LETTER

Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys

Erica N. Borducchi1, Crystal Cabral1, Kathryn E. Stephenson1, Jinyan Liu1, Peter Abbink1, David Ng’ang’a1, Joseph P. Nkolola1, Amanda L. Brinkman1, Lauren Peter1, Benjamin C. Lee1, Jessica Jimenez1, David Jetton1, Jade Mondersir1, Shanell Mojtahed1, Abishek Chandrashekar1, Katherine Molloy1, Galit Alter2, Jeffrey M. Gerold3, Alison L. Hill3, Mark G. Lewis4, Maria G. Pau5, Hanneke Schuitemaker5, Joseph Hesselgesser6, Romas Geleižiūnas6, Jerome H. Kim7,†, Merlin L. Robb7, Nelson L. Michael7 & Dan H. Barouch1,2

The development of immunologic interventions that can target the viral reservoir in HIV-1-infected individuals is a major goal of HIV-1 research1,2. However, little evidence exists that the viral reservoir can be sufficiently targeted to improve virologic control following discontinuation of antiretroviral therapy. Here we show that therapeutic vaccination with Ad26/MVA (recombinant adenovirus serotype 26 (Ad26) prime, modified vaccinia Ankara (MVA) boost)3,4 and stimulation of TLR7 (Toll-like receptor 7) improves virologic control and delays viral rebound following discontinuation of antiretroviral therapy in SIV-infected rhesus monkeys that began antiretroviral therapy during acute infection. Therapeutic vaccination with Ad26/MVA resulted in a marked increase in the magnitude and breadth of SIV-specific cellular immune responses in virologically suppressed, SIV-infected monkeys. TLR7 agonist administration led to innate immune stimulation and cellular immune activation. The combination of Ad26/MVA vaccination and TLR7 stimulation resulted in decreased levels of viral DNA in lymph nodes and peripheral blood, and improved virologic control and delayed viral rebound following discontinuation of antiretroviral therapy. The breadth of cellular immune responses correlated inversely with set point viral loads and correlated directly with time to viral rebound. These data demonstrate the potential of therapeutic vaccination combined with innate immune stimulation as a strategy aimed at a functional cure for HIV-1 infection.

The critical barrier to the discovery of a cure for HIV-1 is the viral reservoir in latently infected CD4+ T lymphocytes3–8, which leads to viral rebound in the vast majority of HIV-1-infected individuals following discontinuation of antiretroviral therapy (ART)9,10. Enhancing antiviral immune responses, potentially together with activation of the viral reservoir, might be able to eliminate these cells1,2,11. However, it is currently unknown whether immunologic interventions can affect the viral reservoir in vivo. In particular, it is unclear if a therapeutic vaccine will be able to induce cellular immune responses with sufficient potency and breadth to control viral rebound following ART discontinuation12. We therefore evaluated a strategy consisting of Ad26/MVA therapeutic vaccination3–6 and TLR7 agonist GS-986 administration in ART-suppressed, SIV-infected rhesus monkeys.

We infected 36 Indian origin rhesus monkeys (Macaca mulatta) with SIVmac239 (refs 4, 13) by a single intrarectal exposure and initiated daily subcutaneous administration of a pre-formulated ART cocktail (tenofovir disoproxil fumarate, emtricitabine, dolutegravir)14 on day 7 of acute infection. Animals had median plasma SIV RNA levels of 7.10 log copies per ml (range 6.04–7.88 log copies per ml) on the day of ART initiation (Fig. 1a). SIV RNA levels were controlled in the majority of animals by day 56 and in all animals by day 224 (Fig. 1a). SIV RNA levels were comparable among the different groups, and the animals that took longer to control the virus had higher starting plasma viral loads on day 7 (P = 0.04; data not shown).

Following 24 weeks of suppressive ART, groups of monkeys received the following interventions: (1) Ad26/MVA vaccines alone; (2) Ad26/MVA vaccines and TLR7 agonist GS-986; (3) TLR7 agonist GS-986 alone; or (4) sham (n = 9 animals per group). In groups 1–2, animals were vaccinated by the intramuscular route with 3 × 10^9 viral particles of Ad26 vectors3,5 expressing SIVsmE543 gag–pol–env at weeks 24 and 36, and were boosted with 108 plaque-forming units of MVA vectors4 expressing SIVsmE543 gag–pol–env at weeks 48 and 60. In groups 2–3, animals received 10 administrations of 0.3 mg kg^-1 GS-986 (Gilead Sciences) by oral gavage every 2 weeks from weeks 50–70. In the combination intervention group, animals began GS-986 treatment at peak immunity 2 weeks after the first MVA boost immunization. TLR7 triggering is known to activate dendritic cells and lymphocytes and to lead to innate immune activation, including secretion of cytokines and chemokines16,17. We observed nonspecific activation of CD8+ and CD4+ T cells, as measured by CD69 expression 1–2 days following each GS-986 administration (Extended Data Figs 1, 2), and increased plasma levels of IFN-α (Extended Data Fig. 3), thus confirming the immunostimulatory activity of GS-986. Other proinflammatory cytokines and chemokines were also induced by GS-986, including IL-1RA, IL-6, IL-23, CCL5 (MIG), CXCL11 (I-TAC), CCL4 (MIP-1β) and CCL11 (Eotaxin) (data not shown).

We next evaluated the immunogenicity of the Ad26/MVA vaccine. The groups that received the vaccine demonstrated a robust >100-fold increase in the magnitude of Gag-, Pol- and Env-specific cellular immune responses, as compared with pre-vaccination responses by IFN-γ ELISPOT assays (Fig. 1b). Cellular immune responses increased substantially at week 28 after priming with Ad26 and further increased at week 50 after administration of MVA boost against both vaccine-matched SIVsmE543 peptides and virus-matched SIVmac239 peptides. We also observed induction of robust Gag-, Pol- and Env-specific CD8+ and CD4+ T-cell responses, as measured by multiparameter intracellular cytokine staining assays (Extended Data Figs 4, 5). These responses were higher magnitude than those elicited with this same vaccine in SIV-uninfected rhesus monkeys6.

Ad26/MVA vaccination also expanded cellular immune breadth by at least 9.2-fold, as measured by IFN-γ ELISPOT assays using subpools of 10 peptides spanning Gag, Pol, and Env (Fig. 1c, Extended Data Fig. 6). These responses were higher magnitude than those elicited with this same vaccine in SIV-uninfected rhesus monkeys6.

Ad26/MVA vaccination also expanded cellular immune breadth by at least 9.2-fold, as measured by IFN-γ ELISPOT assays using subpools of 10 peptides spanning Gag, Pol, and Env (Fig. 1c, Extended Data Fig. 6). Total breadth was defined as the number of Gag-, Pol- and Env-positive subpools. We were unable to fine map individual epitopes owing to insufficient availability of cells given the number of positive subpools. Before vaccination at week 24, we observed an average of 1.9 positive
immunogenicity. Consistent with this hypothesis is the observation that cellular immune breadth correlated inversely with pre-ART day 7 SIV RNA levels (Extended Data Fig. 7), which may be a surrogate marker for immunologic damage. In contrast with robust cellular immune responses, relatively modest humoral immune responses were observed following vaccination, including binding antibody responses by ELISA and functional antibody-dependent cellular phagocytosis, neutrophil phagocytosis, and natural killer cell activation (data not shown).

We next assessed viral DNA levels in lymph nodes and PBMCs using a reverse transcription (RT)–PCR assay with a sensitivity of 3 DNA copies per 10^6 CD4+ T cells (Fig. 2a, b). In sham controls, viral DNA declined slightly between weeks 20 and 48, presumably reflecting the effect of suppressive ART, but no further decline was observed at week 70. In contrast, the two groups that received the Ad26/MVA vaccine demonstrated marked reductions of viral DNA to undetectable levels in the majority of animals by week 70 in both lymph nodes (Fig. 2a) and PBMCs (Fig. 2b), suggesting that vaccination led to substantial reductions in SIV-infected CD4+ T cells in these tissue compartments. It is possible that a larger fraction of proviruses might be intact following ART initiation during acute infection as compared with chronic infection, although this remains to be determined. Viral outgrowth assays using 20 million PBMCs were negative in all animals.

![Figure 1](image1.png)  
**Figure 1 | SIV RNA and vaccine immunogenicity before ART discontinuation.** a. Rhesus monkeys were infected with SIVmac239 on day 0 and began ART on day 7 (n = 9 animals per group). Ad26 and MVA vaccination time points are shown with vertical arrows. The timeframe for TLR7 agonist administration (10 doses every 2 weeks) is shown by the horizontal bar. log SIV RNA copies per ml are shown (limit of detection, 2.3 log RNA copies per ml). b. IFN-γ ELISPOT responses in response to Gag, Pol and Env peptide pools from SIVmac239 and SIVsmE541. Group numbers and time points are denoted on the x-axis. Group 1 received the Ad26/MVA vaccine alone, and group 2 received the Ad26/MVA vaccine plus TLR7 agonist. P values indicate two-sided Wilcoxon rank-sum tests compared with week 24 (pre-vaccination). c. Cellular immune breadth in the vaccinated animals as measured by subpools of 10 peptides spanning the SIVmac239 Gag, Pol, and Env proteins, as well as total proteins (Gag+Pol+Env), at week 0 (naive), week 20 (pre-vaccination), week 29 (Ad26), and week 50 (MVA). The numbers of positive subpools are indicated in red. P values indicate two-sided Wilcoxon rank-sum tests.

Subpools per animal (1.0 Gag, 0.4 Pol, 0.6 Env). After priming with Ad26, cellular immune breadth markedly expanded to an average of 10.1 positive subpools per animal (4.4 Gag, 3.0 Pol, 3.1 Env). Following MVA boost administration, cellular immune breadth further expanded to an average of 17.5 positive subpools per animal (5.4 Gag, 5.9 Pol, 6.2 Env) (Fig. 1c), which probably represents an underestimate of breadth, as some positive subpools may have contained more than one epitope. Several animals developed Gag–Pol–Env-specific T cells that targeted more than 50 epitopes. The expanded cellular immune breadth involved induction of a large number of new epitopes and did not simply reflect expansion of previously established responses, as the vast majority of the epitopes following vaccination were not observed before vaccination in conventional IFN-γ ELISPOT assays or in assays using peripheral blood mononuclear cells (PBMCs) stimulated in vitro with these peptides for enhanced sensitivity (data not shown). We speculate that early initiation of ART induced preserved CD4+ T-cell help (Extended Data Fig. 5), which probably contributed to vaccine
Figure 3 | SIV RNA following ART discontinuation. a, log SIV RNA copies per ml are shown (limit of detection, 2.3 log RNA copies per ml) following ART discontinuation at study week 72. Days following ART discontinuation are shown on the x axis. b, Median log SIV RNA in each group. c, Statistical analysis of set point levels of SIV RNA and time to viral rebound in each group. Vac, vaccination with Ad26/MVA. P values indicate two-sided Wilcoxon rank-sum tests.

Figure 4 | Correlations of cellular immune breadth with set point viral loads and time to viral rebound. a, b, Correlations are shown for the breadth of Gag, Pol, Env and total (Gag+Pol+Env) cellular immune responses, as defined as the number of positive subpools at the time of ART discontinuation at week 72 and set point log SIV RNA (a) or time to viral rebound (b) following ART discontinuation. P values indicate two-sided Spearman rank-correlation tests. R values indicate correlation coefficients.

including controls at week 70 (data not shown), presumably as a result of early initiation of ART.

To evaluate the therapeutic efficacy of the interventions, we discontinued ART at week 72. Viral rebound was observed in all animals (Fig. 3a). All sham controls rebounded by day 10–14 following ART discontinuation in a stereotypical fashion and exhibited median set point plasma SIV RNA levels of 4.89 log copies per ml (range 4.27–5.57 log copies per ml) on day 168 following ART discontinuation. The monkeys that received GS-986 alone did not demonstrate any discernible delay or control of viral rebound, indicating that TLR7 stimulation alone exerted no detectable antiviral effect in this study.

Animals that received the Ad26/MVA vaccine alone exhibited a 0.66 log reduction of median set point plasma SIV RNA levels to 4.23 log copies per ml (range 2.70–4.91 log copies per ml) (P = 0.002, Wilcoxon rank-sum test) but only a marginal delay of viral rebound (P = 0.01, Wilcoxon rank–sum test), as compared with controls (Fig. 3b, c). By contrast, monkeys that received both the Ad26/MVA vaccine and GS-986 showed a notable 1.74 log reduction of median set point plasma SIV RNA levels to 3.15 log copies per ml (range <2.30–4.09 log copies per ml) (P < 0.0001) and a 2.5-fold delay of viral rebound from a median of 10 to 25 days, as compared with controls (P = 0.003) (Fig. 3b, c). Moreover, 33% (3 of 9) of the monkeys in the combination intervention group showed effective virologic control to undetectable set point viral loads (<2.30 log copies per ml) following ART discontinuation. These data demonstrate that the combination of Ad26/MVA vaccination and TLR7 stimulation improved virologic control and delayed viral rebound following ART discontinuation.

We next evaluated the immunologic and virologic correlates of virologic control. Cellular immune breadth immediately before ART discontinuation (Fig. 4a) and at peak immunity (Extended Data Fig. 8) correlated inversely with set point viral loads following ART discontinuation, particularly the breadth of Gag, Env, and total cellular immune responses (P < 0.0001, Spearman rank-correlation tests). Gag, Env, and total cellular immune breadth also correlated directly with the time to viral rebound (Fig. 4b, Extended Data Fig. 9; P = 0.0001 to P = 0.001). Consistent with the correlates analyses, mathematical modelling of viral dynamics further suggested that the combination of changes in the reservoir exit rate and the early viral growth rate accounted for the differences in the time to viral rebound, whereas the virus-specific immune proliferation rate was probably responsible for virologic control (Extended Data Fig. 10).

Viral DNA levels in lymph nodes and PBMCs correlated poorly with virologic control following ART discontinuation and time to viral rebound (P = 0.03, data not shown), presumably because all animals with undetectable viral DNA still experienced rebound. These findings suggest that these viral DNA assays are not sufficiently sensitive to predict functional cure, consistent with recent clinical observations. Moreover, there was no correlation between day 7 pre-ART SIV RNA levels or time to initial virologic suppression on ART and virologic control following ART discontinuation (P value not significant).
In this study, we demonstrate that Ad26/MVA therapeutic vaccinon robustly augmented cellular immune magnitude and breadth in ART-suppressed, SIV-infected rhesus monkeys and that the TLR7 agonist GS-986 led to innate immune stimulation and cellular activation. The combination of Ad26/MVA vaccination and GS-986 resulted in a significant 1.74 log reduction in median set point viral loads and a 2.5-fold delay in the time to viral rebound following ART discontinuation, as compared with sham controls. Moreover, 3 of 9 animals in this group demonstrated virologic control to undetectable levels in the absence of ART. These three animals were characterized by high cellular immune magnitude and breadth and negative viral DNA before ART discontinuation. Taken together, these data demonstrate the proof-of-concept that the combination of therapeutic vaccinon and innate immune stimulation can affect viral rebound following active ART discontinuation.

The Ad26/MVA vaccine induced remarkably potent IFN-γ ELISPOT responses in the ART-suppressed, SIV-infected monkeys in the present study, consistent with a previous prophylactic vaccinon study in uninfected monkeys. We speculate that preserved CD4+ T-cell help, primed by short-term viral replicon following SIV infection before initiation of ART on day 7, probably enhanced vaccine immunogenicity. The Ad26/MVA vaccine also expanded cellular immune breadth by nearly tenfold, including induction of responses to a large number of epitopes that were not detectable after SIV infection. This may be critical for a therapeutic vaccinon, as the viral reservoir typically contains viruses with T-cell-epitope escape mutations. We did not detect evidence for viral ‘blipping’ on day 1 or day 2 following TLR7 agonist administration (J. Whitney, unpublished data), which may reflect the early initiation of ART on day 7 of infection and thus the limited size of the viral reservoir in this study. We were therefore unable to determine whether the beneficial effect of the TLR7 agonist reflected its potential role as a vaccine adjunct, a latency reversing agent, or both. Future studies should be performed using SIV-infected monkeys for whom ART is initiated during chronic infection, as these would be more representative of the majority of HIV-1-infected individuals. Moreover, future studies could explore longer periods of ART suppression, potentially to reduce residual viral replication, although the majority of animals appear to have a stable reservoir after 24–72 weeks of ART. The capacity of immunologic interventions to target follicular helper CD4+ T cells in lymph nodes should also be explored.

Previous studies of poxvirus-, adenovirus- and DNA-based therapeutic vaccinons have typically shown only a modest effect on viral rebound following ART discontinuation in both rhesus monkeys and humans. The present study extends these previous observations by combining therapeutic vaccinon with innate immune stimulation. Of note, the combination of Ad26/MVA vaccinon and TLR7 stimulation proved more potent than either component alone. This finding is consistent with a previous in vitro study that showed that robust CD8+ T cells may be able to facilitate elimination of the viral reservoir following reactivation. The present study also demonstrates that the breadth of Gag-, Pol- and Env-specific T-cell responses correlated inversely with set point viral loads following ART discontinuation, suggesting that the mechanism underlying the therapeutic efficacy of the vaccine involved expansion of cellular immune breadth and immunologic control of virus rebounding from the reservoir.

In summary, our data suggest the potential of combining therapeutic vaccinon with innate immune stimulation as an HIV-1 cure strategy. Our findings show that these interventions can improve virologic control and delay viral rebound following ART discontinuation in SIV-infected rhesus monkeys that began ART during acute infection. Additional preclinical and clinical studies with Ad26/MVA vaccinon and TLR7 stimulation should be performed to explore this strategy in greater detail.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions D.H.B., N.L.M., J.H.K., M.L.R., M.G.P., H.S., and R.G. designed the studies. J.H. and R.G. developed the ART formulation and TLR7 agonist. E.N.B., C.C., K.E.S., J.L., J.P.N., A.L.B., L.R., B.C.L., J.J., D.J., J.M., S.M., A.C., K.M., and G.A. performed the immunologic assays. P.A. and D.N. conducted the virologic assays. J.M.G. and A.L.H. performed the viral dynamics modelling. M.G.L. led the clinical care of the rhesus monkeys. D.H.B. wrote the paper with all co-authors.

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METHODS

Animals. 36 outbred Indian-origin, young adult male and female rhesus monkeys (Macaca mulatta) were genotyped and selected as negative for the protective MHC class I alleles Mamu-A*01, Mamu-B*08, and Mamu-B*17. Animals expressing susceptible and resistant TRIM5α alleles were distributed among the groups. Animals were otherwise randomly allocated to groups. All monkeys were housed at Bioqual, Rockville, Maryland. Animals were infected with 500 TCID50 (50% tissue culture infective dose) of our SIVmac239,4,25 by the intrarectal route. Monkeys were bled to two times per week for viral load determinations. Immunologic and virologic assays were performed blinded. All animal studies were approved by the appropriate Institutional Animal Care and Use Committee (IACUC). The sample size was estimated to achieve 80% power to detect a 1.0 log difference in setpoint viral loads.

ART regimen. The preformulated antiretroviral therapy (ART) cocktail contained 5.1 mg ml<sup>−1</sup> tenofovir disoproxil fumarate (TDF), 40 mg ml<sup>−1</sup> emtricitabine (FTC), and 2.5 mg ml<sup>−1</sup> dolutegravir (DTG) in a solvent containing 15% (v/v) kleptose (Bio In Vitro). Body weight was monitored weekly. ART was administered once daily at 1 ml kg<sup>−1</sup> body weight via the subcutaneous route.

Ad26/MVA vaccination and TLR7 agonist GS-986 administration. In groups 1–2, monkeys were primed by the intramuscular route with 3 × 10<sup>10</sup> viral particles of Ad26 vectors<sup>4,15</sup> expressing SIV<sub>mac239</sub> gag–pol–env at weeks 24 and 36, and were boosted with 10<sup>7</sup> plaque-forming units of MVA vectors<sup>4</sup> expressing SIV<sub>mac239</sub> gag–pol–env at weeks 48 and 60. In groups 2–3, animals received 10 administrations of 0.3 mg per kg GS-986 (Gilead Sciences) by oral gavage every 2 weeks from weeks 50–70.

Cellular immune assays. SIV-specific cellular immune responses were assessed by IFN-γ ELISPOT assays and multiparametric intracellular cytokine staining assays essentially as described.<sup>4</sup> Estimates of cellular immune breadth involved IFN-γ ELISPOT assays using subpools of 10 peptides across the Gag, Pol, and Env proteins. 12-colour intracellular cytokine staining assays were performed with the Aqua green–fluorescent reactive dye (Invitrogen, L23101) and predetermined titres of monoclonal antibodies (Becton Dickinson) against CD3 (SP34; Alexa Fluor 700), CD4 (OKT4; BV711, Biologend), CD8 (SK1; allopurinocyanine–cytine 7 (APC–Cy7)), CD28 (L293; BV610), CD95 (DX2; allopurinocyanine (APC)), CD99 (TP1; 55.3; phycoerythin–Texas red (energy-coupled dye); ECD); Beckman Coulter), gamma interferon (IFN-γ) (B27; phycoerythin–cytine 7 (PE–Cy7)), K67 (B56; fluorescein isothiocyanate (FITC)), CCR5 (3A9; phycoerythin (PE)); CCR7(3D12; Pacific Blue), and PD-1(EH21.1; peridinin chlorophyll-A–cyanine 7.5 (PerCP–Cy5.5)). IFN-γ backgrounds were consistently <0.01% in PBMCs.

Viral RNA assays. Viral RNA was isolated from cell-free plasma using a viral RNA extraction kit (Qiagen) and was quantitated essentially as described.<sup>14</sup>

Viral DNA assays. Levels of proviral DNA were quantitated as previously described.<sup>14</sup> Total cellular DNA was isolated from 5 × 10<sup>6</sup> cells using a QIAamp DNA Blood Mini kit (Qiagen). The absolute quantification of viral DNA in each sample was determined by quantitative PCR (qPCR) using primers specific to a conserved region of SIV<sub>mac239</sub>. All samples were directly compared to a linear virus standard and the simultaneous amplification of a fragment of human GAPDH gene. PCR assays were performed with 100–200 ng sample DNA.

Statistical analyses. Analysis of virologic and immunologic data was performed using GraphPad Prism v6.03 (GraphPad Software). Comparisons of groups was performed using two-sided Wilcoxon rank–sum tests without Bonferroni adjustments. Correlations were assessed by two-sided Spearman rank–correlation tests without Bonferroni adjustments.

Viral dynamics modelling. We employed viral dynamics modelling to better characterize the kinetics of viral rebound and to gain insight into the mechanism behind the improved control of rebound in certain groups of treated animals. We were particularly interested in whether the effect of the vaccine and TLR7 agonist on rebound could be explained by a reduction in the latent reservoir size, or an antiviral immune response, or both.

Most existing viral dynamics models for HIV-1/SIV or other infections are not able to explain the diversity of kinetics seen in different animals in this study—such as rebound to a high set point, rebound to a high peak and lower set point, and rebound to a medium or high peak followed by control to undetectable levels—with mechanisms that are realistic for HIV-1/SIV. Specifically, for HIV-1 and SIV infection, exhaustion of target cells cannot alone explain the decline from peak viraemia, which instead requires an immune response that can markedly reduce the viral load set point without significant changes in the death rate of virus-producing infected cells.<sup>25</sup> On the basis of other modelling work,<sup>26–29</sup> we developed a new model to explain all of these observations.

The model we used is described by a system of ordinary differential equations that track changes in the levels of uninfected (T) and infected (I) target cells, free virus (V), and precursor (P) and effector (E) immune responses over time (Extended Data Fig. 10):

\[ T = \lambda - \beta V T - d_I T \]

\[ I = a - \frac{\beta V T}{1 + (E/N_E)} - d_I I \]

\[ V = k I - c V \]

\[ P = m + (1 - f) \frac{V}{V + N_p} - d_P P \]

\[ E = p f' \frac{V}{V + N_p} - d_E E \]

All variables are expressed as concentrations per ml of plasma. Parameter λ is the rate of production of susceptible uninfected cells (cells per ml per day), β is the viral infectivity (per (copies per ml) per day), d<sub>I</sub> is the death rate of infected cells (per day), d<sub>P</sub> is the death rate of death of infected cells (per day), a is the rate at which latent cells reactivate to become productively infected (cells per day), k is the viral burst size (virions per cell per day) and c is the viral clearance rate (per day).

Our model of the immune response is general enough to describe either humoral or cellular effects. Precursor immune cells (naive and memory) are produced at a baseline rate m (cells per ml per day), proliferate at an antigen-dependent rate, and die at a rate d<sub>P</sub> (per day). The maximum proliferate rate is p (per day) and half-maximal proliferation occurs at viral load N<sub>p</sub> (copies per ml). A fraction, f, of all proliferating cells differentiate into effectors, which die at a rate d<sub>E</sub> (per day). Effectors reduce the rate at which actively infected cells are produced (either by inactivating free virus or killing early-stage infected cells<sup>30,31</sup>), with half-maximal inhibition occurring at a concentration N<sub>E</sub> (cell per ml).

During ART, β = 0, and virus and cells reach steady states at values \( T_0 = \lambda/d_I, I_0 = a/d_I, V_0 = (a/d_I)k/c, P_0 = (m/d_P), E_0 = 0 \) (as \( V_0 < N_p \)), which we take as the initial conditions at the time of ART interruption.

As we only have longitudinal observations of viral load, we cannot uniquely identify all the parameters of this model. We conducted extensive analytic and numeric investigation of the model to determine which parameters were most important for the dynamics and which could be estimated from the available data. On the basis of this knowledge and our previous work,<sup>4</sup> we fixed the parameters \( d_I = 0.4 \) (per day)<sup>31</sup>, \( d_P = 0.05 \) (per day), \( d_E = 0.001 \) (per day), \( d_F = 1 \) (per day), \( f = 0.9 \). As only the ratio m/N<sub>p</sub> can be identified from the viral load data, we fixed N<sub>p</sub> = 10<sup>5</sup>. The ratio k/c was fixed to 2,000 viruses per cell based on a burst size of \( k = 5 \times 10^4 \) (virions per cell per day) and \( c = 23 \) per day for SIV<sup>31–33</sup>. The other parameters (λ, a, m, p, N<sub>E</sub>) were fitted. We also fitted results to a simpler model with no antigen-dependent immune response (\( P, E = 0 \)) to ensure that the fits were improved by adding in the extra terms. We modelled the effect of antiretroviral drug decay by including a three-day washout period, during which the virus could not productively reproduce. Varying this period between 0 and 6 days did not change conclusions from the model.

Models were fit to each animal individually using a Bayesian framework. Briefly, a joint posterior distribution was estimated for the fitted parameters using a likelihood function for the viral load at each time point and very weakly informative priors on the fitted parameters (see below). We parameterized the model using log-transformed parameters to account for the possibility of large parameter variations between monkeys. The likelihood assumes that the observed viral load is log-normally distributed around the true viral load, and that measurements include an error with variance \( \sigma^2 \) (which we fixed at 0.1 based on previous work). The estimated posterior distribution was sampled using the Metropolis–Hastings algorithm implemented in R using the package MHadaptive<sup>34</sup> and the differential equations were numerically integrated using deSolve<sup>35</sup>. After a burn-in of 10<sup>4</sup> iterations, 10<sup>5</sup> updates were run and 1 in 10 samples were collected. The resulting fits were visually inspected to make sure they were consistent with the observed viral load. We specified the prior for each parameter independently according to:

\[ \log_{10}(p) - 1\text{Laplace}(\mu_p, 1) \]

with the means for each parameter \( \log_{10}(\lambda) = 1.3, \log_{10}(a) = -6, \log_{10}(m) = -4.5, \log_{10}(N_p) = 0 \), \( \log_{10}(\beta) = -4 \), and \( \log_{10}(\sigma) = 4 \).

Beyond the individual parameters, we also calculated a composite parameter describing the exponential growth rate of the virus immediately following rebound, \( r \), as

\[ r = \frac{\lambda (k/c) - d_I}{d_I} \]
We also calculated the time of rebound ($\tau_r$) as the time at which viral load was expected to be exactly equal to the detection limit of $v_d = 200$ copies per ml by numerically integrating the differential equations until $V(t) = v_d$.

In Extended Data Fig. 10, we show results of the fits. Values shown represent the median and 95% credible intervals of the posterior of the mean of the group. The posterior mean was constructed by repeatedly sampling one value from the posterior of each animal in the group, and then taking the mean. $P$ values reported for an increase in the posterior mean represent the probability that a random sample from the posterior mean in one group is smaller than a sample from the posterior mean in the other.

**Data availability.** All data generated and analysed in this study are available from the corresponding author upon reasonable request.

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Extended Data Figure 1 | Activation of CD8⁺ T cells following GS-986 administration. Representative data on days 0, 1, and 2 following GS-986 administration. Activation was assessed by CD69 expression on CD3⁺CD8⁺ T cells. Red bars represent mean values for each group.
Extended Data Figure 2 | Activation of CD4+ T cells following GS-986 administration. Representative data on days 0, 1, and 2 following GS-986 administration. Activation was assessed by CD69 expression on CD3+CD4+ T cells.
Extended Data Figure 3 | Innate immune stimulation following GS-986 administration. Plasma IFN-α (pg ml⁻¹) levels are shown on day 1 following GS-986 administration. Red bars represent mean values for each group. Data points reflect all animals following all GS-986 administrations combined with pre-dose levels subtracted.
Extended Data Figure 4 | CD8⁺ T cells following Ad26/MVA vaccination.

SIVmac239 Gag-, Pol- and Env-specific, IFN-γ⁺ CD3⁺ CD8⁺ central memory T cells were assessed by multiparameter intracellular cytokine staining assays.
Extended Data Figure 5 | CD4⁺ T cells following Ad26/MVA vaccination. SIVmac239 Gag-, Pol- and Env-specific, IFN-γ⁺CD3⁺CD4⁺ central memory T cells were assessed by multiparameter intracellular cytokine staining assays.
Extended Data Figure 6 | Cellular immune breadth. Responses to subpools of 10 peptides spanning SIVmac239 Gag, Pol, and Env are shown before vaccination (week 20, blue), after Ad26 prime (week 29, red), and after MVA boost (week 50, green). Coloured squares indicate positive responses. ‘x’ indicates missing data as a result of insufficient PBMCs.
Extended Data Figure 7 | Correlations of cellular immune breadth to day 7 SIV RNA. Correlations are shown for the breadth of Gag, Pol, Env and total (Gag+Pol+Env) cellular immune responses at the time of ART discontinuation at week 72 and pre-ART day 7 log SIV RNA. P value indicates two-sided Spearman rank-correlation test. R value indicates correlation coefficient.

R = -0.56
P = 0.0005
Extended Data Figure 8 | Correlations of cellular immune breadth to set point viral loads. Correlations are shown for the breadth of Gag, Pol, Env and total (Gag+Pol+Env) cellular immune responses at peak immunity at week 50 and set point log SIV RNA following ART discontinuation. \( P \) values indicate two-sided Spearman rank-correlation tests. \( R \) values indicate correlation coefficients.
Extended Data Figure 9 | Correlations of cellular immune breadth to time to viral rebound. Correlations are shown for the breadth of Gag, Pol, Env and total (Gag+Pol+Env) cellular immune responses at peak immunity at week 50 and time to viral rebound following ART discontinuation. P values indicate two-sided Spearman rank-correlation tests. R values indicate correlation coefficients.
Extended Data Figure 10 | Rebound kinetic parameters estimated from viral dynamics modelling. Viral load values following ART discontinuation in each animal were fit to a viral dynamics model using a Bayesian framework. a–d, Plots show the median and 95% credible intervals for estimations of the rate of reactivation of cells from the latent reservoir (a), the initial exponential growth rate (b), the immune proliferation rate (c), and the time at which viral load reaches the detection threshold of 200 copies per ml for each treatment group (d). Monkeys treated with both the vaccine and TLR7 agonist exhibited slower viral growth rates and stronger immune responses than all other groups (P < 0.01 for each comparison). These monkeys and monkeys treated with only the vaccine exhibited a lower reservoir exit rate than untreated monkeys (P < 0.05 for each comparison).