2. Connor EM, Sperling RS, Gelber R, Kiselev P, Scott G, O'Sullivan MJ, et al. **Reduction of maternal–infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group.** N Engl J Med 1994; **331**:1173–1180.

3. Cardo DM, Culver DH, Ciesielski CA, Srivastava PU, Marcus R, Abiteboul D, et al. **A case–control study of HIV seroconversion in health care workers after percutaneous exposure. Centers for Disease Control and Prevention Needlestick Surveillance Group.** N Engl J Med 1997; **337**:1485–1490.

4. Braitstein P, Chan K, Beardsell A, McLeod A, Montaner JSG, O'Shaughnessy MV, Hogg RS. **Another reality check: the direct costs of providing post-exposure prophylaxis in a population-based programme.** AIDS 2001; **15**:2345–2347.

5. Sociales MdlSedA. **Circular DGS/DH/DRT/DSS no. 98/228 of 9 April about recommendations for the implementation of antiretroviral treatment after exposure to the risk of transmission of HIV [in French].** Paris, France: Assistance Publique Hopitaux de Paris; 1998.

6. Lot F, Larsen C, Basselier B, Laporte A. **Evaluation of the therapeutic assumption of responsibility for exposures to HIV; July 1999–December 2001 [in French].** BEH 2003; **36**:173–175.

7. Pinkerton SD, Martin JN, Roland ME, Katz MH, Coates TJ, Kahn JO. **Cost-effectiveness of postexposure prophylaxis after sexual or injection-drug exposure to human immunodeficiency virus.** Arch Intern Med 2004; **164**:46–54.

8. Sonder GJB, Regez RM, Brinkman K, Prins JM, Mulder JW, Spaargaren J, et al. **Prophylaxis and follow-up after possible exposure to HIV, hepatitis B virus, and hepatitis C virus outside hospital: evaluation of policy 2000–3.** BMJ 2005; **330**:825–829.

9. Schechter M, do Lago RF, Mendelsohn AB, Moreira RI, Moulton LH, Harrison LH, et al. **Behavioral impact, acceptability, and HIV incidence among homosexual men with access to post-exposure chemoprophylaxis for HIV.** J Acquir Immune Defic Syndr 2004; **35**:519–525.

10. Vittinghoff E, Douglas J, Judson F, McKirnan D, MacQueen K, Buchbinder SP. **Per-contact risk of human immunodeficiency virus transmission between male sexual partners.** Am J Epidemiol 1999; **150**:306–311.

11. Nachega JB, Stein DM, Lehman DA, Hlatshwayo D, Mothopeng R, Chaisson RE, Karstaedt AS. **Adherence to antiretroviral therapy in HIV-infected adults in Soweto, South Africa.** AIDS Res Hum Retroviruses 2004; **20**:1053–1056.

A randomized, placebo-controlled phase I trial of DNA prime, recombinant fowlpox virus boost prophylactic vaccine for HIV-1

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An HIV-vaccine consisting of a DNA prime, recombinant fowlpox virus (rFPV) boost was evaluated in a double-blind placebo controlled trial. One milligram of pHIS–HIV-B expressing mutated *gag, pol, env, vpu, tat* and *rev* was administered at weeks 0 and 4 boosted by 5×10^7 pfu rFPV–HIV-B expressing *gag/pol* at week 8. The vaccine regimen was safe, but there was no difference between vaccine (*n* = 18) and placebo recipients (*n* = 6) for Gag or Pol-specific T-cell immune responses at week 9.

The need for an effective HIV-1 prophylactic vaccine is clear. The design of a vaccine inducing neutralizing antibodies remains problematical [1]. T-cell responses are implicated in the prevention [2,3] and control of HIV-1 infection [4]. DNA plasmids and viral vectors offer alternatives to attenuated viruses for the induction of T-cell responses, but either used alone have limited immunogenicity [5–7]. Priming immune responses with DNA and boosting with a viral vector encoding identical antigens has proved efficient for inducing T-cell responses effective in modifying infection post-challenge in mice and non-human primates [5–7].

We conducted a single-centre, randomized, placebo-controlled, double blind, phase I trial, the primary objectives of which were to assess the safety and immunogenicity of a vaccine strategy employing a DNA prime (pHIS–HIV-B) and a recombinant fowlpox virus (rFPV–HIV-B) boost. These constructs encode complementary HIV antigens and had proved safe, highly immunogenic and effective in controlling viral challenges in mice and non-human primates [6,8].

The design of pHIS–HIV-B is reported elsewhere [6]. Based on pHIS-64 (Coley Pharmaceutical Group, Boston, Massachusetts, USA) it contains 65% of the HIV-1 genome including *gag, pol*, truncated *env, tat, vpu* and *rev* with various mutations introduced to enhance safety, humanized CpG motifs to enhance immunogenicity and a synthetic intron before HIV gene coding sequences to optimize expression. The construct synthesized to standards of good manufacturing practices (GMP) was resuspended in 0.9% saline at 1 mg/ml (Qiagen, Germany). rFPV–HIV-B, based on attenuated strain FPV-M3, expressing identical *gag* and *pol* sequences [9], was manufactured to GMP (Virax, Melbourne, Australia), and diluted to 5×10^7 plaque forming units (pfu)/ml in 0.9% saline/10% glycerol. Identical placebos for both constructs contained diluent only.

A total of 28 volunteers were screened, and 24 eligible healthy volunteers aged between 18 and 55 years, at low risk of HIV infection, in good health and who had never received active HIV vaccines were randomly assigned in a 3 : 1 ratio to receive either active vaccine or placebo. Effective contraception was mandatory. Volunteers received 1 ml pHIS–HIV-B to the dominant deltoid at weeks 0 and 4 and 1 ml rFPV–HIV-B to the non-dominant deltoid at week 8. Safety and immunogenicity were assessed at vaccine visits and at weeks 9 and 12. HIV status was determined by routine serology and pro-viral DNA (Amplicor; Roche Diagnostics, Pleasanton, California, USA). Pre and post-test counselling was performed at each visit. All procedures were approved by the local Research Ethics Committee.

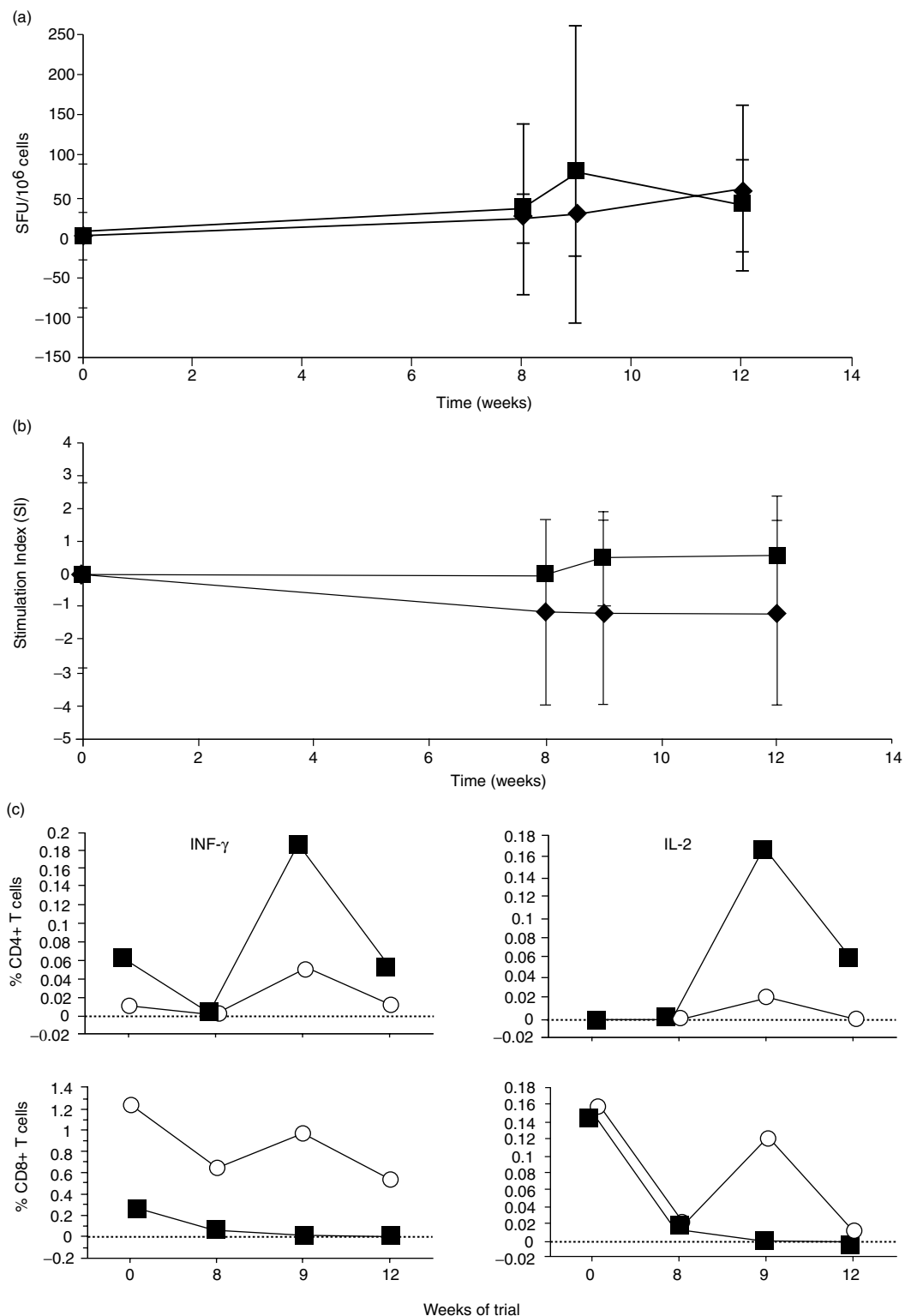Co-primary immunogenicity endpoints were comparisons at week 9 of the mean change from the baseline of

Fig. 1. HIV-1-specific T-cell responses to vaccine administration. (a) IFN-γ responses to single pool of 123 Gag peptides as measured by enzyme-linked immunospot assay. SFU, Spot-forming units. ——■—— Vaccine recipients ($n = 18$); ——◆—— placebo recipients ($n = 6$). (b) Proliferative responses to p24. Mean responses for vaccine and placebo groups are shown, error bars represent 1 SD. (c) Vaccine-induced responses are mediated by CD4 T cells: responses to the same pool of Gag peptides in volunteer no. 108 in vaccine recipient group, one week after recombinant fowlpox virus (rFPV)–HIV-B boost measured by intracellular cytokine staining. Both IL-2 and IFN-γ responses were detected from CD4 cells but not CD8 cells. Responses in the other responder at week 9 were similar in nature. ——○—— Cytomegalovirus/cytomegalovirus/Epstein–Barr virus/'flu; ——■—— Gag.

the IFN-γ enzyme-linked immunospot assay (ELIspot) response to a single pool of 123 15-mer Gag peptides (NIH AIDS Research and Reference Reagent Program, catalogue no. 5107) and the proportion of volunteers responding to this antigen. Analyses were intention-to-treat and included all randomized volunteers receiving any vaccines. An ELIspot result was defined as positive if it was greater than twice the background and greater than 50 sfu/10^6 cells after substraction of the background. Safety data, including adverse events and clinical laboratory data, were listed by treatment group. Adverse event severity was graded using the Division of AIDS/Community Programs for Clinical Research on AIDS toxicity scale.

All volunteers received each vaccine per schedule, attending all specified visits. All participants were Caucasian, predominately male (15/24), and were well matched between groups for age (mean 39 years). Five out of six randomly assigned to placebo were women.

There were no serious adverse events. The vaccines were well tolerated, with no local, systemic adverse events or laboratory abnormalities rated greater than grade 2. No erythema or ulceration was observed at injection sites. Systemic effects were mild and non-specific. Adverse events, local or systemic, were distributed equally between the two groups.

No significant difference in immunogenicity could be demonstrated in response to pooled Gag peptides with the mean (SD) time weighted IFN-γ ELIspot response at week 9 being 6 sfu/10^6 (250) and −3 sfu/10^6 (7.7) cells in the vaccine and placebo groups, respectively ($P = 0.28$, Fig. 1a). There was no significant difference in the number of volunteers with positive responses (4/18 versus 1/6) at week 9 ($P = 0.99$). These results were consistent across an exhaustive range of additional ELIspot and lymphoproliferative assays (Fig. 1b) in which various forms of Gag and Pol antigen were used as stimulating antigens. An intracellular cytokine assay demonstrated that any positive responses to Gag were mediated by CD4 T cells producing both IL-2 and IFN-γ (Fig. 1c). One vaccine recipient developed a faint p24 band on Western blot by week 12. No other bands developed in any individual. Fowlpox antibodies developed in 25% of vaccine recipients by week 12.

This combination of DNA-prime, pox-virus boost induces effective CD4 and CD8 T-cell responses in animal models. Lower peak and steady state viral loads were observed after SHIVmn229 challenge in macaques [7]. However, these vaccines administered via the same route and schedule are poorly immunogenic in humans. Recent reports suggest that other DNA vaccines boosted with other pox viruses, such as MVA, delivering similar HIV antigens, are also poorly immunogenic in humans [10]. The postulated causes of this discrepancy between

responses in animal models and humans include: differences in the route of administration; the relative dose−response curves determined by differences in body size; species-specific responses to DNA vaccines; and the effects of regulatory genes such as *vpu* and *tat* on antigen presentation in humans. Being smaller than humans the relative dose normalized for body weight or surface area is much larger (6−20-fold depending on the metric used) in macaques. When a dose approximately equivalent to the dose administered here is given to macaques (0.2 mg pHIS−HIV-B), it is non-immunogenic. In contrast, doses of 1 and 4.5 mg of pHIS−HIV-B induce equivalent high levels of immunogenicity in macaques, suggesting a steep dose−response curve. On this basis, doses of 5−6 mg of pHIS−HIV-B may be immunogenic in humans. Recent unpublished reports from a number of groups suggest that DNA vaccines become immunogenic in humans at this dosage range.

The results of this trial are consistent with others exploring similar strategies of DNA prime, viral vector boost to induce T-cell immunity. The next trial of these constructs should determine whether maximal doses induce consistent immune responses. Such an approach is justified given the safety profile of these vaccines.

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References

1. Haigwood NL, Stamatatos L. **Role of neutralizing antibodies in HIV infection.** *AIDS* 2003; **17 (Suppl. 4)**:S67–S71.
2. Rowland-Jones S, Sutton J, Ariyoshi K, Dong T, Gotch F, McAdam S, *et al.* **HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women.** *Nat Med* 1995; **1**:59–64.
3. Clerici M, Giorgi JV, Gudeman VK, Chou C-C. **Cell-mediated immune responses to human immunodeficiency virus (HIV) type 1 in seronegative homosexual men with recent sexual exposure to HIV.** *J Infect Dis* 1992; **165**:1012–1019.

4. Pantaleo G, Koup RA. **Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know.** *Nat Med* 2004; **10**:806–810.
5. Kent SJ, Zhao A, Best S, Chandler JD, Boyle D, Ramshaw IA. **Enhanced T cell immunogenicity and protective efficacy of a HIV-1 vaccine regimen consisting of consecutive DNA priming and recombinant fowlpox virus boosting.** *J Virol* 1998; **72**:10180–10188.
6. Dale CJ, De Rose R, Wilson K, Croom HA, Thomson S, Coupar BE, *et al.* **Evaluation in macaques of HIV-1 DNA vaccines containing primate CpG motifs and fowlpoxvirus vaccines co-expressing IFNg or IL-12.** *Vaccine* 2004; **23**:188–197.
7. Dale CJ, De Rose R, Stratov I, Chea S, Montefiori DC, Thomson S, *et al.* **Efficacy of DNA and fowlpoxvirus prime/boost vaccines for simian/human immunodeficiency virus.** *J Virol* 2004; **78**:13819–13828.
8. Ramsay AJ, Kent SJ, Strugnell RA, Suhrbier A, Thomson SA, Ramshaw IA. **Genetic vaccination strategies for enhanced cellular, humoral and mucosal immunity.** *Immunol Rev* 1999; **171**:27–44.
9. Boyle DB, Anderson M, Amos R, Voysey R, Coupar BE. **Construction of recombinant fowlpox viruses carrying multiple vaccine antigens and immunomodulatory molecules.** *BioTechniques* 2004; **37**:104–111.
10. Cohen J. **AIDS vaccines. HIV dodges one-two punch.** *Science* 2004; **305**:1545–1547.

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Enfurvitide prevents vertical transmission of multidrug-resistant HIV-1 in pregnancy but does not cross the placenta

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The use of previously successful antiretroviral regimens in mother-to-child transmission (MTCT) prevention will be increasingly challenged by the rising prevalence of multidrug-resistant (MDR) HIV. We used enfurvitide together with an optimized antiretroviral backbone to prevent the MTCT prevention of MDR HIV in two pregnant women. The measurement of maternal and foetal peripheral blood levels of enfurvitide showed no evidence of transplacental transfer.

The suppression of maternal HIV viraemia is integral to the prevention of mother-to-child transmission (MTCT) of HIV-1. For pregnant women with viral loads below 10 000 copies/ml zidovudine monotherapy may suffice [1], but in women with a high viral load, HAART is indicated because of its greater efficacy in reducing both viraemia and MTCT [2].

The growing use of antiretroviral drugs has led to increasing numbers of patients harbouring resistant virus; surveillance in the UK [3] has indicated increasing rates of multidrug-resistant (MDR) HIV-1. HAART has also been associated with the development of antiretroviral resistance [4] in pregnancy with the transmission of drug-resistant virus to infants.

With increasing rates of HIV-1 seroprevalence among pregnant women in the UK [5], the treatment of MDR HIV-1 in pregnancy is likely to become an increasingly common problem. Here we describe the use of the HIV-1 fusion inhibitor enfurvitide to prevent MTCT of MDR HIV-1 in two pregnant women, with no evidence of the transplacental transfer of enfurvitide.

Case one

A 36-year-old woman presented at 16 weeks' gestation. Two previous children had died of lymphoma associated with MTCT of HIV-1. Previous treatment exposure included zidovudine, zalcitabine, stavudine, didanosine, lamivudine, nevirapine, indinavir, nelfinavir, ritonavir, saquinavir and lopinavir. She was intolerant of ritonavir, reporting persistent perioral paraethesia and loss of taste. Her CD4 cell count was 98 cells/mm^3 and her viral load was 20 000 copies/ml, and she was started on a regimen of abacavir, delavirdine, fosamprenavir and atazanavir. Previous genotypic resistance tests revealed multiple mutations (reverse transcriptase M41 L/M, D67N, L74V mix, K70R, M184V, T215Y/F, V106A and K219E/Q; protease M36I, L33F, I54M and L90M, subtype D/A/B).

Although initially achieving full virological suppression on this regimen (viral load < 50 copies/ml) at 24 weeks' gestation, she subsequently experienced viral rebound to 1362 copies/ml at 32 weeks' gestation. Whereas the risk of transmission was low, we could not predict viral