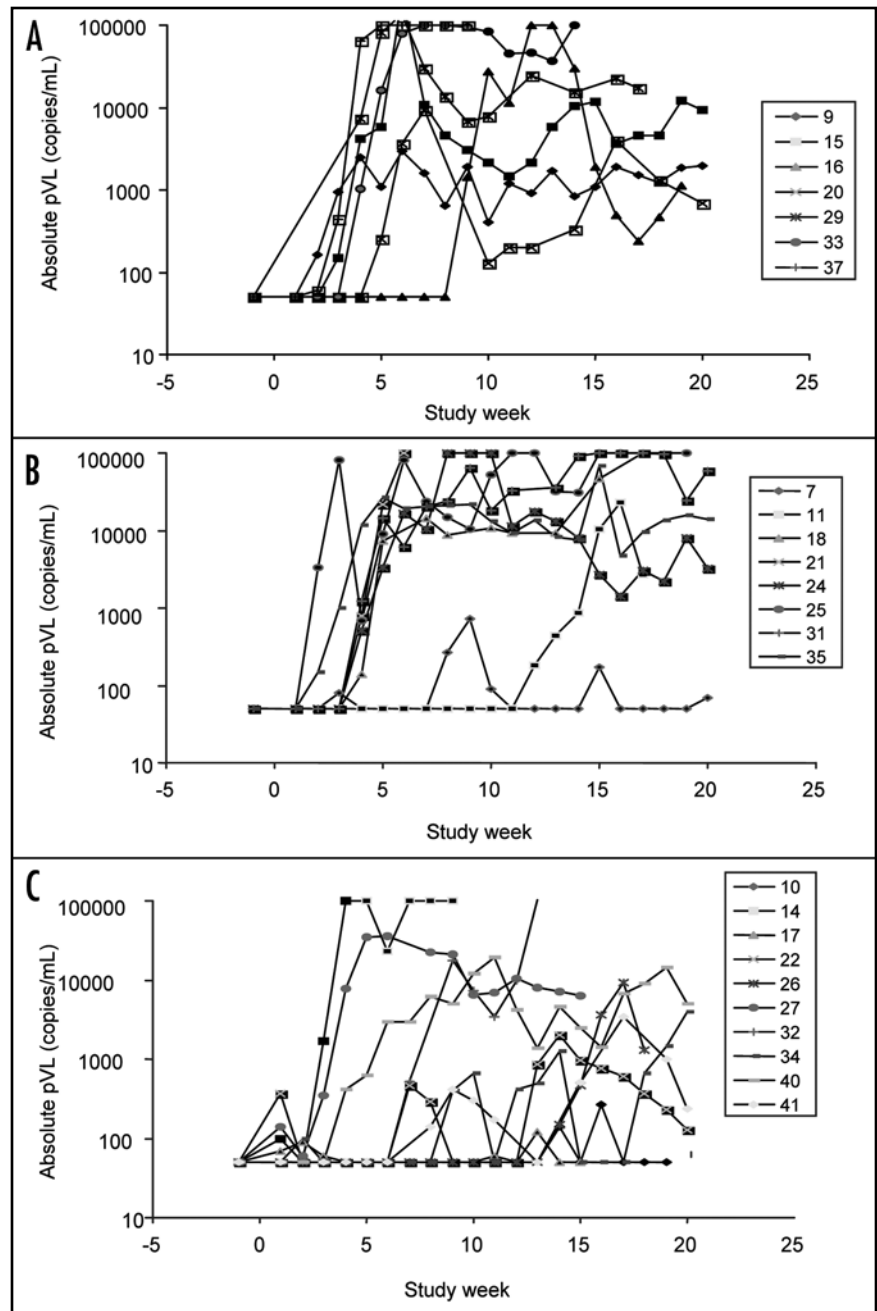


Figure 3. Analysis of HIV rebound for individual patients upon discontinuation of antiretroviral therapy (ART) in the analytical treatment interruption. The individual plasma HIV-1 RNA levels are grouped by treatment arm. (A–C) show placebo, PC and FC recipients respectively. Data obtained upon antiretroviral re-initiation is not shown.

week 0 lymphoproliferative response of FC when compared to combination of PC and placebo groups [mean = 3.68 (95% CI = -1.63, 9.00), $p = 0.164$] or upon comparison of FC and placebo [mean = 2.1 (95% CI = -5.1, 9.4), $p = 0.5342$]. No significant changes were seen in response to Nef antigen (Table 2). Similarly no differences between groups were observed for the control CMV antigen (Table 2).

Innate immune responses. There was no significant increase in ex vivo production of either IRF-1 or CIITA from PBMC at week 2 for any of the groups (data not shown). There was no significant difference in ex vivo IFN α mRNA expression from

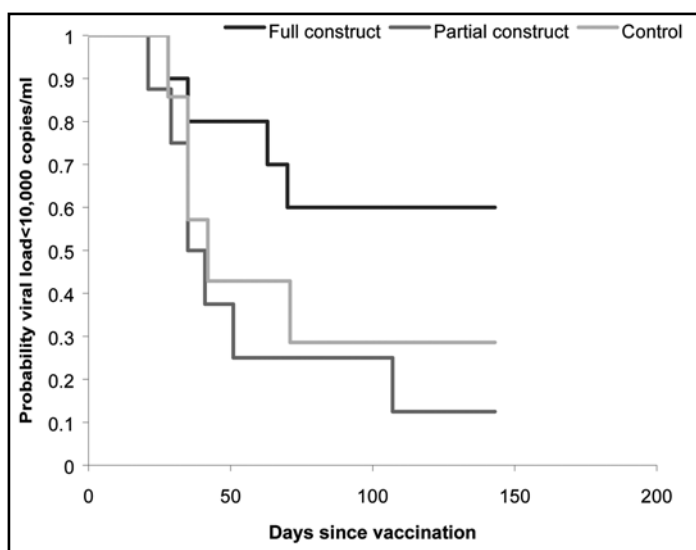


Figure 4. Time to plasma HIV RNA $>10,000$ copies/ml for each treatment group upon discontinuation of antiretroviral therapy (ART) in the analytical treatment interruption as summarised using Kaplan Meier plots.

PBMC between FC and PC ($p = 0.931$) or FC and Placebo ($p = 0.135$) (data not shown).

Host genetic factors influencing control of viral load. The carriage of CCR5, CCR2, and SDF1-polymorphisms is summarized in Table 1. The distribution of these polymorphisms was not different between treatment groups and there was no correlation between the carriage of any of these polymorphisms and the ability to control viral load (data not shown). The sample size of this study is too small to make any conclusions about the effects of HLA alleles on outcome. Alleles were divided into those that have been reported to have a negative effect on viral load (homozygosity of class I allele, carriage of HLA-B35) and into those that have been reported to have a positive outcome on viral load (HLA-B27 and HLA-B57).²⁴ The distribution of these alleles was not different across the three vaccination groups (data not shown).

Toxicities and safety data. There were no serious adverse events reported in this study. The distribution of adverse events, and severity was consistent across each randomly assigned vaccine group (data not shown). Treatment emergent adverse events that occurred within the first week after vaccination prior to analytical treatment interruption were placebo (43%), PC (25%) and FC (10%). Of note, only one PC recipient reported a moderate localized reaction to vaccination.

DISCUSSION

Both rFPV-based vaccines (PC and FC) were shown to be safe and well tolerated when given to subjects who previously received three doses of the construct in a previous trial.¹⁴

The primary study endpoint (mean time-weighted change from baseline in viral load) was approximately one log lower in those receiving the FC compared to those that received either the PC or placebo. This suggestion of an effect of the FC was consistently demonstrated across a variety of analytical approaches including covariate analysis although not attaining statistical significance. This is possibly due to the small study size. In the multivariate modelling age was the only covariate other than vaccine associated

with differential effect on viral load. There was a statistically significant reduction in time to $pVL \geq 10,000$ and when adjusted for age, multivariate analysis demonstrated that PC recipients were approximately 4.5 times more likely to reach $pVL \geq 10,000$ than FC recipients.

The major effect of FC seems to be a blunted recrudescence of pVL . This recrudescence appears to follow very similar patterns in the placebo and the PC groups. In both groups, after the initial rise there is a consistent pattern of equilibration to a variable set-point that appears relatively stable thereafter. In the FC group, there appear to be two discernable patterns of pVL recrudescence. Two patients (20%) experienced a rapid increase in pVL within the first 5–7 weeks of ceasing CART, while the remainder (80%), appeared to exert varying degrees of control over HIV replication throughout the period of study with some patients experiencing negligible recrudescence. In addition, and potentially important to our understanding of the immunopathology in this group, is a small transient peak within two weeks of cessation of antiretroviral drugs during which, pVL levels increased to about 200–300 copies/mL before dissipating. Thereafter the rate of increase of these curves was highly variable but appeared to be consistently lower than the experience of subjects in the other two groups.

The observed antiretrovirological effect of FC was manifest over the 20 week period of the clinical study. It is therefore unclear if vaccination with FC had only a transient antiretrovirological effect which would manifest in no long term clinical benefits for vaccinated individuals. Future studies should evaluate potential long term clinical benefits of vaccination by employing longer ATI periods. In addition it will be critical to ascertain if boosting with FC during ATI prolongs the period of viral suppression.

No clear immunological correlate could be detected between arms to explain these results on the basis of the measures of T-cell immunity or down stream effects of $IFN\gamma$ or innate immunity performed on PBMC. Importantly, although these experiments and their analysis do not provide an immune correlate of this effect, the data presented here exclude the premise that the improved response seen in the FC arm could be explained by host factors associated with good or poor viral control across the treatment arms.

The reason for the failure to measure an immunologic correlate in this study is unclear. The FC was designed to induce a strong cell-mediated immune response, as previously observed in macaques.¹³ There remains the possibility that FC vaccination has primed the immune system appropriately prior to the Analytical Treatment Interruption (ATI), the treatment interruption effectively boosting other unmeasured responses to autologous virus.¹² Alternatively as fowlpox persists for greater than a week, $IFN\gamma$ expression may be affecting viral replication directly via modulation of the immune response upon uptake of the FC by antigen presenting cells and release of the wildtype HIV upon ATI.

It is interesting to compare these results with those of previous studies where ATI was performed after therapeutic vaccination. In the Quest study patients who had commenced ART very soon after primary infection were vaccinated with either the canarypox vector vCP1452 alone or in combination with Remune (Inactivated HIV Immunogen). There was no significant difference between placebo and active groups in their ability to control HIV viral replication in the ATI.¹¹ Comparable results were observed upon vaccination of a similar patient group with vCP1452 + gp160.²⁵ Despite measurable immune responses being induced in both trials

upon vaccination there was no correlation between immunogenicity and viral control during ATI.^{11,25} Contrary to these results upon vaccination of chronically HIV-infected patients with the canarypox vector vCP1433 and the lipopeptides HIV-LIPO-6T followed by three cycles of IL-2 enhanced viral control of the vaccinated group was observed that correlated with the measured immune response.²⁶

In summary the FC was demonstrated to be safe and well tolerated in patients with HIV-1 infection. Results suggest that the FC may have the potential to control viral replication during ATI. It is noted that these studies have been conducted in a small number of patients and a further study of these constructs is required in broader populations with HIV disease. Such studies could include a dose ranging design with an expanded array of assays of the innate and adaptive immune system so as to further probe the virological effect and mechanism of action of this promising immunotherapeutic vaccine. To support further late stage development of FC such trials would have to demonstrate significant viral load reduction relative to the placebo arm that might retard decline in CD4 cell count. A promising application for a HIV therapeutic vaccines possessing such characteristics would be the lengthening the time between primary HIV infection and the need to commence CART.

Acknowledgements

The National Centre in HIV Epidemiology and Clinical Research is funded by the Australian Government Department of Health and Ageing and is affiliated with the Faculty of Medicine at the University of New South Wales. Virax Immunotherapeutics Pty Ltd. provided the candidate vaccines for this trial and funded the research.

National Institute of Allergy and Infectious Diseases for provision of sets of overlapping peptides derived from HIV-1 sequences for the conduct of ELIspot assays.

Other members of the NCHVR02 study team are: Mark Kelly, Jacinta Perram, Janice Forrester, Jeff Hudson, Ground Zero Medical Centre; Robert Fielden, John Miller, Andrew Carr, Sarah Pett, Patrick Mallon, St Vincent's Hospital; David Austin, Andrew Gowers, Holdsworth House General Practice; Julie Patching, Carlton Clinic; Claire McCormack, The Alfred Hospital; Helen Thomson, Sahar Bassal, Virax Development Pty Ltd.; Chloe Brereton, The National Centre in HIV Epidemiology and Clinical Research; Australian Red Cross Blood Service, Southern Division.

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