



A Phase I study evaluating the safety and immunogenicity of MVA85A, a candidate TB vaccine, in HIV-infected adults

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5 **A Phase I study evaluating the safety and immunogenicity of**
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7 **MVA85A, a candidate TB vaccine, in HIV-infected adults**
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ABSTRACT

OBJECTIVES: Control of the TB epidemic is a global health priority and one that is only likely to be achieved through vaccination. The critical overlap with the HIV epidemic requires any effective TB vaccine regimen to be safe in individuals who are infected with HIV. The objectives of this clinical trial were to evaluate the safety and immunogenicity of a leading candidate TB vaccine, MVA85A, in healthy, HIV-infected adults.

DESIGN: This was an open-label Phase I trial, performed in 20 healthy HIV-infected, antiretroviral-naïve subjects. Two different doses of MVA85A were each evaluated as a single immunisation in 10 subjects, with 24 weeks of follow-up. Safety of MVA85A was assessed by clinical and laboratory markers, including regular CD4 counts and HIV RNA load measurements. Vaccine immunogenicity was assessed by *ex-vivo* IFN- γ ELISpot assays and flow cytometric analysis.

RESULTS: MVA85A was safe in subjects with HIV infection, with an adverse event profile comparable to previous trials in HIV-uninfected subjects. There were no clinically significant vaccine-related changes in CD4 count or HIV RNA load in any subjects, and no evidence from qPCR analyses to indicate that MVA85A vaccination leads to widespread preferential infection of vaccine-induced CD4+ T cell populations. Both doses of MVA85A induced an antigen-specific IFN- γ response that was durable for 24 weeks, although of a lesser magnitude compared to HIV-uninfected subjects. The functional quality of the vaccine-induced T cell response in HIV-infected subjects was remarkably comparable, although less durable, to that observed in healthy HIV-uninfected controls.

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3 **CONCLUSION:** MVA85A is safe and immunogenic in healthy adults infected with
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5 HIV. Further safety and efficacy evaluation of this candidate vaccine in TB and HIV
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7 endemic areas is therefore merited.
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10 11 12 **ARTICLE SUMMARY SECTION**

13 14 **Article focus**

- 15
16 • HIV infection increases susceptibility to TB, and globally, TB is the cause of
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18 death in up to half of AIDS deaths
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22 • There is an urgent need for a safe and effective TB vaccine in HIV-infected
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24 people
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27 **Key messages**

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29 • MVA85A, a leading candidate TB vaccine, is safe and well tolerated in HIV-
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31 infected people and does not induce changes in either CD4 count or HIV RNA
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33 load
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37 • MVA85A is immunogenic in HIV-infected people, and induces a similar
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39 immune profile to that seen in HIV-uninfected people, but the immunogenicity
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41 is less durable in HIV-infected people.
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44 **Strengths and limitations of this study**

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46 • This is a Phase I study with 20 subjects, and further studies are needed in TB
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48 endemic countries in this important target population.
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59 Oxford Biomedical Research Centre.
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INTRODUCTION

Tuberculosis (TB) and HIV are inextricably linked. At the end of 2007, approximately 33.2 million persons were living with Human Immune Deficiency Virus-1 (HIV) infection, an estimated one-third of whom were co-infected with *Mycobacterium tuberculosis* (*M. tuberculosis*)¹. TB is the cause of death for up to half of all AIDS patients² and the increasing incidence of drug-resistant strains of *M. tuberculosis* poses a significant threat to a susceptible HIV-infected population³.

Mycobacterium bovis (*M. bovis*) Bacille Calmette Guérin (BCG) fails to protect consistently against the adult pulmonary form of TB, whilst providing reliable protection against disseminated infection in childhood⁴. An improved vaccine strategy is thus essential for global control of this disease⁵. MVA85A (modified vaccinia virus Ankara expressing antigen 85A) is a leading candidate TB vaccine, designed to enhance the effect of BCG; it is safe and highly immunogenic in healthy BCG-naïve and BCG-vaccinated subjects, and in subjects latently infected with *M. tuberculosis* in the UK and Africa⁶⁻¹¹.

It is essential that any new TB vaccine is safe in an HIV-infected population. Subunit vaccines are an ideal choice for an immuno-compromised population in which the safety of replicating whole organism vaccines may be a concern. Although MVA is a live viral vaccine vector, it cannot replicate in human cells^{12, 13}. There are now safety data from a number of clinical trials with recombinant MVAs in HIV-infected subjects, which demonstrate no sustained effect on either HIV load or CD4 count¹⁴⁻¹⁶. Most of these studies assessed immune-reconstituted HIV-infected individuals on anti-retroviral therapy (ARV); however, some ARV-naïve subjects with more advanced HIV infection have also been vaccinated with a recombinant MVA, with no

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3 significant rise in HIV load or fall in CD4 count over a 4-week follow-up period ¹⁷.
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5 Preclinical studies in severely immuno-suppressed macaques have also documented
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7 safety in this model ¹⁸. To date, MVA85A has been administered to more than 1,000
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9 individuals with no vaccine-related serious adverse events ^{6, 8-11} (McShane,
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11 unpublished data).
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15 Here we present the first clinical trial of a subunit TB vaccine in an HIV-infected
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17 population. This trial was designed to evaluate the safety and immunogenicity of two
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19 doses of MVA85A in healthy HIV-infected subjects in the UK.
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METHODS

Trial design and participants

The protocol for this multi-site study was approved by the Medicines and Healthcare products Regulatory Agency (MHRA) and the Gene Therapy Advisory Committee (GTAC). Participants were recruited from the Genitourinary Medicine (GUM) departments at the Oxford Radcliffe Hospitals NHS Trust, University Hospitals Birmingham NHS Foundation Trust (Selly Oak Hospital), Great Western Hospitals NHS Foundation Trust, and Imperial College Healthcare NHS Trust, London.

Potentially eligible subjects were referred to the clinical trial team. Vaccination visits took place in Oxford; screening and follow-up visits took place at local NHS Trust sites. Written informed consent was obtained from all subjects prior to enrolment in the trial, and the referring HIV physicians were notified.

Eligibility required participants to be generally healthy, aged 18-55 years, with a normal chest radiograph and no clinical or radiological evidence of TB disease, a current CD4 count (measured at screening) of at least $350 \times 10^6/L$, a nadir CD4 count of not less than $300 \times 10^6/L$ and an HIV RNA load at screening of less than 100,000 copies/mL. All subjects were diagnosed with HIV infection at least six months before screening and had not received any ARVs within the preceding six months (full inclusion/exclusion criteria, Supplemental Methods). In all cases, testing for latent *M. tuberculosis* infection (LTBI) was conducted at screening using an in-house *ex-vivo* interferon gamma (IFN- γ) ELISpot assay for ESAT6 and CFP10 peptides. Subjects found to be latently infected were referred back to their consultants for possible treatment at the end of the trial.

The trial was registered on a clinical trials database: ClinicalTrials.gov ID NCT00395720.

Vaccine

Clinical grade MVA was produced under Good Manufacturing Practice standard by IDT Biologika GmbH.

Procedures

Participants were vaccinated intradermally with either 5×10^7 plaque-forming units (pfu; first group of 10 subjects) or 1×10^8 pfu (second group of 10 subjects). A diary card was completed by all subjects recording local and systemic adverse events and body temperature for days 1 to 7 post-vaccination. All subjects were followed up regularly for 12 months; blood samples taken at each time-point for CD4 count, HIV RNA load measurement and cellular immunological assays. CD4 count and HIV RNA load results were communicated to the patient's HIV physician at regular intervals. Routine haematology and biochemistry assays were performed at weeks 1 and 12 after vaccination. All subjects attended their usual NHS follow-up appointments and received routine clinical care throughout the trial.

Immunological assays

Ex vivo IFN- γ ELISpot assays were performed at screening and at weeks 1, 2, 4, 12, and 24 post-vaccination using fresh peripheral blood mononuclear cells (PBMCs), as described previously¹⁰. A single pool of 15-mer peptides spanning the Ag85A protein (66, overlapping by 10 amino acids, 2 μ g/mL final concentration of each peptide in each well), and 7 pools of non-overlapping 9-10 Ag85A peptides (final concentration of 10 μ g/mL in each well) were used to stimulate for the IFN- γ ELISpot assays. For the 7 peptide pool wells, the results were summed across all peptide pools for each time-point. This potentially resulted in duplicate counting of T cells that responded to any of the 10mer overlap regions, since any 10mer occurred in two pools with

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3 adjacent peptides, but allows direct comparison with immunogenicity data from
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5 previous trials.
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8 Intracellular cytokine staining of PBMCs was performed as described previously ¹⁹.
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10 Briefly, cells were stained with the amine reactive LIVE/DEAD fixable dye ViViD
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12 (Molecular Probes, Invitrogen) and then surface stained with monoclonal antibodies
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14 against CD4, CD14, CD19, CD27, CD45RO, CD57 and CCR5. After washing, cells
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16 were permeabilized (Cytofix/cytoperm kit; BD Pharmingen) and stained
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18 intracellularly for CD3, CD8, IFN- γ , TNF- α , IL-2 and MIP-1 β . Naïve CD4+ memory
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20 cells were identified as CD45RO-CD27+CD57+; memory CD4 T cell as
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22 CD45RO+CD27 \pm CD57 \pm . CD4+ T cells producing two or more cytokines (IFN- γ , IL-
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24 2 or TNF- α) following stimulation with Ag85A peptides were sorted to greater than
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26 98% purity using a modified FACSaria flow cytometer (BD Biosciences); CMV-
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28 specific CD4+ T cells, defined similarly, and bulk resting memory CD4+ T cells were
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30 sorted simultaneously. Cytokine production and the expression of surface markers
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32 were analysed using FlowJo version 8.7 (Tree Star Inc), Pestle version 1.5 and SPICE
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34 version 4.1 (both from Mario Roederer, Vaccine Research Center, NIAID, NIH,
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36 USA).
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44 Real-time quantitative PCR (qPCR) was used to determine the proportion of HIV-
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46 infected Ag85A-specific CD4+ T cells; resting memory CD4+ T cells (non-Ag85A-
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48 specific) and CMV-specific CD4+ T cells were used as intra-individual controls.
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50 HIV-1 gag proviral DNA within sorted CD4+ T cell populations was quantified by
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52 qPCR, as described previously ²⁰. qPCR was performed simultaneously for albumin
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54 gene copy number in all sorted CD4+ T cell populations to quantify cell number in
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56 each reaction.
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3 CD4+ T cells from 12 subjects (4/10 in the low-dose group and 8/10 in the high-dose
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5 group) were analysed for HIV *gag* content; low sort yields (less than 250 antigen-
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7 specific CD4+T cells) prevented similar analyses in the remaining 8 subjects.
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10 Serum levels of MIP-1 α , MIP-1 β , RANTES, TNF- α and IL-2 were measured pre-
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12 vaccination and at weeks one, two and 24 post-vaccination using FlowCytomix
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14 simplex kits (Bender MedSystems). Serum was thawed and plated out in duplicate in
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16 round-bottom 96-well plates. Samples were used neat for MIP-1 α , MIP-1 β , TNF- α
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18 and IL-2, and diluted 1/10 for RANTES. Standards (provided) were reconstituted as
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20 specified, and 1/3 serial dilutions plated in duplicate. Bead and biotin-conjugate
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22 cocktails were added to each well in 25 μ L and 50 μ L volumes, respectively, and
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24 incubated for two hours in the dark at room temperature. Samples were then washed
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26 twice in 100 μ L Assay Buffer. Streptavidin-PE (50 μ L) and Assay Buffer (100 μ L) were
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28 added and plates were incubated for one hour in the dark at room temperature.
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30 Samples were then washed again and transferred to cluster tubes in 4%
31
32 paraformaldehyde in PBS (400 μ L) and acquired using an LSRII flow cytometer (BD
33
34 Biosciences) set up with the beads provided and the highest concentration standard;
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36 the stopping gate was set so that at least 300 events were collected for each sample.
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38 Data were analysed using FlowCytomixPro software (Bender MedSystems).
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48 **Statistical analysis**

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50 Peak (1 week) and plateau (24 weeks) CD4+ T cell responses were compared with
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52 those at baseline using the Wilcoxon signed rank test, Stata Statistical Software,
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54 Release 9.0. 2005 (Stata Corporation, College Station, TX). Area under the curve
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56 (AUC) analysis was performed to compare the magnitude of vaccine-induced CD4+ T
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58 cell responses in each dose group. Comparisons between this trial and previous trials
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3 of MVA85A, and between low and high dose groups were conducted using the Mann-
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6 Whitney U test (Stata).

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8 Within-subject CD4 counts and HIV RNA load levels at weeks 1 and 24 post-
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10 vaccination were compared with baseline levels, using a repeated measures analysis
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12 of variance (*p*-values obtained from the F-test, Stata).

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15 Non-parametric Spearman rank analyses were used to correlate immune assays and
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18 CD4/HIV RNA load with CD4+ T cell responses.
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RESULTS

Thirty-five individuals attended for screening between October 2006 and June 2009; 20 subjects were enrolled (Fig. 1, consort). The demographics of the 20 participants are shown in Table 1, and were comparable across the two groups.

Local reactions related to the MVA vaccine occur during the first week after vaccination and have been reported previously^{6, 7, 10}. The local adverse event (AE) profile recorded in the diary cards of the HIV-infected subjects in this study was comparable to that observed in HIV-uninfected subjects, although pruritis appeared slightly higher in the HIV-infected group (Table 2a).

One subject in the low dose group experienced a single episode of fever greater than 37.5 °C (documented at 38.1°C). All other subjective systemic symptoms occurred with similar frequencies in the low dose and high dose groups (Table 2b).

Pre-vaccination CD4 counts and HIV RNA loads for all 20 subjects over a time period of one to six years before enrolment are shown in Figure 2. These longitudinal data illustrate the levels of natural fluctuation that occur over time and represent important baseline measurements for the interpretation of these parameters post-vaccination, also shown in Figure 2, in the absence of an unvaccinated control group. A repeated measures analysis of variance showed no evidence of a difference in either CD4 counts or HIV RNA load levels pre- and post- vaccination (low dose, CD4 count: $p=0.35$; low dose, HIV RNA load: $p=0.30$; high dose, CD4 count: $p=0.83$; high dose, HIV RNA load: $p=0.48$).

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3 A major concern with respect to the vaccination of HIV-infected individuals is the
4 possibility that responding antigen-specific CD4+ T cells will act as preferential
5 targets for HIV infection as a function of their activated status, thereby enhancing the
6 propagation of HIV and accelerating disease progression. In this study, however, there
7 was no evidence for preferential HIV infection of Ag85A-specific CD4+ T cells. The
8 median number of CD4+ T cells in Ag85A-specific qPCR reactions was 73 (95% CI:
9 15-130 CD4+ T cells). Where possible, multiple time-points throughout the
10 vaccination course for each subject were analyzed, to determine the proportion of
11 Ag85A-specific CD4+ T cells that were infected with HIV. Of 32 Ag85A-specific
12 CD4+ T cell samples in total (12 subjects), only three samples from two subjects
13 showed a positive signal for HIV *gag* DNA by qPCR: subject 1004 (week 1 post-
14 vaccination) and subject 1029 (weeks 2 and 8 post-vaccination) (data not shown). In
15 all 12 subjects tested, resting HIV-specific memory cell populations showed a positive
16 signal in all assays (data not shown). For CMV-specific CD4+ T cell populations,
17 HIV *gag* DNA was detected in subjects 1004, 1029 and 1035 at the pre-vaccination
18 time-points only (data not shown). All other samples were negative. In addition,
19 vaccination had no effect on levels of MIP-1 α , MIP-1 β , RANTES, TNF- α and IL-2 in
20 unstimulated serum (data not shown).

21
22 MVA85A induced significant antigen-specific T cell responses as measured by IFN- γ
23 ELISpot assay (Fig. 3a-d, Table 3a). In the low dose group, the frequency of IFN- γ -
24 secreting T cells responding to the single 85A peptide pool at week one post-
25 vaccination, increased by a median of 393 sfc/million compared to baseline
26 ($p=0.009$); there a significant increase remained until 24 weeks post-vaccination
27 ($p=0.032$). In the high dose group, a median increase of 502 sfc/million was apparent
28 at week 1 post-vaccination ($p=0.005$); again, this response was maintained until 24
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3 weeks ($p=0.048$). In both groups, T cell responses to the summed 85A peptide pools
4 followed a similar pattern with a significant increase above baseline at week 1 (738
5 and 1730 sfc/million for low and high dose groups, respectively), although, the week
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weeks ($p=0.048$). In both groups, T cell responses to the summed 85A peptide pools followed a similar pattern with a significant increase above baseline at week 1 (738 and 1730 sfc/million for low and high dose groups, respectively), although, the week 24 response was only maintained significantly above baseline in the high dose group (low dose $p=0.17$; high dose $p=0.007$). There were no significant differences in the magnitude of the IFN- γ response between low and high dose groups at any time-point ($p=0.29$ and $p=0.68$ for summed and single peptide pools, respectively; AUC analysis, data not shown). However the responder rate was higher in the high dose group with no observed non-responders compared with 1-2 non-responders (depending on antigen) in the low dose group. Overall, the kinetics of the Ag85A-specific T cell responses were very similar to those previously reported in similar studies of HIV-uninfected subjects⁶⁻¹¹.

Ag85A-specific T cell responses in the low-dose group were significantly lower compared to those observed in previous studies of HIV-uninfected subjects (AUC analysis, $p=0.02$; Table 3b). This difference was not significant at week 1 $p=0.12$, which represents the peak response, but was significant at week 24 post-vaccination ($p=0.004$; Table 3b, Figure 3e-g). There were no significant differences in baseline responses between the HIV-infected and HIV-uninfected groups ($p=0.85$; Table 3b). Similarly, HIV-infected subjects in the high dose group exhibited significantly lower responses compared to HIV-uninfected subjects who received the same dose of MVA85A (AUC analysis, $p=0.0001$; week 1 and week 24 $p = 0.0001$; Table 3b, Figure 3e-g). Again, baseline responses were comparable ($p=0.24$) (Fig. 3).

There was a weak positive correlation between the CD4 count at screening and the peak summed 85A peptide pool response ($R=0.04$, $p=0.09$), and a weak negative correlation between HIV RNA load at screening and the peak immune response ($R= -$

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3 0.04, $p=0.08$). There was a strong negative correlation between viral load at screening
4 and the plateau immune response at week 24 ($R= -0.8$, $p=0.002$).
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8 Four subjects were found to have LTBI. However, there were no significant post-
9 vaccination changes in the magnitude of ESAT-6/CFP10 responses in these subjects
10 (data not shown).
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14 Ag85A-specific cytokine/chemokine production by CD4+ T cells prior to MVA85A
15 vaccination was undetectable and increased significantly post-vaccination (Fig. 4a).
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17 One week following MVA85A vaccination, more than 40% of Ag85A-specific CD4+
18 T cells produced IFN- γ , IL-2, MIP-1 β and TNF- α simultaneously ($p<0.01$); this
19 remained significant at week 2 ($p<0.05$). By week 8 post-vaccination, 60% of Ag85A-
20 specific CD4+ T cells were still producing IFN- γ , IL-2 and TNF- α simultaneously;
21 production of IL-2 and IFN- γ was maintained until 24 weeks. Production of MIP-1 β
22 was not detected beyond week 2 post-vaccination (Fig. 4a, 5a). In the first two weeks
23 post-vaccination, Ag85A-specific CD4+ T cells exhibited similar functional profiles
24 in the HIV-infected groups, both low dose and high dose, and the "control" low dose
25 HIV-uninfected group; in all cases, the responses were dominated by four functionally
26 distinct subsets (Fig. 4b, c). IFN- γ production measured by ICS correlated closely
27 with IFN- γ secretion observed in the ELISpot assay ($R= 0.75$, $p<0.0001$, $n=45$; data
28 not shown).
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32 Surface expression of the HIV M-tropic co-receptor CCR5 was monitored throughout
33 the course of vaccination (Fig. 5). At week 1 post-vaccination, the MFI for CCR5 was
34 highest in the activated Ag85A-specific CD4+ T cells, and lowest in the naïve CD4+
35 T cell subsets. Using the integrated MFI (iMFI) function to account for cell
36 frequencies, expression of CCR5 was highest in the memory CD4+ T cell pool (Fig.
37 5d). No significant increase in CCR5 expression was detected in either the memory
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3 (Fig. 5e) or the total (Fig. 5f) CD4+ T cell populations as a result of MVA85A
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DISCUSSION

This is the first Phase I trial of a subunit vectored TB vaccine in HIV-infected individuals. Similar studies in South Africa (*Tameris et al, unpublished data*) and Senegal (*Mboup et al, unpublished data*) are ongoing. The two main findings of this study are that MVA85A is safe in healthy subjects infected with HIV (a proportion of whom are co-infected with *M. tuberculosis*), and that MVA85A is immunogenic in this population, albeit at a lower level than in HIV-uninfected subjects.

The local and systemic AE profiles in this trial were comparable to those observed in HIV-uninfected subjects previously. Importantly, there were no statistically significant effects of vaccination on either CD4 count or HIV RNA load. It is important to understand the variability in these parameters during stable untreated HIV infection, to enable meaningful interpretation of fluctuations throughout the trial period. A recent quantitative review of cohort studies of HIV-infected adults suggests that, during stable untreated infection, intra-individual HIV RNA measurements can fluctuate over short periods of time by as much as 0.2-0.5 log₁₀ copies/mL, with measurement error and physiological fluctuation each contributing about 50% of this variation. CD4 counts can also vary within patients by 60-130 cells/uL within weeks, but there is less measurement error, and during stable infection the overall within-subject variation is proportionally larger for HIV RNA than for CD4 count²¹. In this study, we also observed greater intra-individual variations in HIV RNA load compared to CD4 counts. Only one subject in each group demonstrated a more than 0.5 log rise in HIV RNA load at week 24 post-vaccination. The first of these (low dose group, 008) had displayed a relatively high baseline HIV RNA load and low baseline CD4 count at screening, just within the inclusion criteria. As the rise in HIV

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3 RNA load was only detectable 24 weeks after vaccination, it was considered highly
4 unlikely that this was due to MVA85A vaccination. This subject commenced ARVs
5 before week 24; all other subjects remained ARV-free throughout the trial period. The
6 second subject (high dose group, 035) developed a rise in HIV RNA load between
7 week 12 and week 24, peaking at just greater than a 0.5 log increase over baseline.
8 However, this subject's HIV RNA load had been stable prior to this, so it was again
9 deemed unlikely that this was a vaccination-related effect. Other fluctuations in the
10 HIV RNA loads were observed, but any sustained rise was well within the expected
11 0.5 log variation.
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24 HIV preferentially infects memory CD4+ T cells^{22, 23}, in particular HIV-specific
25 memory CD4+ T cells,²⁰ and other activated antigen-specific CD4+ T cells²⁴. Using
26 a sensitive qPCR method, we detected HIV *gag* DNA in Ag85A-specific CD4+ T
27 cells from only 2/12 subjects post-vaccination. This low positivity rate concurs with
28 the stable CD4 count and HIV RNA load parameters in most subjects throughout the
29 trial. Although the qPCR assay is sensitive, the low yield of Ag85A-specific CD4+ T
30 cells entering the assay is an important limitation and provides just a snapshot of the
31 HIV burden within the Ag85A-specific CD4+ T cell pool. However, these data
32 suggest that MVA85A vaccination of healthy HIV-infected individuals does not lead
33 to widespread preferential infection and depletion of vaccine-induced CD4+ T cell
34 populations in the periphery. In addition, the lack of effect of vaccination on
35 chemokine and cytokine levels in unstimulated serum supports the interpretation that
36 vaccination with MVA85A did not lead to widespread immune activation in this
37 subject group.
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3 We have shown that vaccine-induced immunogenicity, while of moderate magnitude,
4 is significantly lower than in healthy HIV-uninfected subjects given the same dose of
5 MVA85A. Thus, additional strategies may be required to augment immune responses
6 to MVA85A vaccination in the context of HIV infection. Partial reconstitution of the
7 immune system with ARVs may improve the response to vaccination. Indeed, this
8 study shows that the baseline HIV RNA level is a strong negative predictor of
9 summed 85A peptide pool responses at week 24 post-vaccination. In addition, a
10 booster vaccination with MVA85A may be required to enhance the vaccine-induced
11 immune response.
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27 The functional profile of Ag85A-specific CD4+ T cells observed in this study was
28 remarkably comparable to that of healthy HIV-uninfected subjects¹⁹ and *M.*
29 *tuberculosis*-infected subjects¹⁰, although the response in HIV-infected subjects is
30 less durable. IFN- γ and TNF- α are known to be important for protective immunity to
31 TB, and these cytokines dominated the MVA85A vaccine-induced CD4+ T cell
32 response elicited in the current study cohort. In this study, production of MIP-1 β by
33 Ag85A-specific CD4+ T cells was not detectable after week 2 post-vaccination. The
34 production of multiple cytokines including IL-2 and MIP-1 β by HIV-specific CD8+ T
35 cells is associated with long-term non-progression, albeit with uncertain causality²⁵.
36 The production of β -chemokines by CD8+ T cells was not assessed here as there were
37 no detectable Ag-85A specific CD8+ T cell responses pre- or post-vaccination.
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56 Vaccine-induced bystander activation of CD4+ T cells in HIV-infected subjects is
57 likely to be undesirable because activated CD4+ T cells are at increased risk of HIV
58 infection. The overall pattern of cytokine production and the frequency of CMV-
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3 specific CD4+ T cell producing cytokines remained both relatively stable over the 24-
4 week period following MVA85A vaccination, and comparable to pre-vaccination
5 responses (Supplemental Figure 1). These data suggest that MVA85A vaccination
6 does not induce substantial bystander activation of antigen-specific CD4+ T cell
7 populations.
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17 Demonstrating the safety and immunogenicity of a new TB vaccine in an important
18 target population is a crucial step, but does not mean that the vaccine will be effective.
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20 However, the data presented here support further safety and immunogenicity studies
21 of this candidate vaccine, together with proof-of-concept efficacy trials, in TB and
22 HIV endemic areas.
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Authors' Contributions:

Concept and design of study, and obtaining funding: HMcS.

Protocol writing: HMcS, CRS, AMM.

Recruitment, vaccination and follow-up of volunteers: AMM, RR, MH, AW, JR.

Immunology assays and analysis: AAP, NERB, IS, SH, HP.

Collaboration and contribution of reagents/materials/analysis tools: DRA, JPC, DCD, RAK, MR.

Data analysis: AMM, RR, AAP, NW, NERB, HMcS.

Writing of the paper: AMM, HMcS, with contributions from all authors.

Conflict of Interest Statement: AAP and HMcS are named inventors on a composition of matter patent for MVA85A filed by the University of Oxford, and are shareholders in a Joint Venture formed for the further development of this vaccine.

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TABLE and FIGURE LEGENDS

Table 1 *Subject demographics*: Comparison of low and high dose vaccination groups.

Tables 2a and b *Local and systemic adverse events*: Comparison of low and high dose vaccination groups with dose-matched HIV-uninfected subjects from previous trials of MVA85A. There were significantly fewer systemic AEs per person, (and a lower frequency of systemic AEs overall,) in the 10 HIV-infected subjects receiving high dose MVA85A compared with HIV-uninfected subjects receiving the same dose of vaccine ($p=0.026$, data not shown and Pathan *et al*, unpublished)

Table 3a *Ex-vivo IFN- γ ELISpot statistics (1)*: Comparison of screening, week 1 and week 24 responses within low and high dose groups, for both summed and single pooled peptides, using Wilcoxon signed rank test. Median (range), median difference (range, of week 1 and week 24 responses compared to screening,) and p -values are shown.

Table 3b *Ex-vivo IFN- γ ELISpot statistics (2)*: Comparison of responses between HIV-infected and HIV-uninfected subjects, at each time-point and each vaccine dose, using Mann-Whitney U test. Median (range), difference in medians (with 95% confidence intervals), and p -values are shown. AUC analysis is also shown.

Figure 1 *Consort flowcharts*. Follow-up was conducted for 24 weeks post-vaccination in both the low dose MVA85A (5×10^7 pfu; left panels) and high dose MVA85A (1×10^8 pfu; right panels) groups. Of the volunteers excluded at screening,

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3 5/15 had CD4 counts $<350 \times 10^6/L$ and/or exhibited progressively decreasing CD4
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6 counts, 2/15 had HIV RNA loads of $>100,000$ copies/mL, 2/15 had abnormal chest
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8 radiographs, 1/15 had a current history of active eczema, and 1/15 had a history of
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10 illicit drug usage and unstable depression; the remaining 4/15 were eligible but failed
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12 to attend for vaccination (2 in each dose group).
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17 **Figure 2 (a-d)** *Comparison of pre and post-vaccination CD4 counts:* Longitudinal
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19 CD4 counts of subjects in the low dose (5×10^7 pfu MVA85A) group pre-vaccination,
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21 **(a)**; and high dose (1×10^8 pfu MVA85A) group **(c)** pre-vaccination. Longitudinal CD4
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23 counts post-vaccination (up to 24 weeks) in the low dose **(b)** and high dose **(d)**
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25 groups. NB X-axis for the pre-vaccination data is not standardised for time but varies
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27 from 1-6 years depending on the individual subject. **(e-h)** *Comparison of pre and post-*
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29 *vaccination HIV RNA loads:* Longitudinal HIV RNA loads of subjects in the low dose
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31 **(e)** and high dose **(g)** groups pre-vaccination. Longitudinal HIV RNA loads post-
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33 vaccination (up to 24 weeks) in the low dose **(f)** and high dose **(h)** groups.
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35 Anonymous number codes (001-036) shown in key. NB X-axis for the pre-
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37 vaccination data is not standardised for time but varies from 1-6 years depending on
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39 the individual subject.
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48 **Figure 3 (a-d)** *IFN- γ ELISpot responses in the low dose and high dose groups.*
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50 *Longitudinal* responses to the single 85A peptide pool are shown for the low dose
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52 (5×10^7 pfu MVA85A) group, **(a)** and high dose (1×10^8 pfu MVA85A) group **(b)**.
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54 Longitudinal responses to the summed 85A peptide pools are shown for the low dose
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56 **(c)** and high dose **(d)** groups. Horizontal bars represent the median response.
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4 *Comparison of IFN- γ ELISpot responses in HIV-infected and HIV-uninfected subjects*
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6 (e-g). Responses to summed 85A peptide pools for low dose and high dose vaccine
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8 regimes at screening (e); week 1 post-vaccination (f) and week 24 post-vaccination
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10 (g). Subjects with LTBI are denoted with grey symbols. Statistically significant
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12 differences in the responses between HIV-infected and HIV-uninfected subjects were
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14 assessed using the Mann-Whitney U test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Horizontal
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16 bars represent the median response.
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22 **Figure 4** *MVA85A induces polyfunctional Ag85A-specific CD4+ T cells in HIV-*
23 *infected individuals.* MVA85A vaccination-induced production of IFN- γ , IL-2, MIP-
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25 1 β and TNF- α by antigen-specific CD4+ T cells was assessed following Ag85A
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27 peptide stimulation of cryopreserved PBMC using polychromatic flow cytometry. (a)
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29 Individual data points are shown with median line, interquartile range (open bars) and
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31 range (whiskers) at baseline and at each time-point post-vaccination for every
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33 possible combination of cytokine/chemokine production. High dose and low dose
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35 groups were analyzed together (n=17). (b) The functional profile of the Ag85A-
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37 specific CD4+ T cell response is summarized in the pie charts (n=17). CD4+ T cells
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39 producing a given number of cytokines/chemokines are grouped and colour-coded
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41 together. Pie charts are shown for the high dose HIV-infected group (n=9), the low
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43 dose HIV-infected group (n=8) and low dose healthy controls (n=6). (c) Absolute
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45 percentages of the highest frequency CD4+ T cell subsets producing specific
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47 combinations of chemokine/cytokines at week 1 post-vaccination across the different
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49 groups; * $p < 0.05$, ** $p < 0.01$.
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Figure 5 *Cytokine/chemokine production and receptor expression*

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4 (a) Absolute percent of Ag85A-specific CD4+ T cells producing IL-2 or MIP-1 β at
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6 week 1 post-MVA85A vaccination in HIV-infected subjects (low dose and high dose,
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8 n=17) versus healthy controls (n=6). Representative histogram (b) and scatter plot (c)
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10 showing CCR5 median fluorescence intensity (MFI) in naïve CD4+ T cells, memory
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12 CD4+ T cells and activated Ag85A-specific CD4+ T cells at week 1 post-
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14 vaccination (n=16 in all groups). (d) Scatter plot showing CCR5 integrated MFI
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16 (iMFI) for naïve, memory and Ag85A-specific CD4+ T cells (n=16). Line charts
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18 showing CCR5 MFI in memory CD4+ T cells (e) and the total CD4+ T cell
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20 population (f) across the MVA85A vaccination time-course (n=16). * $p<0.05$, **
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22 $p<0.01$.
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TABLE 1

Demographics and screening results		Low dose 5x10 ⁷ pfu	High dose 1x10 ⁸ pfu
Sex	Male	9 (90%)	8 (80%)
	Female	1 (10%)	2 (20%)
Median age (range)		35.8 (21-52)	35.1 (27-46)
Continent of Birth	Africa	4 (40%)	4 (40%)
	Asia	0	1 (10%)
	Europe	4 (40%)	5 (40%)
	North America	1 (10%)	0
	South America	1 (10%)	0
BCG	Definite	7 (70%)	10 (100%)
	Uncertain	3 (30%)	0 (0%)
Latent infection (ESAT-6/CFP-10 +)		2 (20%)	3 (30%)
Median CD4 count (range)		570 (430-1200)	625 (410-840)
Median HIV RNA load (range)		6069 (39- 41890)	14805 (49- 71090)

TABLE 2

(a)

Local Adverse Events	HIV-infected		HIV-negative		
	Dose Number of subjects	5x10 ⁷ pfu n=10	1x10 ⁸ pfu n=10	5x10 ⁷ pfu n=43	1x10 ⁸ pfu n=12
Redness		10 (100%)	10 (100%)	42 (98%)	12 (100%)
Pruritis		10 (100%)	9 (90%)	22 (51%)	8 (67%)
Pain		8 (80%)	7 (70%)	36 (84%)	12 (100%)
Induration		10 (100%)	10 (100%)	42 (98%)	12 (100%)

(b)

Systemic Adverse Events	HIV-infected		HIV-negative		
	Dose Number of subjects	5x10 ⁷ pfu n=10	1x10 ⁸ pfu n=10	5x10 ⁷ pfu n=43	1x10 ⁸ pfu n=12
Fever		1 (10%)	0 (0%)	3 (7%)	5 (42%)
Feverish		3 (30%)	2 (20%)	17 (40%)	9 (75%)
Arthralgia		2 (20%)	3 (30%)	6 (14%)	7 (58%)
Headache		6 (60%)	4 (40%)	21 (49%)	10 (83%)
Myalgia		1 (10%)	2 (20%)	18 (42%)	9 (75%)
Nausea		1 (10%)	0 (0%)	4 (9%)	4 (33%)
Vasovagal syncope		0 (0%)	1 (10%)	0 (0%)	0 (0%)
Axillary lymphadenopathy		1 (10%)	0 (0%)	2 (5%)	2 (17%)
Change in haematology/biochemistry		0 (0%)	0 (0%)	0 (0%)	0 (0%)

TABLE 3

(a)

Summed pooled peptides – within dose changes from screening to peak and plateau

Dose		Screening (n=10)	Week 1 (n=10)	Week 24 (n=10)
Low	Median (range)	10 (3, 20)	738 (109, 4398)	20 (0, 237) n=9
	Median difference (range) (compared to screening)		730 (109, 4383)	17 (-8, 218) n=9
	<i>p</i> -value*		0.007	0.17
High	Median (range)	3 (0, 14)	1730 (758, 2138)	42 (33, 256)
	Median difference (range) (compared to screening)		1721 (743, 2135)	42 (27, 247)
	<i>p</i> -value*		0.005	0.007

*Wilcoxon signed rank test

Single pooled peptides – within dose changes from screening to peak and plateau

Dose		Screening (n=10)	Week 1 (n=10)	Week 24 (n=10)
Low	Median (range)	0 (0, 8) n=9	393 (67, 1275) n=9	13 (2, 103) n=9
	Median difference (range) (compared to screening)		393 (60, 1272) n=9	13 (2, 97) n=9
	<i>p</i> -value*		0.009	0.032
High	Median (range)	0 (0, 8)	502 (312, 677)	14 (0, 68)
	Median difference (range) (compared to screening)		502 (294, 677)	14 (0, 66)
	<i>p</i> -value*		0.005	0.048

*Wilcoxon signed rank test

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(b)

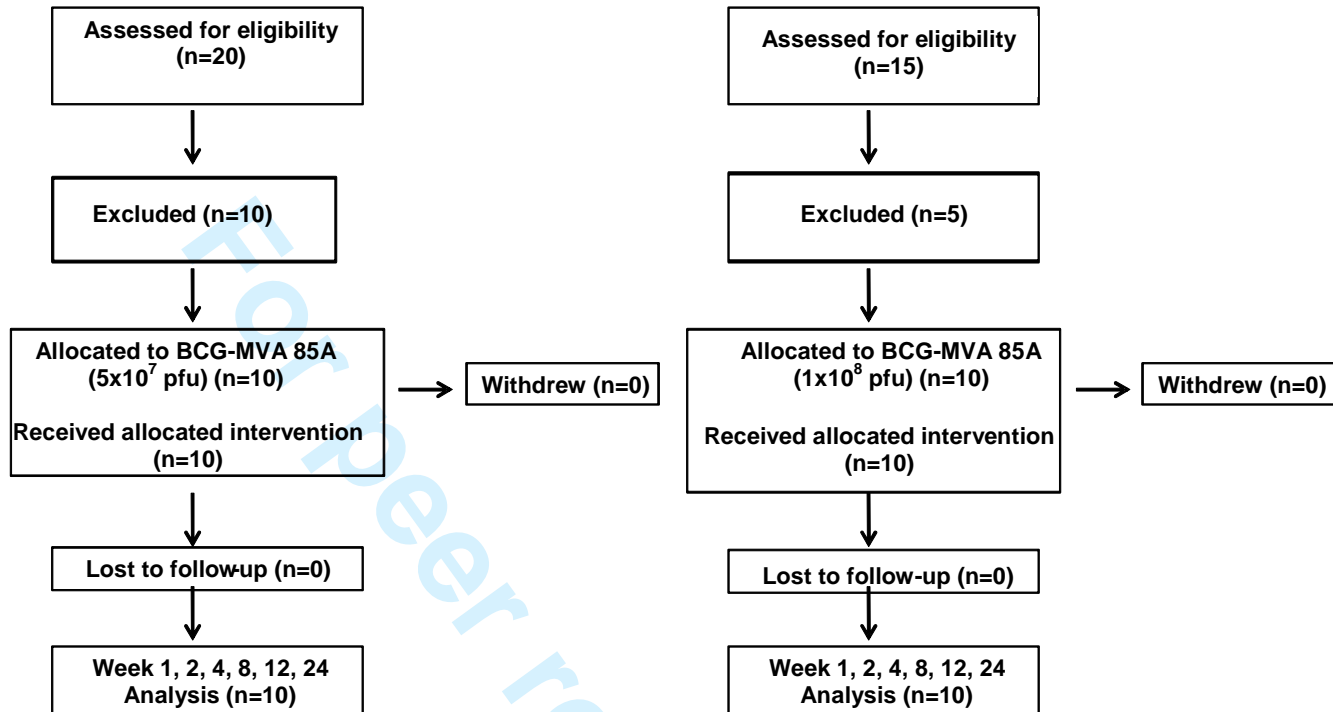
HIV-infected vs. HIV-negative group

	Summed Pooled peptides	Median (range) HIV+	Median (range) HIV-	Difference in medians^ (95% confidence interval)	p-value*
Week 1	Low dose	738 (109, 4398) n=10	2147 (1173, 5085) n=21	1101 (-393, 2873)	0.12
	High dose	1730 (758, 2138) n=10	6493 (4854, 7312) n=12	4557 (3038, 5904)	0.0001
Week 24	Low dose	20 (0, 237) n=9	385 (228, 1010) n=20	339 (161, 534)	0.004
	High dose	42 (33, 256) n=10	970 (655, 1199) n=12	820 (569, 1050)	0.0001
Area under the curve	Low dose	2162 (519, 23348) n=9	16317 (9129, 36418) n=20	11884 (3191, 17387)	0.02
	High dose	5929 (3167, 11607) n=10	41575 (26919, 53807) n=12	31384 (20778, 45504)	0.0001

*Mann Whitney U test

^The method used to calculate the 'difference in medians' does not simply subtract one median from the other. All possible pairwise differences between the 2 groups are calculated and the median of these differences is obtained. Hence the 'difference in medians' are not exactly the differences between the entries in the 'median' columns.

Figure 1



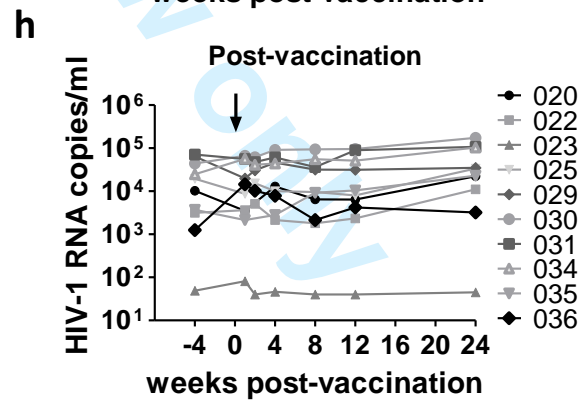
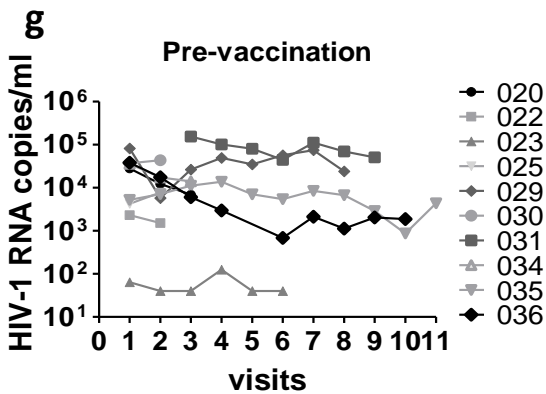
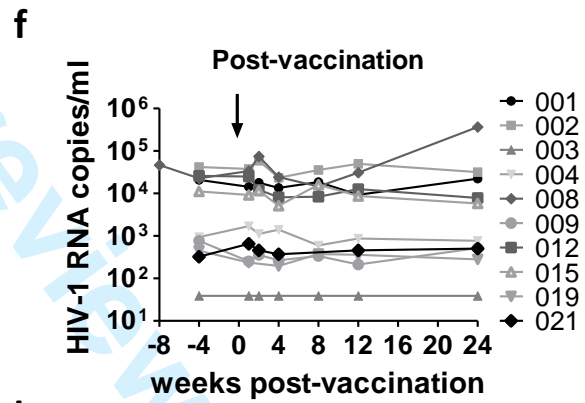
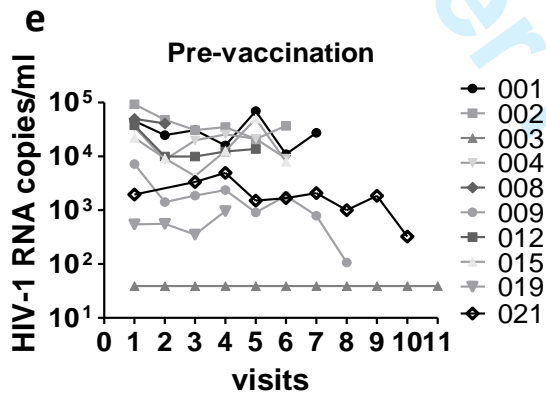
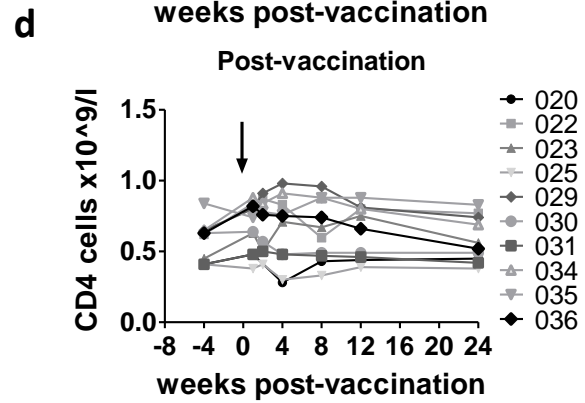
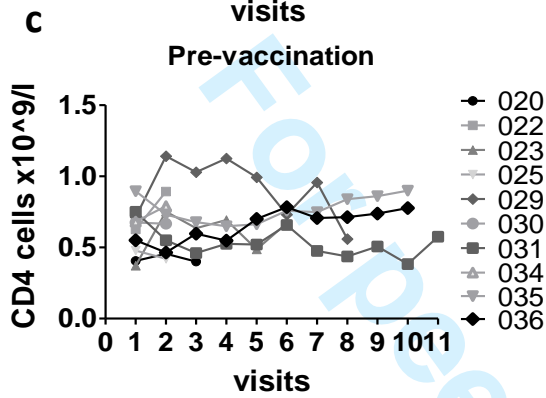
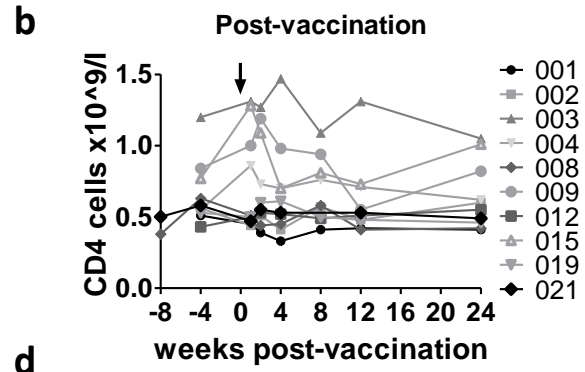
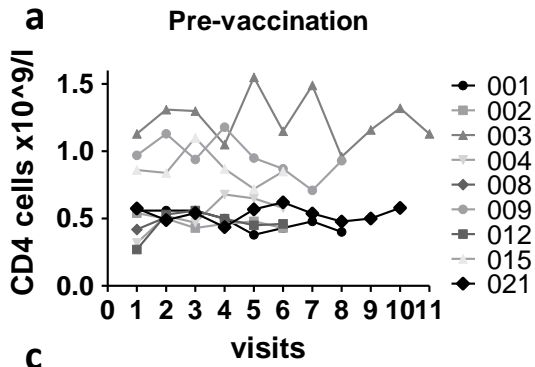


Figure 3

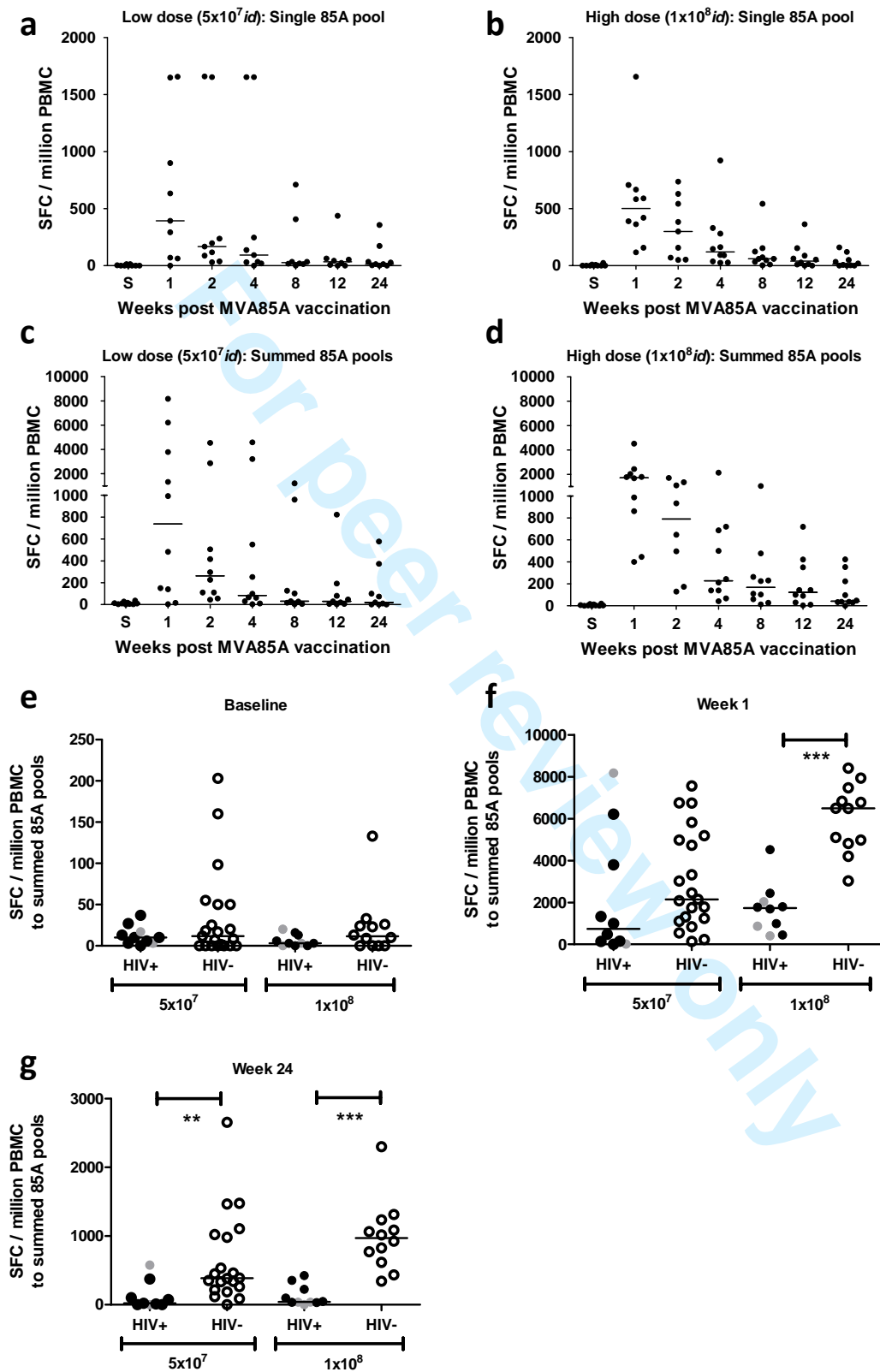


Figure 4

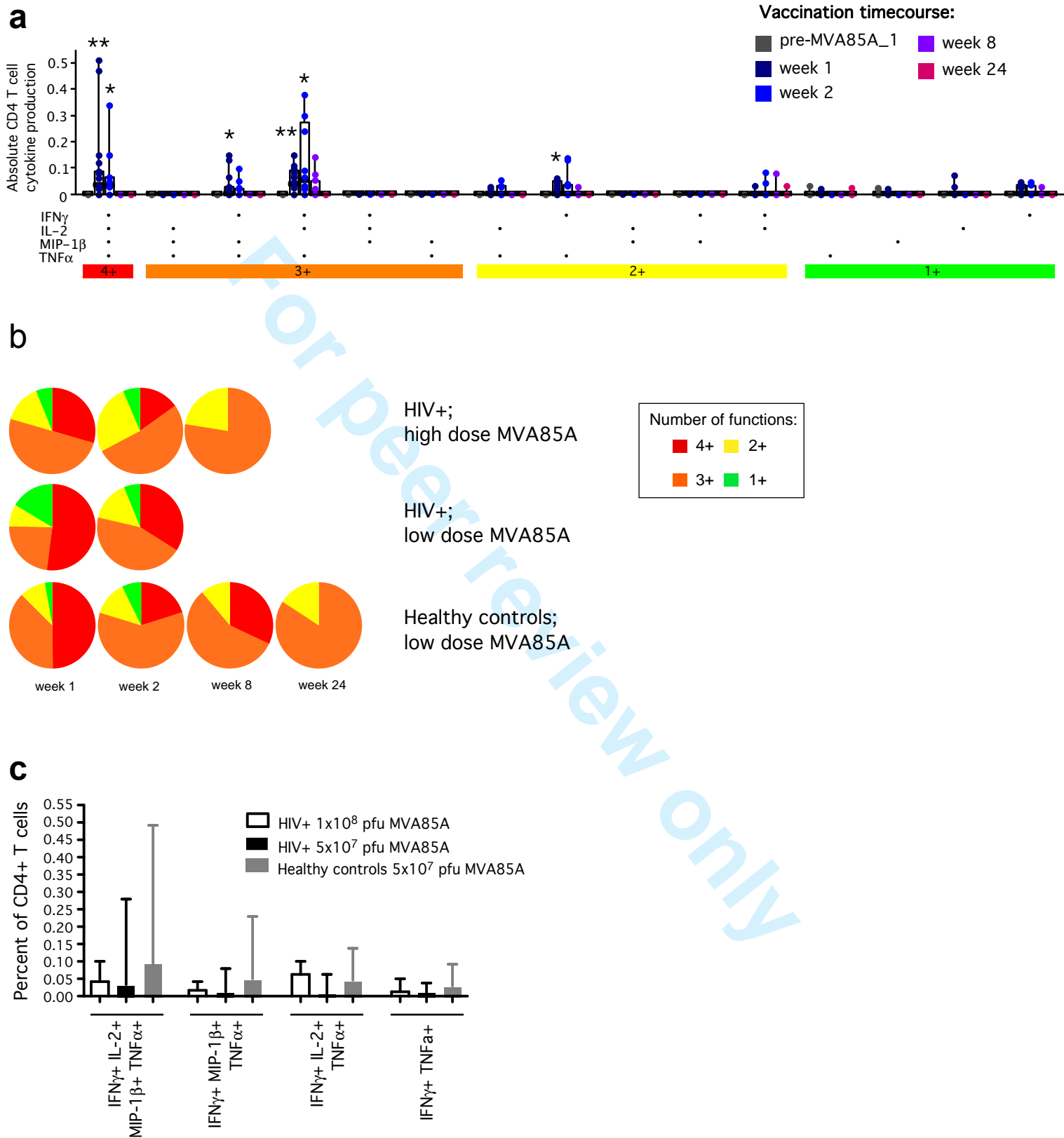
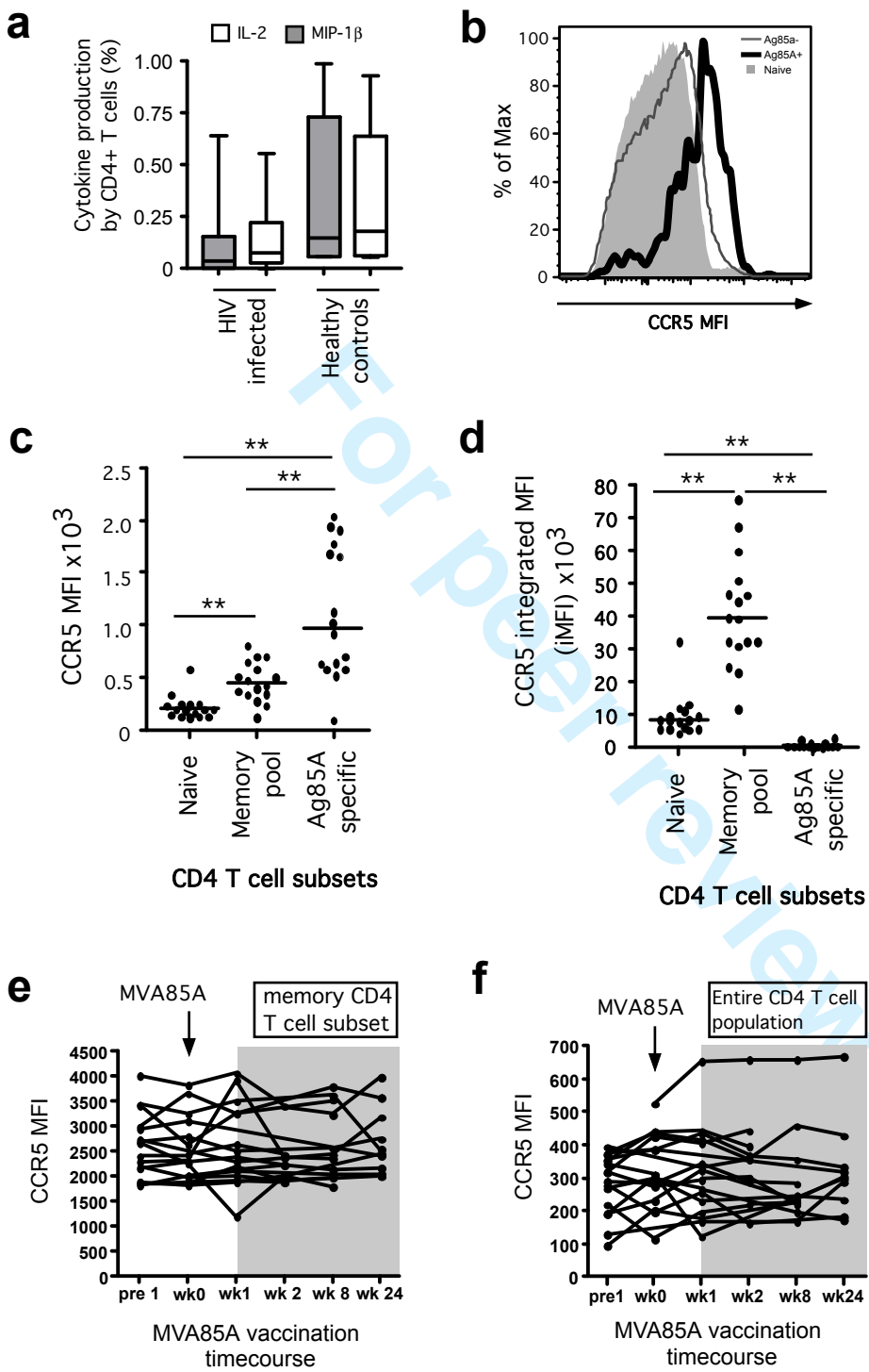


Figure 5





A Phase I study evaluating the safety and immunogenicity of MVA85A, a candidate TB vaccine, in HIV-infected adults

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4 **A Phase I study evaluating the safety and immunogenicity of**
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6 **MVA85A, a candidate TB vaccine, in HIV-infected adults**
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Short title: Phase I study with MVA85A in HIV

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ABSTRACT

OBJECTIVES: Control of the TB epidemic is a global health priority and one that is only likely to be achieved through vaccination. The critical overlap with the HIV epidemic requires any effective TB vaccine regimen to be safe in individuals who are infected with HIV. The objectives of this clinical trial were to evaluate the safety and immunogenicity of a leading candidate TB vaccine, MVA85A, in healthy, HIV-infected adults.

DESIGN: This was an open-label Phase I trial, performed in 20 healthy HIV-infected, antiretroviral-naïve subjects. Two different doses of MVA85A were each evaluated as a single immunisation in 10 subjects, with 24 weeks of follow-up. Safety of MVA85A was assessed by clinical and laboratory markers, including regular CD4 counts and HIV RNA load measurements. Vaccine immunogenicity was assessed by *ex-vivo* IFN- γ ELISpot assays and flow cytometric analysis.

RESULTS: MVA85A was safe in subjects with HIV infection, with an adverse event profile comparable to previous trials in HIV-uninfected subjects. There were no clinically significant vaccine-related changes in CD4 count or HIV RNA load in any subjects, and no evidence from qPCR analyses to indicate that MVA85A vaccination leads to widespread preferential infection of vaccine-induced CD4+ T cell populations. Both doses of MVA85A induced an antigen-specific IFN- γ response that was durable for 24 weeks, although of a lesser magnitude compared to HIV-uninfected subjects. The functional quality of the vaccine-induced T cell response in HIV-infected subjects was remarkably comparable, although less durable, to that observed in healthy HIV-uninfected controls.

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3 **CONCLUSION:** MVA85A is safe and immunogenic in healthy adults infected with
4 HIV. Further safety and efficacy evaluation of this candidate vaccine in TB and HIV
5 endemic areas is merited.

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10 **ARTICLE SUMMARY SECTION**

12 **Article focus**

- 14 • HIV infection increases susceptibility to TB, and globally, TB is the cause of
15 death in up to half of AIDS deaths
- 16 • There is an urgent need for a safe and effective TB vaccine in HIV-infected
17 people

22 **Key messages**

- 24 • MVA85A, a leading candidate TB vaccine, is safe and well tolerated in HIV-
25 infected people and does not induce changes in either CD4 count or HIV RNA
26 load
- 27 • MVA85A is immunogenic in HIV-infected people, and induces a similar
28 immune profile to that seen in HIV-uninfected people, but the immunogenicity
29 is less durable in HIV-infected people.

36 **Strengths and limitations of this study**

- 37 • This is a Phase I study with 20 subjects, and further studies are needed in TB
38 endemic countries in this important target population.

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47 **Funding:** This study was supported by The Wellcome Trust (Grant number
48 WT076943MA), TBVAC (an EU 6th Framework programme grant) and The NIHR
49 Oxford Biomedical Research Centre.
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INTRODUCTION

Tuberculosis (TB) and HIV are inextricably linked. At the end of 2007, approximately 33.2 million persons were living with Human Immune Deficiency Virus-1 (HIV) infection, an estimated one-third of whom were co-infected with *Mycobacterium tuberculosis* (*M. tuberculosis*)¹. TB is the cause of death for up to half of all AIDS patients² and the increasing incidence of drug-resistant strains of *M. tuberculosis* poses a significant threat to a susceptible HIV-infected population³.

Mycobacterium bovis (*M. bovis*) Bacille Calmette Guérin (BCG) fails to protect consistently against the adult pulmonary form of TB, whilst providing reliable protection against disseminated infection in childhood⁴. An improved vaccine strategy is thus essential for global control of this disease⁵. MVA85A (modified vaccinia virus Ankara expressing antigen 85A) is a leading candidate TB vaccine, designed to enhance the effect of BCG; it is safe and highly immunogenic in healthy BCG-naïve and BCG-vaccinated subjects, and in subjects latently infected with *M. tuberculosis* in the UK and Africa⁶⁻¹¹.

It is essential that any new TB vaccine is safe in an HIV-infected population. Subunit vaccines are an ideal choice for an immuno-compromised population in which the safety of replicating whole organism vaccines may be a concern. Although MVA is a live viral vaccine vector, it cannot replicate in human cells^{12, 13}. There are now safety data from a number of clinical trials with recombinant MVAs in HIV-infected subjects, which demonstrate no sustained effect on either HIV load or CD4 count¹⁴⁻¹⁶. Most of these studies assessed immune-reconstituted HIV-infected individuals on anti-retroviral therapy (ARV); however, some ARV-naïve subjects with more advanced HIV infection have also been vaccinated with a recombinant MVA, with no

1
2 significant rise in HIV load or fall in CD4 count over a 4-week follow-up period ¹⁷.

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4 Preclinical studies in severely immuno-suppressed macaques have also documented
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6 safety in this model ¹⁸. To date, MVA85A has been administered to more than 1,000
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8 individuals with no vaccine-related serious adverse events ^{6, 8-11} (McShane,
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10 unpublished data).

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12 Here we present the first clinical trial of a subunit TB vaccine in an HIV-infected

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14 population. The primary endpoint was to evaluate the safety of two doses of

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16 MVA85A in healthy HIV-infected subjects in the UK and the secondary endpoint was

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18 to evaluate the immunogenicity of this vaccine regimen.

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healthy HIV-infected subjects in the UK

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51 **METHODS**
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Trial design and participants

The protocol for this multi-site study was approved by the Medicines and Healthcare products Regulatory Agency (MHRA) and [ethical approval was obtained from](#) the Gene Therapy Advisory Committee (GTAC). Participants were recruited from the Genitourinary Medicine (GUM) departments at the Oxford Radcliffe Hospitals NHS Trust, University Hospitals Birmingham NHS Foundation Trust (Selly Oak Hospital), Great Western Hospitals NHS Foundation Trust, and Imperial College Healthcare NHS Trust, London.

Potentially eligible subjects were referred to the clinical trial team. Vaccination visits took place in Oxford; screening and follow-up visits took place at local NHS Trust sites. Written informed consent was obtained from all subjects prior to enrolment in the trial, and the referring HIV physicians were notified.

Eligibility required participants to be generally healthy, aged 18-55 years, with a normal chest radiograph and no clinical or radiological evidence of TB disease, a current CD4 count (measured at screening) of at least $350 \times 10^6/L$, a nadir CD4 count of not less than $300 \times 10^6/L$ and an HIV RNA load at screening of less than 100,000 copies/mL. All subjects were diagnosed with HIV infection at least six months before screening and had not received any ARVs within the preceding six months (full inclusion/exclusion criteria, Supplemental Methods). In all cases, testing for latent *M. tuberculosis* infection (LTBI) was conducted at screening using an in-house *ex-vivo* interferon gamma (IFN- γ) ELISpot assay for ESAT6 and CFP10 peptides. Subjects found to be latently infected were referred back to their consultants for possible treatment at the end of the trial.

The trial was registered on a clinical trials database: ClinicalTrials.gov ID NCT00395720.

Vaccine

Clinical grade MVA was produced under Good Manufacturing Practice standard by IDT Biologika GmbH.

Procedures

Participants were vaccinated intradermally with either 5×10^7 plaque-forming units (pfu; first group of 10 subjects) or 1×10^8 pfu (second group of 10 subjects). Subjects were sequentially allocated first to the low dose group and then once safety had been demonstrated, to the high dose group. A diary card was completed by all subjects recording local and systemic adverse events and body temperature for days 1 to 7 post-vaccination. All subjects were followed up regularly for 12 months; blood samples taken at each time-point for CD4 count, HIV RNA load measurement and cellular immunological assays. CD4 count and HIV RNA load results were communicated to the patient's HIV physician at regular intervals. Routine haematology and biochemistry assays were performed at weeks 1 and 12 after vaccination. All subjects attended their usual NHS follow-up appointments and received routine clinical care throughout the trial.

Immunological assays

Ex vivo IFN- γ ELISpot assays were performed at screening and at weeks 1, 2, 4, 12, and 24 post-vaccination using fresh peripheral blood mononuclear cells (PBMCs), as described previously¹⁰. A single pool of 15-mer peptides spanning the Ag85A protein (66, overlapping by 10 amino acids, 2 μ g/mL final concentration of each peptide in each well), and 7 pools of non-overlapping 9-10 Ag85A peptides (final concentration of 10 μ g/mL in each well) were used to stimulate for the IFN- γ ELISpot assays. For the 7 peptide pool wells, the results were summed across all peptide pools for each

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time-point. This potentially resulted in duplicate counting of T cells that responded to any of the 10mer overlap regions, since any 10mer occurred in two pools with adjacent peptides, but allows direct comparison with immunogenicity data from previous trials.

Intracellular cytokine staining of PBMCs was performed as described previously¹⁹. Briefly, cells were stained with the amine reactive LIVE/DEAD fixable dye ViViD (Molecular Probes, Invitrogen) and then surface stained with monoclonal antibodies against CD4, CD14, CD19, CD27, CD45RO, CD57 and CCR5. After washing, cells were permeabilized (Cytotfix/cytoperm kit; BD Pharmingen) and stained intracellularly for CD3, CD8, IFN- γ , TNF- α , IL-2 and MIP-1 β . Naïve CD4⁺ memory cells were identified as CD45RO-CD27+CD57+; memory CD4 T cell as CD45RO+CD27 \pm CD57 \pm . CD4⁺ T cells producing two or more cytokines (IFN- γ , IL-2 or TNF- α) following stimulation with Ag85A peptides were sorted to greater than 98% purity using a modified FACS Aria flow cytometer (BD Biosciences); CMV-specific CD4⁺ T cells, defined similarly, and bulk resting memory CD4⁺ T cells were sorted simultaneously. Cytokine production and the expression of surface markers were analysed using FlowJo version 8.7 (Tree Star Inc), Pestle version 1.5 and SPICE version 4.1 (both from Mario Roederer, Vaccine Research Center, NIAID, NIH, USA).

Real-time quantitative PCR (qPCR) was used to determine the proportion of HIV-infected Ag85A-specific CD4⁺ T cells; resting memory CD4⁺ T cells (non-Ag85A-specific) and CMV-specific CD4⁺ T cells were used as intra-individual controls. HIV-1 gag proviral DNA within sorted CD4⁺ T cell populations was quantified by qPCR, as described previously²⁰. qPCR was performed simultaneously for albumin

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2 gene copy number in all sorted CD4+ T cell populations to quantify cell number in
3 each reaction.
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6 CD4+ T cells from 12 subjects (4/10 in the low-dose group and 8/10 in the high-dose
7 group) were analysed for HIV *gag* content; low sort yields (less than 250 antigen-
8 specific CD4+T cells) prevented similar analyses in the remaining 8 subjects.
9

10 Serum levels of MIP-1 α , MIP-1 β , RANTES, TNF- α and IL-2 were measured pre-
11 vaccination and at weeks one, two and 24 post-vaccination using FlowCytomix
12 simplex kits (Bender MedSystems). Serum was thawed and plated out in duplicate in
13 round-bottom 96-well plates. Samples were used neat for MIP-1 α , MIP-1 β , TNF- α
14 and IL-2, and diluted 1/10 for RANTES. Standards (provided) were reconstituted as
15 specified, and 1/3 serial dilutions plated in duplicate. Bead and biotin-conjugate
16 cocktails were added to each well in 25 μ L and 50 μ L volumes, respectively, and
17 incubated for two hours in the dark at room temperature. Samples were then washed
18 twice in 100 μ L Assay Buffer. Streptavidin-PE (50 μ L) and Assay Buffer (100 μ L) were
19 added and plates were incubated for one hour in the dark at room temperature.
20 Samples were then washed again and transferred to cluster tubes in 4%
21 paraformaldehyde in PBS (400 μ L) and acquired using an LSRII flow cytometer (BD
22 Biosciences) set up with the beads provided and the highest concentration standard;
23 the stopping gate was set so that at least 300 events were collected for each sample.
24 Data were analysed using FlowCytomixPro software (Bender MedSystems).
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45 **Statistical analysis**

46 Continuous outcomes were presented as a median (range) for each group. Median
47 difference was calculated to assess difference between two groups and the
48 corresponding 95% confidence interval was computed using the robust method
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proposed by Newson²¹. The overall magnitude of vaccine-induced CD4+ T cell responses over the 24 weeks was summarised using area under the curve (AUC) for each dose group. Peak (1 week) and plateau (24 weeks) CD4+ T cell responses were compared with those at baseline (pre-vaccination) using the Wilcoxon signed rank test, Stata Statistical Software, Release 9.0. 2005 (Stata Corporation, College Station, TX). Mann-Whitney U test was used to compare between the vaccine groups. Comparisons between specific time points in this trial and previously published trials of MVA85A^{6, 22}, and between low and high dose groups were conducted using the Mann-Whitney U test (Stata). Non-parametric Spearman rank analyses were used to correlate immune assays and CD4/HIV RNA load with CD4+ T cell responses.

Deleted: Area under the curve (AUC) analysis was performed to compare the magnitude of vaccine-induced CD4+ T cell responses in each dose group (groups compared using the Mann-Whitney U test).

Deleted: Within-subject CD4 counts and HIV RNA load levels at weeks 1 and 24 post-vaccination were compared with baseline pre-vaccination levels, using a repeated measures analysis of variance (*p*-values obtained from the F-test, Stata). ¶

RESULTS

Thirty-five individuals attended for screening between October 2006 and June 2009; 20 subjects were enrolled (Fig. 1, [CONSORT](#)). The demographics of the 20 participants are shown in Table 1, and were comparable across the two groups.

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Safety of MVA85A in HIV-infected subjects

Local reactions related to the MVA vaccine occur during the first week after vaccination and have been reported previously^{6, 7, 10}. The local adverse event (AE) profile recorded in the diary cards of the HIV-infected subjects in this study was comparable to that observed in HIV-uninfected subjects, although pruritis appeared slightly higher in the HIV-infected group (Table 2a). There were no severe local reactions and most local AEs were mild.

One subject in the low dose group experienced a single episode of fever greater than 37.5 °C (documented at 38.1°C). All other subjective systemic symptoms occurred with similar frequencies in the low dose and high dose groups (Table 2b). There was one moderate systemic AE and all others were mild.

Pre-vaccination CD4 counts and HIV RNA loads for all 20 subjects over a time period of one to six years before enrolment are shown in Figure 2. These longitudinal data illustrate the levels of natural fluctuation that occur over time and represent important baseline measurements for the interpretation of these parameters post-vaccination, also shown in Figure 2, in the absence of an unvaccinated control group.

No clinically significant changes in CD4 counts or HIV RNA load was seen post-vaccination.

Deleted: A repeated measures analysis of variance showed no evidence of a difference in either CD4 counts or HIV RNA load levels pre- and post-vaccination (low dose, CD4 count: $p=0.35$; low dose, HIV RNA load: $p=0.30$; high dose, CD4 count: $p=0.83$; high dose, HIV RNA load: $p=0.48$).

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A major concern with respect to the vaccination of HIV-infected individuals is the possibility that responding antigen-specific CD4+ T cells will act as preferential targets for HIV infection as a function of their activated status, thereby enhancing the propagation of HIV and accelerating disease progression. In this study, however, there was no evidence for preferential HIV infection of Ag85A-specific CD4+ T cells. The median number of CD4+ T cells in Ag85A-specific qPCR reactions was 73 (95% CI: 15-130 CD4+ T cells). Where possible, multiple time-points throughout the vaccination course for each subject were analyzed, to determine the proportion of Ag85A-specific CD4+ T cells that were infected with HIV. Of 29 Ag85A-specific CD4+ T cell samples in total (11 subjects), only one subject showed a positive signal for HIV *gag* DNA by qPCR at two different post-vaccination timepoints (weeks 2 and 8 post-vaccination; data not shown). In the 11 subjects tested, resting HIV-specific and CMV-specific memory cell populations showed a positive signal in all assays (data not shown). In addition, vaccination had no effect on levels of MIP-1 α , MIP-1 β , RANTES, TNF- α and IL-2 in unstimulated serum (data not shown).

Immunogenicity of MVA85A in HIV-infected subjects

MVA85A induced significant antigen-specific T cell responses as measured by IFN- γ ELISpot assay (Fig. 3a-d, Table 3a). In the low dose group, the frequency of IFN- γ -secreting T cells responding to the single 85A peptide pool at week one post-vaccination, increased by a median of 393 sfc/million compared to baseline ($p=0.009$); there a significant increase remained until 24 weeks post-vaccination ($p=0.032$). In the high dose group, a median increase of 502 sfc/million was apparent at week 1 post-vaccination ($p=0.005$); again, this response was maintained until 24 weeks ($p=0.048$). In both groups, T cell responses to the summed 85A peptide pools followed a similar pattern with a significant increase above baseline at week 1 (738

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2 and 1730 sfc/million for low and high dose groups, respectively), although, the week
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4 24 response was only maintained significantly above baseline in the high dose group
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6 (low dose $p=0.17$; high dose $p=0.007$). There were no significant differences in the
7
8 overall magnitude (i.e. AUC) of the IFN- γ response between low and high dose
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10 groups across the follow-up period ($p=0.29$ and $p=0.68$ for summed and single
11
12 peptide pools, respectively; data not shown). However the responder rate was higher
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14 in the high dose group with no observed non-responders (ie those subjects with no
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16 measurable vaccine induced immune response) compared with 1-2 non-responders
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18 (depending on antigen) in the low dose group. Overall, the kinetics of the Ag85A-
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20 specific T cell responses were very similar to those previously reported in similar
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22 studies of HIV-uninfected subjects⁶⁻¹¹.

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24 Ag85A-specific T cell responses in the low-dose group were significantly lower
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26 compared to those observed in previously published studies of HIV-uninfected
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28 subjects (AUC analysis, $p=0.02$; Table 3b)^{6, 22}. This difference was not significant at
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30 week 1 (MWU analysis, $p=0.12$), which represents the peak response, but was
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32 significant at week 24 post-vaccination (MWU analysis, $p=0.004$; Table 3b, Figure
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34 3e-g). There were no significant differences in baseline (pre-vaccination) responses
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36 between the HIV-infected and HIV-uninfected groups (MWU analysis, $p=0.85$;
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38 Figure 3e). Similarly, HIV-infected subjects in the high dose group exhibited
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40 significantly lower responses compared to HIV-uninfected subjects who received the
41
42 same dose of MVA85A (AUC analysis, $p=0.0001$; week 1 and week 24 MWU
43
44 analysis, $p = 0.0001$; Table 3b, Figure 3e-g). Again, baseline responses were
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46 comparable ($p=0.24$) (Fig. 3e).

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48 There was no significant correlation between the CD4 count at screening and the peak
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50 summed 85A peptide pool response ($R=0.04$, $p=0.09$), nor between HIV RNA load at
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1
2 screening and the peak immune response ($R = -0.04$, $p = 0.08$). There was a strong
3
4 negative correlation between viral load at screening and the plateau immune response
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6 at week 24 ($R = -0.8$, $p = 0.002$).
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9 Four subjects were found to have LTBI. However, there were no significant post-
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11 vaccination changes in the magnitude of ESAT-6/CFP10 responses in these subjects
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13 (data not shown).

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15 Ag85A-specific cytokine/chemokine production by CD4+ T cells prior to MVA85A
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17 vaccination was undetectable and increased significantly post-vaccination (Fig. 4a).
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19 One week following MVA85A vaccination, more than 40% of Ag85A-specific CD4+
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21 T cells produced IFN- γ , IL-2, MIP-1 β and TNF- α simultaneously ($p < 0.01$); this
22
23 remained significant at week 2 ($p < 0.05$). By week 8 post-vaccination, 60% of Ag85A-
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25 specific CD4+ T cells were still producing IFN- γ , IL-2 and TNF- α simultaneously;
26
27 production of IL-2 and IFN- γ was maintained until 24 weeks. Production of MIP-1 β
28
29 was not detected beyond week 2 post-vaccination (Fig. 4a, 5a). In the first two weeks
30
31 post-vaccination, Ag85A-specific CD4+ T cells exhibited similar functional profiles
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33 in the HIV-infected groups, both low dose and high dose, and the "control" low dose
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35 HIV-uninfected group; in all cases, the responses were dominated by four functionally
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37 distinct subsets (Fig. 4b, c). IFN- γ production measured by ICS correlated closely
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39 with IFN- γ secretion observed in the ELISpot assay ($R = 0.75$, $p < 0.0001$, $n = 45$; data
40
41 not shown).
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44 Surface expression of the HIV M-tropic co-receptor CCR5 was monitored throughout
45
46 the course of vaccination (Fig. 5). At week 1 post-vaccination, the MFI for CCR5 was
47
48 highest in the activated Ag85A-specific CD4+ T cells, and lowest in the naïve CD4+
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50 T cell subsets. Using the integrated MFI (iMFI) function [calculated using both MFI](#)
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52 [and cell frequencies](#), expression of CCR5 was highest in the [much larger](#) memory
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2 CD4+ T cell pool (Fig. 5d). No significant increase in CCR5 expression was detected
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4 in either the memory (Fig. 5e) or the total (Fig. 5f) CD4+ T cell populations as a
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6 result of MVA85A vaccination.
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DISCUSSION

This is the first Phase I trial of a subunit vectored TB vaccine in HIV-infected individuals. Similar studies in South Africa (*Scriba et al, submitted*) and Senegal (*Mboup et al, unpublished data*) are ongoing. The two main findings of this study are that MVA85A is safe in healthy subjects infected with HIV (a proportion of whom are co-infected with *M. tuberculosis*), and that MVA85A is immunogenic in this population, albeit at a lower level than in HIV-uninfected subjects.

Deleted: Tameris et al, unpublished data

The local and systemic AE profiles in this trial were comparable to those observed in HIV-uninfected subjects previously. Whilst we have not evaluated the safety of this vaccine in HIV-infected subjects with lower CD4 counts, we would not expect the safety profile of this vaccine to be altered by progression of HIV disease.

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Importantly, there were no clinically significant effects of vaccination on either CD4 count or HIV RNA load. It is important to understand the variability in these parameters during stable untreated HIV infection, to enable meaningful interpretation of fluctuations throughout the trial period. A recent quantitative review of cohort studies of HIV-infected adults suggests that, during stable untreated infection, intra-individual HIV RNA measurements can fluctuate over short periods of time by as much as 0.2-0.5 log₁₀ copies/mL, with measurement error and physiological fluctuation each contributing about 50% of this variation. CD4 counts can also vary within patients by 60-130 cells/uL within weeks, but there is less measurement error, and during stable infection the overall within-subject variation is proportionally larger for HIV RNA than for CD4 count²³. In this study, we also observed greater intra-individual variations in HIV RNA load compared to CD4 counts. Only one subject in each group demonstrated a more than

1
2 0.5 log rise in HIV RNA load at week 24 post-vaccination. The first of these (low
3 dose group, 008) had displayed a relatively high baseline HIV RNA load and low
4 baseline CD4 count at screening, just within the inclusion criteria. As the rise in HIV
5 RNA load was only detectable 24 weeks after vaccination, it was considered highly
6 unlikely that this was due to MVA85A vaccination. This subject commenced ARVs
7 before week 24; all other subjects remained ARV-free throughout the trial period. The
8 second subject (high dose group, 035) developed a rise in HIV RNA load between
9 week 12 and week 24, peaking at just greater than a 0.5 log increase over baseline.
10 However, this subject's HIV RNA load had been stable prior to this, so it was again
11 deemed unlikely that this was a vaccination-related effect. Other fluctuations in the
12 HIV RNA loads were observed, but any sustained rise was well within the expected
13 0.5 log variation.
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15 HIV preferentially infects memory CD4+ T cells^{24, 25}, in particular HIV-specific
16 memory CD4+ T cells,²⁰ and other activated antigen-specific CD4+ T cells²⁶. Using
17 a sensitive qPCR method, we detected HIV *gag* DNA in Ag85A-specific CD4+ T
18 cells from only 1/11 subjects post-vaccination (3 low dose and 8 high dose). This
19 subject received high-dose MVA85A vaccination. This low positivity rate concurs
20 with the stable CD4 count and HIV RNA load parameters in most subjects throughout
21 the trial. Although the qPCR assay is sensitive, the low yield of Ag85A-specific
22 CD4+ T cells entering the assay is an important limitation and provides just a
23 snapshot of the HIV burden within the Ag85A-specific CD4+ T cell pool and
24 warrants further future investigation. However, whilst small effects of MVA85A on
25 HIV infection of CD4+ target cells cannot be excluded given the small sample size,
26 these data suggest that MVA85A vaccination of healthy HIV-infected individuals
27 does not lead to widespread preferential infection and depletion of vaccine-induced
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CD4+ T cell populations in the periphery. [These data are supported by no change in surface expression of the HIV co-receptor CCR5 following MVA85A vaccination.](#) In addition, the lack of effect of vaccination on chemokine and cytokine levels in unstimulated serum supports the interpretation that vaccination with MVA85A did not lead to widespread immune activation in this subject group.

We have shown that vaccine-induced immunogenicity, while of moderate magnitude, is significantly lower than in healthy HIV-uninfected subjects given the same dose of MVA85A. Thus, additional strategies may be required to augment immune responses to MVA85A vaccination in the context of HIV infection. Partial reconstitution of the immune system with ARVs may improve the response to vaccination. Indeed, this study shows that the baseline HIV RNA level is a strong negative predictor of summed 85A peptide pool responses at week 24 post-vaccination. In addition, a booster vaccination with MVA85A may be required to enhance the vaccine-induced immune response.

The functional profile of Ag85A-specific CD4+ T cells observed in this study was remarkably comparable to that of healthy HIV-uninfected subjects¹⁹ and *M. tuberculosis*-infected subjects¹⁰, although the response in HIV-infected subjects is less durable. IFN- γ and TNF- α are known to be important for protective immunity to TB, and these cytokines dominated the MVA85A vaccine-induced CD4+ T cell response elicited in the current study cohort. In this study, production of MIP-1 β by Ag85A-specific CD4+ T cells was not detectable after week 2 post-vaccination. The production of multiple cytokines including IL-2 and MIP-1 β by HIV-specific CD8+ T cells is associated with long-term non-progression, albeit with uncertain causality²⁷.

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2 The production of β -chemokines by CD8+ T cells was not assessed here as there were
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4 no detectable Ag-85A specific CD8+ T cell responses pre- or post-vaccination.
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8 Vaccine-induced bystander activation of CD4+ T cells in HIV-infected subjects is
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10 likely to be undesirable because activated CD4+ T cells are at increased risk of HIV
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12 infection. The overall pattern of cytokine production and the frequency of CMV-
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14 specific CD4+ T cell producing cytokines remained both relatively stable over the 24-
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16 week period following MVA85A vaccination, and comparable to pre-vaccination
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18 responses (Supplemental Figure 1). These data suggest that MVA85A vaccination
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20 does not induce substantial bystander activation of antigen-specific CD4+ T cell
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22 populations.
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26 Demonstrating the safety and immunogenicity of a new TB vaccine in an important
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28 target population is a crucial step, but does not mean that the vaccine will be effective.
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30 However, the data presented here support further safety and immunogenicity studies
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32 of this candidate vaccine, together with proof-of-concept efficacy trials, in TB and
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34 HIV endemic areas.
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Authors' Contributions:**Authors' Contributions:**

Concept and design of study, and obtaining funding: HMcS.

Protocol writing: HMcS, CRS, AMM, AML.

Recruitment, vaccination and follow-up of volunteers: AMM, RR, IDP, MH, AW, JR, JS, GR.

Immunology assays and analysis: AAP, NERB, IS, SH, HAF, HP.

Collaboration and contribution of reagents/materials/analysis tools: DRA, DAP, JPC, DCD, RAK, MR.

Data analysis: AMM, RR, AAP, NA, NERB, HMcS.

Writing of the paper: AMM, HMcS, with contributions from all authors.

Conflict of Interest Statement: AAP and HMcS are named inventors on a composition of matter patent for MVA85A owned by the University of Oxford, and are shareholders in a Joint Venture formed for the further development of this vaccine.

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TABLE and FIGURE LEGENDS

Table 1 *Subject demographics*: Comparison of low and high dose vaccination groups.

Tables 2a and b *Local and systemic adverse events*: Comparison of low and high dose vaccination groups with dose-matched HIV-uninfected subjects from previous trials of MVA85A. There were significantly fewer systemic AEs per person, (and a lower frequency of systemic AEs overall,) in the 10 HIV-infected subjects receiving high dose MVA85A compared with HIV-uninfected subjects receiving the same dose of vaccine ($p=0.026$, data not shown and Pathan *et al*, unpublished)

Table 3a *Ex-vivo IFN- γ ELISpot statistics (1)*: Comparison of screening, week 1 and week 24 responses within low and high dose groups, for both summed and single pooled peptides, using Wilcoxon signed rank test. Median (range), median difference (range, of week 1 and week 24 responses compared to screening,) and p -values are shown.

Table 3b *Ex-vivo IFN- γ ELISpot statistics (2)*: Comparison of responses between HIV-infected and HIV-uninfected subjects, at each time-point and each vaccine dose, using Mann-Whitney U test. Median (range), difference in medians (with 95% confidence intervals), and p -values are shown. AUC analysis is also shown.

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Figure 1 *CONSORT diagram*. Follow-up was conducted for 24 weeks post-vaccination in both the low dose MVA85A (5×10^7 pfu; left panels) and high dose MVA85A (1×10^8 pfu; right panels) groups. Of the volunteers excluded at screening, 5/15 had CD4 counts $< 350 \times 10^6/L$ and/or exhibited progressively decreasing CD4 counts, 2/15 had HIV RNA loads of $> 100,000$ copies/mL, 2/15 had abnormal chest radiographs, 1/15 had a current history of active eczema, and 1/15 had a history of illicit drug usage and unstable depression; the remaining 4/15 were eligible but failed to attend for vaccination (2 in each dose group).

Figure 2 (a-d) *Comparison of pre and post-vaccination CD4 counts*: Longitudinal CD4 counts of subjects in the low dose (5×10^7 pfu MVA85A) group pre-vaccination, (a); and high dose (1×10^8 pfu MVA85A) group (c) pre-vaccination. Longitudinal CD4 counts post-vaccination (up to 24 weeks) in the low dose (b) and high dose (d) groups. NB X-axis for the pre-vaccination data is not standardised for time but varies from 1-6 years depending on the individual subject. (e-h) *Comparison of pre and post-vaccination HIV RNA loads*: Longitudinal HIV RNA loads of subjects in the low dose (e) and high dose (g) groups pre-vaccination. Longitudinal HIV RNA loads post-vaccination (up to 24 weeks) in the low dose (f) and high dose (h) groups. Anonymous number codes (001-036) shown in key. NB X-axis for the pre-vaccination data is not standardised for time but varies from 1-6 years depending on the individual subject.

Figure 3 (a-d) *IFN- γ ELISpot responses in the low dose and high dose groups*. Longitudinal responses to the single 85A peptide pool are shown for the low dose (5×10^7 pfu MVA85A) group, (a) and high dose (1×10^8 pfu MVA85A) group (b).

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Longitudinal responses to the summed 85A peptide pools are shown for the low dose (c) and high dose (d) groups. Horizontal bars represent the median response.

Comparison of IFN- γ ELISpot responses in HIV-infected and HIV-uninfected subjects (e-g). Responses to summed 85A peptide pools for low dose and high dose vaccine regimes at screening (e); week 1 post-vaccination (f) and week 24 post-vaccination (g). Subjects with LTBI are denoted with grey symbols. Statistically significant differences in the responses between HIV-infected and HIV-uninfected subjects were assessed using the Mann-Whitney U test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Horizontal bars represent the median response.

Figure 4 *MVA85A induces polyfunctional Ag85A-specific CD4+ T cells in HIV-infected individuals.* MVA85A vaccination-induced production of IFN- γ , IL-2, MIP-1 β and TNF- α by antigen-specific CD4+ T cells was assessed following Ag85A peptide stimulation of cryopreserved PBMC using polychromatic flow cytometry. (a) Individual data points are shown with median line, interquartile range (open bars) and range (whiskers) at baseline and at each time-point post-vaccination for every possible combination of cytokine/chemokine production. High dose and low dose groups were analyzed together (n=17). (b) The functional profile of the Ag85A-specific CD4+ T cell response is summarized in the pie charts (n=17). CD4+ T cells producing a given number of cytokines/chemokines are grouped and colour-coded together. Pie charts are shown for the high dose HIV-infected group (n=9), the low dose HIV-infected group (n=8) and low dose healthy controls (n=6). (c) Absolute percentages of the highest frequency CD4+ T cell subsets producing specific combinations of chemokine/cytokines at week 1 post-vaccination across the different groups; * $p < 0.05$, ** $p < 0.01$.

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5 **Figure 5** *Cytokine/chemokine production and receptor expression*

6 (a) Absolute percent of Ag85A-specific CD4+ T cells producing IL-2 or MIP-1 β at
7 week 1 post-MVA85A vaccination in HIV-infected subjects (low dose and high dose,
8 n=17) versus healthy controls (n=6). Representative histogram (b) and scatter plot (c)
9 showing CCR5 median fluorescence intensity (MFI) in naïve CD4+ T cells, memory
10 CD4+ T cells and activated Ag85A-specific CD4+ T cells at week 1 post-
11 vaccination (n=16 in all groups). (d) Scatter plot showing CCR5 integrated MFI
12 (iMFI) for naïve, memory and Ag85A-specific CD4+ T cells (n=16). Line charts
13 showing CCR5 MFI in memory CD4+ T cells (e) and the total CD4+ T cell
14 population (f) across the MVA85A vaccination time-course (n=16). * $p < 0.05$, **
15 $p < 0.01$.

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TABLE 1

Demographics and screening results		Low dose 5x10 ⁷ pfu	High dose 1x10 ⁸ pfu
Sex	Male	9 (90%)	8 (80%)
	Female	1 (10%)	2 (20%)
Median age (range)		35.8 (21-52)	35.1 (27-46)
Continent of Birth	Africa	4 (40%)	4 (40%)
	Asia	0	1 (10%)
	Europe	4 (40%)	5 (40%)
	North America	1 (10%)	0
	South America	1 (10%)	0
BCG	Definite	7 (70%)	10 (100%)
	Uncertain	3 (30%)	0 (0%)
Latent infection (ESAT-6/CFP-10 +)		2 (20%)	3 (30%)
Median CD4 count (range)		570 (430-1200)	625 (410-840)
Median HIV RNA load (range)		6069 (39- 41890)	14805 (49- 71090)

TABLE 2

(a)

Local Adverse Events	HIV-infected		HIV-negative	
	5x10 ⁷ pfu n=10	1x10 ⁸ pfu n=10	5x10 ⁷ pfu n=43	1x10 ⁸ pfu n=12
Redness	10 (100%)	10 (100%)	42 (98%)	12 (100%)
Pruritis	10 (100%)	9 (90%)	22 (51%)	8 (67%)
Pain	8 (80%)	7 (70%)	36 (84%)	12 (100%)
Induration	10 (100%)	10 (100%)	42 (98%)	12 (100%)

(b)

Systemic Adverse Events	HIV-infected		HIV-negative	
	5x10 ⁷ pfu n=10	1x10 ⁸ pfu n=10	5x10 ⁷ pfu n=43	1x10 ⁸ pfu n=12
Measured fever	1 (10%)	0 (0%)	3 (7%)	5 (42%)
Subjective fever	3 (30%)	2 (20%)	17 (40%)	9 (75%)
Arthralgia	2 (20%)	3 (30%)	6 (14%)	7 (58%)
Headache	6 (60%)	4 (40%)	21 (49%)	10 (83%)
Myalgia	1 (10%)	2 (20%)	18 (42%)	9 (75%)
Nausea	1 (10%)	0 (0%)	4 (9%)	4 (33%)
Vasovagal syncope	0 (0%)	1 (10%)	0 (0%)	0 (0%)
Axillary lymphadenopathy	1 (10%)	0 (0%)	2 (5%)	2 (17%)
Change in haematology/biochemistry	0 (0%)	0 (0%)	0 (0%)	0 (0%)

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TABLE 3

(a)
Summed pooled peptides – within dose changes from screening to peak and plateau

Dose		Screening (n=10)	Week 1 (n=10)	Week 24 (n=10)
Low	Median (range)	10 (3, 20)	738 (109, 4398)	20 (0, 237) n=9
	Median difference (range) (compared to screening)		730 (109, 4383)	17 (-8, 218) n=9
	<i>p</i> -value*		0.007	0.17
High	Median (range)	3 (0, 14)	1730 (758, 2138)	42 (33, 256)
	Median difference (range) (compared to screening)		1721 (743, 2135)	42 (27, 247)
	<i>p</i> -value*		0.005	0.007

*Wilcoxon signed rank test

Single pooled peptides – within dose changes from screening to peak and plateau

Dose		Screening (n=10)	Week 1 (n=10)	Week 24 (n=10)
Low	Median (range)	0 (0, 8) n=9	393 (67, 1275) n=9	13 (2, 103) n=9
	Median difference (range) (compared to screening)		393 (60, 1272) n=9	13 (2, 97) n=9
	<i>p</i> -value*		0.009	0.032
High	Median (range)	0 (0, 8)	502 (312, 677)	14 (0, 68)
	Median difference (range) (compared to screening)		502 (294, 677)	14 (0, 66)
	<i>p</i> -value*		0.005	0.048

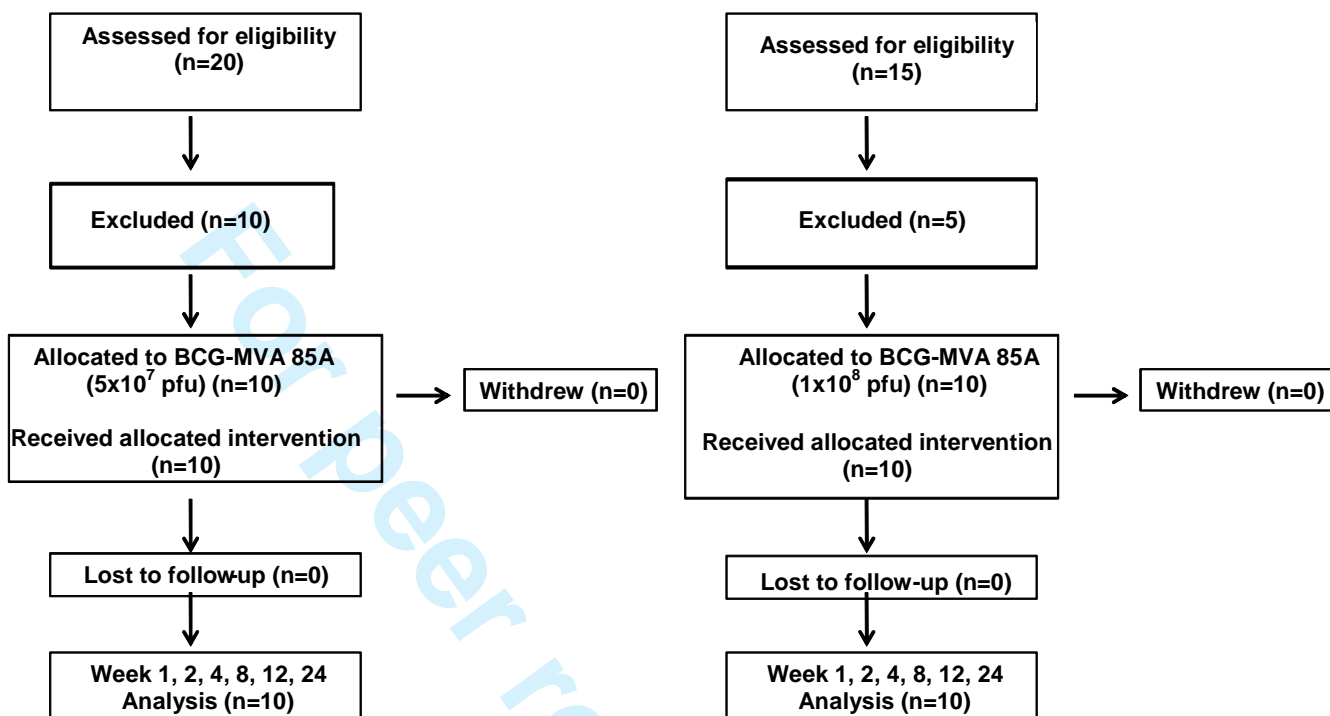
*Wilcoxon signed rank test

(b)

HIV-infected vs. HIV-negative group

	Summed Pooled peptides	Median (range) HIV+	Median (range) HIV-	Difference in medians (95% confidence interval)	<i>p</i> -value*
Week 1	Low dose	738 (109, 4398) n=10	2147 (1173, 5085) n=21	1101 (-393, 2873)	0.12
	High dose	1730 (758, 2138) n=10	6493 (4854, 7312) n=12	4557 (3038, 5904)	0.0001
Week 24	Low dose	20 (0, 237) n=9	385 (228, 1010) n=20	339 (161, 534)	0.004
	High dose	42 (33, 256) n=10	970 (655, 1199) n=12	820 (569, 1050)	0.0001
Area under the curve	Low dose	2162 (519, 23348) n=9	16317 (9129, 36418) n=20	11884 (3191, 17387)	0.02
	High dose	5929 (3167, 11607) n=10	41575 (26919, 53807) n=12	31384 (20778, 45504)	0.0001

Figure 1



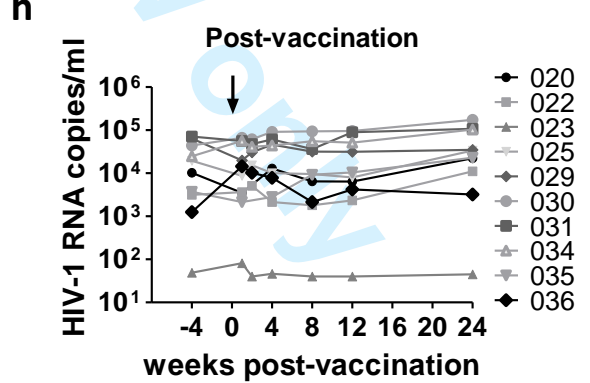
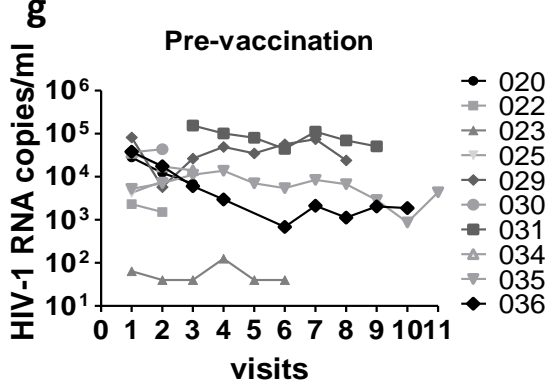
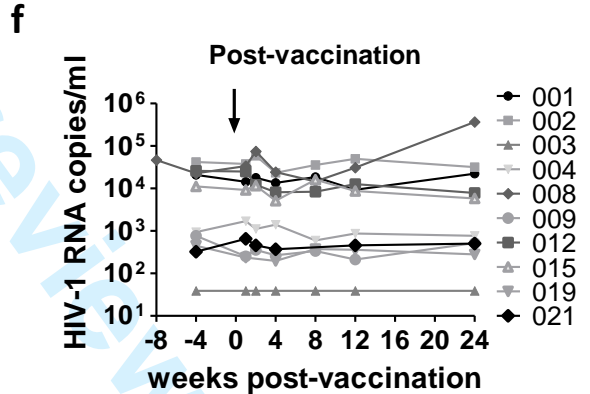
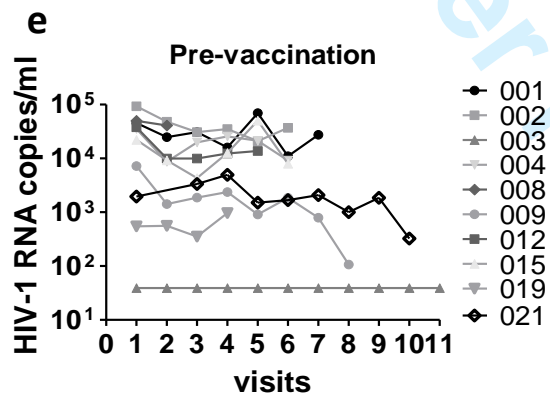
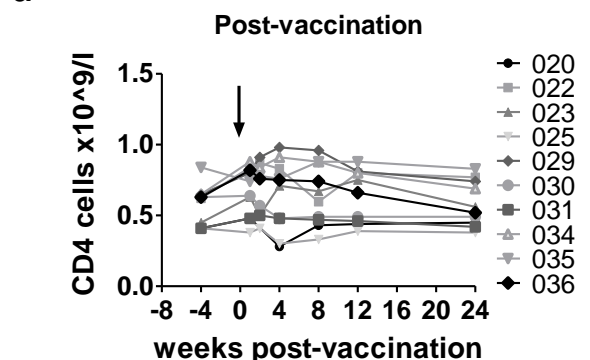
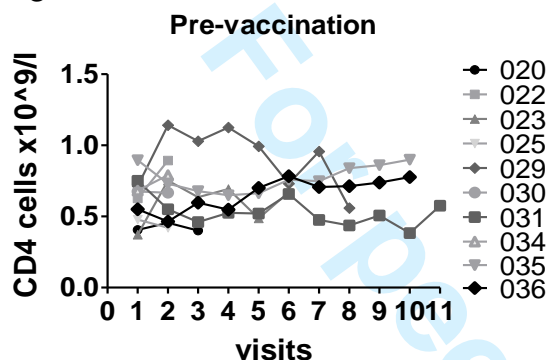
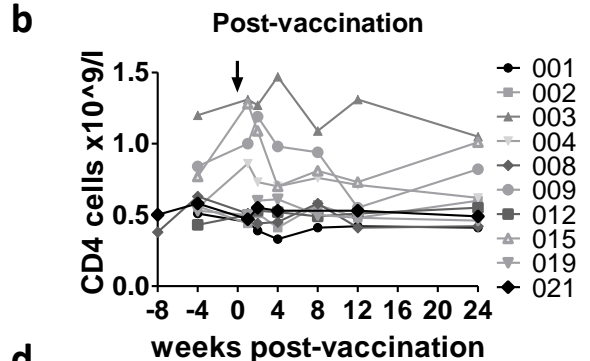
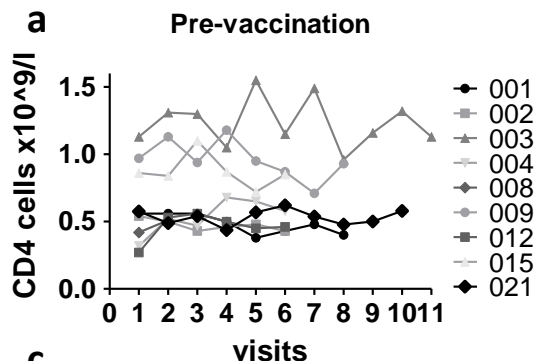


Figure 3

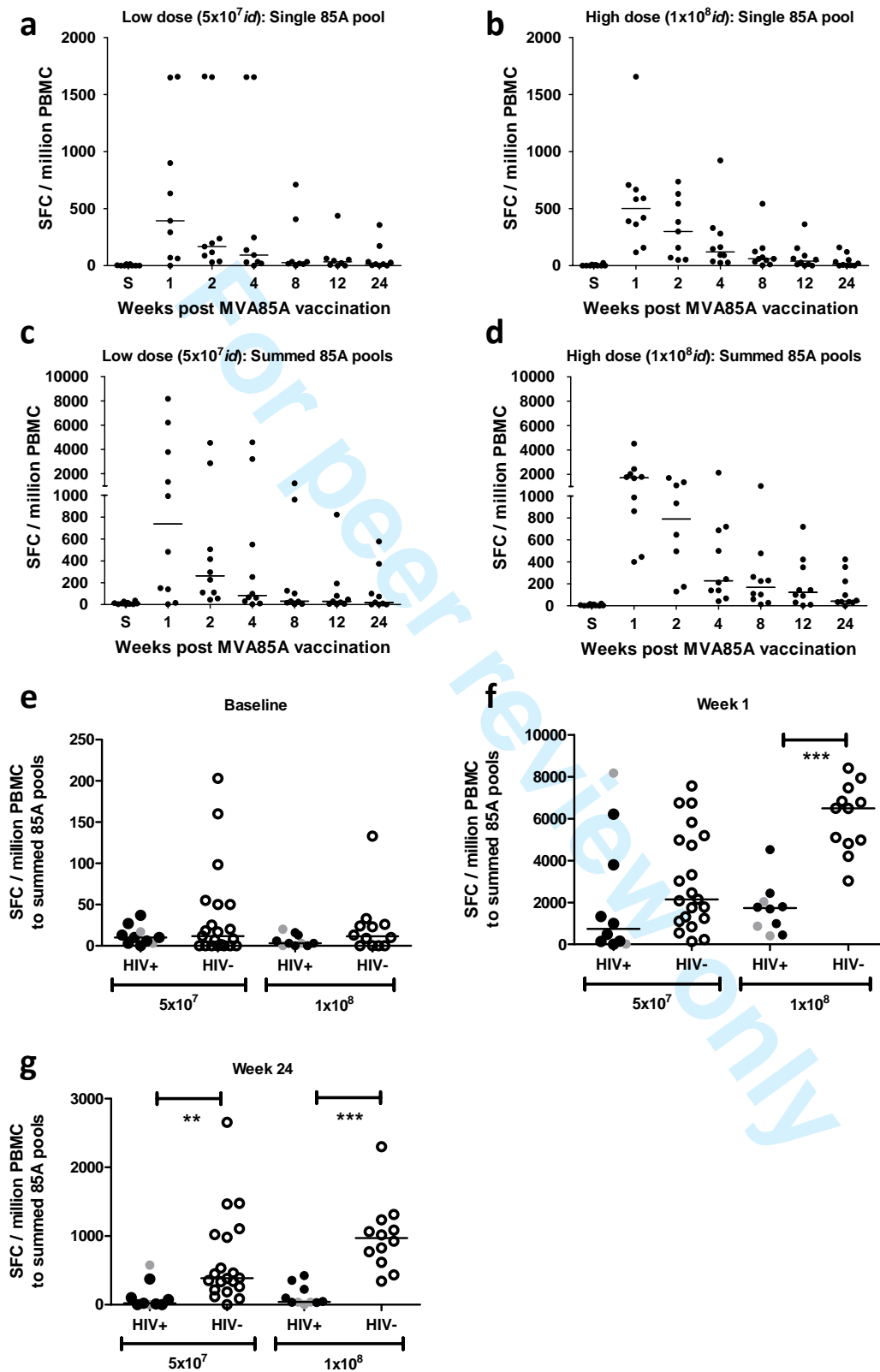


Figure 4

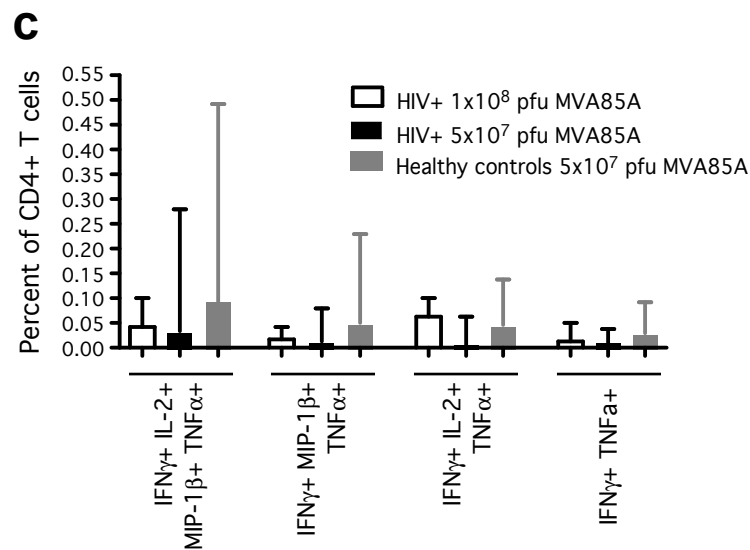
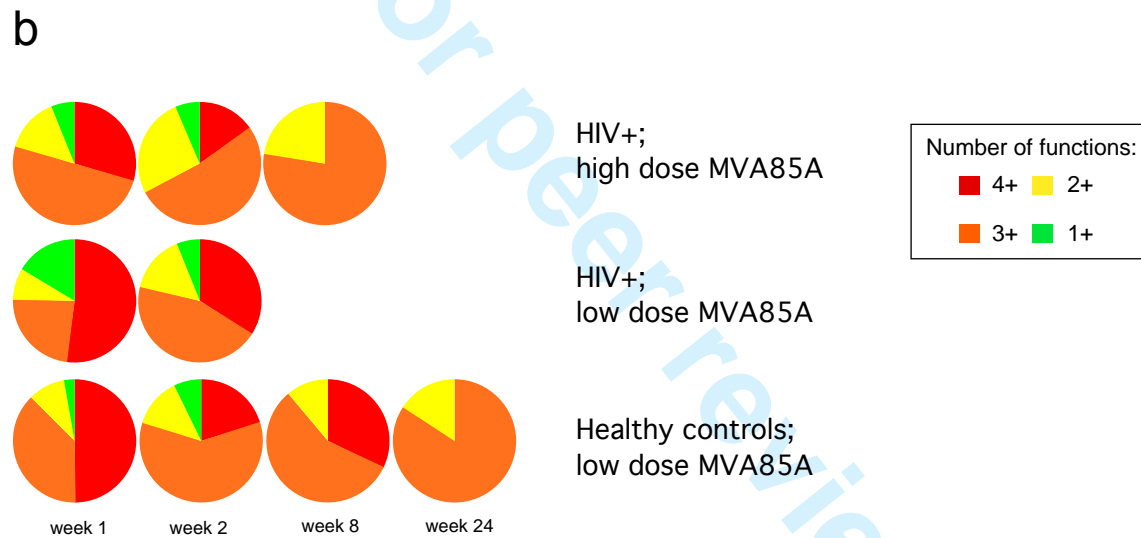
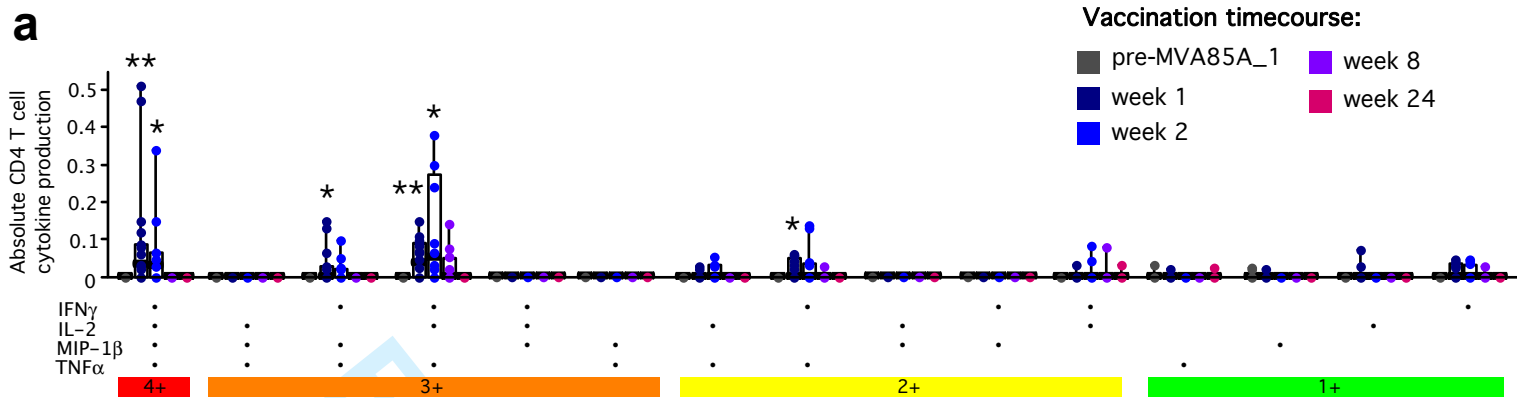
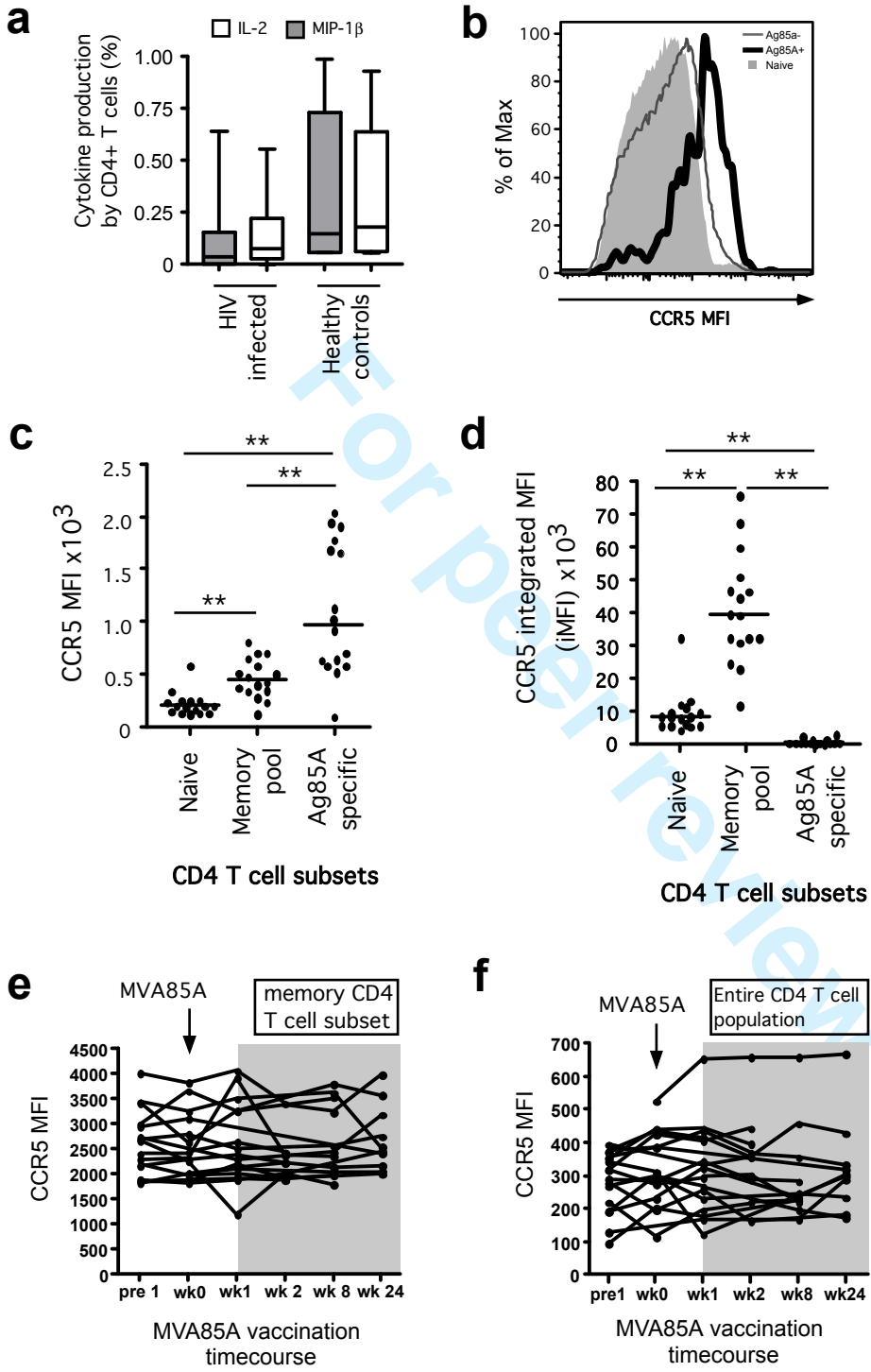


Figure 5





A Phase I study evaluating the safety and immunogenicity of MVA85A, a candidate TB vaccine, in HIV-infected adults

Journal:	<i>BMJ Open</i>
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5 **A Phase I study evaluating the safety and immunogenicity of**
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7 **MVA85A, a candidate TB vaccine, in HIV-infected adults**
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Short title: Phase I study with MVA85A in HIV

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ABSTRACT

OBJECTIVES: Control of the TB epidemic is a global health priority and one that is only likely to be achieved through vaccination. The critical overlap with the HIV epidemic requires any effective TB vaccine regimen to be safe in individuals who are infected with HIV. The objectives of this clinical trial were to evaluate the safety and immunogenicity of a leading candidate TB vaccine, MVA85A, in healthy, HIV-infected adults.

DESIGN: This was an open-label Phase I trial, performed in 20 healthy HIV-infected, antiretroviral-naïve subjects. Two different doses of MVA85A were each evaluated as a single immunisation in 10 subjects, with 24 weeks of follow-up. Safety of MVA85A was assessed by clinical and laboratory markers, including regular CD4 counts and HIV RNA load measurements. Vaccine immunogenicity was assessed by *ex-vivo* IFN- γ ELISpot assays and flow cytometric analysis.

RESULTS: MVA85A was safe in subjects with HIV infection, with an adverse event profile comparable to historical data from previous trials in HIV-uninfected subjects. There were no clinically significant vaccine-related changes in CD4 count or HIV RNA load in any subjects, and no evidence from qPCR analyses to indicate that MVA85A vaccination leads to widespread preferential infection of vaccine-induced CD4+ T cell populations. Both doses of MVA85A induced an antigen-specific IFN- γ response that was durable for 24 weeks, although of a lesser magnitude compared to historical data from HIV-uninfected subjects. The functional quality of the vaccine-induced T cell response in HIV-infected subjects was remarkably comparable to that observed in healthy HIV-uninfected controls, but less durable.

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3 **CONCLUSION:** MVA85A is safe and immunogenic in healthy adults infected with
4
5 HIV. Further safety and efficacy evaluation of this candidate vaccine in TB and HIV
6
7 endemic areas is merited.
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10 11 12 **ARTICLE SUMMARY SECTION**

13 14 **Article focus**

- 15
16 • HIV infection increases susceptibility to TB, and globally, TB is the cause of
17
18 death in up to half of AIDS deaths
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- 21
22 • There is an urgent need for a safe and effective TB vaccine in HIV-infected
23
24 people
25
26

27 **Key messages**

- 28
29 • MVA85A, a leading candidate TB vaccine, is safe and well tolerated in HIV-
30
31 infected people and does not induce changes in either CD4 count or HIV RNA
32
33 load
34
35
- 36
37 • MVA85A is immunogenic in HIV-infected people, and induces a similar
38
39 immune profile to that seen in HIV-uninfected people, but the immunogenicity
40
41 is less durable in HIV-infected people.
42
43

44 **Strengths and limitations of this study**

- 45
46 • This is a Phase I study with 20 subjects, and further studies are needed in TB
47
48 endemic countries in this important target population.
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56 **Funding:** This study was supported by The Wellcome Trust (Grant number
57
58 WT076943MA), TBVAC (an EU 6th Framework programme grant) and The NIHR
59
60 Oxford Biomedical Research Centre.

INTRODUCTION

Tuberculosis (TB) and Human Immune Deficiency Virus (HIV) are inextricably linked. At the end of 2007, approximately 33.2 million persons were living with HIV-1 infection, an estimated one-third of whom were co-infected with *Mycobacterium tuberculosis* (*M. tuberculosis*)¹. TB is the cause of death for up to half of all AIDS patients² and the increasing incidence of drug-resistant strains of *M. tuberculosis* poses a significant threat to a susceptible HIV-infected population³.

Mycobacterium bovis (*M. bovis*) Bacille Calmette Guérin (BCG) fails to protect consistently against the adult pulmonary form of TB, whilst providing reliable protection against disseminated infection in childhood⁴. An improved vaccine strategy is thus essential for global control of this disease⁵. MVA85A (modified vaccinia virus Ankara expressing antigen 85A) is a leading candidate TB vaccine, designed to enhance the effect of BCG; it is safe and highly immunogenic in healthy BCG-naïve and BCG-vaccinated subjects, and in subjects latently infected with *M. tuberculosis* in the UK and Africa⁶⁻¹¹.

It is essential that any new TB vaccine is safe in an HIV-infected population. Subunit vaccines are an ideal choice for an immuno-compromised population in which the safety of replicating whole organism vaccines may be a concern. Although MVA is a live viral vaccine vector, it cannot replicate in human cells^{12, 13}. There are now safety data from a number of clinical trials with recombinant MVAs in HIV-infected subjects, which demonstrate no sustained effect on either HIV load or CD4 count¹⁴⁻¹⁶. Most of these studies assessed immune-reconstituted HIV-infected individuals on anti-retroviral therapy (ARV); however, some ARV-naïve subjects with more advanced HIV infection have also been vaccinated with a recombinant MVA, with no

1
2
3 significant rise in HIV load or fall in CD4 count over a 4-week follow-up period ¹⁷.
4
5 Preclinical studies in severely immuno-suppressed macaques have also documented
6
7 safety in this model ¹⁸. To date, MVA85A has been administered to more than 1,000
8
9 individuals with no vaccine-related serious adverse events ^{6, 8-11} (McShane,
10
11 unpublished data).
12
13

14
15 Here we present the first clinical trial of a subunit TB vaccine in an HIV-infected
16
17 population. The primary endpoint was to evaluate the safety of two doses of
18
19 MVA85A in healthy HIV-infected subjects in the UK and the secondary endpoint was
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21 to evaluate the immunogenicity of this vaccine regimen. .
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METHODS

Trial design and participants

The protocol for this multi-site study was approved by the Medicines and Healthcare products Regulatory Agency (MHRA) and ethical approval was obtained from the Gene Therapy Advisory Committee (GTAC). Participants were recruited from the Genitourinary Medicine (GUM) departments at the Oxford Radcliffe Hospitals NHS Trust, University Hospitals Birmingham NHS Foundation Trust (Selly Oak Hospital), Great Western Hospitals NHS Foundation Trust, and Imperial College Healthcare NHS Trust, London.

Potentially eligible subjects were referred to the clinical trial team. Vaccination visits took place in Oxford; screening and follow-up visits took place at local NHS Trust sites. Written informed consent was obtained from all subjects prior to enrolment in the trial, and the referring HIV physicians were notified.

Eligibility required participants to be generally healthy, aged 18-55 years, with a normal chest radiograph and no clinical or radiological evidence of TB disease, a current CD4 count (measured at screening) of at least $350 \times 10^6/L$, a nadir CD4 count of not less than $300 \times 10^6/L$ and an HIV RNA load at screening of less than 100,000 copies/mL. All subjects were diagnosed with HIV infection at least six months before screening and had not received any ARVs within the preceding six months (full inclusion/exclusion criteria, Table 1). In all cases, testing for latent *M. tuberculosis* infection (LTBI) was conducted at screening using an in-house *ex-vivo* interferon gamma (IFN- γ) ELISpot assay for ESAT6 and CFP10 peptides. Subjects found to be latently infected were referred back to their consultants for possible treatment at the end of the trial.

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2
3 The trial was registered on a clinical trials database: ClinicalTrials.gov ID
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5 NCT00395720.
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7

8 **Vaccine**

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10 Clinical grade MVA was produced under Good Manufacturing Practice standard by
11
12 IDT Biologika GmbH.
13
14

15 **Procedures**

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17 The first group of 10 subjects were vaccinated intradermally with MVA85A at a dose
18
19 of 5×10^7 plaque-forming units (pfu); the second group of 10 subjects were
20
21 subsequently vaccinated with a dose of 1×10^8 pfu MVA85A. Subjects were
22
23 sequentially allocated first to the low dose group and then once safety had been
24
25 demonstrated, to the high dose group. A diary card was completed by all subjects
26
27 recording local and systemic adverse events and body temperature for days 1 to 7
28
29 post-vaccination. All subjects were followed up regularly for 12 months; blood
30
31 samples taken at each time-point for CD4 count, HIV RNA load measurement and
32
33 cellular immunological assays. CD4 count and HIV RNA load results were
34
35 communicated to the patient's HIV physician at regular intervals. Routine
36
37 haematology and biochemistry assays were performed at weeks 1 and 12 after
38
39 vaccination. All subjects attended their usual NHS follow-up appointments and
40
41 received routine clinical care throughout the trial.
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51 **Immunological assays**

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53 *Ex vivo* IFN- γ ELISpot assays were performed at screening and at weeks 1, 2, 4, 12,
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55 and 24 post-vaccination using fresh peripheral blood mononuclear cells (PBMCs), as
56
57 described previously¹⁰. A single pool of 15-mer peptides spanning the Ag85A protein
58
59 (66, overlapping by 10 amino acids, 2 μ g/mL final concentration of each peptide in
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1
2
3 each well), and 7 pools of non-overlapping 9-10 Ag85A peptides (final concentration
4 of 10µg/mL in each well) were used to stimulate for the IFN-γ ELISpot assays. For
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9 the 7 peptide pool wells, the results were summed across all peptide pools for each
10
11
12 time-point. This potentially resulted in duplicate counting of T cells that responded to
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14
15 any of the 10mer overlap regions, since any 10mer occurred in two pools with
16
17
18 adjacent peptides, but allows direct comparison with immunogenicity data from
19
20 previous trials.

21 Intracellular cytokine staining of PBMCs was performed as described previously¹⁹.
22
23 Briefly, cells were stained with the amine reactive LIVE/DEAD fixable dye ViViD
24
25 (Molecular Probes, Invitrogen) and then surface stained with monoclonal antibodies
26
27 against CD4, CD14, CD19, CD27, CD45RO, CD57 and CCR5. After washing, cells
28
29 were permeabilized (Cytofix/cytoperm kit; BD Pharmingen) and stained
30
31 intracellularly for CD3, CD8, IFN-γ, TNF-α, IL-2 and MIP-1β. Naïve CD4+ memory
32
33 cells were identified as CD45RO-CD27+CD57+; memory CD4 T cell as
34
35 CD45RO+CD27±CD57±. CD4+ T cells producing two or more cytokines (IFN-γ, IL-
36
37 2 or TNF-α) following stimulation with Ag85A peptides were sorted to greater than
38
39 98% purity using a modified FACS Aria flow cytometer (BD Biosciences); CMV-
40
41 specific CD4+ T cells, defined similarly, and bulk resting memory CD4+ T cells were
42
43 sorted simultaneously. Cytokine production and the expression of surface markers
44
45 were analysed using FlowJo version 8.7 (Tree Star Inc), Pestle version 1.5 and SPICE
46
47 version 4.1 (both from Mario Roederer, Vaccine Research Center, NIAID, NIH,
48
49 USA).

50
51
52 Real-time quantitative PCR (qPCR) was used to determine the proportion of HIV-
53
54 infected Ag85A-specific CD4+ T cells; resting memory CD4+ T cells (non-Ag85A-
55
56 specific) and CMV-specific CD4+ T cells were used as intra-individual controls.
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3 HIV-1 gag proviral DNA within sorted CD4+ T cell populations was quantified by
4 qPCR, as described previously²⁰. qPCR was performed simultaneously for albumin
5 gene copy number in all sorted CD4+ T cell populations to quantify cell number in
6 each reaction. HIV-1 gag primers for qPCR: Gag clade B primer position and
7 sequence were 795gagB-F: ggtgcgagagcgtcagtattaag, 911gagB-R:
8 agtcacctgctgcccata, and probe was 841gagB-P: FAM-aaaattcgggtaaggccagggggaagaa-
9 QSY7 (MegaBases). Gag clade C primer position and sequence were 689gagC-F:
10 ggggaagtgaYatagcagga, 841gagC-R: ggYccttgtYttatgtccaa, and probe was 710gagC-
11 P: ctactagtaVccttcaRgaacaRataRcatggatga. Albumin primer sequences were Alb-F:
12 tgcattgagaaaacgccagtaa, Alb-R: atggcgcctgttcaccaa, and probe: Alb-P: FAM-
13 tgacagagtcaccaaattgctgcacagaa-QSY7. Degenerate HIV gag clade A/G primer position
14 and sequence were 783gagA/G-F: 5'-gagagagatgggtgcgagagcgtc-3'
15 (GAGAGAGATGGGTGCGAGAGCGTC), 895gagA/G-R: 5'-
16 cttccagctcctgctgccc-3', and probe was 844gagA/G-P: 5'-
17 attcgggtaaggccagggggaagaaaaaat-3'.

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43 CD4+ T cells from 12 subjects (4/10 in the low-dose group and 8/10 in the high-dose
44 group) were analysed for HIV gag content; low sort yields (less than 250 antigen-
45 specific CD4+T cells) prevented similar analyses in the remaining 8 subjects.

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50 Serum levels of MIP-1 α , MIP-1 β , RANTES, TNF- α and IL-2 were measured pre-
51 vaccination and at weeks one, two and 24 post-vaccination using FlowCytomix
52 simplex kits (Bender MedSystems). Serum was thawed and plated out in duplicate in
53 round-bottom 96-well plates. Samples were used neat for MIP-1 α , MIP-1 β , TNF- α
54 and IL-2, and diluted 1/10 for RANTES. Standards (provided) were reconstituted as
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3 specified, and 1/3 serial dilutions plated in duplicate. Bead and biotin-conjugate
4 cocktails were added to each well in 25µL and 50µL volumes, respectively, and
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6 incubated for two hours in the dark at room temperature. Samples were then washed
7
8 twice in 100µL Assay Buffer. Streptavidin-PE (50µL) and Assay Buffer (100µL) were
9
10 added and plates were incubated for one hour in the dark at room temperature.
11
12 Samples were then washed again and transferred to cluster tubes in 4%
13
14 paraformaldehyde in PBS (400uL) and acquired using an LSRII flow cytometer (BD
15
16 Biosciences) set up with the beads provided and the highest concentration standard;
17
18 the stopping gate was set so that at least 300 events were collected for each sample.
19
20 Data were analysed using FlowCytomixPro software (Bender MedSystems).
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30 **Statistical analysis**

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32 Continuous outcomes were presented as a median (range) for each group. Median
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34 difference was calculated to assess difference between two groups and the
35
36 corresponding 95% confidence interval was computed using the robust method
37
38 proposed by Newson²¹. The overall magnitude of vaccine-induced CD4+ T cell
39
40 responses over the 24 weeks was summarised using area under the curve (AUC) for
41
42 each dose group. Peak (1 week) and plateau (24 weeks) CD4+ T cell responses were
43
44 compared with those at baseline (pre-vaccination) using the Wilcoxon signed rank
45
46 test, Stata Statistical Software, Release 9.0. 2005 (Stata Corporation, College Station,
47
48 TX). Mann-Whitney U test was used to compare between the vaccine groups.
49
50
51 Comparisons between specific time points in this trial and previously published trials
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53 of MVA85A^{6, 22}, and between low and high dose groups were conducted using the
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58 Mann-Whitney U test (Stata).
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Non-parametric Spearman rank analyses were used to correlate immune assays and CD4/HIV RNA load with CD4+ T cell responses.

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RESULTS

Thirty-five individuals attended for screening between October 2006 and June 2009; 20 subjects were enrolled (Fig. 1, Flowchart). The demographics of the 20 participants are shown in Table 2, and were comparable across the two groups.

Safety of MVA85A in HIV-infected subjects

Local reactions related to the MVA vaccine occur during the first week after vaccination and have been reported previously^{6, 7, 10}. The local adverse event (AE) profile recorded in the diary cards of the HIV-infected subjects in this study was comparable to that observed in HIV-uninfected subjects^{6, 7, 10} (Pathan et al, unpublished data), although pruritis appeared slightly higher in the HIV-infected group (Table 3a). There were no severe local reactions and most local AEs were mild.

One subject in the low dose group experienced a single episode of fever greater than 37.5 °C (documented at 38.1°C). All other subjective systemic symptoms occurred with similar frequencies in the low dose and high dose groups (Table 3b). There was one moderate systemic AE and all others were mild.

Pre-vaccination CD4 counts and HIV RNA loads for all 20 subjects over a time period of one to six years before enrolment are shown in Figure 2. These longitudinal data illustrate the levels of natural fluctuation that occur over time and represent important baseline measurements for the interpretation of these parameters post-vaccination, also shown in Figure 2, in the absence of an unvaccinated control group. No clinically significant changes in CD4 counts or HIV RNA load was seen post-vaccination.

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3 A major concern with respect to the vaccination of HIV-infected individuals is the
4 possibility that responding antigen-specific CD4+ T cells will act as preferential
5 targets for HIV infection as a function of their activated status, thereby enhancing the
6 propagation of HIV and accelerating disease progression. In this study, however, there
7 was no evidence for preferential HIV infection of Ag85A-specific CD4+ T cells. The
8 median number of CD4+ T cells in Ag85A-specific qPCR reactions was 73 (95% CI:
9 15-130 CD4+ T cells). Where possible, multiple time-points throughout the
10 vaccination course for each subject were analyzed, to determine the proportion of
11 Ag85A-specific CD4+ T cells that were infected with HIV. Of 29 Ag85A-specific
12 CD4+ T cell samples in total (11 subjects), only one subject showed a positive signal
13 for HIV *gag* DNA by qPCR at two different post-vaccination timepoints (weeks 2 and
14 8 post-vaccination; data not shown). In the 11 subjects tested, resting HIV-specific
15 and CMV-specific memory cell populations showed a positive signal in all assays
16 (data not shown). In addition, vaccination had no effect on levels of MIP-1 α , MIP-1 β ,
17 RANTES, TNF- α and IL-2 in unstimulated serum (data not shown).
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41 **Immunogenicity of MVA85A in HIV-infected subjects**

42 MVA85A induced significant antigen-specific T cell responses as measured by IFN- γ
43 ELISpot assay (Fig. 3a-d, Table 4a). In the low dose group, the frequency of IFN- γ -
44 secreting T cells responding to the single 85A peptide pool at week one post-
45 vaccination, increased by a median of 393 sfc/million compared to prevaccination
46 baseline measured on the day of screening ($p=0.009$); there a significant increase
47 remained until 24 weeks post-vaccination ($p=0.032$). In the high dose group, a median
48 increase of 502 sfc/million was apparent at week 1 post-vaccination ($p=0.005$); again,
49 this response was maintained until 24 weeks ($p=0.048$). In both groups, T cell
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3 responses to the summed 85A peptide pools followed a similar pattern with a
4 significant increase above baseline at week 1 (738 and 1730 sfc/million for low and
5 high dose groups, respectively), although, the week 24 response was only maintained
6 significantly above baseline in the high dose group (low dose $p=0.17$; high dose
7 $p=0.007$). There were no significant differences in the overall magnitude (i.e. AUC)
8 of the IFN- γ response between low and high dose groups across the follow-up period
9 ($p=0.29$ and $p=0.68$ for summed and single peptide pools, respectively; data not
10 shown). However the responder rate was higher in the high dose group with no
11 observed non-responders (ie those subjects with no measurable vaccine induced
12 immune response) compared with 1-2 non-responders (depending on antigen) in the
13 low dose group. Overall, the kinetics of the Ag85A-specific T cell responses were
14 very similar to those previously reported in similar studies of HIV-uninfected subjects
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17 Ag85A-specific T cell responses in the low-dose group were significantly lower
18 compared to those observed in previously published studies of HIV-uninfected
19 subjects (AUC analysis, $p=0.02$; Table 4b)^{6, 22}. This difference was not significant at
20 week 1 (MWU analysis, $p=0.12$), which represents the peak response, but was
21 significant at week 24 post-vaccination (MWU analysis, $p=0.004$; Table 4b, Figure
22 3e-g). There were no significant differences in baseline (pre-vaccination) responses
23 between the HIV-infected and HIV-uninfected groups (MWU analysis, $p=0.85$;
24 Figure 3e). Similarly, HIV-infected subjects in the high dose group exhibited
25 significantly lower responses compared to HIV-uninfected subjects who received the
26 same dose of MVA85A (AUC analysis, $p=0.0001$; week 1 and week 24 MWU
27 analysis, $p = 0.0001$; Table 4b, Figure 4e-g). Again, baseline responses were
28 comparable ($p=0.24$) (Fig. 3e).

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3 There was no significant correlation between the CD4 count at screening and the peak
4 summed 85A peptide pool response ($R=0.04$, $p=0.09$), nor between HIV RNA load at
5 screening and the peak immune response ($R= -0.04$, $p=0.08$). There was a strong
6 negative correlation between viral load at screening and the plateau immune response
7 at week 24 ($R= -0.8$, $p=0.002$).

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15 Four subjects were found to have LTBI. However, there were no significant post-
16 vaccination changes in the magnitude of ESAT-6/CFP10 responses in these subjects
17 (data not shown).

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22 Ag85A-specific cytokine/chemokine production by CD4+ T cells prior to MVA85A
23 vaccination was undetectable and increased significantly post-vaccination (Fig. 4a).
24 One week following MVA85A vaccination, more than 40% of Ag85A-specific CD4+
25 T cells produced IFN- γ , IL-2, MIP-1 β and TNF- α simultaneously ($p<0.01$); this
26 remained significant at week 2 ($p<0.05$). By week 8 post-vaccination, 60% of Ag85A-
27 specific CD4+ T cells were still producing IFN- γ , IL-2 and TNF- α simultaneously;
28 production of IL-2 and IFN- γ was maintained until 24 weeks. Production of MIP-1 β
29 was not detected beyond week 2 post-vaccination (Fig. 4a, 5a). In the first two weeks
30 post-vaccination, Ag85A-specific CD4+ T cells exhibited similar functional profiles
31 in the HIV-infected groups, both low dose and high dose, and the "control" low dose
32 HIV-uninfected group; in all cases, the responses were dominated by four functionally
33 distinct subsets (Fig. 4b, c). IFN- γ production measured by ICS correlated closely
34 with IFN- γ secretion observed in the ELISpot assay ($R= 0.75$, $p<0.0001$, $n=45$; data
35 not shown).

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Surface expression of the HIV M-tropic co-receptor CCR5 was monitored throughout the course of vaccination (Fig. 5). At week 1 post-vaccination, the MFI for CCR5 was highest in the activated Ag85A-specific CD4+ T cells, and lowest in the naïve CD4+

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3 T cell subsets. Using the integrated MFI (iMFI) function calculated using both MFI
4 and cell frequencies, expression of CCR5 was highest in the much larger memory
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8 CD4+ T cell pool (Fig. 5d). No significant increase in CCR5 expression was detected
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10 in either the memory (Fig. 5e) or the total (Fig. 5f) CD4+ T cell populations as a
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12 result of MVA85A vaccination.
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For peer review only

DISCUSSION

This is the first Phase I trial of a subunit vectored TB vaccine in HIV-infected individuals. The two main findings of this study are that MVA85A is safe in healthy subjects infected with HIV (a proportion of whom are co-infected with *M. tuberculosis*), and that MVA85A is immunogenic in this population, albeit at a lower level than in HIV-uninfected subjects. As a result of these promising findings, similar studies in South Africa (*Scriba et al, submitted*) and Senegal (*Mboup et al, unpublished data*) are ongoing.

The local and systemic AE profiles in this trial were comparable to those observed in HIV-uninfected subjects previously. Whilst we have not evaluated the safety of this vaccine in HIV-infected subjects with lower CD4 counts, we would not expect the safety profile of this vaccine to be altered by progression of HIV disease. Importantly, there were no clinically significant effects of vaccination on either CD4 count or HIV RNA load. It is important to understand the variability in these parameters during stable untreated HIV infection, to enable meaningful interpretation of fluctuations throughout the trial period. A recent quantitative review of cohort studies of HIV-infected adults suggests that, during stable untreated infection, intra-individual HIV RNA measurements can fluctuate over short periods of time by as much as 0.2-0.5 log₁₀ copies/mL, with measurement error and physiological fluctuation each contributing about 50% of this variation. CD4 counts can also vary within patients by 60-130 cells/uL within weeks, but there is less measurement error, and during stable infection the overall within-subject variation is proportionally larger for HIV RNA than for CD4 count²³. In this study, we also observed greater intra-individual variations in HIV RNA load compared to CD4 counts. Only one subject in

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3 each group demonstrated a more than 0.5 log rise in HIV RNA load at week 24 post-
4 vaccination. The first of these (low dose group, 008) had displayed a relatively high
5 baseline HIV RNA load and low baseline CD4 count at screening, just within the
6 inclusion criteria. As the rise in HIV RNA load was only detectable 24 weeks after
7 vaccination, it was considered highly unlikely that this was due to MVA85A
8 vaccination. This subject commenced ARVs before week 24; all other subjects
9 remained ARV-free throughout the trial period. The second subject (high dose group,
10 035) developed a rise in HIV RNA load between week 12 and week 24, peaking at
11 just greater than a 0.5 log increase over baseline. However, this subject's HIV RNA
12 load had been stable prior to this, so it was again deemed unlikely that this was a
13 vaccination-related effect. Other fluctuations in the HIV RNA loads were observed,
14 but any sustained rise was well within the expected 0.5 log variation.
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31 HIV preferentially infects memory CD4+ T cells^{24, 25}, in particular HIV-specific
32 memory CD4+ T cells,²⁰ and other activated antigen-specific CD4+ T cells²⁶. Using
33 a sensitive qPCR method, we detected HIV *gag* DNA in Ag85A-specific CD4+ T
34 cells from only 1/11 subjects post-vaccination (3 low dose and 8 high dose). This
35 subject received high-dose MVA85A vaccination. This low positivity rate concurs
36 with the stable CD4 count and HIV RNA load parameters in most subjects throughout
37 the trial. Although the qPCR assay is sensitive, the low yield of Ag85A-specific
38 CD4+ T cells entering the assay is an important limitation and provides just a
39 snapshot of the HIV burden within the Ag85A-specific CD4+ T cell pool and
40 warrants further future investigation. However, whilst small effects of MVA85A on
41 HIV infection of CD4+ target cells cannot be excluded given the small sample size,
42 these data suggest that MVA85A vaccination of healthy HIV-infected individuals
43 does not lead to widespread preferential infection and depletion of vaccine-induced
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3 CD4+ T cell populations in the periphery. These data are supported by no change in
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5 surface expression of the HIV co-receptor CCR5 following MVA85A vaccination. In
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7 addition, the lack of effect of vaccination on chemokine and cytokine levels in
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9 unstimulated serum supports the interpretation that vaccination with MVA85A did not
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11 lead to widespread immune activation in this subject group.
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17 We have shown that vaccine-induced immunogenicity, while of moderate magnitude,
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19 is significantly lower than in healthy HIV-uninfected subjects given the same dose of
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21 MVA85A. Thus, additional strategies may be required to augment immune responses
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23 to MVA85A vaccination in the context of HIV infection. Partial reconstitution of the
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25 immune system with ARVs may improve the response to vaccination. Indeed, this
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27 study shows that the baseline HIV RNA level is a strong negative predictor of
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29 summed 85A peptide pool responses at week 24 post-vaccination. In addition, a
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31 booster vaccination with MVA85A may be required to enhance the vaccine-induced
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33 immune response.
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41 The functional profile of Ag85A-specific CD4+ T cells observed in this study was
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43 remarkably comparable to that of healthy HIV-uninfected subjects¹⁹ and *M.*
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45 *tuberculosis*-infected subjects¹⁰, although the response in HIV-infected subjects is
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47 less durable. IFN- γ and TNF- α are known to be important for protective immunity to
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49 TB, and these cytokines dominated the MVA85A vaccine-induced CD4+ T cell
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51 response elicited in the current study cohort. In this study, production of MIP-1 β by
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53 Ag85A-specific CD4+ T cells was not detectable after week 2 post-vaccination. The
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55 production of multiple cytokines including IL-2 and MIP-1 β by HIV-specific CD8+ T
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57 cells is associated with long-term non-progression, albeit with uncertain causality²⁷.
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3 The production of β -chemokines by CD8+ T cells was not assessed here as there were
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5 no detectable Ag-85A specific CD8+ T cell responses pre- or post-vaccination.
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11 Vaccine-induced bystander activation of CD4+ T cells in HIV-infected subjects is
12 likely to be undesirable because activated CD4+ T cells are at increased risk of HIV
13 infection. The overall pattern of cytokine production and the frequency of CMV-
14 specific CD4+ T cell producing cytokines remained both relatively stable over the 24-
15 week period following MVA85A vaccination, and comparable to pre-vaccination
16 responses (Supplemental Figure 1). These data suggest that MVA85A vaccination
17 does not induce substantial bystander activation of antigen-specific CD4+ T cell
18 populations.
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32 Demonstrating the safety and immunogenicity of a new TB vaccine in an important
33 target population is a crucial step, but does not mean that the vaccine will be effective.
34 However, the data presented here support further safety and immunogenicity studies
35 of this candidate vaccine, together with proof-of-concept efficacy trials, in TB and
36 HIV endemic areas.
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Authors' Contributions:**Authors' Contributions:**

Concept and design of study, and obtaining funding: HMcS.

Protocol writing: HMcS, CRS, AMM, AML.

Recruitment, vaccination and follow-up of volunteers: AMM, RR, IDP, MH, AW, JR, JS, GR.

Immunology assays and analysis: AAP, NERB, IS, SH, HAF, HP.

Collaboration and contribution of reagents/materials/analysis tools: DRA, DAP, JPC, DCD, RAK, MR.

Data analysis: AMM, RR, AAP, NA, NERB, HMcS.

Writing of the paper: AMM, HMcS, with contributions from all authors.

Conflict of Interest Statement: AAP and HMcS are named inventors on a composition of matter patent for MVA85A owned by the University of Oxford, and are shareholders in a Joint Venture formed for the further development of this vaccine.

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TABLE and FIGURE LEGENDS

Table 1 *Full inclusion and exclusion criteria.* Subjects were required to meet all of the inclusion criteria to participate in the study:

Table 2 *Subject demographics:* Comparison of low and high dose vaccination groups.

Tables 3a and b *Local and systemic adverse events:* Comparison of low and high dose vaccination groups with dose-matched HIV-uninfected subjects from previous trials of MVA85A^{6, 7, 10} (Pathan et al, unpublished data). There were significantly fewer systemic AEs per person, (and a lower frequency of systemic AEs overall,) in the 10 HIV-infected subjects receiving high dose MVA85A compared with HIV-uninfected subjects receiving the same dose of vaccine ($p=0.026$, data not shown and Pathan *et al*, unpublished)

Table 4a *Ex-vivo IFN- γ ELISpot statistics (1):* Comparison of screening, week 1 and week 24 responses within low and high dose groups, for both summed and single pooled peptides, using Wilcoxon signed rank test. Median (range), median difference (range, of week 1 and week 24 responses compared to screening,) and p -values are shown.

Table 4b *Ex-vivo IFN- γ ELISpot statistics (2):* Comparison of responses between HIV-infected and HIV-uninfected subjects, at each time-point and each vaccine dose,

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3 using Mann-Whitney U test. Median (range), difference in medians (with 95%
4 confidence intervals), and *p*-values are shown. AUC analysis is also shown.
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10 **Figure 1 Flowchart.** Follow-up was conducted for 24 weeks post-vaccination in both
11 the low dose MVA85A (5×10^7 pfu; left panels) and high dose MVA85A (1×10^8 pfu;
12 right panels) groups. Of the volunteers excluded at screening, 5/15 had CD4 counts
13 $< 350 \times 10^6/L$ and/or exhibited progressively decreasing CD4 counts, 2/15 had HIV
14 RNA loads of $> 100,000$ copies/mL, 2/15 had abnormal chest radiographs, 1/15 had a
15 current history of active eczema, and 1/15 had a history of illicit drug usage and
16 unstable depression; the remaining 4/15 were eligible but failed to attend for
17 vaccination (2 in each dose group).
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32 **Figure 2 (a-d) Comparison of pre and post-vaccination CD4 counts:** Longitudinal
33 CD4 counts of subjects in the low dose (5×10^7 pfu MVA85A) group pre-vaccination,
34 (a); and high dose (1×10^8 pfu MVA85A) group (c) pre-vaccination. Longitudinal CD4
35 counts post-vaccination (up to 24 weeks) in the low dose (b) and high dose (d)
36 groups. NB X-axis for the pre-vaccination data is not standardised for time but varies
37 from 1-6 years depending on the individual subject. (e-h) *Comparison of pre and post-*
38 *vaccination HIV RNA loads:* Longitudinal HIV RNA loads of subjects in the low dose
39 (e) and high dose (g) groups pre-vaccination. Longitudinal HIV RNA loads post-
40 vaccination (up to 24 weeks) in the low dose (f) and high dose (h) groups.
41 Anonymous number codes (001-036) shown in key. NB X-axis for the pre-
42 vaccination data is not standardised for time but varies from 1-6 years depending on
43 the individual subject.
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3 **Figure 3 (a-d)** *IFN- γ ELISpot responses in the low dose and high dose groups.*
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5 *Longitudinal responses to the single 85A peptide pool are shown for the low dose*
6 *(5×10^7 pfu MVA85A) group, (a) and high dose (1×10^8 pfu MVA85A) group (b).*
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10 *Longitudinal responses to the summed 85A peptide pools are shown for the low dose*
11 *(c) and high dose (d) groups. Horizontal bars represent the median response.*

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15 *Comparison of IFN- γ ELISpot responses in HIV-infected and HIV-uninfected subjects*
16 *(e-g). Responses to summed 85A peptide pools for low dose and high dose vaccine*
17 *regimes at screening (e); week 1 post-vaccination (f) and week 24 post-vaccination*
18 *(g). Subjects with LTBI are denoted with grey symbols. Statistically significant*
19 *differences in the responses between HIV-infected and HIV-uninfected subjects were*
20 *assessed using the Mann-Whitney U test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Horizontal*
21 *bars represent the median response^{6, 7, 10} (Pathan et al, unpublished data).*
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34 **Figure 4** *MVA85A induces polyfunctional Ag85A-specific CD4+ T cells in HIV-*
35 *infected individuals. MVA85A vaccination-induced production of IFN- γ , IL-2, MIP-*
36 *1 β and TNF- α by antigen-specific CD4+ T cells was assessed following Ag85A*
37 *peptide stimulation of cryopreserved PBMC using polychromatic flow cytometry. (a)*
38 *Individual data points are shown with median line, interquartile range (open bars) and*
39 *range (whiskers) at baseline and at each time-point post-vaccination for every*
40 *possible combination of cytokine/chemokine production. High dose and low dose*
41 *groups were analyzed together (n=17). (b) The functional profile of the Ag85A-*
42 *specific CD4+ T cell response is summarized in the pie charts (n=17). CD4+ T cells*
43 *producing a given number of cytokines/chemokines are grouped and colour-coded*
44 *together. Pie charts are shown for the high dose HIV-infected group (n=9), the low*
45 *dose HIV-infected group (n=8) and low dose healthy controls (n=6). (c) Absolute*
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3 percentages of the highest frequency CD4+ T cell subsets producing specific
4 combinations of chemokine/cytokines at week 1 post-vaccination across the different
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8 groups; * $p < 0.05$, ** $p < 0.01$.
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12 **Figure 5** *Cytokine/chemokine production and receptor expression*
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15 (a) Absolute percent of Ag85A-specific CD4+ T cells producing IL-2 or MIP-1 β at
16 week 1 post-MVA85A vaccination in HIV-infected subjects (low dose and high dose,
17 n=17) versus healthy controls (n=6). Representative histogram (b) and scatter plot (c)
18 showing CCR5 median fluorescence intensity (MFI) in naïve CD4+ T cells, memory
19 CD4+ T cells and activated Ag85A-specific CD4+ T cells at week 1 post-
20 vaccination (n=16 in all groups). (d) Scatter plot showing CCR5 integrated MFI
21 (iMFI) for naïve, memory and Ag85A-specific CD4+ T cells (n=16). Line charts
22 showing CCR5 MFI in memory CD4+ T cells (e) and the total CD4+ T cell
23 population (f) across the MVA85A vaccination time-course (n=16). * $p < 0.05$, **
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 $p < 0.01$.

Table 1 Full inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none"> • Healthy adults aged 18 to 55 years • Willingness to allow the investigators to discuss the volunteer's medical history with the volunteer's HIV lead physician (and GP, if appropriate) • HIV antibody positive; diagnosed at least 6 months previously • CD4 count >350; nadir CD4 not < 300 • HIV viral load not > 100,000 copies per millilitre • Written informed consent 	<ul style="list-style-type: none"> • Any clinically significant abnormal finding on screening biochemistry or haematology blood tests or on urinalysis • Any ARV therapy within the past 6 months • Any AIDS defining illness • CXR showing TB or evidence of other active infection • Prior receipt of a recombinant MVA or Fowlpox vaccine • Use of any investigational or non-registered drug, live vaccine or medical device other than the study vaccine within 30 days preceding dosing of study vaccine, or planned use during the study period • Administration of chronic (defined as more than 14 days) immunosuppressive drugs or other immune modifying drugs within six months of vaccination. (For corticosteroids, this will mean prednisolone, or equivalent, ≥ 0.5 mg/kg/day. Inhaled and topical steroids are allowed.) • History of allergic disease or reactions likely to be exacerbated by any component of the vaccine, e.g. egg products • Presence of any underlying disease that compromises the diagnosis and evaluation of response to the vaccine (including evidence of cardiovascular disease, history of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ), history of insulin requiring diabetes mellitus, any ongoing chronic illness requiring ongoing specialist supervision (e.g., gastrointestinal), and chronic or active neurological disease) • History of ≥ 2 hospitalisations for invasive bacterial infections

	<p>(pneumonia, meningitis)</p> <ul style="list-style-type: none">• Suspected or known current drug and/or alcohol abuse (as defined by an alcohol intake of >42 units a week)• Seropositive for hepatitis B surface antigen (HBsAg) and/or hepatitis C (HCV antibodies)• Evidence of serious psychiatric condition• Any other on-going chronic illness requiring hospital specialist supervision• Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate• Pregnant/lactating female and any female who is willing or intends to become pregnant during the study• Any history of anaphylaxis in reaction to vaccination• PI assessment of lack of willingness to participate and comply with all requirements of the protocol, or identification of any factor felt to significantly increase the participant's risk of suffering an adverse outcome
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TABLE 2

Demographics and screening results		Low dose 5x10 ⁷ pfu	High dose 1x10 ⁸ pfu
Sex	Male	9 (90%)	8 (80%)
	Female	1 (10%)	2 (20%)
Median age (range)		35.8 (21-52)	35.1 (27-46)
Continent of Birth	Africa	4 (40%)	4 (40%)
	Asia	0	1 (10%)
	Europe	4 (40%)	5 (40%)
	North America	1 (10%)	0
	South America	1 (10%)	0
BCG	Definite	7 (70%)	10 (100%)
	Uncertain	3 (30%)	0 (0%)
Latent infection (ESAT-6/CFP-10 +)		2 (20%)	3 (30%)
Median CD4 count (range)		570 (430-1200)	625 (410-840)
Median HIV RNA load (range)		6069 (39- 41890)	14805 (49- 71090)

TABLE 3

(a)

Local Adverse Events	HIV-infected		HIV-negative		
	Dose Number of subjects	5x10 ⁷ pfu n=10	1x10 ⁸ pfu n=10	5x10 ⁷ pfu n=43	1x10 ⁸ pfu n=12
Redness		10 (100%)	10 (100%)	42 (98%)	12 (100%)
Pruritis		10 (100%)	9 (90%)	22 (51%)	8 (67%)
Pain		8 (80%)	7 (70%)	36 (84%)	12 (100%)
Induration		10 (100%)	10 (100%)	42 (98%)	12 (100%)

(b)

Systemic Adverse Events	HIV-infected		HIV-negative		
	Dose Number of subjects	5x10 ⁷ pfu n=10	1x10 ⁸ pfu n=10	5x10 ⁷ pfu n=43	1x10 ⁸ pfu n=12
Measured fever		1 (10%)	0 (0%)	3 (7%)	5 (42%)
Subjective fever		3 (30%)	2 (20%)	17 (40%)	9 (75%)
Arthralgia		2 (20%)	3 (30%)	6 (14%)	7 (58%)
Headache		6 (60%)	4 (40%)	21 (49%)	10 (83%)
Myalgia		1 (10%)	2 (20%)	18 (42%)	9 (75%)
Nausea		1 (10%)	0 (0%)	4 (9%)	4 (33%)
Vasovagal syncope		0 (0%)	1 (10%)	0 (0%)	0 (0%)
Axillary lymphadenopathy		1 (10%)	0 (0%)	2 (5%)	2 (17%)
Change in haematology/biochemistry		0 (0%)	0 (0%)	0 (0%)	0 (0%)

TABLE 4

(a)
Summed pooled peptides – within dose changes from screening to peak and plateau

Dose		Screening (n=10)	Week 1 (n=10)	Week 24 (n=10)
Low	Median (range)	10 (3, 20)	738 (109, 4398)	20 (0, 237) n=9
	Median difference (range) (compared to screening)		730 (109, 4383)	17 (-8, 218) n=9
	<i>p</i> -value*		0.007	0.17
High	Median (range)	3 (0, 14)	1730 (758, 2138)	42 (33, 256)
	Median difference (range) (compared to screening)		1721 (743, 2135)	42 (27, 247)
	<i>p</i> -value*		0.005	0.007

*Wilcoxon signed rank test

Single pooled peptides – within dose changes from screening to peak and plateau

Dose		Screening (n=10)	Week 1 (n=10)	Week 24 (n=10)
Low	Median (range)	0 (0, 8) n=9	393 (67, 1275) n=9	13 (2, 103) n=9
	Median difference (range) (compared to screening)		393 (60, 1272) n=9	13 (2, 97) n=9
	<i>p</i> -value*		0.009	0.032
High	Median (range)	0 (0, 8)	502 (312, 677)	14 (0, 68)
	Median difference (range) (compared to screening)		502 (294, 677)	14 (0, 66)
	<i>p</i> -value*		0.005	0.048

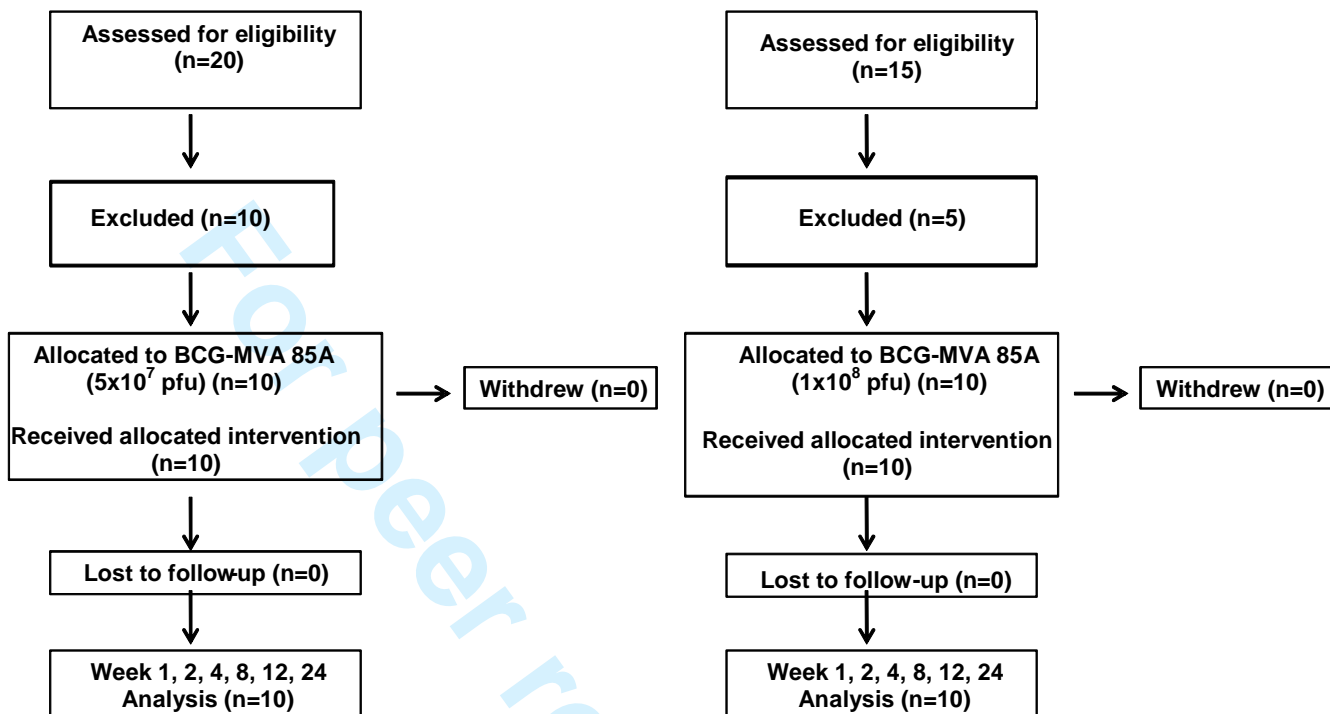
*Wilcoxon signed rank test

(b)

HIV-infected vs. HIV-negative group

	Summed Pooled peptides	Median (range) HIV+	Median (range) HIV-	Difference in medians (95% confidence interval)	p-value*
Week 1	Low dose	738 (109, 4398) n=10	2147 (1173, 5085) n=21	1101 (-393, 2873)	0.12
	High dose	1730 (758, 2138) n=10	6493 (4854, 7312) n=12	4557 (3038, 5904)	0.0001
Week 24	Low dose	20 (0, 237) n=9	385 (228, 1010) n=20	339 (161, 534)	0.004
	High dose	42 (33, 256) n=10	970 (655, 1199) n=12	820 (569, 1050)	0.0001
Area under the curve	Low dose	2162 (519, 23348) n=9	16317 (9129, 36418) n=20	11884 (3191, 17387)	0.02
	High dose	5929 (3167, 11607) n=10	41575 (26919, 53807) n=12	31384 (20778, 45504)	0.0001

Figure 1



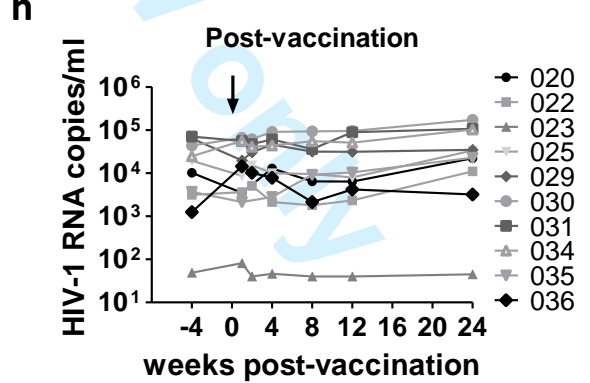
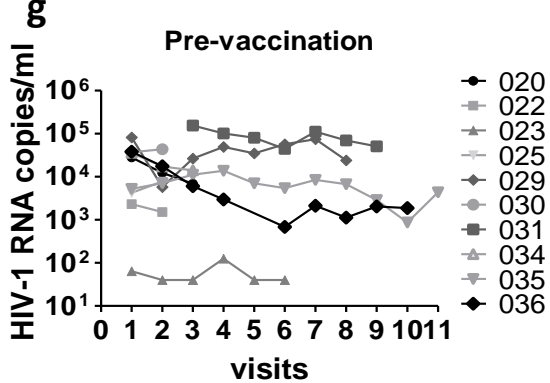
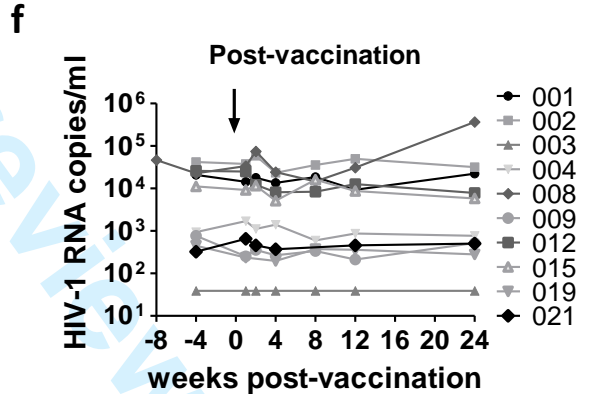
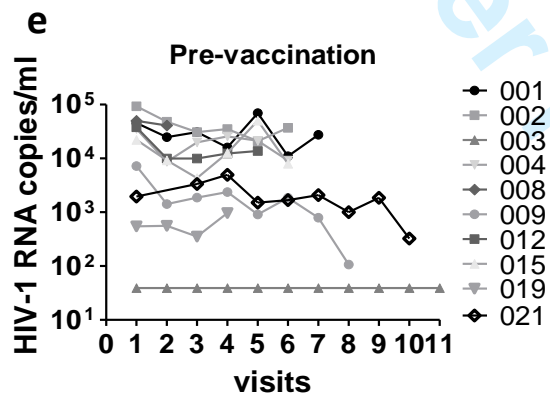
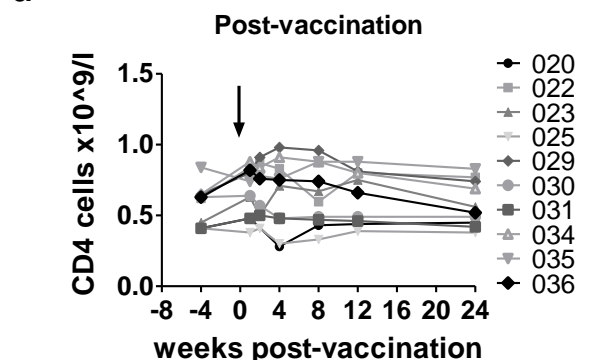
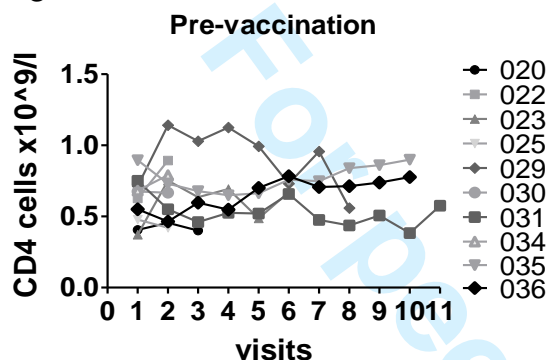
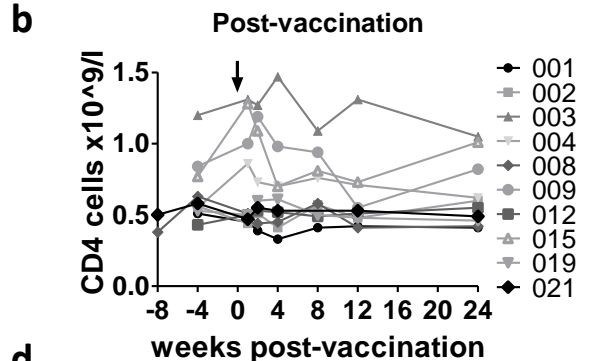
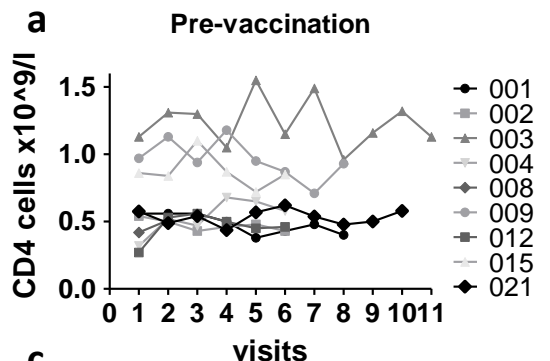


Figure 3

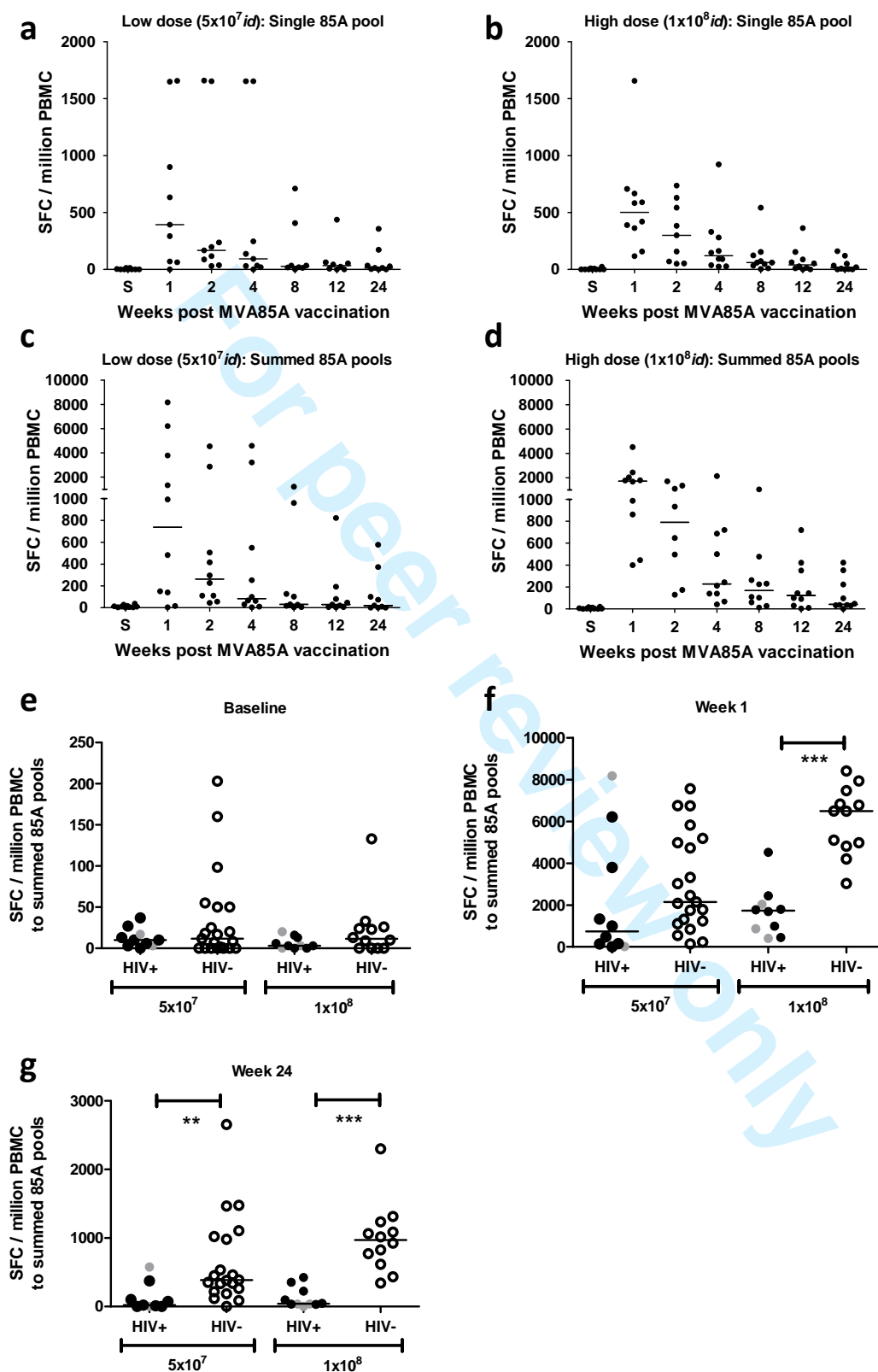


Figure 4

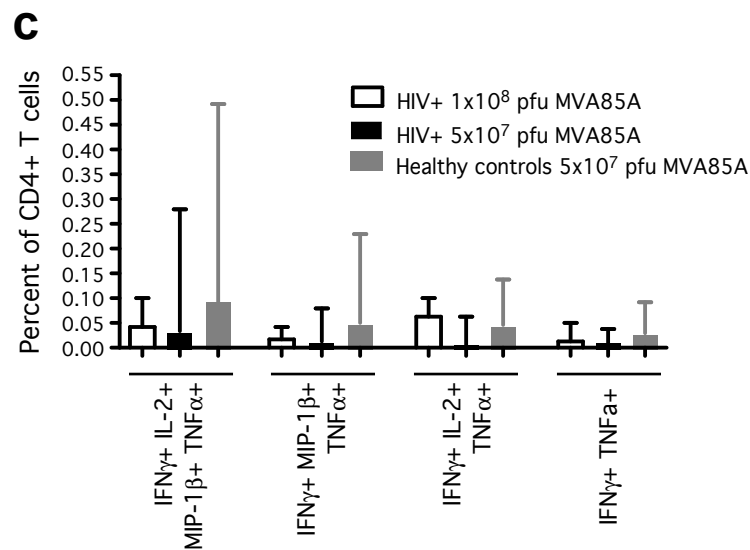
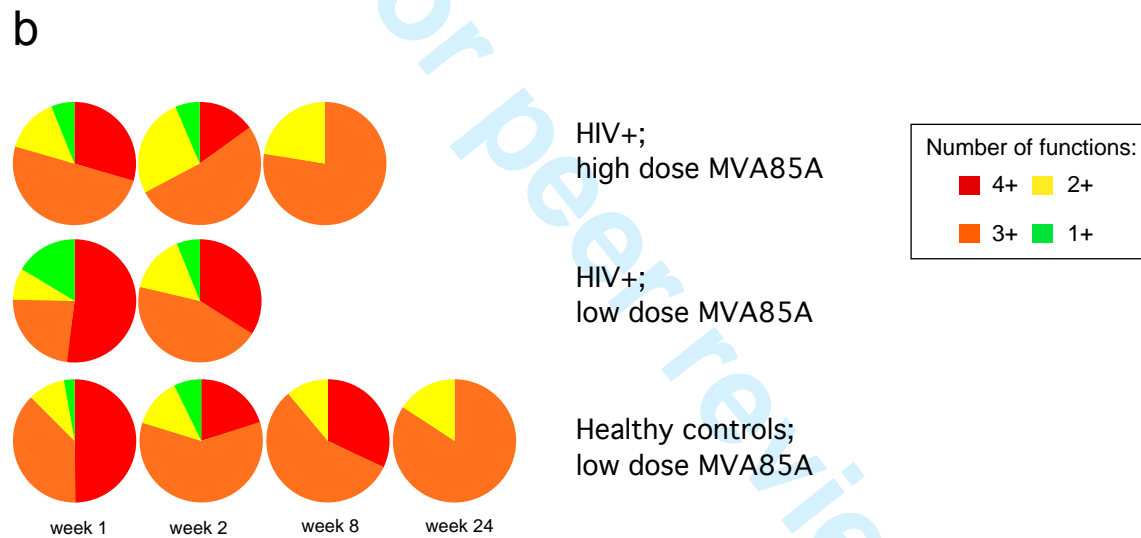
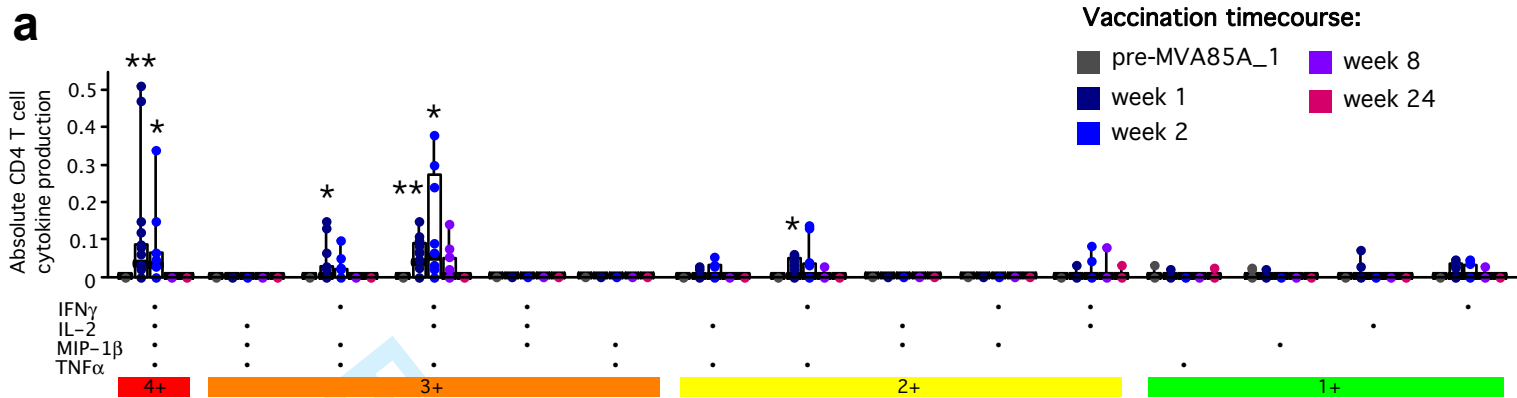


Figure 5

