Characterizing the immunogenicity of a therapeutic DNA vaccine and determinants of viral control in SIV-infected rhesus macaques with variable responses to antiretroviral therapy

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Abstract

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A therapeutic vaccine that induces lasting control of HIV infection could eliminate the need for lifelong adherence to antiretroviral therapy. This study investigated a therapeutic DNA vaccine delivered with a single adjuvant or a novel combination of adjuvants to augment T cell immunity in the blood and gut-associated lymphoid tissue in SIV-infected rhesus macaques. Animals that received DNA vaccines expressing SIV proteins, combined with plasmids expressing adjuvants designed to increase peripheral and mucosal T cell responses, including the catalytic subunit of the *E. coli* heat-labile enterotoxin, IL-12, IL-33, retinaldehyde dehydrogenase 2, soluble PD-1 and soluble CD80, were compared to mock-vaccinated controls. Following treatment interruption, macaques exhibited variable levels of viral rebound, with four animals from the vaccinated groups and one animal from the control group controlling virus at median levels of 10^3 RNA copies/ml or lower (controllers) and nine animals, among all groups, exhibiting immediate viral rebound and median viral loads greater than 10^3 RNA copies/ml (non-controllers). Although there was no significant difference between the vaccinated and control groups in protection from viral rebound, the variable virological outcomes during treatment

interruption enabled an examination of immune correlates of viral replication in controllers versus non-controllers regardless of vaccination status. Lower viral burden in controllers correlated with increased polyfunctional SIV-specific $CD8^+$ T cells in mesenteric lymph nodes and blood prior to and during treatment interruption. Notably, higher frequencies of colonic $CD4^+$ T cells and lower Th17/Treg ratios prior to infection in controllers correlated with improved responses to ART and control of viral rebound. These results indicate that mucosal immune responses, present prior to infection, can influence efficacy of antiretroviral therapy and the outcome of immunotherapeutic vaccination, suggesting that therapies capable of modulating host mucosal responses may be needed to achieve HIV cure.

Table of Contents

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List of Figures

Chapter 1: Introduction

HIV virology and current progress towards an HIV therapeutic vaccine

HIV virology and current progress towards an HIV therapeutic vaccine

The early HIV epidemic

At the outset of the 1980s, a sudden, dramatic increase in cases of a rare lung infection called *Pneumocystis carinii* pneumonia (PCP) [1] and unusually aggressive cases of Kaposi's sarcoma in young, previously healthy men who have sex with men (MSM) [2] alarmed and confounded clinicians. By year's end, 270 reports of severe immunodeficiency among MSM – 121 of these cases ending in death – revealed the gravity of the situation. Initially, the cause of this immune deficiency was thought to be sexual, and the syndrome was inaccurately termed gay-related immune deficiency (GRID). The Center for Disease Control (CDC) later renamed the syndrome Acquired Immune Deficiency Syndrome (AIDS), although its causative agent remained unknown. In 1983, a team of doctors at the Pasteur Institute in France isolated an uncharacterized virus from a lymph node biopsy obtained from an AIDS patient, which they named "Lymphadenopathy-Associated Virus (LAV)" and suggested that this novel virus could be the cause of AIDS [3]. These findings were later confirmed by the National Cancer Institute in 1984, through the development of a diagnostic test measuring virus-specific humoral immunity [4], and the virus was officially named human immunodeficiency virus (HIV) in 1986.

The HIV life cycle

Further research revealed that HIV is an enveloped virus with a single-stranded, positive sense RNA genome, making it part of the *Retroviridae* family. Each virion contains two copies of the RNA genome, which is enclosed in the capsid (p24). The capsid and other internal structural components of the virion, namely the matrix and nucleocapsid, are derived from the main HIV structural protein, Gag (p55 for HIV), following cleavage by the viral protease. The viral envelope (Env) is a heterodimeric glycoprotein comprised of the surface antigen (gp120) and transmembrane proteins (gp41), which facilitates viral fusion and entry by binding first to CD4 on $CD4^+$ T cells and then to the coreceptor CCR5 [5] (Although depending on the strain, HIV may also use CXCR4 as a coreceptor, among others [6]). Upon fusion of the viral and cellular

membranes, the capsid containing the RNA genome, error-prone reverse transcriptase (RT), and integrase enzymes enters the cytoplasm, where the RNA genome is reverse transcribed to complimentary DNA (cDNA). The cDNA then travels to the nucleus of the cell, where the viral protein integrase inserts the viral cDNA into the host cell genome. Once the viral cDNA is integrated with the host DNA, the virus will either utilize the cell replication machinery to replicate and produce new virions, or the cell will enter into a resting, or latent, state. These cells, which harbor viral cDNA but do not produce viral proteins, make up the "latent reservoir," which remains a major barrier to HIV cure due to its incredibly long half-life and inability to be targeted by antiretroviral therapy (ART) [7].

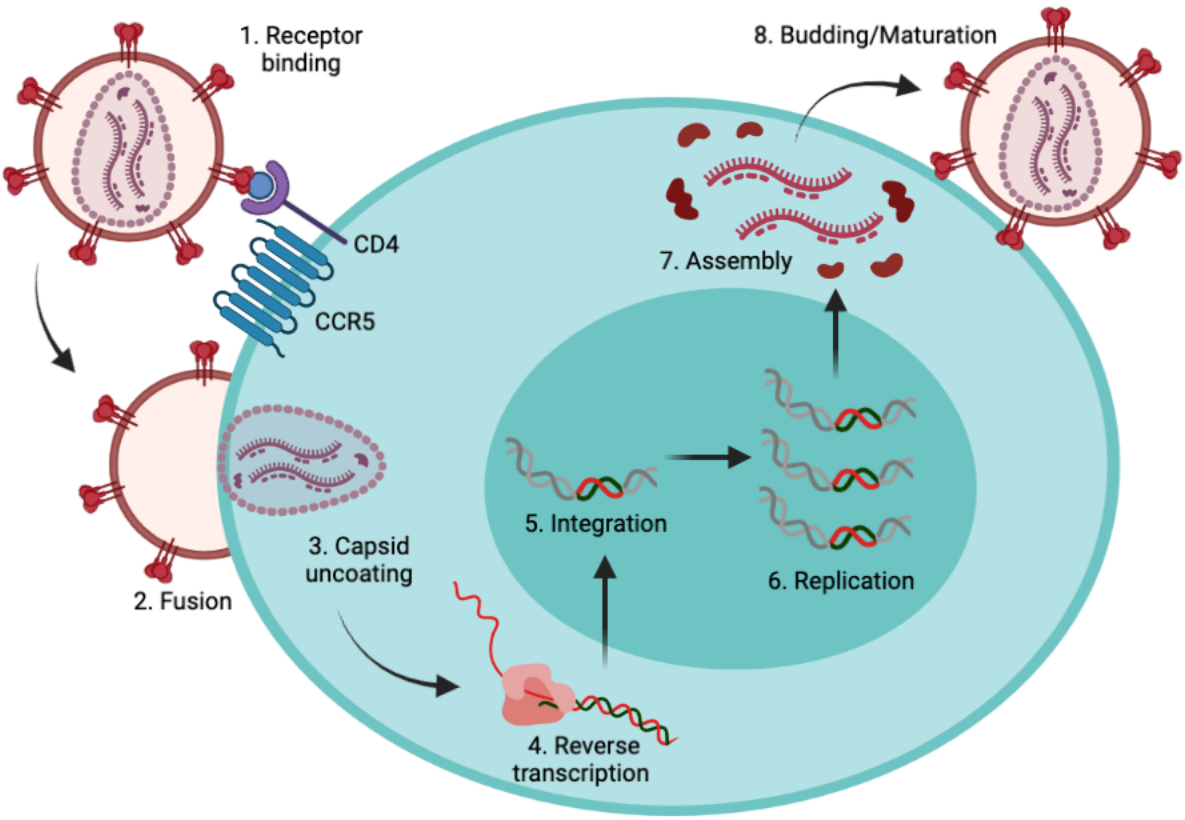


Figure 1.1 Human immunodeficiency virus (HIV) life cycle. HIV Env binds CD4 and the co-receptor CCR5, triggering fusion of the viral and cellular membranes. After the viral capsid enters the cytoplasm, the viral RNA genome is reverse transcribed into cDNA. The cDNA genome traffics to the nucleus, where it is integrated into cellular DNA. Replication of the viral genome occurs and new virions are produced. (Figure produced using Biorender.com)

HIV epidemiology and pathogenesis

Since the beginning of the HIV pandemic, the World Health Organization (WHO) and UNAIDS estimate that between 55.9 million and 100 million people have been infected with HIV, and up to 42.2 million people have died from AIDS-related complications. As of 2019, the UNAIDS estimates that 38 million people globally are living with HIV, with 1.7 million new infections and 690,000 deaths due to HIV-related co-morbidities occurring in that year alone. Fortunately, AIDS-related deaths have been reduced by 60% since the peak in 2004, and AIDS-related mortality has continued to decline by 39% since 2010 due to vast improvements in HIV treatment. However, effective HIV prevention and treatment is not universally accessible, and HIV remains a significant public health concern and economic burden.

In most individuals, HIV infection gradually depletes $CD4^+$ T cells [8, 9] and leads to immunodeficiency, ultimately resulting in death from opportunistic infections and AIDS-related complications. However, a small number of individuals living with HIV, termed "elite controllers," maintain undetectable viremia without antiretrovirals, likely due to a combination of immunologic and virologic mechanisms that are not yet fully characterized [10-13]. The Centers for Disease Control (CDC) classifies HIV infection into three stages of disease progression. The first stage, acute HIV replication, occurs within two to four weeks of initial infection, and is characterized by detectable HIV RNA in the blood [14]. During this stage, infected individuals may experience flu-like symptoms, such as fever, headache, or sore throat, although some people may experience no symptoms [14]. The second stage is termed clinical latency, otherwise known as asymptomatic HIV infection or chronic HIV infection. After the initial burst of replication, the virus enters a phase of active but low-level replication [15, 16], wherein infected individuals may only experience occasional fatigue or transient weight loss, or may not experience any symptoms at all [14]. If left untreated, chronic HIV infection will progress to the third stage of disease progression, acquired immunodeficiency syndrome (AIDS) [17]. AIDS is the most severe form of HIV infection and is characterized by a $CD4^+$ T cell count of less than 200 $CD4^+$ T cells per

microliter of blood, or the presence of opportunistic infections such as *Cytomegalovirus*, *Mycobacterium tuberculosis*, or *Pneumocystis jirovecii* pneumonia [18]. People with AIDS also commonly experience chills, fever, swollen lymph nodes, fatigue, and weight loss [14]. Without treatment, the life expectancy for an HIV-infected individual who has progressed to AIDS is around three years [19].

Effectiveness and limitations of antiretroviral therapy (ART)

The first drug to treat HIV, azidothymidine, or AZT, was approved by the FDA in 1987 for use in patients with advanced HIV. A nucleoside reverse transcriptase inhibitor (NRTI), the drug had originally been developed in the 1960s to treat cancer by blocking tumor cell replication, but was shelved after it was deemed ineffective. AZT was thought to be effective at halting HIV replication and appeared to significantly delay disease progression in clinical trials [20]. Unfortunately, it became apparent that monotherapy with AZT could select for viral escape mutants that were highly resistant to the drug [21, 22]. To combat drug-resistance and prolong viral suppression, new classes of drugs were developed, such as non-nucleoside reverse transcriptase inhibitors (NNRTIs) and drugs targeting other steps in the viral life cycle, including protease inhibitors (PIs), integrase inhibitors, fusion inhibitors, and entry/attachment inhibitors [23-27]. Currently, the standard of care is antiretroviral therapy (ART), which refers to combinations of drugs from two different drug classes at minimum [28], usually consisting of a nucleoside/nucleotide dual “backbone” and a third drug from another class [29]. Combining multiple HIV inhibitors into a single pill, once-daily regimen is highly effective at limiting viral replication and preventing disease progression, while also providing a simpler experience for patients that encourages compliance [30, 31]. Most recently, long-acting ART drugs have been developed that could be administered at monthly intervals by intramuscular injection. These medications could be utilized as maintenance therapy for individuals already using oral ART, or as pre-exposure prophylaxis in HIV-negative individuals [32, 33].

Given these advances in treatment, it may come as a surprise that the global ART coverage rate is only about 67%, according to the WHO and UNAIDS. This is partly due to limited accessibility of ART in resource-poor settings, where barriers hindering access to ART include the high financial costs associated with accessing and receiving treatment, drug shortages, and inability to travel long distances to treatment centers [34, 35]. Unfortunately, problems with ART accessibility are not limited to low-income countries, as even in the United States, only 53% of HIV-positive people have their viral loads suppressed by ART, according to the Center for Disease Control (CDC). However, even in people whose viral loads are fully suppressed, ART cannot fully restore normal immune function, enhance HIV-specific immune responses, or reduce the latent reservoir [36-42]. The latent reservoir is remarkably stable, with an estimated half-life of 44 months, meaning that even after years of effective ART, the latent reservoir will still persist [43-45]. The stability of the latent reservoir necessitates strict, lifelong adherence to ART, as halting therapy usually results in a resurgence of viral replication and disease progression [46]. These limitations are the reason behind the continued interest in developing alternate HIV therapies, including therapeutic vaccines that could induce viral remission without dependence on ART.

Nonhuman primate models for AIDS

A crucial resource in the search for effective HIV therapies is the nonhuman primate (NHP) model. Animal models are invaluable for enabling scientists and physicians to study disease pathogenesis and to test potential cures or therapies without risking the health of infected individuals. Animal models allow for more thorough, comprehensive tissue sampling, and greater experimental control over variables such as immune-genetic factors, timing and route of infection, strain of challenge virus, and initiation and duration of ART. A good animal model also recapitulates the hallmarks of HIV infection in humans but develops disease at a faster rate, which permits completion of experiments in a timely manner.

In order to develop an animal model, it is first crucial to understand the origins of the pathogen in question. The AIDS pandemic is primarily driven by HIV-1, although studies of HIV-

2 provided key insights into the origin of HIV-1. HIV-2 is genetically distant from HIV-1, less pathogenic, and is largely restricted to a few West African countries. Genetic evidence showed that HIV-2 originated from a type of lentivirus found in sooty mangabeys and found to be endemic to many species of African monkeys [47]. These viruses became known as simian immunodeficiency viruses (SIVs), and were the first indication that HIV was the result of cross-species transmission. A key study in 1989 identified an SIV that was serologically identical to HIV-1 in wild chimpanzees [48]. Eventually, subsequent studies strongly suggested that HIV-1 originated from a specific SIV lineage found in the chimpanzee subspecies, *Pan troglodytes troglodytes [49].* Precisely how SIV made the leap into humans is not known, but is thought to have been through unsafe consumption of bushmeat.

The natural hosts of SIV, namely the African green monkeys and sooty mangabeys, are not good animal models for HIV since they do not develop disease [50, 51]. However, SIV infection of Asian macaques shares key pathogenic features with HIV infection in humans, such as robust viral replication, depletion of $CD4^+$ T cells, integration of viral DNA into the target cell genome, and progression to AIDS, albeit over a much shorter timeframe [52, 53]. Common NHP models for AIDS include rhesus macaques (*Macaca mulatta*), pig-tailed macaques (*Macaca nemestrina*) and cynomolgus macaques (*Macaca fascicularis*), though the rhesus macaque is the most widely used model.

A major advantage of the rhesus macaque model is our ability to control for some of the various immune-genetic factors that are known to play a role in SIV viral control. Similar to what is observed in humans, rhesus macaques with certain major histocompatibility complex I (MHC-1) haplotypes are better able to control virus replication due to the repertoire of viral peptides that can engage with those MHC [54-56]. Specifically, the *Mamu-A*01, B*08,* and *B*17* alleles are enriched among animals that robustly control viral replication [54-56]. Another important factor to consider when using the rhesus macaque model is the restriction factor tripartite motif 5 alpha (TRIM5α). TRIM5α restricts replication of SIV and other lentiviruses at the post-entry stage by

preventing uncoating of the viral capsid prior to reverse transcription of the viral RNA genome [57, 58]. Rhesus macaques express three allelic forms of TRIM5α: TRIM5^{TFP}, TRIM5^{Q}, and TRIM5^{CypA} that provide varying degrees of protection from SIV infection [59, 60]. Specifically, TRIM5^{TFP} and TRIM5^{CypA} are considered restrictive, while TRIM5^{Q} is considered permissive [59, 60]. Thus, when designing NHP experiments, researchers must take care to evaluate animals' MHC-1 and TRIM5α genetics and either avoid animals that may be genetically predisposed to viral control, or structure the experiment such that these animals are spread out evenly amongst treatment groups. This ensures that any therapeutic benefit observed during a study can be attributed to the interventions themselves, rather than intrinsic host factors.

Lastly, no discussion of an animal model is complete without considering what viruses can be studied with it. While HIV cannot infect NHPs, several different SIVs and chimeric simian-human immunodeficiency viruses (SHIVs) have been developed for use in the macaque model. SIVs typically replicate to higher titers and are more pathogenic in macaques relative to SHIVs, although some SHIVs have the advantage of expressing HIV *env*. SIVmac239 was the first consistently pathogenic molecular clone to be isolated and is highly resistant to neutralizing antibodies [61]. SIVmac251 is closely related to SIVmac239, but challenge stocks of this virus exhibit more heterogeneity compared to SIVmac239, meaning that it more closely resembles the diversity of HIV seen in humans [62]. Another SIV that has been developed for use in the rhesus macaque model is SIVsmE660, which also exhibits more heterogeneity within challenge stocks and is genetically distant from SIVmac251 and SIVmac239, making it attractive for use in heterologous challenge studies where the vaccine antigens are based off SIVmac239 or SIVmac251 [63]. In contrast to lab-adapted strains of SIV, SIVΔB670 is a highly pathogenic, neurotropic, primary isolate that replicates to extremely high titers in rhesus macaques and can cause AIDS within 10 months of untreated infection, making it a particularly stringent test for experimental therapies [64]. On the other hand, certain SHIVs were designed to carry the HIV *env* gene to directly evaluate Env-targeted therapeutics. SHIV-89.6P was the first SHIV able to

rapidly deplete $CD4^+$ T cells and cause AIDS-like illness in rhesus macaques, but this virus did not recapitulate the tropism of HIV-1 or SIV, and thus is no longer used [65]. SHIV.CH505 is a good example of a modern SHIV that recapitulates the peripheral viral kinetics and mucosal immunopathology typical of HIV-1 infection of humans and pathogenic SIV infection of NHPs [66]. Altogether, there are a plethora of challenge viruses to use with the NHP model for AIDS, and it falls upon researchers to evaluate which SIV or SHIV best fulfills their experimental needs.

The importance of mucosal immunity in HIV infection

The SIV NHP model for AIDS enabled more in-depth study of viral pathogenesis than would have been possible in cell culture or human subjects alone. Notably, the hallmark of HIV infection, depletion of $CD4^+$ T cells, was primarily studied in the peripheral blood for a little more than a decade, despite the vast majority of $CD4^+$ T cells residing in lymphoid tissues. It was not until 1998 when Veazey *et al* reported that the intestine appears to be a major target for SIV infection in rhesus macaques, and till 2004 when Dandekar *et al* demonstrated that severe and persistent depletion of CD4 T cells occurs in the mucosa of HIV-infected individuals, that the importance of the mucosal compartment in HIV pathogenesis became clear [67, 68].

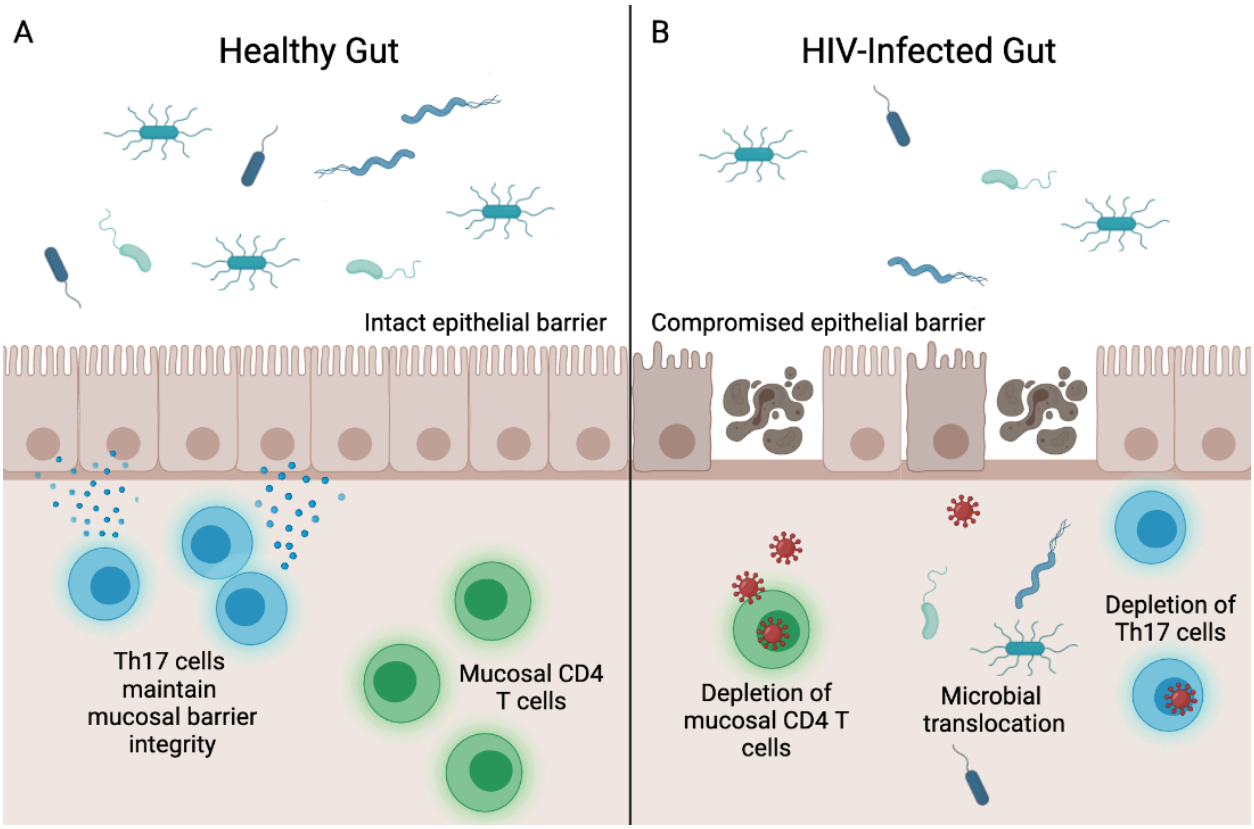


Figure 1.2 Mucosal immunity in HIV infection. **(A)** In a healthy gut, T helper 17 (Th17) cells secrete IL-17 and IL-21 to maintain the integrity of the mucosal epithelial barrier and prevent microbial translocation. **(B)** In an HIV-infected gut, viral replication causes depletion of mucosal $CD4^+$ T cells and Th17 cells, and breakdown of the gut epithelial barrier. This leads to microbial translocation and contributes to chronic inflammation and disease progression. (Figure produced using Biorender.com)

The gastrointestinal (GI, or gut) mucosa is uniquely susceptible to HIV and SIV infection for several reasons: First, the majority of the body's lymphocytes reside in the GI mucosa, rather than the peripheral blood. Second, large numbers of $CD4^+$ T cells in the GI mucosa are activated, $CCR5^+$ memory $CD4^+$ T cells, making them highly susceptible to infection. Finally, high levels of proinflammatory cytokines maintain a state of physiological inflammation in the GI mucosa. These characteristics make the gut a major target for HIV and SIV replication and a primary site of $CD4^+$ T cell depletion in early infection [67, 69, 70]. Loss of $CD4^+$ T cells in the gut occurs rapidly within days of infection, before any changes are observed in peripheral lymphoid tissues [70]. This preferential depletion of mucosal $CD4^+$ T cells is maintained throughout the course of the infection – At any stage of disease, the GI mucosa exhibits the most substantial $CD4^+$ T cell depletion [69]. This results in loss of antigen-presenting cells and innate lymphocytes, and functional alteration of remaining gut mucosal $CD4^+$ T cells.

One particular type of $CD4^+$ T cells, T helper 17 (Th17) cells, play a critical role in defense against microbes and maintenance of gut mucosal barrier integrity. Depletion of Th17 cells during HIV and SIV infection results in compromise of the mucosal barrier and microbial translocation [71]. Microbial translocation, in turn, is associated with systemic immune activation that drives chronic viral infection and disease pathogenesis [37, 72]. Suppression of viral replication with effective antiretroviral drugs appears to partially resolve microbial translocation, but $CD4^+$ T cell populations remain depleted in the GI mucosa, even though suppression of viral replication permits near-total reconstitution of $CD4^+$ T cells in the peripheral blood in nearly all individuals, with the exception of those with severe $CD4^+$ T cell depletion prior to ART initiation [37].

T Cellular Immunity Can Control HIV and SIV Infection

Studies in the SIV/macaque model were also critical for building up our understanding of the importance of virus-specific T cellular immunity in controlling HIV and SIV infection. In brief, $CD8^+$ T cells identify virus-infected cells through recognition of major histocompatibility complex class I (MHC-I) restricted viral 8-11 mer peptides on infected cells. Upon identification, $CD8^+$ T

cells can kill infected cells through the release of the cytotoxins perforin and granzyme, as well as cytokines such as IFN-γ, TNFα, and IL-2.

T cellular immunity was first shown to be important in control of HIV infection in 1986, when Walker *et al* demonstrated that $CD8^+$ T cells could control HIV infection *in vitro* [73]. Further studies in HIV-infected individuals observed that $CD8^+$ T cells arise during acute infection and are temporally associated with the initial decline in viral load following peak viremia, although these studies were only correlative in nature [74, 75]. The first studies demonstrating a causal relationship between $CD8^+$ T cell immunity and control of lentiviral replication were published in the late 90s and utilized the SIV/macaque model to show that depleting $CD8^+$ T cells in macaques infected with SHIV or SIV resulted in increased viremia [76-78]. It is now generally accepted that $CD8^+$ T cells can play a role in control of HIV replication. Although the precise mechanisms behind $CD8^+$ T cell-mediated protection are not fully understood, there is strong evidence for the importance of broad epitope specificity (The ability to recognize a large number of epitopes) [79-81], T cell polyfunctionality (Secretion of multiple cytokines)[82], and potent cytotoxicity [83].

On the other hand, the role of virus-specific $CD4^+$ T cells in HIV and SIV infection is unclear. HIV and SIV primarily infect activated $CD4^+$ T cells and preferentially infect HIV-specific $CD4^+$ T cells [84], but these cells also have direct antiviral properties and provide crucial help to B cells and $CD8^+$ T cells. The earliest evidence suggesting a protective role for HIV-specific $CD4^+$ T cells was reported in 1997 and showed an association between increased HIV-specific $CD4^+$ T cells and viral control [85]. Later on, studies detected HIV-specific, cytolytic $CD4^+$ T cells and demonstrated an association between increased frequencies of these $CD4^+$ T cells and reduced viral loads [86]. However, in spite of this proven antiviral capacity, vaccines generally attempt to avoid eliciting a robust $CD4^+$ T cell response, in order to avoid increasing potential targets for infection.

Taken together, there is much evidence that $CD8^+$ and $CD4^+$ T cellular immunity can control HIV replication to a certain extent. However, a hallmark of HIV is the ability of the virus to

evade T cellular immune responses due to its rapid mutation rate and the resulting enormous genetic diversity. Additionally, chronic activation by viral antigens blunts virus-specific T cell immunity through the upregulation of negative immune regulators, a phenomenon otherwise known as immune exhaustion.

Immune exhaustion

During HIV disease progression, HIV-specific $CD4^+$ and $CD8^+$ T cells undergo a progressive reduction in their ability to persist, proliferate, and produce cytokines, gradually becoming exhausted [87, 88]. This is in part because HIV infection is associated with increased expression of inhibitory receptors, or exhaustion markers, such as programmed death-1 (PD-1) [87] and cytotoxic T lymphocyte antigen-4 (CTLA4) [89].

PD-1 was first identified as a marker of programmed cell death on mouse T cells [90], and was later determined to be an important contributor to T cell exhaustion in the lymphocytic choriomeningitis virus (LCMV) mouse model [91], commonly used for studying chronic viral infections. These studies illustrated the causal role of PD-1 in T cell exhaustion during chronic infections by showing that PD-1 was expressed at high levels on virus-specific $CD8^+$ T cells, and that preventing the interaction between PD-1 and its ligand, PD-L1, improved T cell function and reduced viral loads in infected mice [91]. In the context of HIV infection, the first studies of PD-1 indicated that PD-1 was expressed at high levels on HIV-specific $CD8^+$ T cells, and that elevated expression of PD-1 was correlated with high viral load and low $CD4^+$ T cell counts [87, 92, 93]. Later studies demonstrated that viral replication drives increased PD-1 expression on HIV-specific $CD4^+$ and $CD8^+$ T cells in progressors, but not in long-term non-progressors (LTNPs) [94], otherwise known as individuals infected with HIV who maintain a $CD4^+$ T cell count greater than 500 without ART, despite a detectable viral load. SIV infection in rhesus macaques similarly increases PD-1 expression on SIV-specific T cells and is associated with reduced proliferative capacity in $CD8^+$ T cells and increased plasma viral loads [95-97].

CTLA-4 was first identified as a negative regulator of T cell activation in 1995, when studies found that knocking out the gene in mice resulted in massive lymphoproliferation and tissue destruction [98, 99]. Further studies revealed that CLTA-4 is an immune checkpoint receptor expressed only on T cells, where it competes with the costimulatory molecule CD28 in binding CD80 on antigen-presenting cells (APCs) [100], resulting in inhibition of T cell activity and reduced IL-2 production [101]. During HIV infection, CTLA-4 is selectively upregulated on virus-specific $CD4^+$ T cells and is associated with disease progression and reduced production of IL-2 in response to viral antigens [89].

Ultimately, HIV's rapid mutation rate produces enormous genetic diversity, leading to immune escape and immune exhaustion through chronic overstimulation by viral antigens. Immune escape and immune exhaustion are the primary reasons why HIV viral replication cannot normally be controlled in the absence of ART and represent formidable barriers in the search for an HIV cure. However, understanding how T-cell exhaustion impairs HIV-specific immunity could lead to more potent immunotherapies, potentially through combining therapeutic vaccination with immune checkpoint blockade to augment HIV-specific immunity.

Therapeutic vaccination

In essence, a therapeutic vaccine is a vaccine that is administered after an infection or disease has already occurred. In contrast to a prophylactic vaccine that acts as a precautionary measure against future infection, a therapeutic vaccine aims to activate the immune system in order to combat an existing infection or disease.

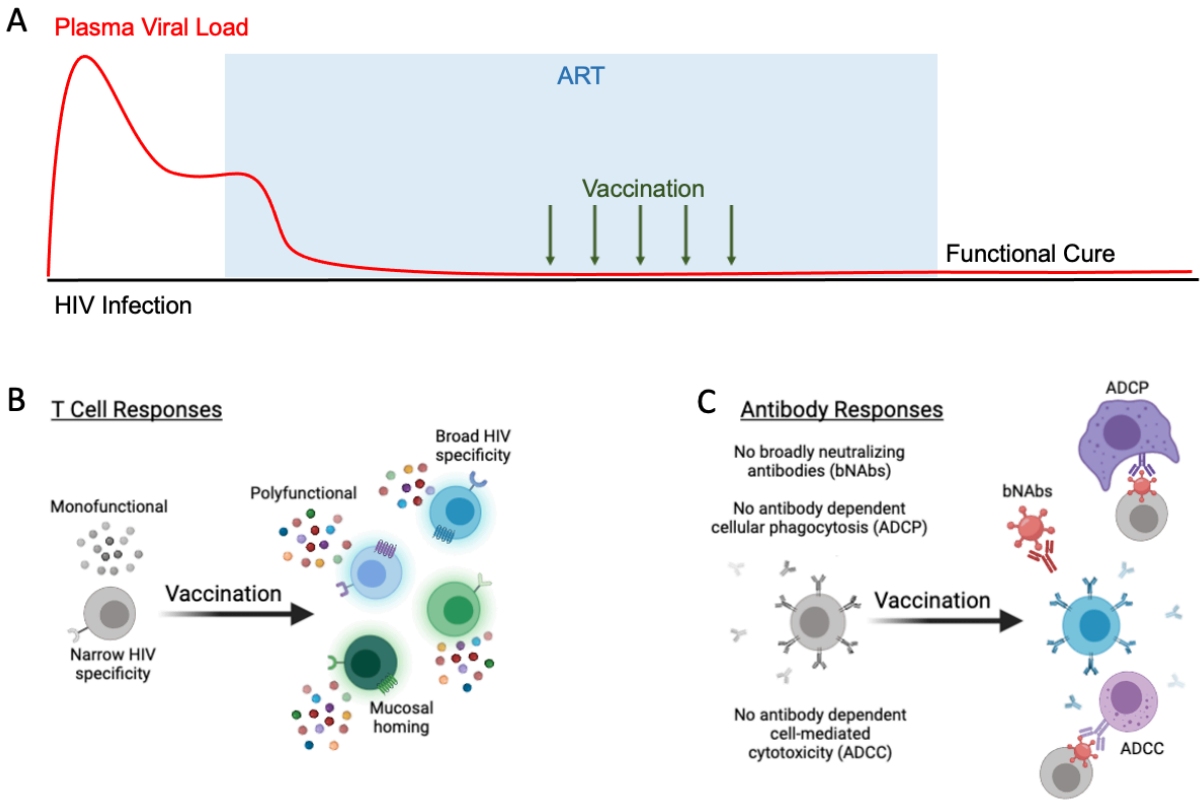


Figure 1.3 The goal of therapeutic vaccination. (A) A therapeutic vaccine could allow an HIV-infected individual to control viral replication in the absence of antiretroviral therapy (ART). **(B-C)** Therapeutic vaccination could induce control of viral replication through enhancement of T cell responses or antibody responses. (Figure produced using Biorender.com)

In the context of HIV, the goal of therapeutic vaccination is to prevent disease progression by augmenting HIV-specific immunity, thereby enabling an HIV-infected individual to control viral replication in the absence of ART. Both T cell and antibody-based approaches have been tried and tested, with varying levels of success. T cell-based vaccines primarily aim to augment T cell effector function [83], increase the breadth of epitopes targeted [80, 102], expand polyfunctional T cell responses [82], and direct virus-specific responses to viral reservoir sites and major sites of viral replication, namely mucosal tissues and lymph nodes [80, 103]. Antibody-based approaches focus on induction of broadly neutralizing antibodies (antibodies capable of neutralizing many diverse strains of HIV) [104] or enhancement of antibody-mediated effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) [105]. Although no therapeutic vaccine strategy to date has been successful at inducing long-term protection from viral rebound in HIV-infected individuals, the feasibility of the concept is supported by the existence of rare elite controllers, whose robust immune responses are able to maintain undetectable levels of plasma viremia in the absence of ART [12, 82, 83, 106, 107].

Vaccination strategies:

In the decades since HIV was identified as the causative agent of AIDS, many different therapeutic vaccine approaches have been tested in both human clinical trials and nonhuman primate studies, including protein subunit vaccines, inactivated or attenuated viruses, viral vectored vaccines, nucleic acid vaccines, and dendritic cell-based approaches.

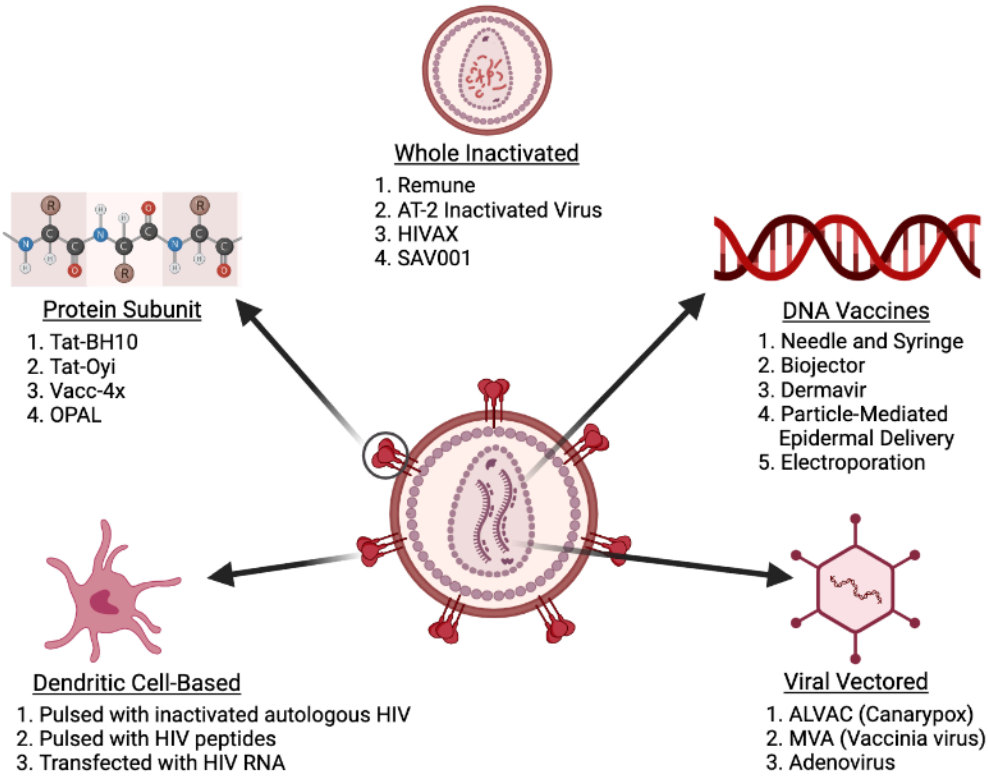


Figure 1.4 HIV therapeutic vaccine strategies. Numerous HIV therapeutic vaccines have been tested in human clinical trials, including whole inactivated virus, protein subunit, dendritic cell-based, viral vectored and DNA vaccines. However, although numerous strategies were reported to be safe and immunogenic, none of these strategies have been able to consistently elicit durable control of HIV replication in the absence of ART. (Figure produced using Biorender.com)

Inactivated/attenuated virus

Inactivated or attenuated pathogens are a classic approach to vaccine development. However, these approaches are considered controversial for HIV vaccine development, due to the risks associated with incomplete inactivation of HIV and the potential for attenuated viruses to establish persistent infection in vaccinated individuals, resulting more virulent and pathogenic progeny. Studies in macaques proved these concerns to be justified: On one occasion, a live-attenuated SIV with large deletions in nef and vpr induced progressive disease in both infant and adult vaccinated macaques [108]. In a separate study, a nef-deleted, live-attenuated SIV (SIVmac239Δnef) was able to mutate *in vivo* and restore normal nef function, resulting in progression to AIDS in vaccinated animals [109]. Current work on inactivated and attenuated HIV vaccines focuses on addressing these safety concerns and improving vaccine efficacy.

Remune/HIV-1 Immunogen

One of the first therapeutic vaccines, HIV-1 immunogen (also known as Remune), was developed by none other than Dr. Jonas Salk [110]. Initially intended as a prophylactic vaccine, HIV-1 immunogen consists of an even mixture of incomplete Freund's adjuvant and whole HIV-1 virions, inactivated via treatment with beta-propiolactone and Cobalt irradiation [110]. Notably, this method of inactivation depletes the outer gp120 surface antigen [110]. HIV-1 immunogen is also one of the most extensively studied therapeutic vaccines for HIV, having been studied in thousands of HIV-infected individuals in more than a dozen clinical trials spanning over three decades, albeit with little clinical success.

Multiple phase 1 clinical studies, in both treated and untreated individuals at various disease stages and including children, demonstrated HIV-1 immunogen to be both immunogenic and safe [111-122]. To date, three phase 2 studies, one phase 2/3 study, and two phase 3 clinical trials of HIV-1 Immunogen have been completed, although some of the reported positive clinical outcomes have been overshadowed by controversies. The first phase 2 clinical trial of HIV-1 Immunogen as a therapeutic vaccine, CTN 173, reported that HIV-1 Immunogen had no effect on

time to viral rebound after treatment interruption or viral setpoint after treatment interruption [121, 122]. However, the results of the second phase 2 clinical trial, P2101B, indicate vaccination with HIV-1 immunogen resulted in a lower rate of clinical disease progression and significant increases in $CD4^+$ T cell counts in the vaccinated cohort, and significantly lower mean viral load in vaccine responders compared to non-responders [118-120]. The third phase 2 clinical trial, STIR-2102, found that vaccination with HIV-1 Immunogen was associated with a trend to delayed viral rebound, elevated HIV-specific $CD4^+$ T cell responses, and increased Gag-specific CTL activity [116, 117]. Notably, the phase 2/3 clinical trial reported that therapeutic vaccination with HIV-1 immunogen significantly reduced viral loads and $CD4^+$ T cell decline, while significantly boosting cell-mediated immunity and humoral responses [114, 115]. In contrast to these promising results, the first phase 3 clinical trial of HIV-1 Immunogen did not demonstrate any effect on disease progression or plasma viremia [113], although a substudy of randomly selected participants observed a significantly greater reduction in viral load in the HIV-1 immunogen group, compared to the placebo group [112]. The last published phase 3 clinical trial, the QUEST study, tested HIV-1 immunogen alone and with the ALVAC vaccine, but found that HIV-1 immunogen had no effect on virus-specific immunity or viral load [111].

Notably, three clinical trials that reported a positive effect of HIV-1 Immunogen on plasma viremia or $CD4^+$ T cell counts have been contested. Glidden *et al* put forward that the $CD4^+$ T cell data from study P2101B, reported by Churdboonchart *et al*, was only one out of ten total analyses of the $CD4^+$ T cell count, and that eight of the ten analyses failed to show any statistically significant differences in the $CD4^+$ T cell count between the HIV-1 Immunogen and placebo groups [120, 123]. The results of the phase 2/3 clinical trial were called into question when the U.S. Food and Drug Administration (FDA) issued a warning letter to the study team due to their undisclosed exclusion of two participants from the published analysis [124]. Lastly, disagreements on analysis methodology led the study team on the first phase 3 clinical trial of HIV-1 Immunogen to report that it had no effect on any study endpoints [113], in direct conflict

with the study sponsor's retrospective analysis declaring some positive effects on viral loads [112].

Altogether, the results of numerous clinical trials prove that HIV-1 Immunogen is safe, well-tolerated, and capable of inducing both humoral and cell-mediated immunity. However, whether the vaccine has any clinical efficacy remains controversial. It is possible that therapeutic vaccination with HIV-1 immunogen would be more effective if combined with a latency reversal agent to eliminate or reduce the viral reservoir, or immunomodulators to reverse T cell exhaustion. These combinatorial approaches merit further study.

AT-2 inactivated virus

Attempts to improve upon the Remune vaccine include improving the method of virus inactivation, such as using Aldrithiol 2 (AT-2) instead of beta-propiolactone and irradiation. In contrast to Remune, which does not contain the gp120 surface antigen due to the method of inactivation used, AT-2 inactivated SIV and HIV maintain the proper structure and antigen processing of all virion-associated proteins [125], making them appealing vaccine immunogens. Initial studies of prophylactic vaccination of rhesus macaques with AT-2 inactivated SIVmac239 induced robust SIV-specific T cell responses and significantly reduced the rate of infection following intrarectal challenge with pathogenic SIVmac239 [126]. Based on this encouraging data, AT-2 inactivated SIVmac239 was tested as a therapeutic vaccine in SIV-infected, ART-treated rhesus macaques. Vaccination significantly boosted neutralizing antibody titers and induced a decrease in plasma viral loads during ATI in comparison to mock-vaccinated controls [127]. However, vaccine efficacy was limited to animals that responded well to ART, and additional booster immunizations in the absence of ART did not further decrease viral loads during ATI [127].

HIVAX

In an attempt to circumvent the safety risks associated with a live-attenuated HIV vaccine, HIVAX was designed as a replication-defective HIV-1, attenuated by multiple deletions of the pol,

vif, env, and nef genes and pseudotyped from Vesicular Stomatitis Virus (VSV) to expand cell tropism to dendritic and Langerhans cells [128]. The resulting virus was able to infect cells but not able to initiate a second round of replication. In a randomized, placebo-controlled clinical trial of HIV-1 infected volunteers receiving ART, HIVAX was found to be immunogenic and well-tolerated [128]. Vaccination reduced immune activation and chronic inflammation [129] and boosted both $CD4^+$ and $CD8^+$ HIV-specific T cell responses [128]. Additionally, vaccinated individuals exhibited significantly lower viral loads during treatment interruption in comparison to their pre-ART levels [128].

SAV001

Most recently, Choi *et al* conducted a Phase I human clinical trial using SAV001, a killed, whole-virus vaccine that consists of inactivated, modified HIV-1 Clade B NL4-3 with the genes for nef and vpu deleted, and the coding sequence for the Env signal peptide replaced with that of the honeybee melittin signal peptide, to produce a less virulent and more replication efficient virus. In an observer-blinded, placebo-controlled trial of 33 HIV-1 positive volunteers receiving ART, SAV001 was well-tolerated and boosted the pre-existing antibody responses to HIV-1 proteins, including broadly neutralizing antibodies [130]. No evidence was found of vaccine virus replication in volunteers [130].

Protein subunit vaccines

Tat

A different approach to therapeutic vaccine design aims to target the viral transactivator of transcription protein (Tat). Tat plays a critical role in HIV transcription, latency, and viral rebound following ART interruption [131], and is fairly conserved among all subtypes in its immunogenic domains [132], making it an attractive vaccine target. In support of this, the presence of anti-Tat antibodies in the serum of HIV-infected individuals is associated with lower viral load and delayed progression to AIDS [133-135]. So far, two Tat-based vaccines have been investigated in both animal studies and human clinical trials: One derived from HIV strain BH10 (Tat-BH10) and one

from HIV strain Oyi (Tat-Oyi). Tat-BH10 is a functional protein, while Tat-Oyi is not. Both types of Tat vaccine were tested in NHP models [136-140] and have since moved on to phase1/2a human clinical trials involving HIV-infected adults. One study, ISS T-002, was an exploratory phase 2 open label therapeutic trial of Tat-BH10 that did not include a double blinded, randomized control arm, but compared vaccinated participants instead to a non-vaccinated, HIV^+ matched group [141]. In this study, Tat-BH10 vaccination was found to be safe and resulted in detectable levels of anti-Tat antibodies in 90% of vaccinees, significant restoration of $CD4^+$ T cells, reduced immune activation, and significant and durable reductions in HIV-1 DNA [141]. A randomized, blinded, placebo-controlled phase 2 trial of Tat-BH10 (ISS T-003) confirmed that the vaccine was safe and induced durable, high titers of anti-Tat antibodies that were associated with increased $CD4^+$ T cells [142]. Notably, containment of viral rebound was observed in ART-noncompliant, vaccinated participants, as compared to ART-noncompliant subjects who received a placebo [142]. The Tat-Oyi phase1/2a clinical trial likewise determined that vaccination was safe, induced anti-Tat antibodies, and was associated with reduced viral rebound following ART interruption [143]. An 8-year follow-up of the ISS T-002 study indicated that anti-Tat antibodies persisted in more than 50% of volunteers, while $CD4^+$ T cell counts continued to increase and HIV proviral DNA continued to decrease [144]. Altogether, the results from these clinical trials demonstrate anti-Tat therapeutic vaccines are promising and will require further investigation in larger cohorts of HIV-infected individuals.

Vacc-4x

The Vacc-4x vaccine is comprised of four synthetic peptides that correspond to conserved amino acid sequences of the HIV major core protein, p24, adjuvanted with granulocyte macrophage colony-stimulating factor (GM-CSF) to ensure optimal presentation of the immunogen to APCs [145]. A small phase I safety study of 11 HIV-1 infected individuals demonstrated that the vaccine was safe, well-tolerated, and induced modest strong cell-mediated and humoral immunity [145]. A phase 2 clinical trial of 40 HIV-1-infected individuals provided

further evidence that the vaccine was immunogenic, with 90% of participants developing Vacc-4x-specific responses as detected via delayed type hypersensitivity (DTH) tests, and 80% of participants demonstrating Vacc-4x-specific $CD4^+$ and $CD8^+$ T cell responses, although this study was not placebo controlled [146]. An analytic treatment interruption in vaccine-responders showed that individuals exhibiting the highest DTH responses to Vacc-4x maintained lower viral loads [147]. Notably, long-term follow up indicated that Vacc-4x-specific immune response were unchanged 1.5 years after the last immunization, and 62% of vaccine-responders did not yet meet the criteria to resume ART [148]. More recently, a randomized, placebo controlled, double blind phase 2 clinical trial sought to assess the safety, immunogenicity, and efficacy of Vacc-4x. The vaccine was safe, well-tolerated, and immunogenic, inducing proliferative responses in both $CD4^+$ and $CD8^+$ T cells [149]. However, after treatment interruption there was no difference between the Vacc-4x and placebo groups either in time to necessary ART resumption or changes in $CD4^+$ T cell counts during treatment interruption [149].

OPAL

Another, more unconventional therapeutic vaccine approach is termed OPAL – Overlapping Peptide-pulsed Autologous Cells. First tested in SIV-infected, ART-treated pigtail macaques, OPAL aimed to induce SIV-specific cellular immune responses by incubating fresh whole blood or peripheral blood mononuclear cells (PBMCs) with overlapping SIV peptides, either from only Gag or spanning the full SIV proteome [150]. The peptide-pulsed blood or PBMCs were then reinfused intravenously into the autologous animal [150]. In comparison to the control group, immunized animals demonstrated significant increases in SIV-specific $CD4^+$ and $CD8^+$ T cell immunity and significantly lower viral loads after treatment interruption, throughout the one year of follow up [150]. There was no difference in therapeutic outcome between animals immunized with blood cells pulsed with Gag peptides or peptides spanning the entire SIV proteome, suggesting that pulsing blood with Gag peptides alone would be an effective immunotherapy [150]. Based on these results, a preclinical study of HIV-infected individuals on ART began to

assess the safety and preliminary immunogenicity of this approach. However, only 2 out of 15 vaccinated participants exhibited increases in HIV-specific $CD8^+$ T cell responses [151]. The study was ultimately terminated early after a serious, potentially life-threatening adverse event occurred in one study participant following a single immunization [152]. The cause of the occurrence was not identified, but the vaccine itself could not be ruled out [152]. As such, authors of the study concluded that the lack of immunogenicity observed warranted emphasis on alternative methods of enhancing T cell immunity [151, 152].

Dendritic cell vaccines

Dendritic cells (DCs) are antigen-presenting cells, or cells that capture and process antigens and present them to naïve T cells on major histocompatibility complex (MHC) molecules, thereby inducing naïve T cell activation and effector differentiation [153]. The essential role that DCs play in priming specific immune responses provides strong rationale for harnessing DCs to treat HIV infection. Generally speaking, DC-based therapeutic vaccine strategies seek to boost HIV-specific immune responses by obtaining autologous monocytes, culturing those monocytes with cytokines to promote their maturation into DCs, and pulsing them with HIV antigens *in vitro,* before administering them to the patient [154]. Several different methods of pulsing DCs with HIV antigens have been investigated: Exposure of DCs to inactivated autologous HIV [155-159], pulsing DCs with HIV peptides [160-162], electroporation of HIV RNA into DCs [163-168], and delivery of HIV viral vectored vaccines to DCs *ex vivo* [165].

Notably, vaccines comprised of DCs exposed to autologous HIV have demonstrated the most therapeutic efficacy. Of particular note is a double-blinded, placebo-controlled clinical trial testing the efficacy of a therapeutic vaccine consisting of DCs pulsed with autologous, heat-inactivated whole HIV in chronically HIV-1 infected, ART-treated individuals [158]. In this study, vaccination resulted in a significant $\geq$1 log reduction of plasma viral load in 55% of participants, compared to 9% of participants in the placebo group [158]. This decrease in viremia was associated with an increase in HIV-specific T cell responses [158]. However, vaccination did not

prevent $CD4^+$ T cell depletion, and control of viremia waned over time [158]. In spite of these drawbacks, this study represents the most effective HIV therapeutic vaccine thus far. Importantly, nearly all clinical trials reporting reductions in plasma viral loads after vaccination (with two exceptions) used autologous virus [155-157, 159], despite differences in terms of the protocol used to obtain DCs, the number of doses, whether patients were ART-treated or untreated, and the immunization route.

Therapeutic efficacy in other trials has been variable. A small-scale trial of (N=12) treatment naïve, HIV-1-infected individuals demonstrated that vaccination with DCs pulsed with peptides consisting of conserved elements in gag, pol, env, vpu and vif elicited a transient decrease in viral load in 5 out of 12 vaccinees [161]. Another phase 1 clinical trial in ART-treated, HIV-1-infected individuals assessed the efficacy of a therapeutic vaccine comprised of autologous DCs pulsed with five HIV-1-derived lipopeptides from gag, pol, and nef, and determined that vaccination boosted both $CD4^+$ and $CD8^+$ T cell responses and 50% of vaccine recipients experienced a $\leq$1 log decrease in viral load following treatment interruption [162]. This decrease in viral load was associated with polyfunctional $CD4^+$ T cells [162]. Other clinical trials, namely those involving DCs transfected with HIV mRNA [163, 164, 166], pulsed with HIV peptides [160], or exposed to HIV viral vectored vaccines [165], demonstrated that these approaches are immunogenic but do not confer any virological benefit.

Most recently, a small pilot study tested the therapeutic efficacy of a "kick and kill" approach by combining a latency-reversal agent (LRA), vorinostat, with the DC-based vaccine AGS-004 [168]. AGS-004 consists of autologous DCs co-electroporated with RNA encoding HIV-1 gag, nef, rev, and vpr amplified from participants' pre-ART plasma, and RNA encoding human CD40 ligand to maximize antigen presentation [167, 168]. By co-administering vorinostat, this cure strategy sought to reduce the latent viral reservoir by exposing latently infected cells through with an LRA (the "kick"), that triggers reactivation and production of virus. A therapeutic vaccine, administered prior to or concurrently with the LRA, would then enable the body's own immune

system to target and kill these reactivated cells (the “kill”). A previous phase 2b, randomized, double-blinded, placebo-controlled clinical trial demonstrated that AGS-004 induced polyfunctional $CD8^+$ T cell responses, although these responses did not translate to reductions in viral load during treatment interruption [167]. When combined with vorinostat, AGS-004 did not elicit any boosting of HIV-specific immune responses, nor did vorinostat induce any reduction in the proviral reservoir [168]. The lack of therapeutic efficacy in this study demonstrates that only using autologous antigens in a DC-based vaccine is not sufficient to induce reductions in viral loads, and that much work remains to be done in optimizing this relatively new vaccine technology.

Viral vectored vaccines

ALVAC

Of the HIV viral vector vaccines, ALVAC-HIV is the most thoroughly studied. ALVAC-HIV vaccines consist of a modified recombinant canarypox that expresses HIV genes but is unable to replicate effectively within mammalian cells [169, 170]. Notably, the prophylactic RV144 phase 3 clinical trial utilized a version of the ALVAC vaccine (vCP1521), followed by a gp120 AIDSVAX B/E protein boost [171]. This regimen was only 31% efficacious, but this was the first study to provide evidence for vaccine-induced protection [171]. However, clinical trials conducted to assess the efficacy of ALVAC-HIV as a therapeutic vaccine have been met with varying levels of success.

A randomized, double blind, placebo-controlled study tested the efficacy of ALVAC (vCP1452) expressing Env and Gag genes as well as CTL epitopes from Nef and Pol administered with or without Remune in HIV-infected individuals who initiated ART during acute HIV infection [122]. This study determined that, independent of Remune co-administration, vaccination delayed viral rebound and extended the time to necessary ART resumption but did not lower the viral set point or reduce the viral reservoir [122, 172]. Another partially blinded, randomized phase 2 study of HIV-infected individuals on ART treatment assessed the effects of vCP1452 vaccination with or without IL-2 [173]. In this study, vaccination with just vCP1452

reduced the mean viral load during treatment interruption, while combining vCP1452 with IL-2 boosted $CD4^+$ T cell counts but did not reduce viral loads during treatment interruption [173]. In contrast to these promising results, a randomized, double blind, placebo-controlled phase 2 study of vCP1452 in chronically HIV-infected individuals on ART reported that vaccination was associated with increased mean viral load during treatment interruption and reduced time to necessary ART resumption [174]. A follow up study determined that the vaccine primarily induced HIV-specific $CD4^+$ T cell responses [175]. Given that HIV preferentially infects HIV-specific $CD4^+$ T cells, this potentially explains the enhancement of viral replication in vaccinated study participants.

An open single-arm study evaluated the safety and immunogenicity of another ALVAC-HIV vaccine (vCP1433) expressing Env, Gag, protease, and CTL epitopes from Nef and Pol, in ART-treated patients chronically infected with HIV [176]. This study determined that vaccination boosted HIV-p24-specific lymphoproliferative responses and Gag-specific $CD8^+$ T cell responses [176]. Notably, lymphoproliferative responses were significantly associated with the probability of remaining off ART [176]. A separate, randomized study of HIV-1 infected individuals treated with ART combined vCP1433 with a LIPO-6T vaccine, composed of the tetanus toxoid TT-830-843 class II restricted universal $CD4^+$ T cell epitope and five HIV-1 peptides from Gag, Nef, and Pol, followed with subcutaneous dosing of IL-2 [177]. These interventions induced both HIV-specific $CD4^+$ and $CD8^+$ T cell responses and were associated with a reduction in immune suppressive T regulatory cells [177-179]. After treatment interruption, 24% of vaccinated participants demonstrated lower set point viral loads, compared to 5% of unvaccinated, ART-treated controls [177, 179]. Control of viral rebound was associated with vaccine-induced immune responses [177, 179]. Interestingly, this same vaccine regimen was used in a randomized study of HIV-infected individuals who initiated ART early during acute infection, where it was reported to have no effect on viral rebound after treatment interruption [180]. The authors speculate that the better

immunological status of these early-treated patients may have obscured any effects of the immune interventions.

MVA

The Modified Vaccinia Virus Ankara (MVA) is another well-tested, safe, and highly immunogenic viral vector. Originally developed towards the end of the campaign for the eradication of smallpox, the virus was developed through more than 500 serial passages of vaccinia viruses in chicken embryo fibroblasts [181]. Through this aggressive serial passaging, MVA lost about 10% of the vaccinia genome and the ability to replicate efficiently in virtually all mammalian cells [181].

The first small scale, single-arm clinical trial of an MVA-nef vaccine in HIV-infected, ART-treated individuals was safe and immunogenic, but did not have any effect on protection from viral rebound in any study participants [182]. Notably, vaccination increased both Nef-specific and MVA-specific T cell immunity, indicating that although anti-vector immunity was induced, it did not interfere with vaccine immunogenicity [182].

A more recent clinical trial of an MVA-vectored HIV therapeutic vaccine sought to target the latent virus reservoir through co-administration of an LRA, disulfiram. To this end, a double-blinded, placebo-controlled phase 1 clinical trial in HIV-1-positive, ART-treated individuals (RISVAC03) tested the safety and immunogenicity of an MVA-based vaccine (MVA-B) expressing HIV-1 antigens from clade B (gp120, gag, pol, and nef) with or without disulfiram [183]. Although disulfiram did not have any effect on the proviral reservoir, vaccination with MVA-B alone was associated with a modest delay in viral rebound, compared to placebo recipients [183, 184]. Vaccine recipients also demonstrated significant increases in Env-specific antibody titers and both the breadth and magnitude of the HIV-specific T cell response during treatment interruption [183].

A slightly different approach sought to use the MVA vector to present the immune system with conserved HIV-1 sequences, to re-direct T cell responses towards these typically subdominant viral epitopes [185]. Mutations in these conserved elements are more likely to

decrease viral fitness, and immune responses to conserved epitopes are associated with HIV-1 elite controllers and long term nonprogressors [186]. To this end, a randomized, double-blinded, placebo-controlled trial, known as the HIV-CORE 001 trial, tested an HIVconsv immunogen vectored by MVA (MVA.HIVconsv) in chronically HIV-infected adults who were virologically suppressed on ART [185]. The HIVconsv immunogen consists of a chimeric protein assembled from 14 highly conserved domains derived from HIV gag, pol, vif, and env [185, 186]. Vaccination with MVA.HIVconsv modestly boosted the $CD8^+$ T cell viral inhibitory capacity in *ex vivo* experiments, but did not induce any reduction in the proviral reservoir [185].

Most recently, an open-label, single-arm, phase 1 clinical trial, enrolling 15 early-treated HIV-1 infected individuals (BCN02) integrated these two approaches by co-administering the LRA romdesipin with the MVA.HIVconsv vaccine [187, 188]. This trial determined that vaccination boosted responses to conserved domains in all vaccinees and that romdesipin elicited a significant reduction of the viral reservoir [187, 188]. All vaccinated participants rebounded after treatment interruption, although 3 out of 15 vaccine recipients maintained plasma viral loads below the criterium for ART resumption for up to 32 weeks [188]. However, this study was not placebo-controlled [188].

Other notable clinical trials of MVA-vectored HIV therapeutic vaccines involve priming with an adenovirus-vectored HIV vaccine and boosting with the MVA-vectored vaccine, and thus will be discussed further in the following section.

Adenovirus

Like the canarypox and MVA viral vectors, adenovirus (Ad) vectors have been widely studied for HIV therapeutic vaccine development. Ad are particularly attractive for vaccine delivery due to their high levels of transgene expression, physical and genetic stability, and ability to infect both dividing and non-dividing cells [189-191]. Additionally, Ad can infect dendritic cells, allowing them to effectively present antigens to the immune system and induce robust immune responses [189-191]. They also have a broad tissue tropism, enabling them to elicit both mucosal

and systemic immunity and making them ideal candidates for HIV vaccine vectors [189-191]. Both replication defective and replication competent Ad vectors have been tested in human clinical trials, although HIV therapeutic vaccine studies have primarily utilized replication defective Ad [189-191].

Two early clinical trials sought to use an Ad-vectored HIV vaccine to boost immune responses to gag, in order to elicit $CD8^+$ T cell-mediated control over virus replication. The first of these trials, a randomized, double-blinded, placebo-controlled clinical study involving HIV-1 infected individuals on effective ART, evaluated a recombinant, replication deficient AD5 HIV-1 gag vaccine [192]. Vaccination with AD5 HIV-1 gag was safe and immunogenic, and a trend was observed towards a reduction in post-ART viral loads [192]. Interestingly, HIV-1 gag-specific $IFN\gamma^+CD4^+$ cells were associated with viral control, although it is unclear if those cells had a direct antiviral effect or provided support for $CD8^+$ T cells or other immune cells [192]. A separate randomized, placebo-controlled trial involving HIV-1 infected participants receiving ART, assessed the efficacy of a recombinant Ad5 (rAD5) HIV-1 gag vaccine [193]. This study reported that therapeutic vaccination with the rAd5-HIV gag vaccine was associated with lower plasma viral load post-ART, although counterintuitively, this control was not associated with gag-specific $CD4^+$ or $CD8^+$ T cell responses [193].

Recently, trials involving Ad-vectored HIV vaccines have utilized a more combinatorial approach. The BCN01 trial was a phase 1, open-label, non-randomized study in HIV-1-positive individuals who initiated ART during early HIV-1 infection [194]. Participants first received a prime vaccination consisting of a replication-defective, modified chimpanzee adenovirus serotype 63 (ChAdV-63) expressing the HIVconsv immunogen, and subsequently received an MVA.HIVconsv boost vaccination [194]. This vaccination regimen was safe and well-tolerated, and significantly boosted T cell responses to conserved epitopes [194]. However, vaccination was not associated with a decrease in proviral DNA, and the study design did not incorporate a treatment interruption [194]. Based on these moderately encouraging results, a phase 2, open-label, randomized,

placebo-controlled follow up study, known as the RIVER trial, was conducted in HIV-positive, ART-treated individuals [195]. This study aimed to employ the "kick and kill" strategy by combining the LRA vorinostat (the kick) with the ChAdV63.HIVconsv-prime and MVA.HIVconsv-boost vaccine regimen (the kill), but this approach did not significantly reduce the HIV proviral reservoir in comparison to ART alone [195].

Finally, a small-scale, randomized, placebo-controlled, double-blind study in HIV-infected individuals who initiated ART during acute infection, tested the safety, immunogenicity, and efficacy of Ad26 and MVA vectors containing mosaic HIV-1 env, gag, and pol antigens (Ad26.Mos.HIV and MVA-Mosaic, respectively), optimized to provide maximal coverage of potential T cell epitopes [196, 197]. Vaccinees received a priming immunization consisting of Ad26.Mos.HIV and a boosting immunization consisting of MVA-Mosaic [197]. The vaccines were safe and significantly increased the titers of antibody-dependent cell-mediated cytotoxicity (ADCC)-mediating antibodies, the titers of antibody-dependent cell-mediated phagocytosis (ADCP)-mediating antibodies, and the breadth and polyfunctionality of the HIV-specific T cell response [197]. In spite of the vaccines' robust immunogenicity however, this regimen did not elicit any protection from viral rebound [197].

DNA vaccines

Fundamentally, DNA vaccines are composed of DNA plasmids encoding antigens of interest. DNA vaccines theoretically have a number of advantages over traditional methods of vaccination: They are relatively simple to mass-produce, can be rapidly adapted to new pathogens, bypass the need for a cold chain, and can be administered multiple times without inducing anti-vector immunity. DNA vaccines also mediate the endogenous production of antigens, ensuring that the antigens are in their natural conformation with appropriate post-translational modifications, and elicit both $CD8^+$ T cell and antibody responses.

Needle and syringe injection

The earliest reports of therapeutic DNA immunization in HIV-infected subjects came from Boyer *et al* in 1997, who performed a proof-of-concept study in two chronically HIV-1-infected chimpanzees (*Pan troglodytes) [198].* One animal received DNA plasmids encoding HIV-1 env and rev, while the other received the empty plasmid vector as a placebo. Each animal received 3 immunizations of either vaccine or placebo, consisting of 100 micrograms of DNA injected into the quadriceps muscle. Post-immunization, the vaccinated animal demonstrated enhanced antibody responses to HIV-1 env and reduced viremia to below the limit of detection, while humoral immunity and viral loads in the control animal remained unchanged [198]. Notably, these results were repeatable: The control animal was also later vaccinated and exhibited a similar increase in humoral immunity and reduction of viremia to undetectable levels [198]. Overall, although this study only consisted of two subjects, the results provide critical evidence for the viability of DNA vaccines as an HIV cure strategy.

This initial success prompted the first human trial of an HIV DNA vaccine, published by MacGregor *et al* in 1998. This study enrolled 15 ART-untreated, asymptomatic HIV-1-infected individuals, who were divided into 3 groups of 5 and received 3 intramuscular injections of a DNA vaccine encoding HIV env and rev, at either 30, 100, or 300 micrograms (μg) per immunization [199]. Vaccination was safe and well-tolerated at all three doses, and immunogenic at 100 and 300 μg. Increases in antibody titers against gp120 were observed at both the 100 and 300 μg doses [199]. While only the 100 μg group exhibited enhanced CTL activity, the 300 μg group demonstrated increased production of MIP-1α, an HIV-1 suppressive factor [199-201]. Furthermore, none of the participants developed anti-DNA antibodies [199], putting to rest one of the primary concerns regarding the use of DNA vaccines. Although vaccination was not associated with consistent trends in $CD4^+$ T cell counts or HIV viral loads [199, 200], this study demonstrated the safety and potential immunogenicity of DNA vaccines and encouraged further investigation of this strategy.

During 1998-2005, subsequent studies of DNA vaccines delivered via intramuscular injection yielded similar results [202-206]. These vaccines were modestly immunogenic at best, and did not significantly reduce viremia, with the notable exception of a Phase 1 clinical trial published by MacGregor *et al* in 2005. In this double-blinded, placebo-controlled study, asymptomatic, ART-treated, HIV-1-infected individuals were vaccinated with DNA plasmids expressing gag/pol and env/rev [207]. In addition to being safe and well-tolerated, the vaccine was comparatively immunogenic, eliciting HIV-specific IFN-γ responses in 8 of 13 vaccinated participants [207]. Remarkably, only 1 of 13 vaccinated participants experienced "viral blips," or transient, detectable elevations of HIV RNA after vaccination, compared to 3 out of 5 participants who received a placebo [207]. The decreased frequency of viral blips in vaccinated participants in this study affirms the therapeutic potential of DNA vaccines and substantiates the idea that augmenting HIV-specific cellular immunity could lead to control of viral replication.

While there is considerable evidence of DNA vaccines' therapeutic potential, the lack of efficacy across these early trials makes it clear that DNA vaccines alone are likely insufficient to elicit a functional cure for HIV. Continuing work in the field is focused in large part on improving vaccine immunogenicity through improved methods of delivery and novel adjuvants.

Biojector

Studies undertaken to improve the physical methods of vaccine delivery aim to increase the uptake of DNA by antigen presenting cells (APCs). One device that could improve DNA vaccine immunogenicity through more efficient delivery of vaccine plasmids into cells is the Biojector 2000. The Biojector is a needle-free injection device that uses compressed carbon dioxide to eject fluid through a small opening to the skin or muscle [208-211].

In 2013, Graham *et al* published the results of a phase I clinical trial utilizing the Biojector to deliver a prophylactic DNA vaccine consisting of four plasmids expressing a fusion of HIV subtype B gag/pol/nef and env from HIV subtypes A, B, and C into the muscle [209]. This study aimed to elicit protection from HIV infection by inducing immune responses against a broad range

of HIV env subtypes. Notably, the magnitude of the IFN-γ response was approximately 3-fold higher in individuals who were vaccinated with the Biojector, compared to individuals who were vaccinated with a needle and syringe [209]. Similarly, Env-specific antibody responses were about 10-fold higher in the Biojector group than in the needle and syringe group [209]. Although not a therapeutic vaccine, this study crucially demonstrated that the physical method of vaccination could greatly affect DNA vaccine immunogenicity.

Building upon this success, Rosenberg *et al* tested this same therapeutic vaccine delivered intramuscularly via Biojector, in a phase I/II study of individuals who initiated ART during the early/acute phase of HIV infection [211]. This study sought to address whether DNA vaccines would be more immunogenic in subjects who initiated ART early, compared to subjects who initiated ART during chronic HIV infection. Theoretically, people who initiated ART early would have less immune dysfunction and be more likely to respond to vaccination, compared to subjects who initiated ART during chronic HIV infection. Contrary to this hypothesis, vaccination did not induce strong cellular immune responses, and there were no differences in viral load between vaccine recipients and placebo recipients during treatment interruption [211].

It is important to note that despite the lack of success seen in the phase I/II clinical trial, the Biojector still improved immune responses to DNA vaccines in a large number of studies, across a wide range of diseases. It is possible that greater efficacy would have been observed if the DNA vaccine had been co-delivered with an adjuvant, or if different immunogens had been chosen. Additional studies should be pursued to thoroughly evaluate the Biojector as a method of therapeutic DNA vaccine delivery.

Dermavir

Vaccines are most commonly delivered via intramuscular injection, although there are relatively few APCs present in muscle tissue. In comparison, the epidermis and dermis are highly populated by APCs, including monocytes, macrophages, and epidermal dendritic cells, also known as Langerhans cells [212, 213]. It is thought that dermal or epidermal delivery of DNA

vaccines will result in uptake of vaccine DNA by Langerhans cells, which then traffic to the draining lymph nodes and stimulate maturation of naïve T cells into cytotoxic T lymphocytes, thus resulting in improved vaccine immunogenicity [214]. Thus, DermaVir was designed to improve DNA vaccine immunogenicity by directly delivering DNA to the skin. DermaVir consists of plasmid DNA encoding nearly the entire HIV genome (excluding integrase) mixed with polyethylenimine mannose and applied topically to exfoliated skin via a patch [215].

This method of vaccination was initially tested in SIV-infected rhesus macaques during chronic infection and AIDS with and without ART. When administered in combination with ART, Dermavir enhanced SIV-specific T cell responses and improved control of viral replication during treatment interruption [216]. Importantly, these results indicated not only that topical vaccination was feasible, but also that therapeutic vaccines could be immunogenic and effective even in immune compromised hosts.

Following the success of this NHP trial, the GIHU004 phase I clinical trial of DermaVir was conducted in HIV-infected individuals on ART [217]. Volunteers received either a low (0.1 mg of DNA), medium (0.4 mg of DNA), or high (0.8 mg of DNA) dose of the vaccine or a placebo [217]. In accordance with studies in animal models, vaccination with DermaVir was safe and well-tolerated, with no serious adverse events reported at any of the doses tested [217]. The medium dose elicited the most robust cellular immunity in comparison to the low dose, high dose, and placebo groups, although these responses decreased by greater than 10-fold in 11 months [217].

A larger clinical study composed of 36 HIV-infected, ART-treated men and women receiving either a low (0.1 mg of DNA), intermediate (0.4 mg of DNA), or high (0.8 mg of DNA) dose of the vaccine or a placebo reported similar results [218]. The vaccine was well tolerated, with no dose-limiting adverse effects [218]. The intermediate dose was the most immunogenic, while DermaVir vaccination was overall associated with a trend towards greater HIV-specific T cell responses, regardless of dose [218]. Although these initial results were certainly

encouraging, these trials were limited to a small number of participants and did not include a treatment interruption to assess therapeutic efficacy.

Another clinical trial of DermaVir attempted to improve upon the original vaccine regimen through co-administration of an immunomodulator, hydroxyurea [219]. A major concern in HIV therapeutic vaccine design is that enhancement of virus-specific $CD8^+$ T cell responses may also drive activation of virus-specific $CD4^+$ T cells, thereby increasing potential targets of infection. Thus, co-administration of a therapeutic vaccine and an immunomodulator such as hydroxyurea could augment virus-specific $CD8^+$ T cell responses while suppressing $CD4^+$ T cell activation. To test this hypothesis, HIV-infected subjects on ART were vaccinated with DermaVir alone or in combination with hydroxyurea [219]. Interestingly, subjects vaccinated with DermaVir, but not DermaVir and hydroxyurea, exhibited increased HIV-specific IFN-γ responses in comparison to subjects who received a placebo [219]. However, neither vaccine group exhibited reduced viral replication during treatment interruption [219].

Particle-mediated epidermal delivery (PMED)

Other vaccine delivery platforms, such as particle-mediated epidermal delivery (PMED, or gene gun), seek to combine epidermal immunization with more efficient delivery of vaccine plasmids into cells. Originally designed to genetically modify plants, the gene gun uses compressed air to propel DNA-coated gold particles into the epidermis [220]. These particles penetrate into the cell nucleus, resulting in much more efficient DNA transduction compared to intramuscular injection with a needle and syringe or intradermal administration with the Biojector [221]. Studies in mice during the early 1990s showed that the gene gun was a viable DNA vaccine platform [222, 223]. Shortly thereafter, a number of studies in nonhuman primates using a variety of DNA vaccines for pathogens including SIV [224, 225], influenza [225, 226], and hepatitis B [225, 227], demonstrated that the gene gun was a viable and effective method of DNA vaccine delivery in large animal models. By 2000, a clinical trial of a hepatitis B DNA vaccine delivered via gene gun demonstrated the safety and efficacy of this technology in humans [228]. Although

the gene gun has yet to be utilized in human clinical trials of therapeutic HIV vaccines, it has been used in therapeutic SIV vaccine studies in nonhuman primates, with notable success.

First, Fuller *et al* used the gene gun to test a DNA vaccine consisting of 19 $CD8^+$ T cell epitopes inserted into the hepatitis B core antigen, in an effort to expand SIV-specific $CD8^+$ T cell breadth and limit immune escape [229]. Rhesus macaques were infected with SIVB670 and treated with PMPA two weeks after infection; macaques that responded to ART were then vaccinated [229]. This DNA vaccine regimen increased SIV-specific CTL breadth, and 17/20 vaccinated macaques controlled viral rebound for 7 months after ART interruption, in comparison to 0/4 macaques in the placebo group [229]. Importantly, this vaccine regimen induced robust $CD8^+$ T cell immunity and antiviral efficacy in the absence of any adjuvants and was the first to demonstrate the viability of a therapeutic DNA vaccine for a pathogenic AIDS virus delivered via gene gun.

A follow-up study by Fuller *et al* sought to improve upon these results by enhancing immunity in the gut mucosa, an important anatomical reservoir of HIV-infected cells and a major site of HIV replication [80]. To this end, rhesus macaques were infected with SIVB670 and treated with ART (PMPA and FTC) starting at 6 weeks post-infection [80]. Animals were then vaccinated with an SIV DNA vaccine co-formulated with a plasmid encoding the heat-labile *E. coli* enterotoxin, LT [80]. Notably, animals receiving the DNA vaccine and LT adjuvant exhibited a substantial 2-4 log fold reduction in mean viral burden in both the gut mucosa and blood, in comparison to mock-vaccinated controls [80]. Furthermore, 5/7 animals in the DNA vaccine and LT adjuvant group controlled virus replication after treatment interruption, compared to 0/4 animals in the mock-vaccinated group[80]. Protection from viral rebound was associated with increased IFN-γ responses in the blood and gut, SIV-specific $CD8^+$ T cells expressing dual effector functions in the blood, and a broader specificity in the mucosal T cell response [80]. Importantly, these results suggest that the quality or breadth of the virus-specific response, as opposed to the magnitude, may be crucial for containment of viral replication in the mucosa.

Altogether, the gene gun remains an exciting prospective vaccine platform, although its clinical use has been limited. Recent efforts made by Orlance, Inc. to develop a more clinic-friendly version of the gene gun represent an important step forward in the continued development of this technology.

Electroporation (intramuscular and intradermal)

Another method used to increase the uptake of vaccine DNA by APCs is electroporation (EP). EP has been used for decades to increase transformation efficiency of cells *in vitro and* works through two complementary mechanisms: The electric pulse generated by the electrodes permeabilizes the cell membrane and also results in electrophoresis of the negatively charged DNA into the cell.

In 2000, Widera *et al* were the first to demonstrate that EP enhanced immune responses to both a weakly immunogenic hepatitis B DNA vaccine and a highly immunogenic HIV gag DNA vaccine in mice [230]. Crucially, these experiments showed that cellular uptake of DNA was a significant barrier to DNA vaccine efficacy, and that EP could further increase immune responses towards an already immunogenic vaccine [230]. Shortly afterward in 2004, Otten *et al* demonstrated the utility of this technology in nonhuman primates, showing that EP enhanced the magnitude and duration of both antibody and cell-mediated immune responses to an HIV DNA vaccine in rhesus macaques [231]. These results were corroborated by additional studies done by Otten *et al*, Luckay *et al,* and Cristillo *et al* [232-234].

By 2011, EP was beginning to be used in human clinical trials. That year, Vasan *et al* used intramuscular EP to deliver a multigenic HIV-1 DNA vaccine, ADVAX, to healthy volunteers in a phase I, double-blinded, randomized, placebo-controlled trial [235]. EP was safe, well-tolerated, and increased the breadth and magnitude of HIV-specific IFN-γ responses by up to 70-fold in comparison to intramuscular injection without EP [235]. Following these promising results, Morrow *et al* were the first to use EP with a therapeutic HIV DNA vaccine, PENNVAX-B, consisting of three optimized synthetic plasmids encoding multiclade HIV Gag and Pol and a consensus

Clade B Env [236]. This phase I clinical study, published in 2015, evaluated the safety and immunogenicity of PENNVAX-B in 12 ART-treated, HIV-infected individuals. This study also found that EP was safe and well-tolerated, and there were no severe adverse effects associated with PENNVAX-B [236]. Notably, increased HIV-specific IFN-γ responses were detected in all 12 study participants, while 8/12 participants exhibited HIV-specific $CD8^+$ T cells expressing the cytolytic effectors granzyme B and perforin [236]. However, this study was not placebo-controlled [236].

Since these first studies, EP remains a useful tool for enhancing DNA vaccine immunogenicity and has been used in numerous therapeutic vaccine trials in both nonhuman primates and humans. However, further improvements to this vaccine delivery platform, such as optimizing the electrode design and electroporation protocol may be necessary to realize its full potential. Shifting from intramuscular EP to intradermal EP could also further improve DNA vaccine potency, due to the large population of APCs present in the skin. While intramuscular EP is still much more widely used, intradermal EP has also been shown to improve cellular and humoral immune responses to DNA vaccines in rhesus macaques [237]. Current studies do not rely on EP alone to maximize vaccine immunogenicity but use it in conjunction with other interventions such as adjuvants or immunomodulators.

Vaccine adjuvants

Cytokines that enhance CTL function are of particular interest as vaccine adjuvants. One of the first cytokines tested as an adjuvant was Interleukin 2 (IL-2). IL-2 is a Th1-associated cytokine that promotes cell activation and expansion and enhances the cytolytic activity of T and NK cells. Previous work using recombinant IL-2 (rIL-2) as an HIV therapy to restore immune system function indicated that rIL-2 in combination with ART significantly increased $CD4^+$ T cell counts in HIV-infected individuals [238]. Studies in mice and rhesus macaques demonstrated that co-immunizing animals with plasmids encoding IL-2 and viral proteins enhanced antigen-specific immune responses in comparison to DNA vaccination alone [239-241]. IL-2 was also tested as

an adjuvant in several clinical trials of HIV therapeutic vaccines, although none were DNA vaccines. First, Pido-Lopez *et al* co-administered IL-2 with Remune in HIV-infected, ART-treated individuals, but saw no effect of vaccination on $CD4^+$ T cell counts or viremia [242]. Similarly, Hardy *et al* also tested co-administration of IL-2 with Remune in HIV-infected, ART-treated individuals but did not observe sustained induction of HIV-specific immunity [243]. Levy *et al* reported moderate success in a clinical trial involving HIV-infected, ART-treated individuals, who received a combination of ALVAC-HIV (vCP1433, a canarypox-vectored vaccine) and LIPO-6T (a peptide-based vaccine) with IL-2 as an adjuvant – 24% of vaccine recipients exhibited lower viral set points during treatment interruption, compared to 5% of volunteers in the control group [177]. However, a different clinical trial utilizing IL-2 as an adjuvant for another ALVAC vaccine (vCP1452) in HIV-infected, ART-treated individuals didn't observe any reduction of viremia after discontinuation of ART [244]. Furthermore, after a clinical trial in HIV-infected adults demonstrated that treatment with rIL-2 was associated with a significant number of serious adverse clinical events [245], studies of IL-2 both as a treatment for HIV and an HIV vaccine adjuvant were largely halted, and other cytokine adjuvants were explored.

A critical potentiator of cell-mediated immunity, IL-12 promotes the differentiation of naïve $CD4^+$ T cells into Th1 cells and mediates enhancement of the cytotoxic activity of NK cells and CTLs. IL-12 initially showed promise as a vaccine adjuvant in nonhuman primate models: Boyer *et al* compared the efficacy of an un-adjuvanted DNA vaccine encoding env, rev, gag, and pol, to the DNA vaccine adjuvanted with a plasmid encoding IL-12, in HIV-1-infected chimpanzees [246]. Co-immunization with the DNA vaccine and IL-12 plasmid enhanced proliferative responses to HIV-1 antigens, compared to immunization with the DNA vaccine alone [246]. Similarly, Halwani *et al* demonstrated that therapeutic vaccination of SIV-infected rhesus macaques with an SIV DNA vaccine and plasmid encoding IL-12 improved T cell polyfunctionality, increased production of IFN-γ, and enhanced SIV-specific memory immune responses [247]. These initial successes led to a randomized, placebo-controlled, partially double-blinded phase 1 clinical trial to assess

the safety, tolerability, and immunogenicity of an HIV DNA vaccine adjuvanted with IL-12 [248]. HIV-1-infected, ART-treated individuals received DNA encoding gag, pol, nef, tat, vif, and env alone or co-delivered with a plasmid encoding IL-12 at a low, intermediate, or high dose [248]. This vaccination regimen was safe and well-tolerated but did not enhance HIV-specific $CD8^+$ T cell responses, regardless of adjuvant dose [248]. However, HIV-specific $CD4^+$ T cell responses were significantly elevated in the cohort that received the DNA vaccine and low-dose IL-12, relative to the placebo group [248].

Another cytokine that has been considered for use as a vaccine adjuvant is IL-15. Similar to IL-2 and IL-12, IL-15 stimulates cytokine production by NK cells and promotes activation and expansion of T cells. However, unlike IL-2 and IL-12, IL15 also promotes the survival of memory cells, potentially making it an even more effective vaccine adjuvant due to its ability to induce long-lasting immunity. Initial studies in SIV-infected rhesus macaques demonstrated that boosting previously vaccinated animals with an SIV DNA vaccine combined with a plasmid expressing IL-15 led to further enhancement of SIV-specific $CD8^+$ effector memory T cell responses [247]. A separate study reported that therapeutic vaccination of SIV-infected rhesus macaques with an SIV DNA vaccine and IL-15 expression plasmid resulted in significant induction of SIV-specific $CD4^+$ and $CD8^+$ T cell responses, as well as a modest 1 log reduction in viremia during treatment interruption, as compared to unvaccinated control animals [249]. The first randomized, placebo-controlled, double-blinded, in human trial of a vaccine co-delivered with an IL-15 plasmid cytokine adjuvant was conducted in HIV-1 uninfected adults, who received a DNA vaccine encoding HIV gag along with a plasmid encoding IL-15 at low, intermediate, or high concentrations [250]. In contrast to what was previously observed in animal models, cellular immunogenicity among human vaccinees was poor, with only 11.5% of those receiving gag DNA and IL-15 exhibiting immune responses to gag, irrespective of what concentration of adjuvant they received [250]. Inclusion of the IL-15 plasmid did not increase the percent of vaccine responders, compared to immunization with gag DNA alone [250]. Notably, this study also assessed the

effects of co-immunization with gag DNA and a plasmid expressing IL-12 at low, intermediate, or high concentrations, and determined that the group receiving gag DNA and an intermediate dose of IL-12 exhibited the greatest percentage of vaccine responders, at 44.4% [250].

In summary, despite the initially encouraging results obtained in the rhesus macaque model, co-administration of DNA vaccines and cytokine adjuvants did not significantly enhance HIV-specific $CD8^+$ T cell responses in human clinical trials. It is possible that co-administration of cytokines had unforeseen downstream effects leading to upregulation of suppressive immune cells, such as T regulatory (Treg) cells or myeloid-derived suppressor cells (MDSCs) or that immune dysfunction occurring during acute HIV and SIV infection could have obscured the effects of the cytokine adjuvants. Further studies are needed to explore these possibilities.

Complementary interventions: exhaustion reversal agents

Large strides have been made in enhancing therapeutic HIV DNA vaccine immunogenicity since the earliest studies in 1997, yet significant protection from viral rebound during ATI has yet to be achieved in human clinical trials. This may be due in part to immune exhaustion caused by HIV infection. As discussed previously, immune exhaustion occurs when chronic activation of $CD8^+$ T cells by viral antigens results in upregulation of negative immune regulators, such as PD-1 or CTLA4, and a progressive loss of their cytolytic effector functions.

It follows then, that obstructing the interaction of PD-1 and its ligand, PD-L1, or blocking CTLA-4 from binding to CD80 on APCs, could prevent inhibition of immune responses and boost HIV-specific immunity. In fact, the PD-1 blockade strategy is already being used as part of the standard therapy for several cancers [251], and a combination of CLTA-4 and PD-1 blockers significantly enhanced anti-tumor immune responses in metastatic melanoma patients over many clinical trials [252]. In further support of this concept, SIV-infected rhesus macaques that received an infusion of α-PD-1 antibodies exhibited improved cellular and humoral immunity, decreased viral loads, and prolonged survival [95, 97]. Another recent study, published by Harper *et al* in 2020, demonstrated that CTLA-4 and PD-1 immune checkpoint blockade was significantly more

effective than PD-1 blockade alone in enhancing T cellular immunity in SIV-infected, ART-treated rhesus macaques [253]. Dual blockade of PD-1 and CTLA4 even resulted in robust latency reversal and reduced total levels of integrated virus, although this degree of reservoir clearance was not sufficient to attain viral control [253]. Notably, these results were achieved in the absence of other interventions. It is possible that co-administration of a therapeutic vaccine or neutralizing antibodies could greatly enhance the virological benefit of immune checkpoint blockade, and it is clear that these results warrant further study.

Discussion

All in all, progress towards an effective therapeutic vaccine for HIV has been modest, despite considerable advancements in both vaccine technology and our understanding of HIV pathology. One of the most successful HIV therapeutic vaccines to date is a dendritic cell-based vaccine, consisting of DCs pulsed with autologous, heat-inactivated HIV and resulting in significant reductions of viremia in vaccinated participants [158]. However, vaccination did not prevent $CD4^+$ T cell depletion and control of viremia diminished over time [158]. Another promising therapeutic vaccine is Tat-BH10, consisting of a functional HIV Tat protein. Clinical trials showed that vaccination with Tat-BH10 was associated with significant restoration of $CD4^+$ T cells, reduced immune activation [141], and reduced viremia [142]. Some DNA vaccines induced potent control of viral replication or even functional cures in SIV-infected rhesus macaques [80, 216, 229], but this level of success has yet to be seen in clinical trials [217, 218]. Altogether, no therapeutic vaccine tested in clinical trials so far has been able to consistently elicit profound, durable control of HIV replication. Efforts to improve upon previous therapeutic vaccines will likely need to incorporate one or more of the following: 1) Improved vaccine delivery platforms capable of targeting of virus-specific immune responses to viral reservoir sites and major sites of viral replication, namely mucosal tissues and lymph nodes, 2) novel or combinatorial adjuvants that induce potent cell-mediated immunity, and 3) immune checkpoint inhibitors to prevent or reverse immune exhaustion.

Many previous therapeutic vaccines, including early DNA vaccines, were delivered into the muscle, which is only sparsely populated with APCs [212-214]. Newer vaccine delivery platforms such as the Biojector, Dermavir, Gene Gun, and intradermal electroporation are primarily focused on delivering vaccine plasmids into the immunocompetent skin. Importantly, epidermal and intradermal vaccination are capable of stimulating potent mucosal immune responses [80]. The gut is a major site of HIV and SIV replication [67, 69, 70], resulting in depletion gut mucosal $CD4^+$ T cells [71], loss of mucosal barrier integrity, chronic immune activation, and disease pathogenesis [37, 72]. Thus, an effective therapeutic vaccine for HIV will likely need to induce potent cytotoxic $CD8^+$ T cell immunity in both the peripheral blood and intestinal mucosa.

A number of adjuvants have been tested with therapeutic HIV vaccines, namely alum, GM-CSF, MF59, IL-2, IL-12, and IL-15 [145, 177, 192, 204, 248, 250], but an effective HIV therapeutic vaccine will likely require adjuvants capable of inducing a greater magnitude and quality of $CD8^+$ T-cells. In particular, co-administration of IL-12 and therapeutic DNA vaccines improved T cell polyfunctionality, increased production of IFN-γ, and enhanced virus-specific memory immune responses in NHP [240, 247]. Although these results were not replicable in human clinical trials [248, 250], it remains to be seen whether combining IL-12 with other cytokine adjuvants could be more effective. Recently, IL-33 was shown to augment DNA vaccine immunogenicity in mice [254, 255]. Studies indicate that IL-33 is a proinflammatory cytokine that promotes development of Th1 responses and cytotoxic $CD8^+$ T cell immunity as well as humoral immunity, making it a promising candidate genetic adjuvant [256, 257]. Combining cytokine adjuvants that promote T cell immunity and proven mucosal adjuvants, such as LT, may also enhance therapeutic vaccine efficacy by directing immune responses to primary sites of HIV replication. Additionally, preventing the interaction of negative immune regulators and their ligands could reverse HIV-associated immune dysfunction and improve therapeutic vaccine immunogenicity and efficacy. In support of this concept, CTLA-4 and PD-1 immune checkpoint

blockade was shown to be effective in enhancing T cellular immunity in SIV-infected, ART-treated rhesus macaques, even in the absence of a vaccine [253].

Finally, numerous studies demonstrated the feasibility of using therapeutic vaccines to enhance control of viral replication in the absence of ART, although efficacy in nonhuman primate models for AIDS rarely exceeds about 50%. This suggests potential interference from unidentified, pre-existing host factors. Notably, the gut mucosa remains a vastly understudied compartment in the context of therapeutic vaccination, despite being a major site of HIV replication and pathogenesis. Devising an effective HIV therapeutic vaccine will likely require a more complete understanding of what host immune parameters, particularly in the gut mucosa, affect vaccine immunogenicity and efficacy.

Chapter 2: The effects of an SIV therapeutic DNA vaccine and novel adjuvant combination and pre-infection determinants of control of viral rebound

Part 1: Immunogenicity of an SIV therapeutic DNA vaccine and novel adjuvant combination

Introduction

ART greatly reduces HIV replication and restores $CD4^+$ T cell counts, thus preventing progression to AIDS and prolonging the lifespan of people living with HIV [258]. However, ART alone is unable to eliminate the latent viral reservoir, which necessitates strict lifelong adherence to a daily ART regimen [259]. For most individuals, ART interruption will lead to a resurgence in viral replication within weeks [260]. However, continuous usage of ART can be prohibitively expensive and may result in side effects that discourage compliance [261, 262]. Furthermore, ART cannot fully reverse the immune dysfunction induced by HIV, particularly in the gut mucosa, that drives chronic immune activation and disease pathogenesis [263, 264]. Thus, although advances in ART have greatly improved the health and life expectancy of people living with HIV, a vaccine or cure for HIV is still urgently needed, especially for people in developing countries that are most affected by this pandemic.

To this end, many cure strategies are in development, including therapeutic HIV vaccines designed to enhance virus-specific T-cellular and humoral immune responses to provide immune control of virus replication after stopping ART. Numerous therapeutic HIV vaccines have been tested, both in the SIV/SHIV nonhuman primate (NHP) model and in human clinical trials, including protein subunit [143, 149], live-attenuated [128], dendritic cell [158], viral vectored [265, 266], and DNA vaccines [80, 250, 267]. Unfortunately, none of these approaches have resulted in durable control of viremia in human clinical trials and immunotherapies in NHP have, at best, achieved approximately 50% efficacy [268-270]. This suggests that inherent host factors may impact the efficacy of therapeutic interventions but, to date, there is an incomplete understanding of what these factors are.

The gut is a major site of HIV and SIV replication [67, 69, 70], resulting in depletion and functional alteration of gut mucosal $CD4^+$ T cells and loss of antigen-presenting cells and innate lymphocytes [71]. These events contribute to structural damage of the gastrointestinal (GI) tract and systemic translocation of GI microbial products that drives chronic immune activation and

disease pathogenesis [37, 72]. A previous study published by this lab showed in the rhesus macaque model that an SIV DNA vaccine expressing SIV Gag, RT, Nef and Env and co-delivered with a plasmid expressing a mucosal adjuvant, the heat-labile *E. coli* enterotoxin (LT) by particle-mediated epidermal delivery (PMED or gene gun), induced durable protection from viral rebound and disease progression after ART withdrawal in approximately 60% of animals. In that study, Gag-specific mucosal T cell responses in the vaccinated animals significantly correlated with reduced viremia [80], suggesting that SIV-specific T cell responses in the gut are important for controlling viral rebound. To further improve therapeutic efficacy, I therefore tested a new multiantigen SIV DNA vaccine (MAG) expressing Gag, Pol and Env, delivered by intradermal electroporation with a novel combination of adjuvants designed to increase both mucosal and systemic immunogenicity in SIV-infected rhesus macaques. Our adjuvant combination (AC) consisted of co-delivered DNA plasmids encoding the catalytic subunit of LT (LTA1), the cytokines IL-12 and IL-33, the enzyme retinaldehyde dehydrogenase 2 (RALDH2), soluble PD-1 (sPD-1), and soluble CD80 (sCD80). LTA1 is a potent adjuvant that performs similarly to LT, through the recruitment and activation of dendritic cells [271, 272]. The IL-12 adjuvant has been widely used in both NHP and human clinical trials [273, 274] and promotes differentiation of naïve $CD4^+$ and $CD8^+$ T cells to Th1 and cytotoxic T lymphocytes (CTLs), respectively. IL-33 has also been shown to augment vaccine immunogenicity in mice [254, 275], and works by directly promoting the activity of Th1 cells and CTLs [257, 276]. RALDH2 has previously been used as an adjuvant to enhance mucosal vaccine immunogenicity in mice [277], and was included to enhance mucosal vaccine immunogenicity through the conversion of retinaldehyde to retinoic acid, the molecule responsible for inducing the expression of the mucosal homing factors CCR9 and $\alpha 4\beta 7$ on activated lymphocytes [277, 278]. Finally, previous studies showed that blocking the PD-1 and CTLA-4 pathways can enhance antigen-specific immunity, reduce immune activation, and reverse immune exhaustion [95, 279]. I therefore co-delivered plasmids expressing rhesus sPD-

1 and sCD80 to block the interaction of $CD8^+$ T cells expressing PD-1 and CTLA-4 with antigen-presenting cells (APCs) expressing PDL-1 and CD80.

Our results show that the vaccine delivered with the adjuvant combination (MAG + AC) significantly increased IFN-γ T cell responses in the blood but not in the gut-associated lymphoid tissue (GALT), and did not achieve a significant improvement in viral control during analytic treatment interruption (ATI, discontinuation of ART) when compared to mock-vaccinated controls. However, a subset of animals among all groups maintained low viremia during ATI, providing an opportunity to investigate immune correlates of this prolonged resistance to viral rebound. Our results show that animals that controlled viral rebound (controllers) during ATI exhibited higher polyfunctional SIV-specific $CD8^+$ T cells in the mesenteric lymph nodes (MLN) and blood. Importantly, increased colonic $CD4^+$ T cells and lower Th17/Treg ratios pre-infection correlated with improved response to ART and lower viral burden during ATI. Together, these data provide new evidence that the state of the mucosal immune system before infection may influence an individual's response to ART and their ability to develop and maintain mucosal and systemic $CD8^+$ T cell responses that can contribute to control of viral rebound during ATI.

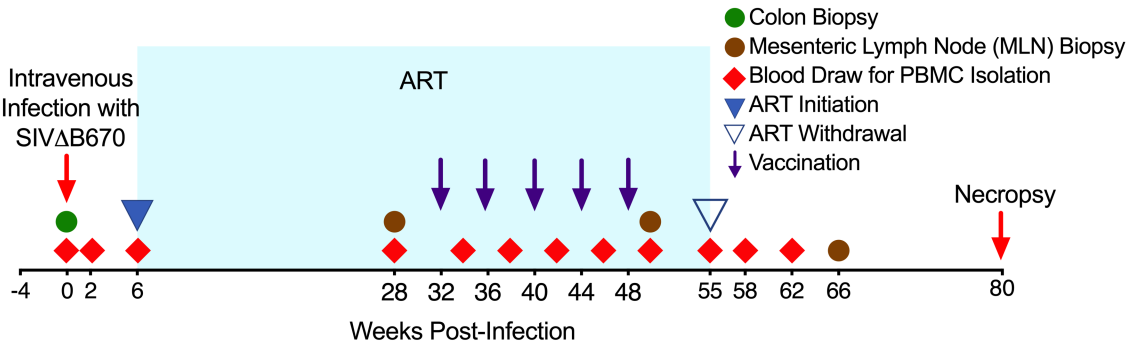
Results

NHP study design

Rhesus macaques were intravenously infected with SIVΔB670, a highly pathogenic, primary isolate that induces AIDS in most rhesus macaques within 5-17 months of infection [280]. This strain was chosen because the 15% divergence between the Env consensus sequence of the SIVΔB670 inoculum and the SIV/17E-Fr Env sequence in the vaccine mimics therapeutic vaccination of humans infected with diverse variants of a given HIV subtype [80, 229, 280].

At six weeks post-infection (wpi), animals began ART, consisting of emtricitabine (FTC), tenofovir (PMPA), and Raltegravir, administered daily. Starting at 32 wpi, the macaques in the vaccine groups received a series of 5 DNA immunizations, spaced 4 weeks apart (Fig 1). Prior to initiating therapeutic immunizations, I stratified the animals so that each group had comparable

levels of plasma viremia and blood $CD4^+$ T cell counts (S1 Fig) during acute infection and ART to help balance the effects of pre-existing virological and host factors among all groups.



	Vaccine	Adjuvants	Immunomodulators	Administration Method
Mock	Noncoding DNA	None	None	Gene Gun
MAG + LT	SIV Gag-Pol-Env + p57 Gag	LT	None	Gene Gun
MAG + AC	SIV Gag-Pol-Env + p57 Gag	LTA1 + IL-12 + IL-33 + RALDH2	sPD-1 + sCD80 (2nd - 5th vaccinations)	Intradermal Electroporation

Fig 1. Therapeutic Vaccine Study Design & Response to Antiretroviral Therapy. Indian origin rhesus macaques were infected with SIVΔB670 at week 0 (red arrow) and were treated with ART starting at 6 weeks post-infection (wpi). Purple arrows indicate a series of 5 DNA immunizations spaced 1 month apart, occurring between 32 wpi and 48 wpi. At week 55, ART was interrupted to assess the efficacy of the therapeutic vaccine on viral control. Animals were necropsied at 80 wpi or earlier in the presence of AIDS-defining conditions. Red triangles indicate blood draws for PBMC isolation and brown circles indicate MLN biopsies to measure systemic and gut-associated immune responses. Prior to administering therapeutic immunizations, macaques were stratified so that each group had comparable viral loads and $CD4^+$ T cell counts prior to and during ART.

The control group (N = 4) received mock DNA immunizations via PMED consisting of the vaccine plasmid backbone, without SIV antigens or adjuvants. The MAG + LT group (N = 5) received the MAG vaccine, a plasmid that encodes SIV/17E-Fr Gag-Pol-Env and expresses virus-like particles [281] co-delivered with plasmids encoding SIV/17E-Fr p57 Gag and the LT adjuvant previously shown to induce mucosal T cell responses [80, 272], also via PMED. A PMED group was included as a comparator because of its previously demonstrated therapeutic efficacy with another DNA vaccine [15]. The MAG vaccine was supplemented with an additional plasmid encoding p57 Gag based on evidence from both human and NHP studies that Gag-specific T cell responses are crucial for control of viral replication [79, 80, 282-286]. Additionally, our previously published therapeutic vaccine study demonstrated that SIVΔB670-infected rhesus macaques vaccinated with a Gag expression plasmid within the context of a multi-epitope SIV DNA vaccine durably controlled viral rebound after stopping ART and consistently demonstrated elevated Gag-specific $CD8^+$ T cell responses [229].

The MAG + AC group (N = 5) received DNA immunizations via intradermal injection followed by electroporation. For the first vaccination, this group of animals received the MAG DNA vaccine co-formulated with plasmids expressing the adjuvants LTA1, IL-12, IL-33, RALDH2. For the subsequent four vaccinations, each macaque in this group received the same vaccine and adjuvants, but also received a plasmid co-expressing sPD-1 and sCD80.

ART reduces viremia and restores $CD4^+$ T cells in the periphery

To provide potent suppression of viral replication during ART, I employed a combination of three antiretrovirals: the integrase inhibitor, Raltegravir, and the non-nucleoside reverse transcriptase inhibitors FTC and PMPA that were previously shown to effectively suppress SIV infection in rhesus macaques [287]. However, impaired kidney function due to prolonged treatment with PMPA necessitated a switch over to tenofovir dispoproxil fumarate (TDF) in four animals: A16144 and A16145 in the MAG + LT group, A16149 in the mock vaccine group, and A16237 in the MAG + AC group. A16144 was switched at 48 wpi, A16145 at 51 wpi, A16149 at

47 wpi, and A16237 at 41 wpi. The switch from FTC to TDF did not cause any notable changes in the level of suppression of viral replication in these animals.

Prior to starting the immunizations at week 32, 12/14 animals responded well to ART, reaching a significant 2.75-4.70 log-fold reductions in median viral loads prior to vaccination ($P < 0.0001$, S1 Fig). Among these, 9/14 animals exhibited undetectable levels of viremia prior to vaccination ($\leq$30 viral copies per mL of plasma), although low levels of transient viral replication during ART were detected in all ART-responders. During acute infection, animals experienced a rapid $CD4^+$ T cell decline that was significantly restored after initiation of ART ($P = 0.040$, S1 Fig). Two animals did not reach our criteria for effective viral suppression on ART (A16234 in the mock vaccine group and A16236 in the MAG + AC group, S1 Fig), maintaining median viral loads greater than 10^4 viral copies per mL of plasma. However, these animals still showed a substantial two log-fold decrease in their plasma viremia during ART and did not experience disease progression prior to ATI. Notably, our previous studies indicate that SIVΔB670 appears to be more resistant to ART [80, 229] than other SIVs commonly used as inoculums in NHP studies, namely SIVmac251 [288, 289] and SIVmac239 [290, 291]. This inherent ART resistance likely contributed to persistent viral replication during ART in 2 out of 14 animals. However, the levels of viral suppression on ART in these animals were superior to our previous therapeutic studies utilizing SIVΔB670, including two studies that reported a significant impact of therapeutic vaccination on viral control in ART responders [80, 229]. This lab's previous therapeutic vaccine studies with SIVΔB670 reported highly variable responses to ART consisting of one or two antiretroviral drugs, with 40-50% of macaques exhibiting no decrease in viral load during ART [80, 229]. Here, using a more potent triple drug combination, 14/14 animals exhibited at least a 3-log decrease in viral load within 4 weeks of ART initiation that was maintained for the duration of ART.

Therapeutic vaccination with MAG + AC increases SIV-specific IFN-γ T cellular responses

To assess the impact of therapeutic vaccination on antibody responses, levels of SIV gp130-specific IgG were measured by ELISA. Peak antibody titers developed after the first vaccine dose (34 wpi) in the MAG + LT animals and after the second dose (38 wpi) in the MAG + AC group, but antibody responses declined by two weeks after the third dose (Fig 2A). No differences in antibody titer were observed between the MAG + LT and MAG + AC groups.

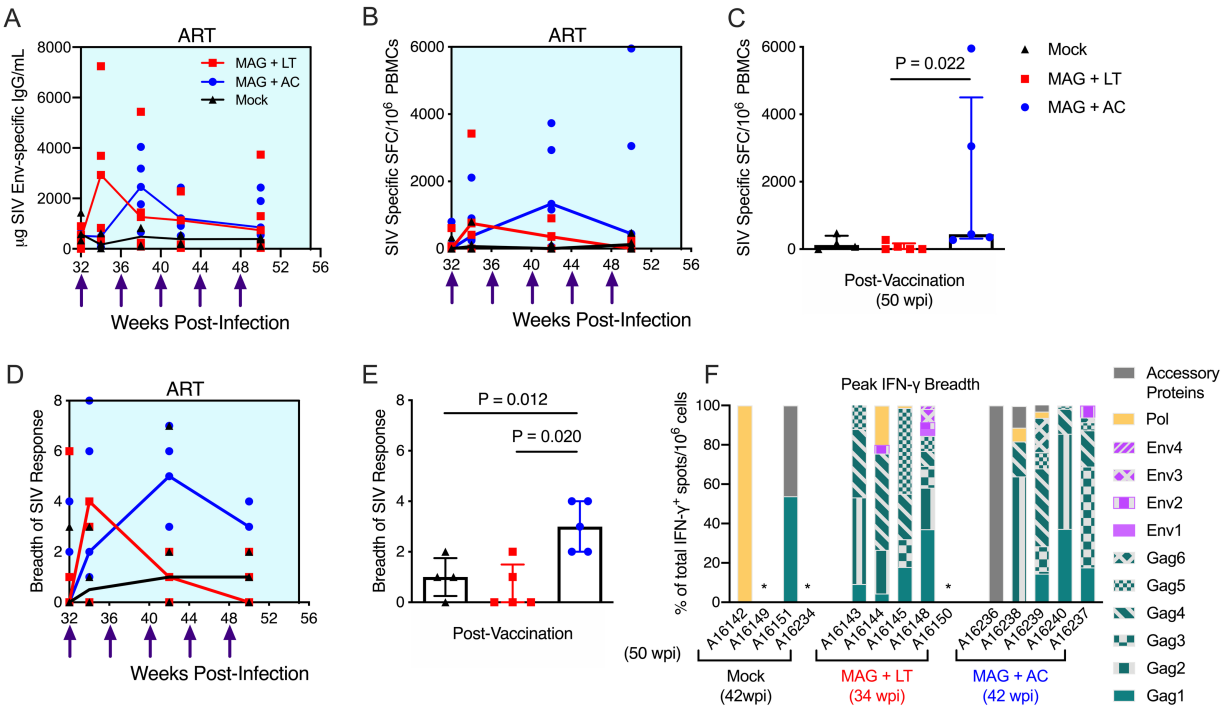


Fig 2. DNA vaccination increases Env-specific antibody responses and total SIV-specific IFN-γ T cell responses during ART. (A) The magnitude of the SIV Env-specific IgG response in the plasma was measured by ELISA, using SIV gp130 as the capture antigen. Shown are medians with individual animals' data layered over. **(B-C)** PBMCs were stimulated with Gag, Env, Pol, Vif, Vpr, Rev, Nef, and Tat peptides to quantify the SIV-specific IFN-γ response. Samples were considered positive if peptide-specific responses were at least twice that of the negative control plus at least 0.01% after background (DMSO) subtraction. **(B)** Shown are medians of the cumulative (sum of response against all peptides) IFN-γ response, with data from individual animals layered over. **(C)** The cumulative SIV-specific IFN-γ response is shown after 5 vaccinations (50 wpi). Shown are medians and interquartile ranges with individual responses layered over each bar. **(D)** The breadth of the SIV-specific IFN-γ response is the number of peptide pools with a positive IFN-γ response. The cumulative breadth of the SIV-specific IFN-γ response post-vaccination (50 wpi) is shown, with medians and interquartile ranges of each group depicted, with individual animals' data layered over each bar. **(E)** The cumulative breadth of the SIV-specific

IFN-γ response post-vaccination (50 wpi) is shown as medians and interquartile ranges with individual responses layered over each bar. **(C, E)** A Dunn's multiple comparisons test was used when making multiple comparisons between vaccine groups and the mock group. Results are considered significant if $P \leq 0.05$. Shown are the medians and individual responses layered over each timepoint. **(F)** The percent of the IFN-γ response specific for Gag, Env and accessory proteins was calculated from the cumulative IFN-γ response at peak breadth (34 wpi for MAG + LT, 42 wpi for MAG + AC and Mock). Asterisks indicate no detectable response.

To determine the impact of the therapeutic vaccines on T cell responses, SIV-specific T cells producing IFN-γ in response to stimulation with peptide pools spanning SIVmac239 $p57^{Gag}$ (Gag), gp130 (Env), Pol, Vif, Vpr, Rev, Nef, and Tat were measured by ELISpot. (These experiments were performed by a previous member of the lab, Dr. Paul Munson, although the conclusions presented here are my own.) While the SIVmac239 peptide pools are not a precise match for the SIVΔB670 inoculum or the SIV/17E-Fr strain used to construct the DNA vaccine, the genetic diversity between the three viruses resembles the intraclade diversity observed for HIV-1 [280] and thus are relevant in the context of human HIV-1 infection. However, I could not rule out the possibility that some vaccine or challenge strain-specific IFN-γ responses were not detected in our T cell assays due to these differences. The median SIV-specific IFN-γ response in the MAG + LT group peaked after the first vaccine dose, but declined afterwards despite additional vaccinations (Fig 2B). In contrast, the median IFN-γ response in the MAG + AC animals increased with each vaccine dose up to the third (Fig 2B), resulting in significantly higher responses compared to the MAG + LT group two weeks after the fifth and final DNA vaccine dose ($P = 0.022$, Fig 2C). This is consistent with our previous study, where a PMED-delivered therapeutic DNA vaccine administered over six doses induced peak IFN-γ responses after the third dose [15]. It was not possible to distinguish between virus-stimulated and vaccine-induced immune responses from these experiments. However, SIV-specific IFN-γ immune responses were mostly undetectable prior to immunization (32 wpi), suggesting that most animals did not have these responses prior to vaccination. I thereby conclude that the increase in SIV-specific immunity detected post-vaccination can be attributed to the vaccine.

The breadth of the IFN-γ T cell response was also measured with IFN-γ ELISpot experiments performed by Dr. Munson, where T cell responses were mapped against pools of overlapping peptides (15-mers overlapping by 11 amino acids) spanning SIVmac239 Gag, Env, Pol, Vif, Vpr, Rev, Nef, and Tat, and defined as the number of peptide pools eliciting an IFN-γ response. In the MAG + LT group, the breadth of the IFN-γ T cell response peaked after the first

vaccine dose (34 wpi) and subsequently declined, while the breadth of the MAG + AC group peaked two weeks after the third dose (42 wpi, Fig 2D). Following the final vaccine dose (50 wpi), the MAG + AC vaccine group sustained significantly greater breadth in T cell responses than both the MAG + LT group (P = 0.020, Fig 2E) and the mock-vaccinated group (P = 0.012, Fig 2E). Interestingly, during the peak IFN-g T cell response post-vaccination, the response was predominantly directed towards Gag in both vaccine groups, with up to 100% of the IFN-g response targeting Gag sequences in both the MAG + LT and MAG + AC groups. Responses to Pol were detected in two out of five animals in both the MAG + LT and MAG + AC groups, accounting for up to 20% of the total IFN-γ response (Fig 2F). Env-specific responses were detected in two out of five animals in the MAG + LT group and one out of five animals in the MAG + AC group, with up to 15% of the total IFN-γ response targeting Env. SIV-specific IFN-γ responses were not observed in one animal in the MAG + LT group (A16150), while one animal in the MAG + AC group (A16236) only exhibited IFN-γ responses to accessory proteins, indicating vaccination had no effect on the SIV-specific IFN-γ response in 1/5 animals in each vaccine group. In contrast, IFN-γ responses in the mock-vaccinated group were only detected in two out of four animals and predominantly targeted either Pol or accessory proteins (Vif, Rev and Nef), with Gag-specific responses detected in only one animal (Fig 2F).

Env- and Gag-specific T cell responses were further characterized in the PBMC and MLN for effector functions, including secretion of IFN-γ, TNF-α, and IL-2, and co-expression of CD107a/Granzyme B as markers of cytolytic function by intracellular cytokine staining (ICS) and flow cytometry. In contrast to the IFN-γ ELISpot assay, that measures the SIV-specific IFN-γ response as a whole in bulk PBMCs, here $CD4^+$ and $CD8^+$ T cell effector functions were assessed separately. There were no significant differences between the three groups in these functions in either the PBMC or MLN (S2-S5 Figs), although the MAG + AC group demonstrated a trend towards higher cumulative IFN-γ^+ $CD4^+$ and $CD8^+$ T cells in the PBMC (S6 Fig). This trend was not statistically significant, likely due to the significant differences in the populations of cells

analyzed by each assay – The ICS analysis consisted of only $CD8^+$ T cells, whereas the ELISpot experiments did not differentiate between $CD4^+$ and $CD8^+$ T cells or other lymphocytes. However, the similar trends observed in our ICS analysis affirms that our ELISpot results are representative of the overall IFN-γ^+ T cell response.

To further characterize the SIV-specific $CD8^+$ T cell response post-vaccination, I assessed the magnitude of the polyfunctional $CD8^+$ T cell response. Here, I define polyfunctionality as the frequency of T cells specific for either Gag or Env and expressing any three or more of the cytokines IFN-γ, TNFα, IL-2, and/or co-expressing the cytolytic markers CD107a/Granzyme B. Three out of five animals in the MAG + AC group exhibited increases in SIV-specific polyfunctional $CD8^+$ T cells post-vaccination in both PBMC and MLN, whereas these responses remained nearly undetectable in both the MAG + LT and mock-vaccinated controls. However, these results were not statistically significant (S7 Fig).

Impact of therapeutic vaccination on protection from viral rebound during analytical treatment interruption (ATI)

To determine if therapeutic vaccination improved viral control, ATI was initiated three weeks after the final vaccine dose (55 wpi), and viral loads were monitored for 6 months. Containment of median viral loads at or below 10^3 copies/mL of plasma during ATI was chosen as the primary criterion for therapeutic efficacy based on previous studies showing that rhesus macaques infected with SIVΔB670 that maintained viral loads at or below this level in the absence of ART consistently exhibit long term (>1 year) protection from progression to AIDS [281]. During ATI, 60% (3/5) of animals in the MAG + AC group maintained viremia below 10^3 RNA copies/mL of plasma for 6 months and sustained $CD4^+$ T cell counts above 50% of pre-infection levels, whereas only one animal in the MAG + LT group (1/5, 20%) and one in the control group (1/4, 25%) exhibited similar viral control and protection from disease progression (Fig 3A-C). However, these differences between groups were not statistically significant. Vaccinated and control animals that exhibited immediate viral rebound during ATI also showed significant $CD4^+$ T cell

decline during ATI (S8 Fig). Overall, there was no significant difference in protection from viral rebound or mean viral loads during ATI between the three groups (Fig 3D), an outcome that is likely due to the small group sizes coupled with variability in response to ART across all three groups.

This lab's previous therapeutic vaccine studies excluded animals with high levels of persistent viral replication on ART and observed a significant impact of therapeutic vaccination in animals categorized as ART responders (Median viral loads < 5 x 10^4 RNA copies/mL of plasma during ART) [80, 229]. Although the animals in this study demonstrated a wide range of virologic suppression on ART, all were considered ART responders based on the criteria used in the two previous therapeutic vaccine studies utilizing SIVΔB670. However, to address the possibility that the two animals with incomplete viral suppression on ART could have skewed the results, I redid the analysis with A16234 from the mock-vaccinated group and A16236 from the MAG + AC group excluded and determined that excluding these animals from analysis of vaccine efficacy did not impact the conclusions.

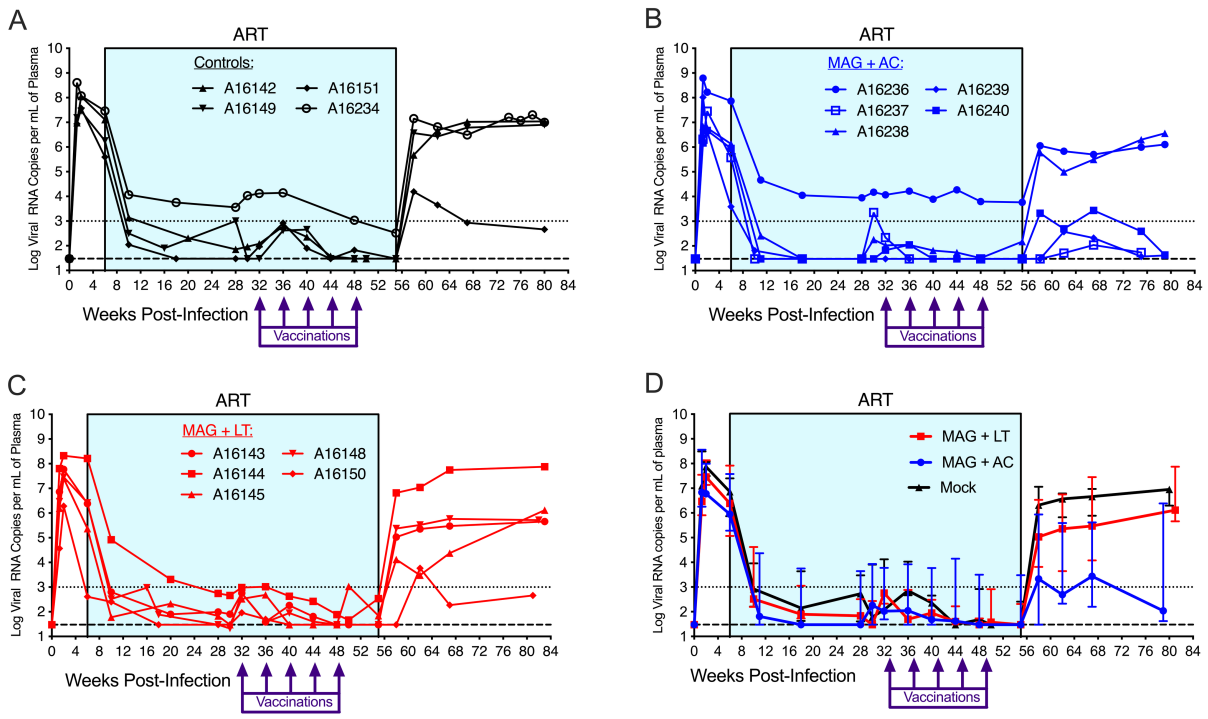
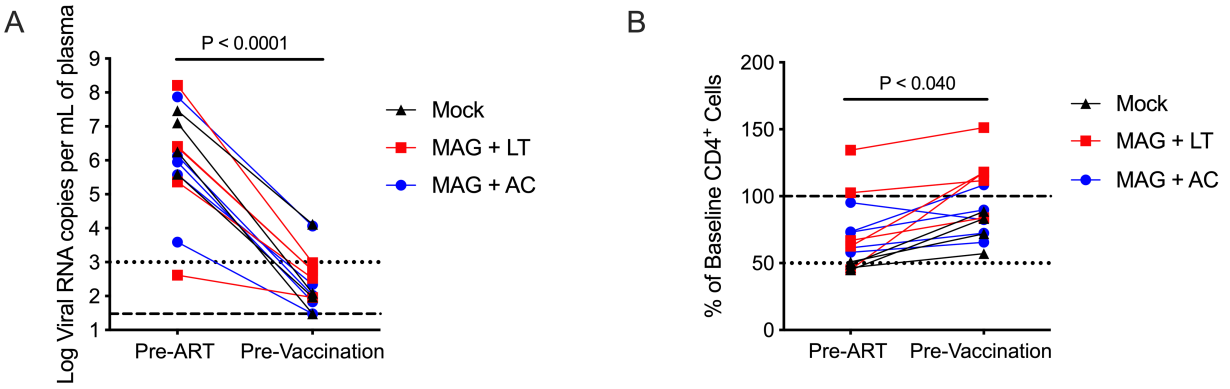
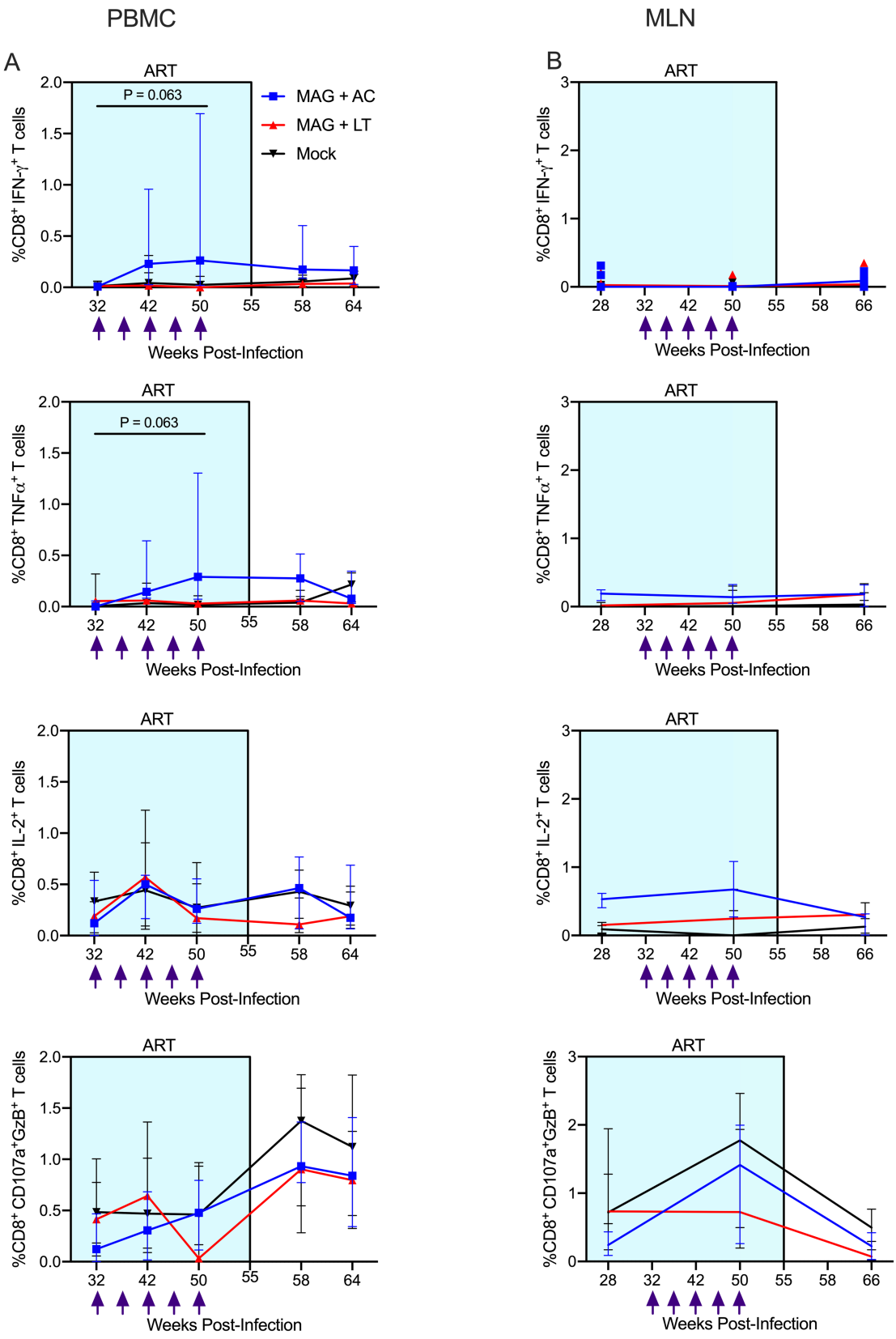


Fig 3. Three out of five animals in the MAG + AC group control virus replication during ATI. **(A-C)** Plasma viral RNA levels were quantified using RT-q-PCR, with a limit of detection of 30 viral RNA copies per 1 mL of plasma, as indicated by the dashed line. **(D)** Shown are the median viral load and interquartile ranges for each treatment group. The dotted line indicates the threshold for control of virus replication, based on previous studies using SIVΔB670.

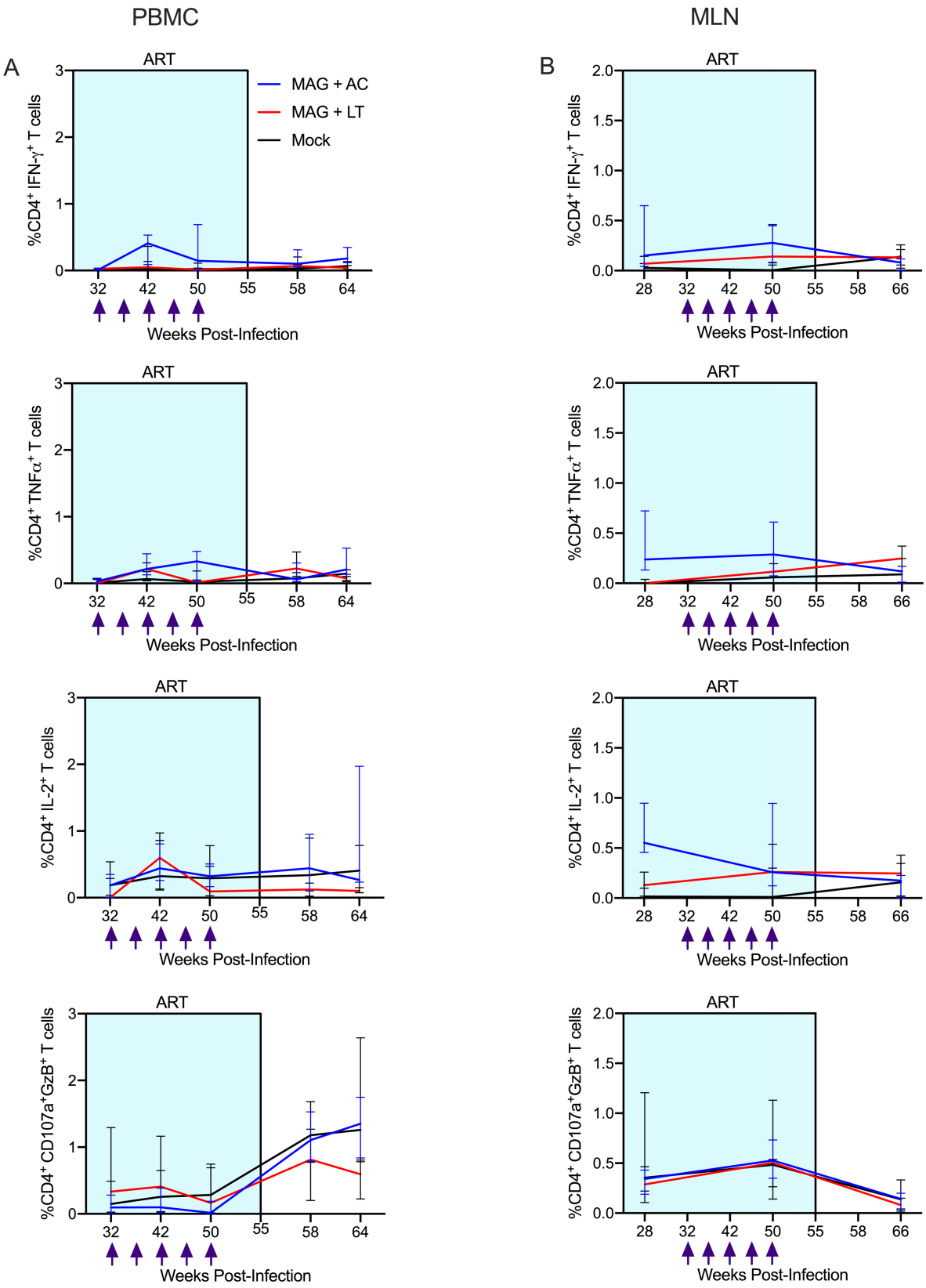
Supplemental Figures:



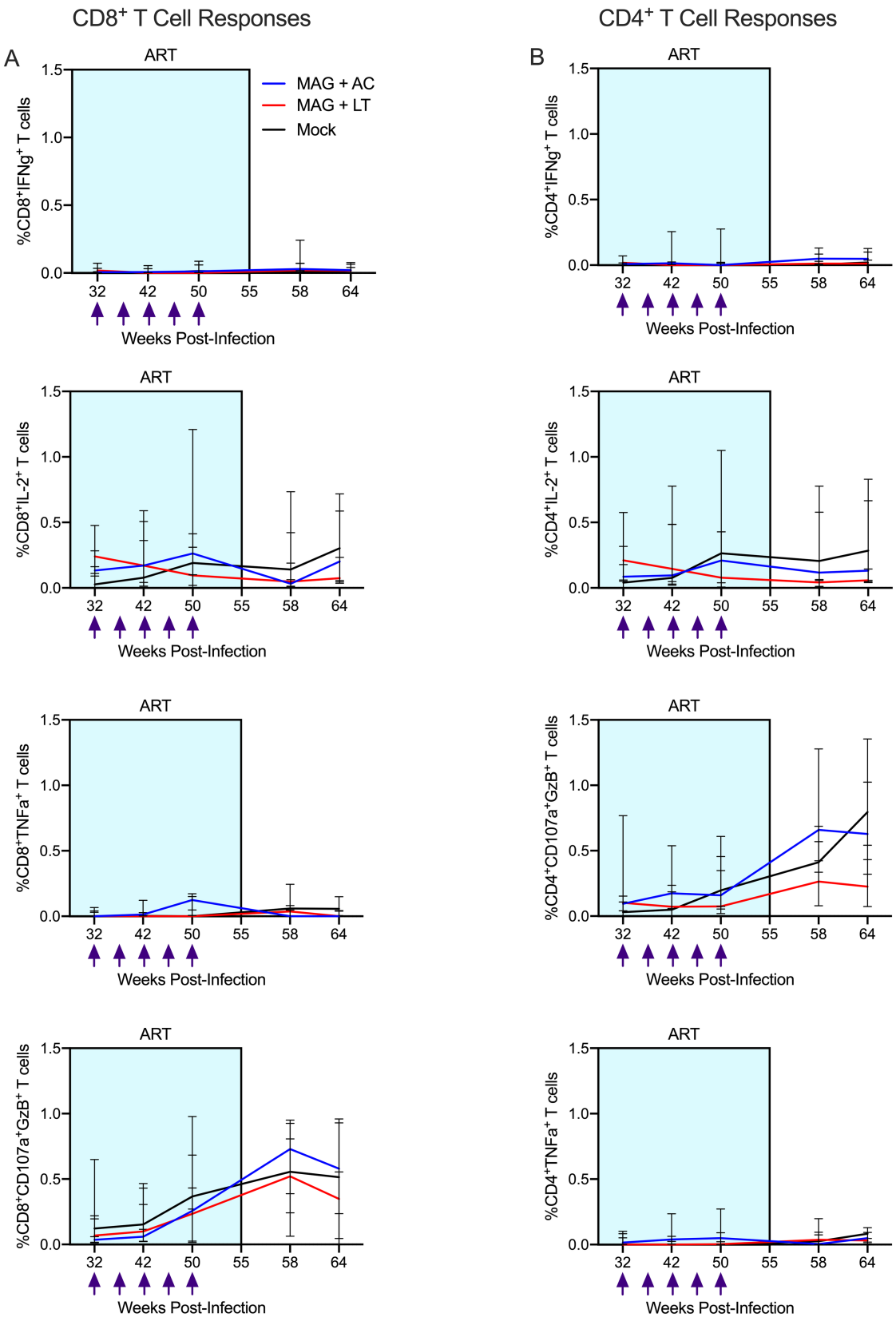
S1 Fig. Animals in each vaccine group demonstrate similar plasma viral loads, CD4$^+$ T cell counts, and ART responsiveness. (A) Plasma viral loads were determined by RT-q-PCR for the mock (black circles), MAG + LT (red squares) and MAG + AC (purple triangles) groups. The dashed line indicates the assay limit of detection (30 viral RNA copies/1mL of plasma) and the dotted line indicates the threshold for control of virus replication**.** Shown is the decrease of each animals' viral loads between pre-ART (6 wpi) and pre-vaccination (32 wpi). Statistical analyses were performed using a Wilcoxon matched-pairs signed rank test; results are considered significant if $P \leq 0.05$. **(B)** Percent of baseline CD4$^+$ T cell counts were calculated for the mock, MAG + LT and MAG + AC groups over time by dividing the absolute CD4$^+$ T cell count at a timepoint by the absolute CD4$^+$ count at 0 wpi and multiplying by 100. The dotted line indicates 50% of baseline CD4$^+$ T cells. CD4$^+$ T cell counts were obtained using a Beckman Coulter® AC*T^{TM} 5diff hematology analyzer. Shown is the restoration of each animals' percent of baseline CD4$^+$ T cell counts between pre-ART (6 wpi) and pre-vaccination (32 wpi). Statistical analyses were performed using a Wilcoxon matched-pairs signed rank test; results are considered significant if $P \leq 0.05$.


PBMC
MLN
A
B
ART
P = 0.063
MAG + AC
MAG + LT
Mock
%CD8+ IFN-γ+ T cells
%CD8+ TNFα+ T cells
%CD8+ IL-2+ T cells
%CD8+ CD107a+GzB+ T cells
Weeks Post-Infection

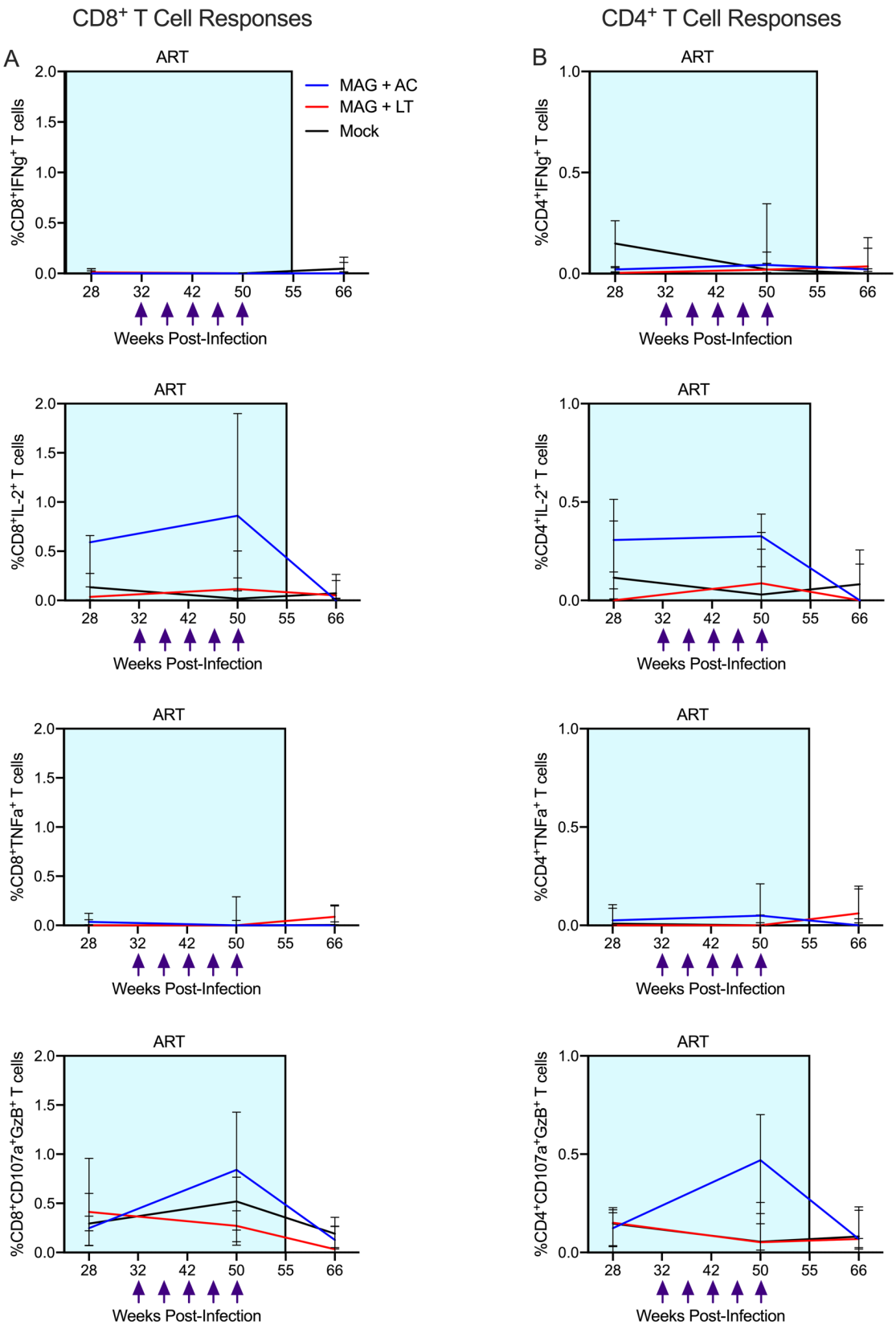
S2 Fig. The MAG + AC group shows a trend towards increased IFN-γ and TNFα $CD8^+$ T cell responses post-vaccination compared to pre-vaccination. (A) PBMCs were thawed and stimulated with Gag peptides, and expression of IL-2, IFN-γ, TNFα and CD107a/GzB were quantified using intracellular cytokine staining. Shown are the medians and interquartile ranges of each group's SIV-Gag specific T cell response. **(B)** Lymphocytes isolated from MLN were thawed and stimulated with Gag peptides, and expression of IL-2, IFN-γ, TNFα and CD107a/GzB were quantified using intracellular cytokine staining. Shown are the medians and interquartile ranges of each group's SIV-Gag specific T cell response. **(A, B) Statistics**. Statistical comparisons between baseline and post-vaccination timepoints within a group were calculated using a Wilcoxon matched-pairs signed rank test. Results are considered significant if $P \leq 0.05$.


PBMC
MLN
A
B
ART
MAG + AC
MAG + LT
Mock
%CD4+ IFN-γ+ T cells
%CD4+ TNFα+ T cells
%CD4+ IL-2+ T cells
%CD4+ CD107a+GzB+ T cells
Weeks Post-Infection

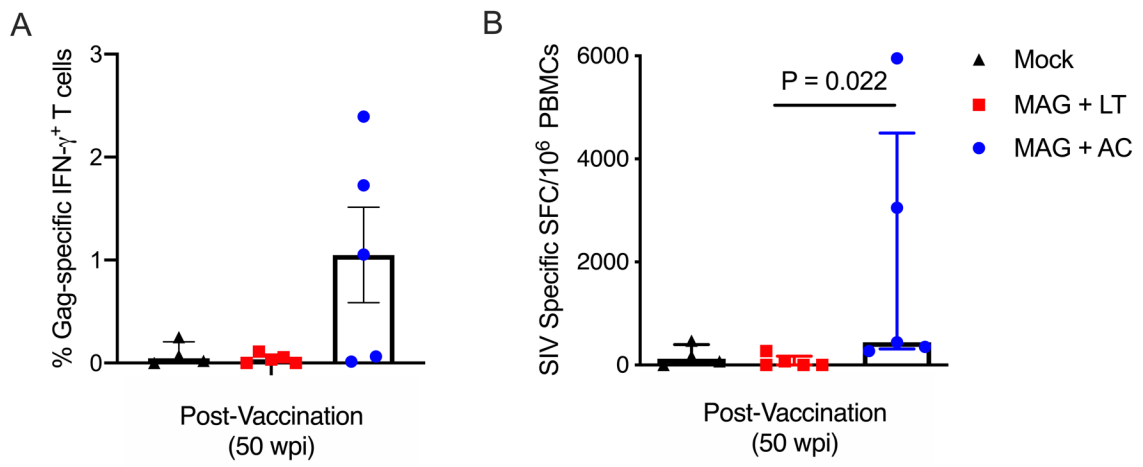
S3 Fig. No differences were observed between groups in Gag-specific $CD4^+$ T cell responses in PBMC and MLN. (A) PBMCs were thawed and stimulated with Gag peptides, and expression of IL-2, IFN-γ, TNFα and CD107a/GzB were quantified using intracellular cytokine staining. Shown are the medians and interquartile ranges of each group's SIV-Gag specific T cell response. **(B)** Lymphocytes isolated from MLN were thawed and stimulated with Gag peptides, and expression of IL-2, IFN-γ, TNFα and CD107a/GzB were quantified using intracellular cytokine staining. Shown are the medians and interquartile ranges of each group's SIV-Gag specific T cell response. **(A, B) Statistics**. Statistical comparisons between baseline and post-vaccination timepoints within a group were calculated using a Wilcoxon matched-pairs signed rank test. A Dunn's multiple comparisons test was used when making multiple comparisons between vaccine groups and the mock group. Results are considered significant if $P \leq 0.05$.


CD8+ T Cell Responses
CD4+ T Cell Responses
A
B
ART
MAG + AC
MAG + LT
Mock
%CD8+IFNg+ T cells
%CD4+IFNg+ T cells
%CD8+IL-2+ T cells
%CD4+IL-2+ T cells
%CD8+TNFa+ T cells
%CD4+CD107a+GzB+ T cells
%CD8+CD107a+GzB+ T cells
%CD4+TNFa+ T cells
0.0
0.5
1.0
1.5
32
42
50
55
58
64
Weeks Post-Infection

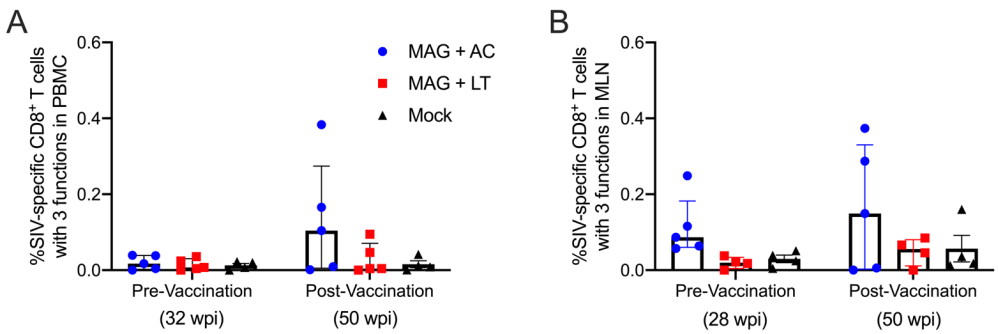
S4 Fig. No differences were observed among groups in Env-specific $CD8^+$ or $CD4^+$ T cell responses in PBMC. (A-B) PBMCs were thawed and stimulated with Env peptides, and expression of IL-2, IFN-γ, TNFα and CD107a/GzB were quantified using intracellular cytokine staining. Shown are the medians and interquartile ranges of each group's SIV-Env specific T cell response. **(A, B) Statistics**. Statistical comparisons between baseline and post-vaccination timepoints within a group were calculated using a Wilcoxon matched-pairs signed rank test. A Dunn's multiple comparisons test was used when making multiple comparisons between vaccine groups and the mock group. Results are considered significant if $P \leq 0.05$.


CD8+ T Cell Responses
CD4+ T Cell Responses
A
B
ART
MAG + AC
MAG + LT
Mock
%CD8+IFNg+ T cells
%CD4+IFNg+ T cells
%CD8+IL-2+ T cells
%CD4+IL-2+ T cells
%CD8+TNFa+ T cells
%CD4+TNFa+ T cells
%CD8+CD107a+GzB+ T cells
%CD4+CD107a+GzB+ T cells
Weeks Post-Infection
28
32
42
50
55
66

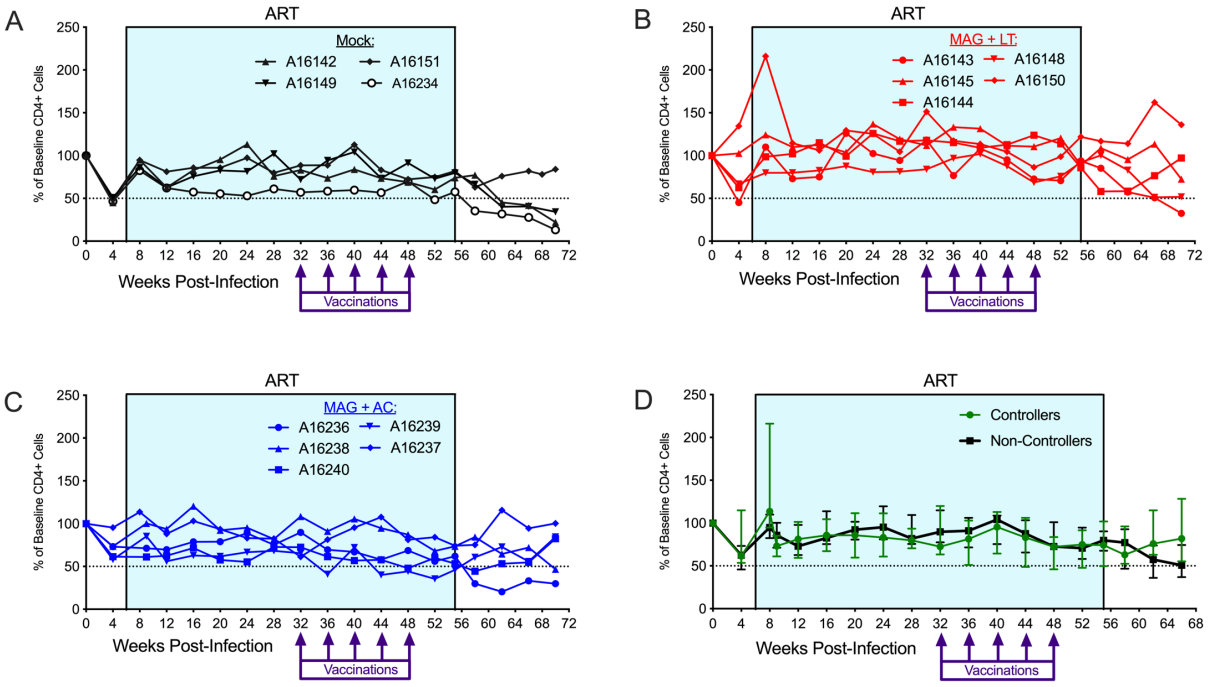
S5 Fig. No differences were observed among groups in Env-specific $CD8^+$ or $CD4^+$ T cell responses in MLN. (A-B) Lymphocytes isolated from MLNs were thawed and stimulated with Env peptides, and expression of IL-2, IFN-γ, TNFα and CD107a/GzB were quantified using intracellular cytokine staining. Shown are the medians and interquartile ranges of each group's SIV-Env specific T cell response. **(A, B) Statistics**. Statistical comparisons between baseline and post-vaccination timepoints within a group were calculated using a Wilcoxon matched-pairs signed rank test. A Dunn's multiple comparisons test was used when making multiple comparisons between vaccine groups and the mock group. Results are considered significant if $P \leq 0.05$.



S6 Fig. The MAG + AC group shows a trend towards increased IFN-γ T cell responses post-vaccination as measured by ELISpot and ICS. **(A)** PBMCs were thawed and stimulated with Gag peptides and expression of IFN-γ was quantified using intracellular cytokine staining. Shown are the medians and interquartile ranges of each group's SIV-Gag specific T cell response. **(B)** Bulk PBMCs were stimulated with Gag peptides to quantify the SIV-specific IFN-γ response. Results were considered positive if peptide-specific responses were at least twice that of the negative control plus at least 0.01% after background (DMSO) subtraction. Shown are medians and interquartile ranges with data from individual animals layered over each bar. **(A-B)** A Dunn's multiple comparisons test was used when making multiple comparisons between vaccine groups and the mock group. Results are considered significant if $P \leq 0.05$.



S7 Fig. The MAG + AC group shows a trend towards increased SIV-specific polyfunctional CD8$^+$ T cell responses post-vaccination in PBMC and MLN. (A-B) PBMCs and lymphocytes from MLNs were thawed and stimulated with Gag and Env peptides, and expression of IL-2, IFN-γ, TNFα and CD107a/GzB were quantified using intracellular cytokine staining. Polyfunctionality is defined as the frequency of T cells specific for Gag or Env and expressing any three or more effector functions. Shown are medians and interquartile ranges with data from individual animals layered over each bar. A Dunn's multiple comparisons test was used when making comparisons between vaccine groups and the mock group. Results are considered significant if $P \leq 0.05$.



S8 Fig. CD4$^+$ T cell counts corresponded with virus burden in plasma. (A-C) Shown are the percent of baseline CD4$^+$ T cell counts for each individual animal in the mock, MAG + LT and MAG + AC groups over time. Percent of baseline CD4$^+$ T cell counts were calculated for the mock, MAG + LT and MAG + AC groups over time by dividing the absolute CD4$^+$ count at a timepoint by the absolute CD4$^+$ count at 0 wpi and multiplying by 100. The dotted line indicates 50% of baseline CD4$^+$ T cells. CD4$^+$ T cell counts were obtained using a Beckman Coulter® AC*T^{TM} 5diff hematology analyzer. **(D)** Graphed are the median and interquartile range of controllers' and non-controllers' percent of baseline CD4$^+$ counts.

Part 2: Immune correlates of control of viral rebound in animals with variable responses to antiretroviral therapy

Viral control during ATI is associated with increased Gag-specific $CD4^+$ and $CD8^+$ T cells in MLN and PBMC

The variability in viral rebound and viremia during ATI among animals in this study enabled further study of immune correlates of viral control. Altogether, there were 5 viral controllers and 9 non-controllers, defined as animals that maintained median viremia at or below 10^3 copies/mL of plasma or greater than 10^3 copies/mL of plasma, respectively, for 5 months after stopping ART (Fig 4A). Viral burden during ATI was significantly different between controllers and non-controllers ($P = 0.0010$, Fig 4B). Certain MHC and TRIM5 genetics have been associated with improved viral control in SIV infected macaques [54-56, 58, 292, 293], however, there was no association between these MHC or TRIM5 alleles and the control of viral rebound (S1 Table) indicating these genotypes did not likely influence viral burden during ATI in this study.

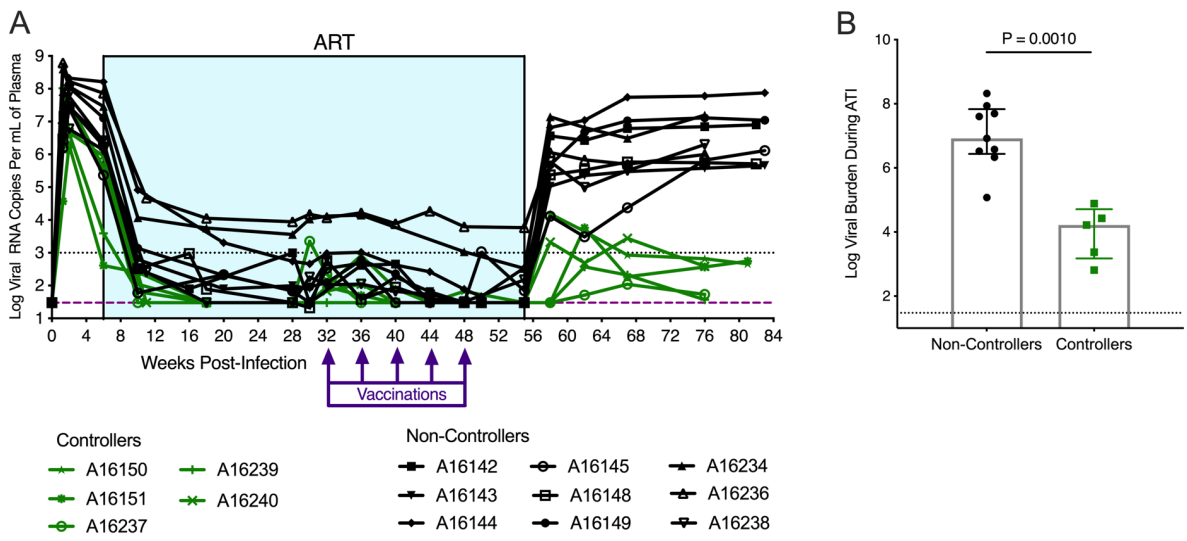


Fig 4. Five controllers maintained significantly lower viral burden during ATI compared to nine non-controllers. (**A)** Plasma viral RNA levels were quantified using RT-q-PCR, with a limit of detection of 30 viral RNA copies per 1 mL of plasma, as indicated by the dashed line. The dotted line denotes the threshold for control of virus replication, based on previous studies using SIVΔB670. Controllers were defined as animals that maintained a median viremia at or below 1000 viral RNA copies per 1 mL of plasma for 5 months post-ART. Non-controllers were defined as animals with a median viremia that exceeded 1000 viral RNA copies per 1mL of plasma for 5 months post-ART. **(B)** Viral burden during ATI was calculated as the area under the curve of each animal's viral load from 55 wpi to 76 wpi, shown are the median viral burden and interquartile ranges. Statistics were calculated using a Mann-Whitney t-test. Results are considered significant if $P \leq 0.05$.

To determine immune correlates of viral control, I first compared Env-specific IgG titers in controllers and non-controllers and found that non-controllers exhibited a consistent trend towards higher titers of Env-specific IgG both during ART treatment and during ATI (S9 Fig), likely due to higher levels of ongoing virus replication.

Next, I compared frequencies of Gag-specific $CD4^+$ and $CD8^+$ T cells expressing IFN-γ, TNFα, IL-2, and/or co-expressing the cytolytic markers CD107a/Granzyme B as detected by flow cytometry in the controllers and non-controllers. Most therapeutic vaccine studies in NHP are limited to investigating immune correlates in the peripheral blood. Here, I sought to establish the role of T cell responses in both the blood and the MLN (as part of the GALT) on viral control. Immune responses in PBMC and MLN were compared prior to ATI (50 wpi) and after viral setpoint was established (62 wpi for PBMC and 66 wpi for MLN). I observed no differences between controllers and non-controllers in terms of Gag-specific $CD4^+$ T cell responses in PBMC or MLN (S10 Fig). However, controllers demonstrated a trend towards higher frequencies of Gag-specific $TNF\alpha^+$ $CD8^+$ T cells in PBMC prior to ATI (50 wpi, Fig 5A, P = 0.056) and exhibited significantly higher frequencies of these cells in PBMC during ATI (62 wpi, Fig 5B, P = 0.0080). In the MLN, controllers had significantly higher frequencies of Gag-specific $IL\text{-}2^+$ $CD8^+$ T cells pre-ATI (50 wpi, Fig 5C, P = 0.037), although this was not sustained during ATI (66 wpi). Importantly, higher frequencies of Gag-specific $IFN\text{-}\gamma^+$ and $TNF\alpha^+$ $CD8^+$ T cell responses in PBMC and Gag-specific $IL\text{-}2^+$ $CD8^+$ T cell responses in the MLN significantly correlated with lower viral burden during ATI (Fig 5D-F), suggesting that SIV Gag-specific T cell responses in both the periphery and GALT contribute to the improved control of virus replication in controllers. Although I could not rule out a role for strain-specific neutralizing or non-neutralizing antibodies, these results suggest viral control during ATI was likely mediated, at least in part, by $CD8^+$ T cell responses.

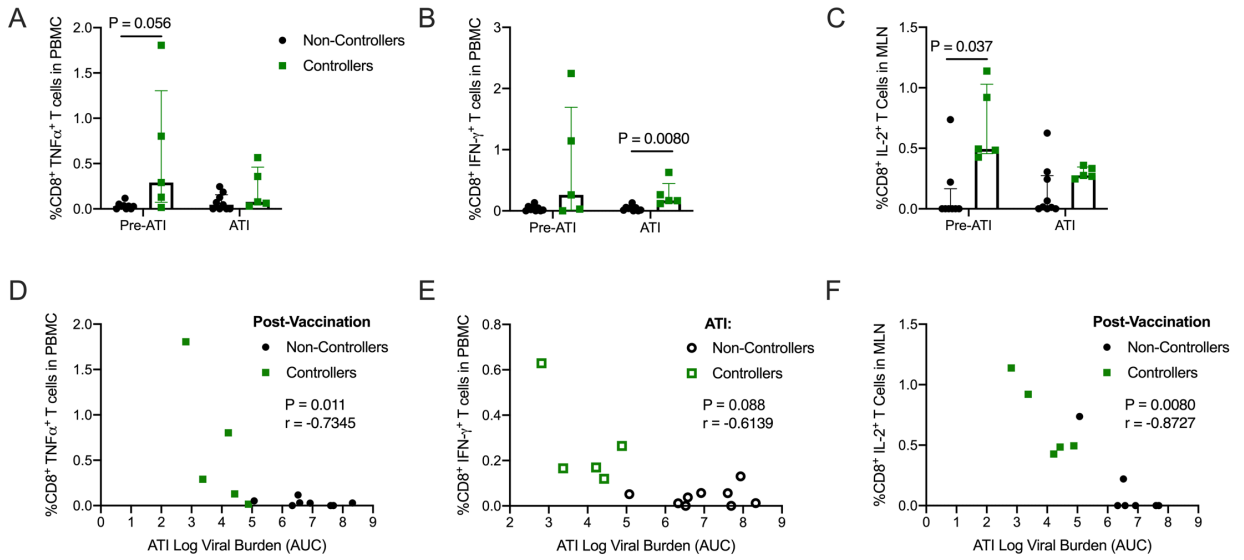


Fig 5. Controllers have higher SIV Gag-specific CD8$^+$ T cell responses in PBMC and MLN post-vaccination and during ATI. (A-C) PBMCs and MLNs were thawed and stimulated with Gag peptides, and expression of cytokines was quantified using intracellular cytokine staining. Shown are the medians and interquartile ranges of the SIV Gag-specific CD8$^+$ T cell responses of controllers and non-controllers, with individual responses layered over each bar at a post-vaccination timepoint (50 wpi) and during ATI (62 wpi for PBMC and 66 wpi for MLN). Statistical differences between controllers and non-controllers at each timepoint were calculated using a Mann-Whitney t-test. Benjamini-Hochberg adjusted P values are shown, results are considered significant if $P \leq 0.05$. **(D)** The SIV Gag-specific TNFα CD8$^+$ T cell responses in PBMC at 50 wpi negatively correlated with the viral burden measured as area under the curve (AUC) during ATI. **(E)** The SIV Gag-specific IFN-γ CD8$^+$ T cell responses in PBMC at 50 wpi negatively correlated with the viral burden measured as area under the curve (AUC) during ATI. **(F)** The SIV Gag-specific IL-2 CD8$^+$ T cell responses in MLN at 62 wpi negatively correlated with the viral burden measured as area under the curve (AUC) during ATI. The P and r values shown were calculated using a Spearman rank correlation test. Benjamini-Hochberg adjusted P values are shown, results are considered significant if $P \leq 0.05$.

Viral control during ATI is associated with increased polyfunctionality in MLN and PBMC

To further elucidate the role of SIV-specific $CD8^+$ T cell responses in viral control, I next compared the magnitude of the polyfunctional $CD8^+$ T cell response, as defined by the frequency of T cells specific for either Gag or Env and expressing any three or more of the cytokines IFN-γ, TNFα, IL-2, and/or co-expressing the cytolytic markers CD107a/Granzyme B. Prior to ATI (50 wpi), higher frequencies of polyfunctional $CD8^+$ T cells expressing three effector functions in the MLN ($P = 0.016$, Fig 6A) and PBMC ($P = 0.013$, Fig 6B) correlated with lower viral burden during ATI. Interestingly, during ATI (62 wpi in PBMC and 66 wpi in MLN), the significant correlation between higher frequencies of polyfunctional $CD8^+$ T cells in MLN and lower ATI viral burden ($P = 0.015$, Fig 6C) persisted, whereas in the PBMC, the correlation fell below statistical significance ($P = 0.12$, Fig 6D). These results indicate that while polyfunctional $CD8^+$ T cell responses in the periphery may have played an integral part in controlling viral recrudescence during ATI, responses in the GALT may be more critical for sustained containment of viral rebound. Notably, there are no differences between controllers and non-controllers in the frequencies of $CD8^+$ T cells expressing one or two effector functions (non-polyfunctional responses) before or during ATI (S12 Fig). Although non-polyfunctional responses make up the majority of the SIV-specific $CD8^+$ T cell response, these results demonstrate that polyfunctional $CD8^+$ T cells likely play a greater role in controlling viral replication. The importance of T cell polyfunctionality in the blood in viral control during ATI is well-established [80, 294, 295]. Our data extends these findings and suggests that polyfunctional T cell responses in the GALT as well as the PBMC are likely key for achieving durable immune control of viral replication.

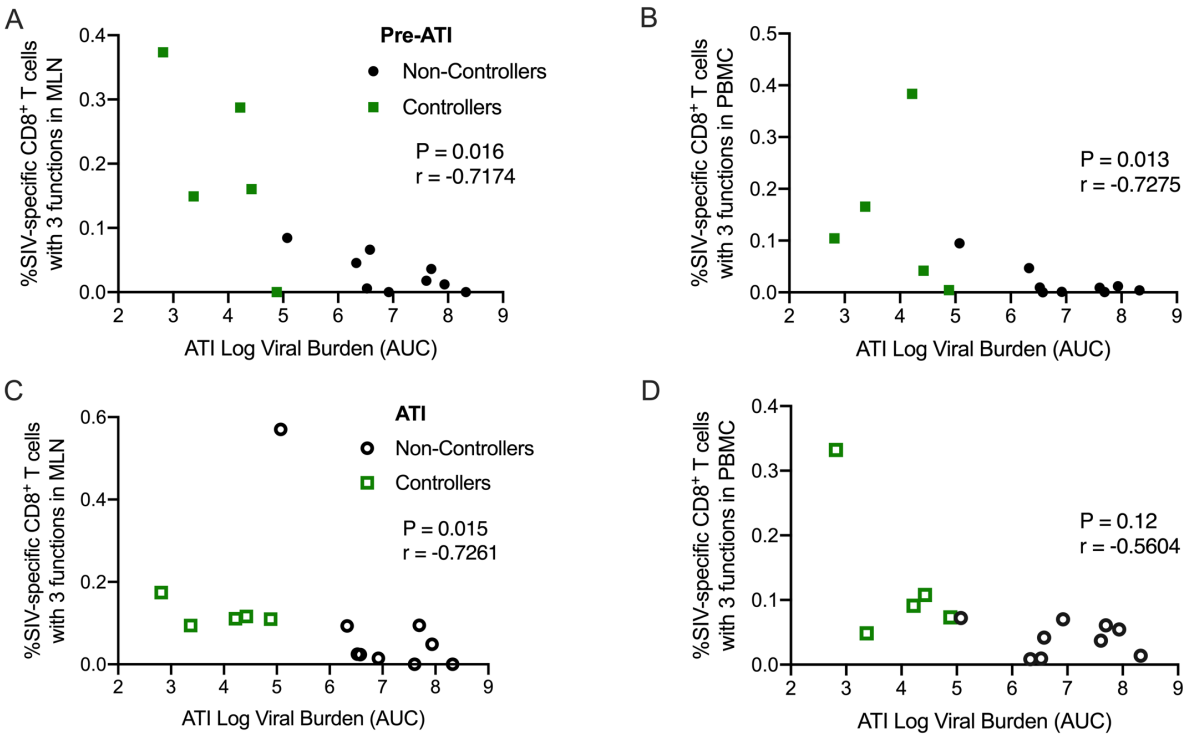


Fig 6. Increased frequencies of polyfunctional CD8$^+$ T cells in MLN and PBMC of controllers. (A-D) The polyfunctional SIV-specific CD8$^+$ T cell responses in MLN and PBMC post-vaccination and during ATI negatively correlated with ATI viral burden. The P and r values shown were calculated using a Spearman rank correlation test. Benjamini-Hochberg adjusted P values are shown, and results are considered significant if $P \leq 0.05$.

ART responsiveness and pre-infection populations of mucosal $CD4^+$ T cells predict control of viremia during ATI

A significant variable in our study is the wide range of acute viral loads and response to ART that occurred prior to initiating therapeutic vaccinations. To determine if these variables influenced viral control during ATI, I compared the viral loads of controllers to non-controllers during acute infection (0-6 wpi) and during ART but prior to vaccinations (6-32 wpi). Controllers demonstrated a trend towards lower viral burden during acute infection ($P = 0.11$, Fig 7A) and significantly lower viral burden while on ART ($P = 0.014$, Fig 7A) when compared to non-controllers.

Importantly, lower acute viral burden was associated with lower residual viral replication during ART ($P = 0.0078$, Fig 7B), and lower viral burden on ART was strongly correlated with better control of viral replication during ATI ($P = 0.00040$, Fig 7C). Together, these data suggest that pre-infection host immune parameters may have influenced the extent of acute viral replication and subsequently affected the extent of residual viral replication on ART, or ART efficacy. Persistent viral replication on ART in turn may have influenced the ability of each animal to develop and maintain polyfunctional $CD8^+$ T cell responses in the blood and GALT during ART that significantly correlated with control of viral replication during ATI (Fig 6A-D).

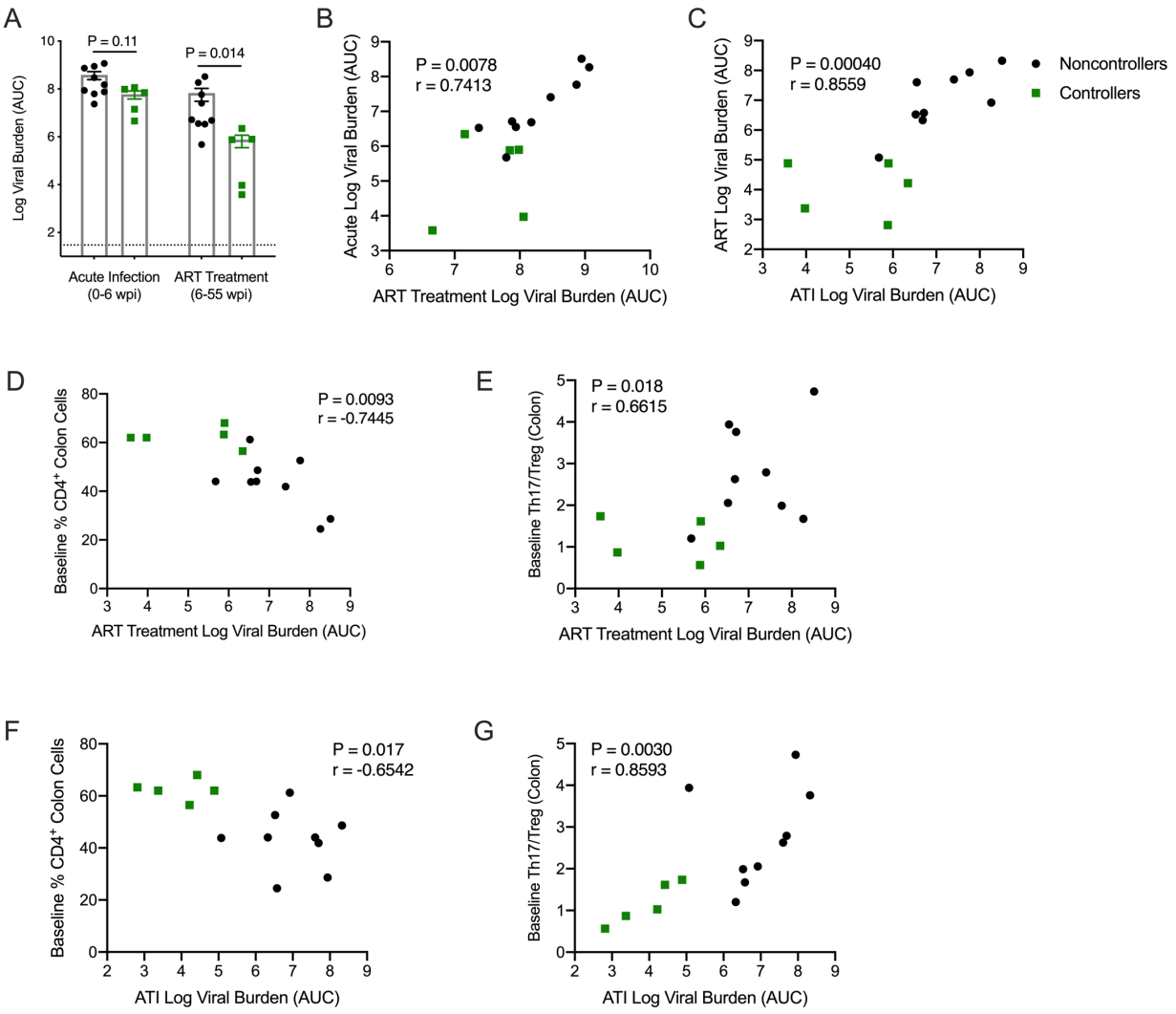


Fig 7. Lower viral replication during acute infection correlates with improved ART responsiveness that in turn is associated with better control of viral rebound during ATI. Improved virological response to ART and better control of viral rebound are associated with the relative populations of mucosal CD4⁺ T cells and Th17/Treg cells before infection. **(A-C)** Viral loads were measured via RT-q-PCR and viral burden was calculated as the area under the curve of animals' viral loads. **(A)** Statistics were calculated using a Mann-Whitney t-test. Benjamini-Hochberg adjusted P values are shown. Results are considered significant if $P \leq 0.05$. **(B-C)** Acute log viral burden (0 – 6 wpi) correlated with log viral burden during ART treatment (6 – 32 wpi). ART log viral burden in turn correlated with log viral burden during ATI (55 wpi – 76

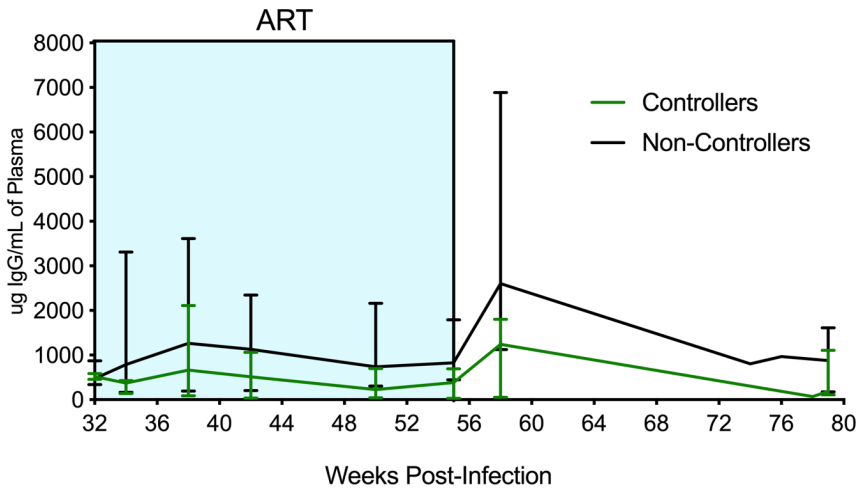
wpi). **(D)** The frequency of $CD4^+$ T cells in the colon at baseline (0 wpi) correlated with viral burden during ART. **(E)** The ratio of Th17 and Treg cells at baseline correlated with viral burden during ART. **(F)** The frequency of $CD4^+$ T cells in the colon at baseline (0 wpi) correlated with viral burden during ATI. **(G)** The ratio of Th17 and Treg cells at baseline correlated with viral burden during ATI. **(B-G)** A Spearman rank correlation test was used to determine P and r values. Shown are Benjamini-Hochberg adjusted P values, results are considered significant if $P \leq 0.05$.

To investigate this theory, I assessed a number of baseline immune factors, including $CD4^+$ T cells, T helper 17 (Th17) and T regulatory (Treg) cells in the colon, that I hypothesized could influence acute viral infection and potentially affect the virological response to ART and outcome of ATI. (The Th17 and Treg data included here were obtained from experiments done by a postdoctoral fellow in the Fuller Lab, Dr. Megan O'Connor, though the conclusions described here are my own.) In particular, depletion of Th17 cells in the gut contributes to immune activation and disease progression [296, 297]. The role of Treg cells in HIV pathogenesis is not as well-characterized, but they could contribute to viral replication by suppressing HIV-specific $CD8^+$ T cell activity [298] or conversely, slow disease progression by decreasing chronic immune activation [299]. In the colon, neither baseline frequencies of Th17 ($P = 0.22$) nor Treg ($P = 0.14$) cells correlated with viral burden during ATI (S13 Fig). However, I observed a correlation between greater $CD4^+$ T cell frequencies ($P = 0.0093$, Fig 7D) and lower Th17/Treg ratios ($P = 0.018$, Fig 7E) and improved virological response to ART. Furthermore, higher frequencies of colonic $CD4^+$ T cells and lower Th17/Treg ratios at baseline were predictive of lower viral burden during ATI ($P = 0.017$, Fig 7F and $P = 0.0030$, Fig 7G). Collectively, these data suggest that during the early stages of infection, the relative proportions of mucosal T cell subsets play an important role in maintenance of gut homeostasis and prevention of immune dysfunction, and may influence responsiveness to ART, therapeutic vaccination and/or control of viremia during ATI.

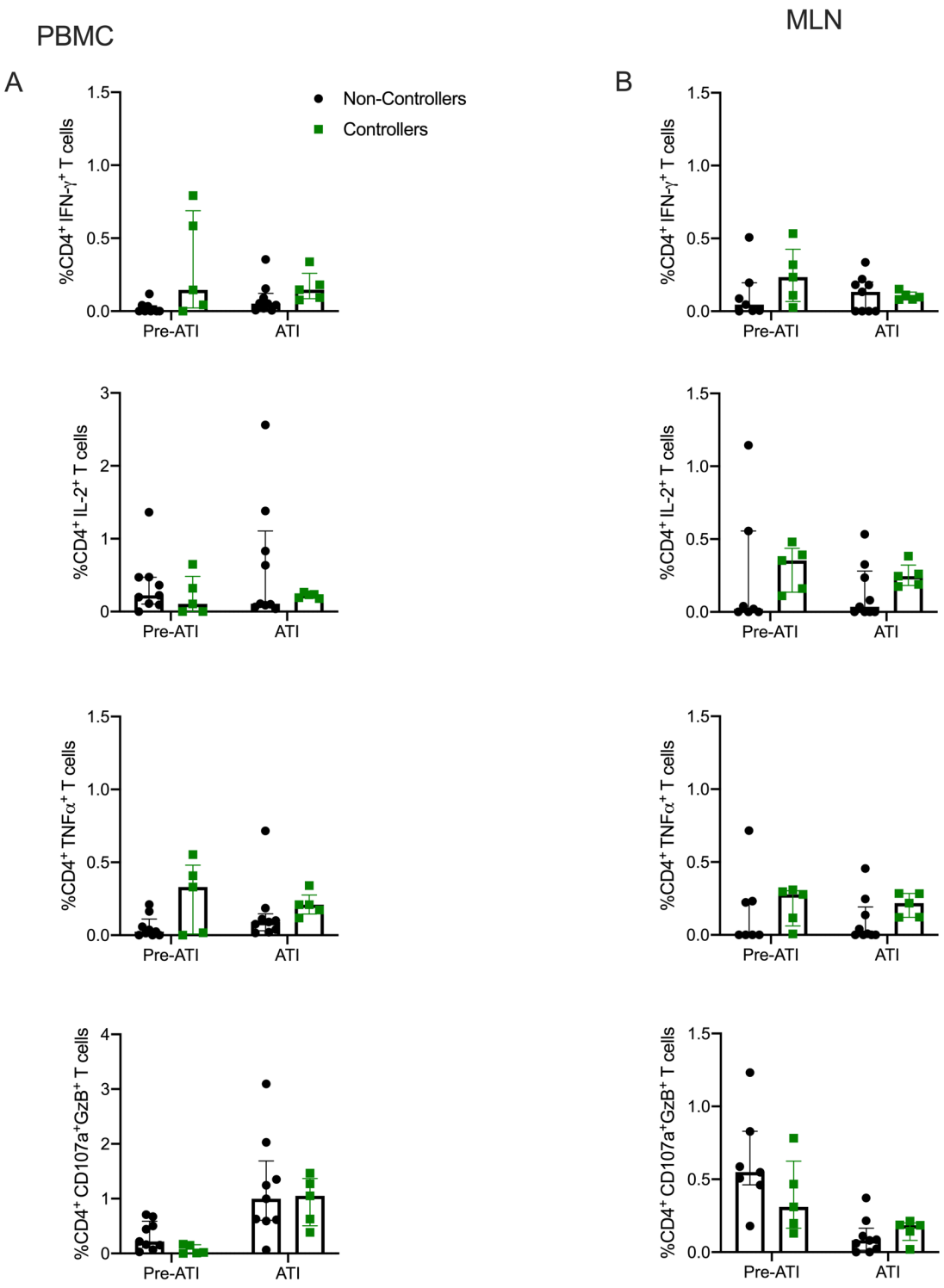
Supplemental Figures:

Animal ID	MHC	TRIM5	Vaccine Group	Status
A16142	-	TFP/TFP	Mock	Non-controller
A16149	A*02	TFP/Cyp	Mock	Non-controller
A16234	B*17	Q/Q	Mock	Non-controller
A16236	-	Q/TFP	MAG + AC	Non-controller
A16238	B*17	TFP/TFP	MAG + AC	Non-controller
A16143	A*02	Q/TFP	MAG + LT	Non-controller
A16144	-	Q/TFP	MAG + LT	Non-controller
A16145	-	TFP/Cyp	MAG + LT	Non-controller
A16148	-	TFP/TFP	MAG + LT	Non-controller
A16237	-	TFP/TFP	MAG + AC	Controller
A16239	B*08	Q/TFP	MAG + AC	Controller
A16240	B*08	TFP/TFP	MAG + AC	Controller
A16150	A*01	TFP/Cyp	MAG + LT	Controller
A16151	-	Q/TFP	Mock	Controller

S1 Table. MHC and TRIM5 genetics. Indicated for each animal are TRIM5 haplotype, the presence or absence of an MHC-1 allele commonly associated with control of viral replication, vaccination group, and virological status during ATI. Animals in gray are non-controllers, while controllers are shown in green.

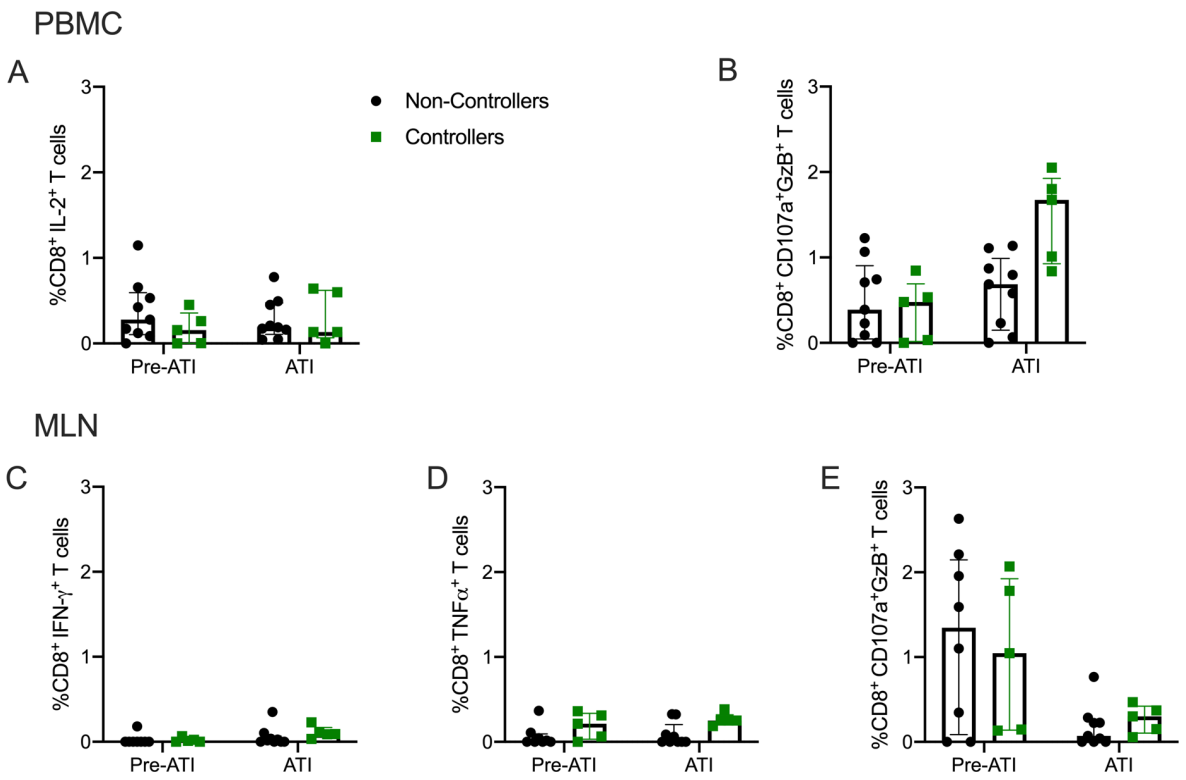


S9 Fig. Non-controllers exhibited a trend towards higher median titers of Env-specific IgG compared to controllers. The magnitude of the SIV Env-specific IgG response in the plasma was measured by ELISA, using SIV gp130 as the capture antigen. Shown are medians and interquartile ranges.

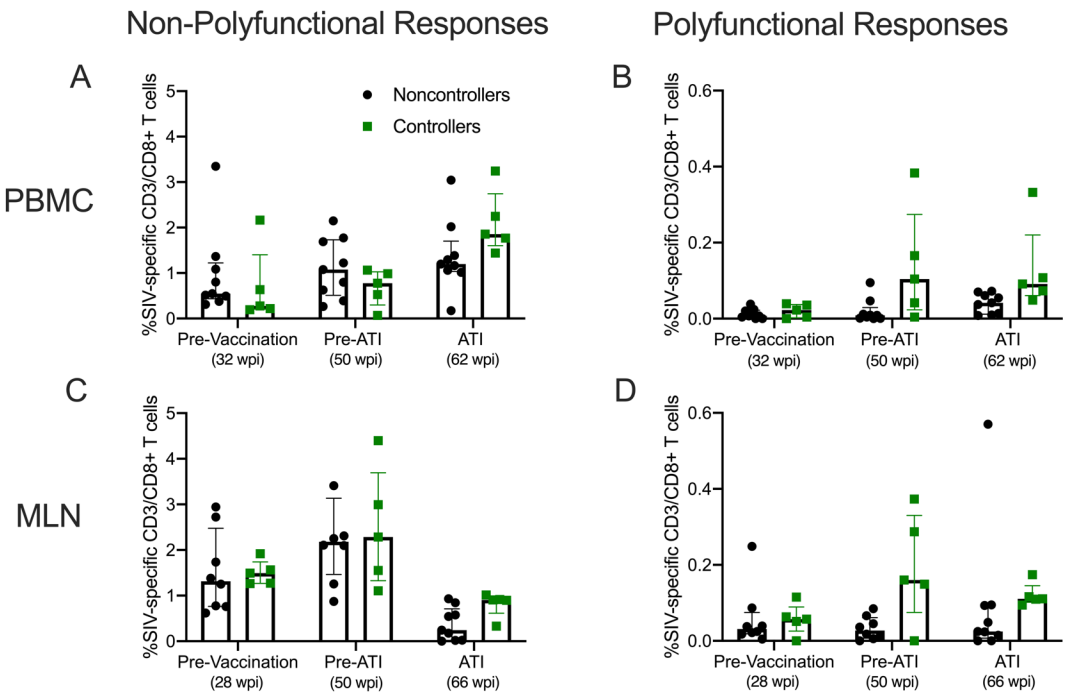


S10 Fig. Controllers and non-controllers demonstrate similar levels of SIV Gag-specific CD4⁺ T cell responses in the PBMC and MLN post-vaccination and during ATI. (A-B) PBMCs and MLNs were thawed and stimulated with Gag peptides, and expression of cytokines was

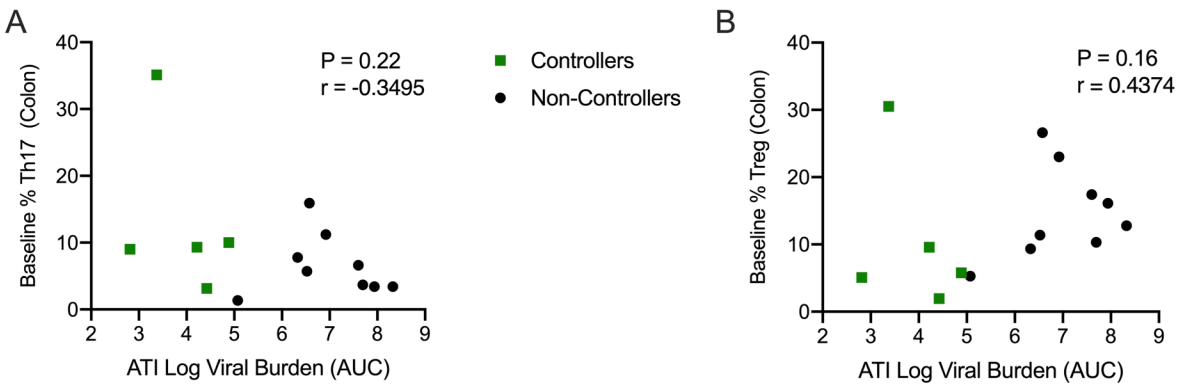
quantified using intracellular cytokine staining. Shown are the medians and interquartile ranges of the SIV Gag-specific $CD4^+$ T cell responses of controllers and non-controllers, with individual responses layered over each pre-ATI (50 wpi) and during ATI (62 wpi for PBMC and 66 wpi for MLN). Statistical differences between controllers and non-controllers at each timepoint were assessed using a Mann Whitney t test and the Benjamini-Hochberg method was used to adjust P values. Results are considered significant if $P \leq 0.05$.



S11 Fig. No difference between controllers/non-controllers in Gag-specific IL-2$^+$ and CD107a$^+$GzB$^+$ CD8$^+$ T cells in PBMC or Gag-specific IFN-γ$^+$, TNFα$^+$, and CD107a$^+$GzB$^+$ CD8$^+$ T cells in MLN. (A-E) PBMCs and MLNs were thawed and stimulated with Gag peptides, expression of cytokines was quantified using intracellular cytokine staining. Shown are the medians and interquartile ranges of the SIV Gag-specific CD8$^+$ T cell responses of controllers and non-controllers, with individual responses layered over each bar pre-ATI (50 wpi) and during ATI (62 wpi for PBMC and 66 wpi for MLN). Statistical differences between controllers and non-controllers at each timepoint were assessed using a Mann Whitney t test and the Benjamini-Hochberg method was used to adjust P values. Results are considered significant if $P \leq 0.05$.



S12 Fig. Non-polyfunctional responses compose the majority of the SIV-specific CD8$^+$ T cell response, but are not elevated in controllers compared to non-controllers. (A-D) PBMCs and lymphocytes from MLNs were thawed and stimulated with Gag and Env peptides, and intracellular cytokine staining was used to quantify expression of IL-2, IFN-γ, TNFα and CD107a/GzB. SIV-specific T cells expressing three or more effector functions are considered polyfunctional, while SIV-specific T cells expressing two or fewer effector functions are considered non-polyfunctional. Shown are medians and interquartile ranges with data from individual animals layered over each bar. At each timepoint, differences between controllers and non-controllers were assessed using a Mann Whitney t test, and the Benjamini-Hochberg method was used to adjust P values. Results are considered significant if $P \leq 0.05$.



S13 Fig. Baseline frequencies of Th17 and Treg cells are not associated with therapeutic outcome during ATI. (A-B) Lymphocytes were isolated from colon biopsies and expression of Th17 and Treg markers was quantified using intracellular cytokine staining on fresh cells. A Spearman rank correlation test was used to determine P and r values. Shown are the Benjamini-Hochberg adjusted P values, results are considered significant if $P \leq 0.05$.

Discussion

The primary goals of this study were to assess the immunogenicity and efficacy of a multi-antigen DNA vaccine (MAG) delivered with a novel genetic adjuvant combination (AC), and to determine what host factors could be influencing the effects of therapeutic vaccination. To this end, I reported that animals in the multi-antigen vaccine and adjuvant combination group (MAG + AC) exhibited significant increases in the breadth of IFN-γ T cell responses when compared to the vaccine group that received MAG with only a single adjuvant (MAG + LT) and mock-vaccinated controls, an outcome that is consistent with other vaccine trials in NHP and mice using the IL-12 and IL-33 adjuvants [273, 275, 300]. However, the RALDH2 adjuvant did not enhance vaccine immunogenicity in the GALT as shown in mice [277], possibly due to SIV infection causing significant mucosal immune dysfunction in the NHP that may have interfered with the effects of this adjuvant. Additionally, I observed that therapeutic vaccination only transiently boosted the magnitude of the IFN-γ T cell responses and Env-specific antibody responses in both the MAG + AC and MAG + LT groups. This is similar to what was reported in our previous study [15] and may be the result of increases in regulatory immune responses, such as myeloid derived suppressor cells (MDSCs), that occur during acute infection and persist despite ART treatment. Three out of five MAG + AC animals controlled viral rebound (60%), although this outcome was not statistically different from the controls. The lack of significant differences between the vaccine and control groups may be due to the small group sizes and the variability in the response to ART within each group. This is consistent with a previous study that reported significant viral control during ATI following therapeutic immunization with a PMED DNA vaccine, but only in a subset of animals that responded well to ART [15]. However, since the three controllers in the MAG + AC group exhibited a better virological response to ART prior to vaccination than the two non-controllers, I cannot conclusively determine whether improved viral control in a subset of animals in this group was due to the vaccine or pre-existing intrinsic host factors that influenced their response to ART.

Overall, five animals from all groups controlled viral rebound and were protected from progression to AIDS, in contrast to nine animals that exhibited immediate viral rebound during ATI. This variability in viral control during ATI enabled further analysis of immune correlates of protection from viral rebound. Notably, I found that increased expression of IL-2 and higher frequencies of polyfunctional, SIV-specific $CD8^+$ T cells in the MLN prior to stopping ART were associated with lower viral burden during ATI. I also observed that $CD8^+$ T polyfunctionality and expression of IFN-γ and TNFα in PBMC were associated with lower viral burden during ATI. The disparity between our observations in the MLN and PBMC is consistent with previous studies reporting significant variations between $CD8^+$ T cells in blood and lymphoid tissues in SIV-infected rhesus macaques [301], and high frequencies of tissue-resident HIV-specific $CD8^+$ T cells in elite controllers that are distinct in their functionality from $CD8^+$ T cell responses in the blood [302]. It is possible that these differences in cytokine expression and polyfunctionality arose because $CD8^+$ T cells were primed *in situ* and did not migrate between peripheral compartments and the GALT during chronic infection. Alternatively, these cells may have been primed in peripheral lymph nodes and trafficked to the GALT, subsequently forming highly stable tissue-resident populations [301]. Collectively, these data provide evidence that polyfunctional $CD8^+$ T cell responses in not just the blood, but also the GALT and gut mucosa are important for controlling reactivating virus in mucosal reservoirs. Therefore, future therapeutic vaccine efforts should be directed towards inducing polyfunctional mucosal responses.

While our data clearly show a protective role for mucosal and systemic polyfunctional $CD8^+$ T cell responses, the precise mechanisms underlying viral control or recrudescence during ATI are still unclear. Although ART significantly reduced viral burden in these animals, low levels of detectable SIV viremia (median viral loads of $<10^3$ viral copies per mL of plasma) persisted in most animals. Importantly, I found controllers exhibited significantly lower viral loads than non-controllers during ART, and this correlated with lower viral burden during ATI. This is accordant with other NHP studies showing correlations between lower acute SIV viremia and lower SIV viral

loads during ATI [303]. Our results extend these findings and show that even low levels of persistent viral replication during ART can affect control of viremia during ATI. Although quantifying viral diversity was outside the scope of this study, persistent viral replication during ART likely increased viral diversity, especially in non-controllers relative to controllers. Higher viral diversity increases the likelihood of immune escape from vaccine-induced immune responses and may have contributed to failure to control viral replication during ATI in some animals. Additional studies are needed to determine how incomplete suppression of viral replication during ART impacted viral diversity in this study and its impact on therapeutic vaccine efficacy.

Another factor that could have influenced post-ART viral rebound is the size of the latent reservoir. Previous studies showed levels of proviral DNA in the GALT correlate with time to viral rebound [304], and increased proviral DNA in PBMC correlates with higher viral loads during ATI [305]. It is therefore possible that the controllers in this study had reduced proviral reservoirs that contributed to improved control of virus replication. Further experiments are needed to quantify cell-associated viral DNA in the GALT and PBMC and to determine how the latent reservoir may have affected virologic outcome during ATI.

To further elucidate mechanisms underlying the response to ART and ultimately viral control during ATI, I investigated a possible role for intrinsic baseline immune factors and found that pre-infection immune responses in the GALT may influence acute viral replication, ART responsiveness, and the ability to control viremia during ATI. Specifically, I found correlations between higher pre-infection frequencies of colonic $CD4^+$ T cells and lower viral burden during ATI, a finding that is consistent with previous studies in humans, where HIV-specific $CD4^+$ T cell proliferation and function during early infection were associated with viral control and slower disease progression in the absence of ART [86, 306]. Together, these data indicate that robust $CD4^+$ T cell responses in the mucosa may play a key role in mitigating acute viral infection. I did not detect an association between baseline frequencies of Treg cells or Th17 cells and viral control during ATI. However, when considered together, I observed that higher ratios of Th17 to

Treg cells pre-infection correlated with higher viral burden during ATI. This result is consistent with a previous NHP study from this lab, where a higher Th17/Treg ratio measured prior to SIV infection predicted higher acute viremia [307]. When considered together, this suggests that the balance between Th17 and Treg cells during the earliest stages of infection impacts acute viremia and the virological response to ART. Furthermore, the disruption of mucosal Th17 and Treg homeostasis, coupled with persistent, low-level viremia during ART, likely compromised the non-controllers' ability to develop or maintain polyfunctional $CD8^+$ T cell responses in the blood and GALT and their subsequent failure to control virus during ATI.

In summary, the results reported here further our understanding of how robust, polyfunctional $CD8^+$ T cell responses in the GALT contribute to control of SIV replication, and highlight the need for therapeutic HIV vaccines that can induce mucosal immunity. Furthermore, our observations provide new insight into the importance of effective ART as a crucial component of therapeutic interventions. Finally, the data presented here show that mucosal $CD4^+$ T cell homeostasis prior to infection can have far-reaching effects on an individual's ability to control viral rebound during ATI, suggesting that monitoring and maintaining the Th17/Treg ratio may be key to the success of immune-based HIV cure strategies. Altogether, these results underscore the need for therapeutic vaccines and adjuvants capable of enhancing polyfunctional $CD8^+$ T cell responses in both the GALT and the periphery, and emphasize the need for additional studies to more clearly define the role of inherent host factors in shaping the response to ART and therapeutic interventions.

Chapter 3: Materials and Methods

Ethics statement and animal care

Fourteen male, adult rhesus macaques (*Macaca mulatta*) of Indian origin were used for this study. These animals were housed at the Washington National Primate Research Center (WaNPRC), which is accredited by the American Association for the Accreditation of Laboratory Animal Care International (AAALAC). At the WaNPRC, animals received the highest standard of care from a team of highly trained, experienced animal technicians, veterinarians, and animal behavior specialists.

Animals were singly housed in stainless-steel cages with a minimum of 6.0sq ft of floor space. In animal housing areas, humidity was maintained between 30 - 70% while temperature was kept at a range of 72 - 82°F. Paired animals were kept in adjacent cages to allow for grooming contact. Waste pans were cleaned daily, while cages, racks, and accessories were sanitized in mechanical cage washers at least once every two weeks. Animals were fed a commercial monkey chow, supplemented daily with fruits and vegetables, and drinking water was available at all times. Environmental enrichment was provided throughout the duration of the study, including grooming contact, perches, toys, foraging experiences, and food enrichment. Animal care staff monitored the health and well-being of all animals on a daily basis. All biopsies, surgeries, and blood draws were performed under ketamine anesthesia (10 mg/kg) and any continuous discomfort or pain was alleviated at the discretion of the veterinary staff to minimize suffering.

Rhesus macaques were challenged intravenously with 100 $TCID_{50}$ of cryopreserved SIVΔB670, diluted in 1 milliliter of RPMI. Following SIV infection, animals were monitored for changes in weight, complete blood counts, blood $CD4^+$ T cell count, and clinical signs of opportunistic infections. Simian AIDS was defined according to WaNPRC guidelines, namely: weight loss exceeding 15%, anemia (sustained hematocrit <15%), $CD4^+$ T cell decline to less than 200 cells per microliter, and presence of opportunistic infections. All four criteria were

evaluated in each animal at least monthly and at each specimen collection time point, or more frequently if two or more signs of disease progression were present. None of the animals required euthanasia prior to their experimental endpoint at 80 weeks post-infection.

At the experimental endpoint, euthanasia was performed by administration of Euthasol® (Virbac Corp., Houston, TX) while the animal was under deep anesthesia, in accordance with the 2007 American Veterinary Medical Association Guidelines on Euthanasia.

The University of Washington's Institutional Animal Care and Use Committee (IACUC) approved all experiments in these macaques (IACUC Protocol # 4266-04).

MHC-I and TRIM5 typing

All macaques were major histocompatibility class I (MHC-1) typed *for Mamu* alleles (A*01, A*02, A*08, A*11, B*01, B*08, B*17, and B*29). DNA was extracted using the Roche© MagNA Pure™ system (Roche, catalog number: 06541089001) and analyzed via PCR by Dr. David Watkins and the MHC Genotyping Service at the University of Miami, as previously described [55, 56]. All animals were also tested for TRIM5 haplotypes, including TFP, Q, and CypA, by PCR of genomic DNA by Dr. David O'Connor at the Wisconsin National Primate Research Center (WNPRC).

Quantification of plasma viral load and complete blood counts (CBCs) and serum chemistries

The Virology Core at the WaNPRC, led by Dr. Shiu-Lok Hu and Dr. Patricia Firpo, quantified viral RNA in the plasma of SIVΔB670-infected animals using a real-time quantitative PCR (RT-q-PCR) assay. The Virology Core also determined complete blood counts, using a Beckman Coulter® AC*T^{TM} 5diff hematology analyzer (Beckman Coulter) as described previously [308].

Antiretroviral therapy

All SIV-infected animals were treated with a combination of 3 antiretroviral therapies:

9-(2-Phosphoryl-methoxypropyly) adenine (PMPA or tenofovir; provided by Gilead Sciences) was resuspended in phosphate-buffered saline (PBS) at 120 mg/mL. To completely dissolve the PMPA, 1 molar NaOH was added until the pH reached 7.4-7.8. The solution was then filter purified, injected into sterile glass vials, and stored at -20°C. PMPA was administered subcutaneously in a once-daily dose of 20 milligrams per kilogram (mg/kg) of animal weight.

2',3'-dideoxy-5-fluoro-3'-thiacytidine (FTC or emtricitabine, provided by Gilead Sciences) was resuspended in PBS at 120 mg/mL. The mixture was heated at 37°C with constant stirring until completely dissolved, and stored at 4°C. FTC was administered once-per-day subcutaneously at 30 mg/kg during the first month of ART (weeks 6-10 post-infection) and at 20 mg/kg once per-day for the remainder of ART. Raltegravir (Isentress, provided by Merck & Co.) was given orally at 250 mg/animal twice daily for the first month of ART, and at 150 mg/animal twice daily for the remainder of ART.

Trained animal technicians administered all ART drugs, and veterinary staff closely monitored animals for adverse side effects, which were treated immediately at their discretion.

A few animals experienced elevated creatinine levels due to prolonged treatment with PMPA, (A16144 and A16145 in the MAG + LT group, A16149 in the mock vaccine group, and A16237 in the MAG + AC group) so these animals were promptly switched to tenofovir disoproxil fumarate (TDF, Fisher Scientific, catalog number: AC-5262), a prodrug of tenofovir that is metabolized to PMPA.

TDF was resuspended in a solution of 15% Kleptose in water, at a concentration of 10.2 mg/mL, and pH adjusted to 4.1-4.3. The solution was then filter purified and stored at 4°C or frozen at -20°C for long-term storage. TDF was administered once per-day subcutaneously at 5.2 mg/kg for the duration of ART.

DNA vaccinations

Particle-mediated epidermal delivery (PMED, or gene gun):

Vaccine and adjuvant plasmids were formulated onto gold particles as previously described, and administered using the PowderJect® XR1 gene delivery device (PowderJect Vaccines, Inc.) [80]. Fur was shaved off of vaccination sites, which were then swabbed with alcohol prior to vaccine administration. Macaques were vaccinated over 16 epidermal sites along the lower abdomen and over the inguinal lymph nodes. Each animal received 32 μg of the MAG or Gag DNA vaccine co-formulated with 3.2 μg of plasmid expressing the LT adjuvant (2 μg MAG or Gag DNA + 0.2 μg LT per site).

Intradermal electroporation (ID EP):

MAG, Gag, and adjuvant plasmids (rhIL-12, LTA1, expressed on one plasmid each, and hRALDH2/rhIL-33 and rhPD-1/rhCD80 co-expressed on one plasmid each) were prepared in a citrate buffer (Sigma-Aldrich) and administered via intradermal injection into the dermis above the quadriceps muscle on each leg. For the first vaccination, each macaque received 900 μg of the MAG or Gag DNA vaccine co-formulated with 900 μg of DNA expressing hRALDH2/rhIL-33, 900 μg of DNA expressing rhIL-12, and 162 μg of DNA expressing LTA1, evenly distributed over 3 injection sites per leg (300 μg MAG or Gag + 300 μg hRALDH2/rhIL-33 + 300 μg rhIL-12 + 54 μg LTA1 per site). For each subsequent vaccination, each macaque received 900 μg of the MAG or Gag DNA vaccine co-formulated with 900 μg of DNA expressing hRALDH2/rhIL-33, 900 μg of DNA expressing rhIL-12, 975 μg of DNA expressing rhPD-1/rhCD80 and 162 μg of DNA expressing LTA1, evenly distributed over 4 injection sites per leg (225 μg MAG or Gag + 225 μg hRALDH2/rhIL-33 + 225 μg rhIL-12 + 244 μg rhPD-1/CD80 + 40.5 μg LTA1 per site). Prior to each vaccination, fur covering the vaccination site was shaved and the skin was swabbed with alcohol. Following injection of vaccine and adjuvant DNA, electrical pulses were delivered using the Agile Pulse device (BTX, catalog number: 47-0400N) according to the device manufacturer's instructions.

Enzyme-linked immunospot assay (ELISpot)

ELISpot was performed to quantify the frequency of SIV-specific IFN-γ spot-forming cells (SFC) in accordance with previously described methods. In brief, PBMCs were isolated from whole blood via Ficoll density gradient separation and stimulated with pools of 15-mer peptides overlapping by 11 amino acids and corresponding to the following SIVmac239 proteins: Gag, Env, Pol, Vif, Vpr, Rev, Nef, and Tat (NIH AIDS Reagent Program, catalog numbers as follows: Gag: 6204, Env: 6883, Pol: 6443, Vif: 6205, Vpr: 6449, Rev: 6448, Nef: 8762, Tat: 6207). As a negative control, samples were stimulated with dimethyl sulfoxide (DMSO). For a positive control, samples were stimulated with concanavalin A (5 μg/mL, Sigma-Aldrich, catalog number: C2272). Samples were considered positive if peptide-specific responses were at least twice that of the negative control plus at least 0.01% after background (DMSO) subtraction.

Enzyme-linked immunosorbent assay (ELISA) for analysis of antibody responses and microbial translocation

SIV Env-specific IgG binding antibody was measured by ELISA, as previously described [80]. In brief, 1μg/mL SIVmac239 gp130 (NIH AIDS Reagent Program, catalog number: 12797) was used as the capture antigen, and a rabbit anti-IgG (heavy and light chains conjugated to horseradish peroxidase) was used to detect antibody bound to the capture antigen.

Intracellular cytokine staining (ICS)

Cryopreserved PBMCs and MLN lymphocytes were thawed and rested at 37°C and 5% CO_2 for 6 hours before stimulation with DMSO, PMA/Ionomycin, or SIVmac239 Gag or Env peptides (1 μg/mL) for 1 hour with CD107a PECy5 (eBioH4A3, Thermofisher, catalog number: 15-1079-42) in R10 media before adding 1 mg/mL of Brefeldin A (Sigma-Aldrich®, catalog number: B7651-5MG). Cells were stimulated overnight (approximately 14 hours) at 37°C and 5% CO_2. After stimulation, cells were washed with PBS and stained using LIVE/DEAD® Aqua (Life Technologies, catalog number: L34957) amine-reactive dye to distinguish live cells, then surface stained with CD3 Brilliant Violet (BV) 711 (Sp34-2, BD Biosciences, catalog number: 740807),

CD4 PerCPCy5.5 (L200, BD Biosciences, catalog number: 552838), CD8 APC-Cy7 (RPA-T8, BD Biosciences catalog number: 557760), CD28 PE-CF594 (CD28.2, BD Biosciences, catalog number: 562296), CD95 BV421 (Dx2, BD Biosciences, catalog number: 562616), in Brilliant Stain buffer (BD Biosciences, catalog number: 566349). Cells were then permeabilized with Cytofix/Cytoperm (BD Biosciences, catalog number: 554722) and stained for intracellular cytokines with an antibody cocktail of IFNγ FITC (B27, BD Biosciences, catalog number: 552887), TNFα PE-Cy8 (Mab11, BD Biosciences, 557647), IL-2 PE (MQ1-17H12, BD Biosciences, catalog number: 500307), and GranzymeB APC (GB12, ThermoFisher®, catalog number: MHGb05), in Perm/Wash™ Buffer (BD Biosciences, catalog number: 554723). Finally, cells were washed with Perm/Wash™ Buffer and fixed with 1% paraformaldehyde. Data were collected on an LSR II (BD Biosciences) and analyzed using FlowJo software (Version 9.7.6, Treestar Inc.).

Intracellular cytokine staining (ICS) of gut mucosa

Intraepithelial and lamina propria lymphocytes were isolated from colon biopsies and stimulated in the presence of brefeldin A (Sigma-Aldrich®, catalog number: B7651-5MG) and CD107a antibody (eBioH4A3; eBioscience, catalog number: 15-1079-42), as previously described [307]. Cells were assessed for viability using LIVE/DEAD® Aqua (Life Technologies, catalog number: L34957) and stained using surface and intracellular/intranuclear markers as previously described [307]. All samples were acquired on an LSRII (BD Biosciences) and analyzed using FlowJo software version 9.9.4 (FlowJo; LLC). Gating schemes are described previously [307]. Briefly, $CD4^+$ Tregs were designated by coexpression of CD25 and FoxP3 and Th17 cells were defined by IL-17 production.

Statistical analyses

Statistical differences between multiple groups were calculated using a Dunn's multiple comparisons test, while statistical comparisons between two groups were determined using a two-sided Mann-Whitney. Statistical differences in viral load, $CD4^+$ T cell counts, or immune

responses between time points were calculated using a Wilcoxon matched-pairs signed-rank test. Viral burden was determined by calculating the area under the curve of each animal's viral load graph. Correlations between immune responses and viral burden were determined by a Spearman's rank correlation test. When necessary, P values were adjusted for multiple comparisons using the Benjamini-Hochberg method. A P value of ≤ 0.05 was considered significant for each test. All calculations were performed using GraphPad Prism software (Version 8, GraphPad Software).

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Chapter 4: Conclusions and Future Directions

In this dissertation, I summarized our knowledge of HIV virology and examined the field's progress towards a therapeutic vaccine for HIV (Chapter 1: Introduction) and evaluated the immunogenicity and efficacy of our therapeutic SIV DNA vaccine and novel genetic adjuvant combination (MAG + AC, Chapter 2: Immunogenicity and Efficacy of an SIV therapeutic DNA vaccine and novel adjuvant combination). To this end, I demonstrated that vaccination with MAG + AC significantly boosted SIV-specific IFN-γ responses in comparison to mock-vaccinated controls and animals that received the same DNA vaccine co-administered with a single adjuvant (MAG + LT). Three out of five animals in the MAG + AC group also exhibited increased SIV-specific polyfunctional $CD8^+$ T cells in blood and gut-associated lymphoid tissue (GALT) post-vaccination, whereas none of the MAG + LT or mock-vaccinated animals did. Notably, the three animals with increased frequencies of SIV-specific polyfunctional $CD8^+$ T cells went on to control viral replication during analytic treatment interruption (ATI), compared to one out of five animals in the MAG + LT group and one of four animals in the mock-vaccinated group. While these results were not statistically significant, likely due to the limited number of animals per group, these data show that co-administration of multiple adjuvants can further boost vaccine immunogenicity, compared to a single adjuvant, and highlight the need for therapeutic vaccines and adjuvants able to elicit polyfunctional $CD8^+$ T cell responses in both the GALT and periphery.

The clear distinction between the five animals that controlled viral replication and the nine animals that exhibited immediate viral rebound also enabled me to determine the immune correlates of virologic control in this study (Chapter 2: Immune Correlates of Protection from Viral Rebound in Animals with Variable Responses to ART). Importantly, I found that low ATI viral burden was associated with the quality of the SIV-specific $CD8^+$ T cell response in the blood and GALT. Furthermore, I showed that although the majority of the SIV-specific $CD8^+$ T cell response is not polyfunctional, these responses are not associated with containment of viral replication, while polyfunctional responses are. This suggests that polyfunctional $CD8^+$ T cells are likely more important for sustained control of viral rebound. The importance of T cell polyfunctionality in the

blood for control of viral rebound is well-established, but this data provides new evidence that that polyfunctional T cell responses in the GALT are also likely key for achieving durable containment of viremia.

Finally, I defined inherent host factors that likely influenced viral control during ATI. I determined that lower viral burden during acute infection was associated with less residual viral replication on ART, and improved virological response to ART was associated with control of viral rebound during ATI. While others have shown that therapeutic vaccination is not effective in nonhuman primates whose viral loads are unaffected by ART, these results go a step further to demonstrate how even low levels of enduring viremia on ART can affect control of viral rebound during ATI. I also identified that increased colonic $CD4^+$ T cells and lower proportions of T helper 17 (Th17) and T regulatory cells (Tregs) pre-infection were predictive of lower viral burden during ATI. Collectively, this data suggests that pre-infection proportions of mucosal T cell subsets likely impact acute viremia, with far-reaching effects on the persistence of viral replication on ART and control of viral rebound during ATI.

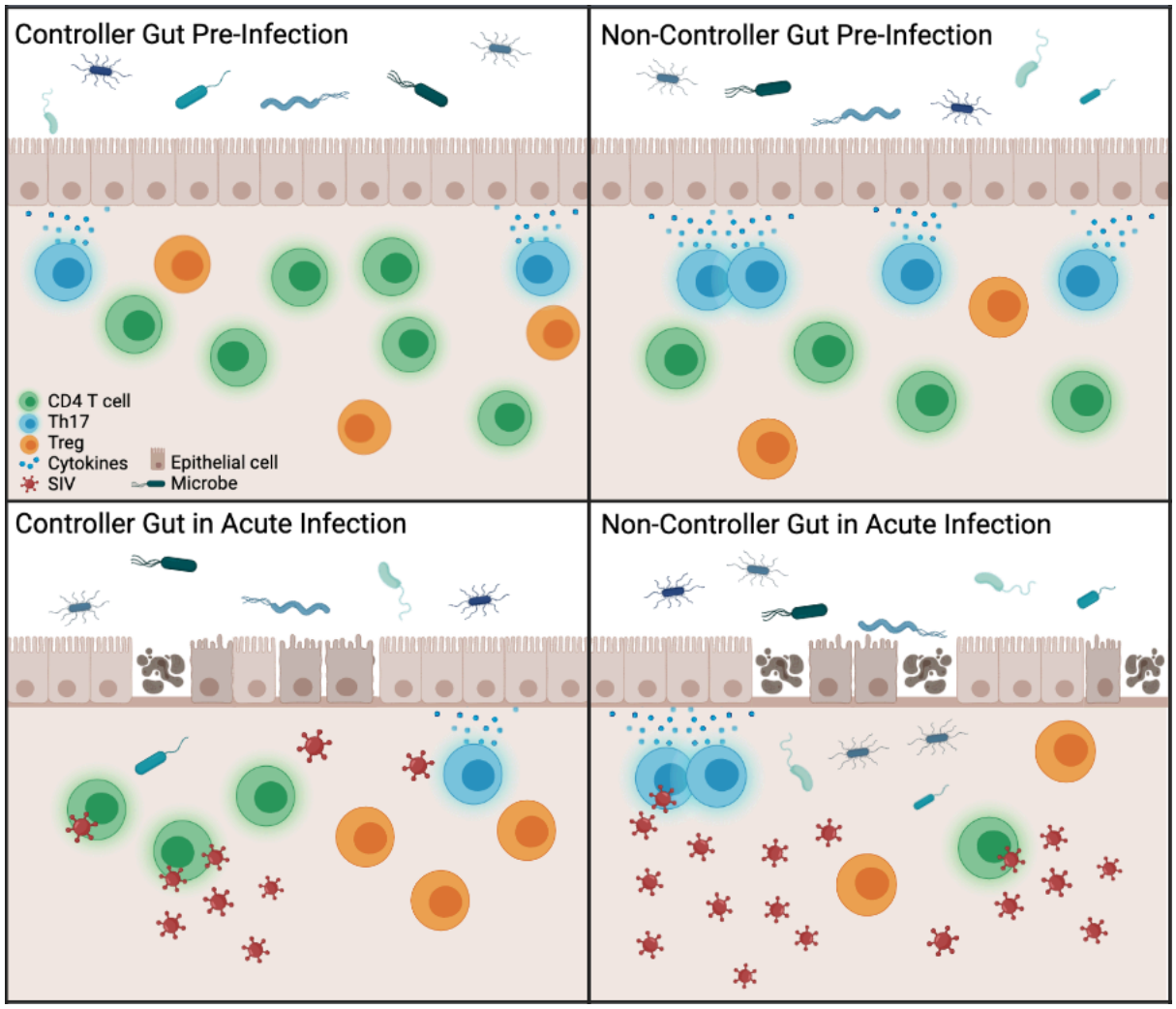

Figure 4.1 Pre-infection mucosal CD4$^+$ T cells can have far-reaching effects on viral replication. Shown is a theoretical representation of how differing proportions of mucosal CD4$^+$ T cells pre-infection could affect viral replication and gut dysbiosis during infection. (Figure produced using Biorender.com)

It is possible that an effective therapeutic vaccine for HIV will need to incorporate immunotherapies that more fully restore gut immune homeostasis than ART alone, as suppression of viral replication with effective ART doesn't result in full reconstitution of mucosal $CD4^+$ T cells or resolution of microbial translocation. Incorporation of anti-inflammatory drugs and probiotics could limit systemic inflammation, reduce mucosal immune dysfunction, and reverse alterations in the microbiome resulting from HIV infection, leading to improved therapeutic outcomes during ATI. There is evidence of probiotic therapies reversing mucosal damage and reducing inflammation following SIV infection in rhesus macaques, although reconstitution of Th17 cells was limited [309, 310]. However, co-administration of probiotics and IL-21 resolves this issue by also improving mucosal immunity and reducing microbial translocation, while enhancing expansion of polyfunctional Th17 cells in SIV-infected macaques [311]. IL-21 and probiotic therapy have not been tested in the context of therapeutic vaccination, but my findings suggest that improving mucosal immune reconstitution could result in improved responses to therapeutic vaccination.

The data that I presented here also indicates that there is an ongoing need for vaccine adjuvants that induce robust mucosal immunity. Although vaccination with MAG + AC boosted SIV-specific $CD8^+$ T cell responses in peripheral blood, the same could not be said for the mucosa. To this end, the adjuvant combination could be improved by substituting other mucosal adjuvants for retinaldehyde dehydrogenase 2, such as IL-7 or plasmid-expressed mucosal chemokines. A recent study showed that local administration of IL-7 triggers immune cell homing to the vaginal mucosa of rhesus macaques [312], suggesting that this cytokine could be a promising mucosal adjuvant. In support of this, a study showed that co-administration of plasmids encoding IL-7 and IL-33 enhanced the immunogenicity of a herpes zoster DNA vaccine in mice, compared to the DNA vaccine alone [313]. This study did not assess mucosal immunity but is an important proof of concept that that IL-7 can enhance the immunogenicity of DNA vaccines.

Alternatively, plasmids encoding mucosal chemokines could also be promising mucosal adjuvants. In particular, thymus-expressed chemokine (TECK, or CCL25) is a chemokine, a protein secreted by cells to induce chemotaxis in other cells through receptor binding. TECK binds to CCR9 and is important for T cell homing to the gut mucosa [310, 314]. TECK and other chemokine adjuvants have not been tested in the context of a therapeutic vaccine, but a prophylactic DNA vaccine co-administered with plasmids expressing TECK conferred significant protection against SIV challenge in rhesus macaques [315]. This suggests that chemokine adjuvants could also enhance the immunogenicity and efficacy of therapeutic DNA vaccines.

All in all, the work presented here demonstrates that co-administration of the novel adjuvant combination with a therapeutic DNA vaccine boosted SIV-specific IFN-γ responses in the peripheral blood, compared to the therapeutic DNA vaccine adjuvanted with plasmids expressing LT. Three out of five vaccinated animals went on to control viral rebound, although this result was not statistically significant, potentially due to the small number of animals per group. I also identified immune correlates of protection from viral rebound, namely elevated frequencies of polyfunctional $CD8^+$ T cells in the GALT and peripheral blood. Finally, I provide evidence that the frequencies of pre-infection mucosal T cell subsets may affect acute viral replication, with long-term effects on control of viremia during ATI. With further refinement, the adjuvant combination could be used in a broad variety of settings to improve the immunogenicity of T cell-based vaccines. Ultimately, these results deepen our understanding of how host mucosal immunity may influence the immunogenicity and efficacy of therapeutic vaccines and may lead to development of more efficacious HIV cure strategies and improved DNA vaccine immunogenicity for other pathogens.

Works Cited:

1. Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, et al. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med. 1981;305(24):1425-31. Epub 1981/12/10. doi: 10.1056/NEJM198112103052401. PubMed PMID: 6272109.
2. Durack DT. Opportunistic infections and Kaposi's sarcoma in homosexual men. N Engl J Med. 1981;305(24):1465-7. Epub 1981/12/10. doi: 10.1056/NEJM198112103052408. PubMed PMID: 6272112.
3. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science. 1983;220(4599):868-71. Epub 1983/05/20. doi: 10.1126/science.6189183. PubMed PMID: 6189183.
4. Brun-Vezinet F, Rouzioux C, Barre-Sinoussi F, Klatzmann D, Saimot AG, Rozenbaum W, et al. Detection of IgG antibodies to lymphadenopathy-associated virus in patients with AIDS or lymphadenopathy syndrome. Lancet. 1984;1(8389):1253-6. Epub 1984/06/09. doi: 10.1016/s0140-6736(84)92444-9. PubMed PMID: 6144973.
5. Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, et al. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell. 1996;85(7):1135-48. Epub 1996/06/28. doi: 10.1016/s0092-8674(00)81313-6. PubMed PMID: 8674119.
6. Schols D, Struyf S, Van Damme J, Este JA, Henson G, De Clercq E. Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4. J Exp Med. 1997;186(8):1383-8. Epub 1997/10/23. doi: 10.1084/jem.186.8.1383. PubMed PMID: 9334378; PubMed Central PMCID: PMCPMC2199084.
7. Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat Med. 1999;5(5):512-7. Epub 1999/05/06. doi: 10.1038/8394. PubMed PMID: 10229227.
8. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature. 1995;373(6510):123-6. Epub 1995/01/12. doi: 10.1038/373123a0. PubMed PMID: 7816094.
9. Doitsh G, Cavrois M, Lassen KG, Zepeda O, Yang Z, Santiago ML, et al. Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue. Cell. 2010;143(5):789-801. Epub 2010/11/30. doi: 10.1016/j.cell.2010.11.001. PubMed PMID: 21111238; PubMed Central PMCID: PMCPMC3026834.
10. Deeks SG, Walker BD. Human immunodeficiency virus controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. Immunity. 2007;27(3):406-16. Epub 2007/09/26. doi: 10.1016/j.immuni.2007.08.010. PubMed PMID: 17892849.
11. Saez-Cirion A, Lacabaratz C, Lambotte O, Versmisse P, Urrutia A, Boufassa F, et al. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. Proc Natl Acad Sci U S A. 2007;104(16):6776-81. Epub 2007/04/13. doi: 10.1073/pnas.0611244104. PubMed PMID: 17428922; PubMed Central PMCID: PMCPMC1851664.
12. Pereyra F, Addo MM, Kaufmann DE, Liu Y, Miura T, Rathod A, et al. Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. J Infect Dis. 2008;197(4):563-71. Epub 2008/02/16. doi: 10.1086/526786. PubMed PMID: 18275276.
13. Lambotte O, Boufassa F, Madec Y, Nguyen A, Goujard C, Meyer L, et al. HIV controllers: a homogeneous group of HIV-1-infected patients with spontaneous control of viral

replication. Clin Infect Dis. 2005;41(7):1053-6. Epub 2005/09/06. doi: 10.1086/433188. PubMed PMID: 16142675.
14. Vanhems P, Dassa C, Lambert J, Cooper DA, Perrin L, Vizzard J, et al. Comprehensive classification of symptoms and signs reported among 218 patients with acute HIV-1 infection. J Acquir Immune Defic Syndr. 1999;21(2):99-106. Epub 1999/06/09. PubMed PMID: 10360800.
15. Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, Fox CH, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature. 1993;362(6418):355-8. Epub 1993/03/25. doi: 10.1038/362355a0. PubMed PMID: 8455722.
16. Piatak M, Jr., Saag MS, Yang LC, Clark SJ, Kappes JC, Luk KC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science. 1993;259(5102):1749-54. Epub 1993/03/19. doi: 10.1126/science.8096089. PubMed PMID: 8096089.
17. Vanhems P, Baratin D, Allard R, Marceillac E, Biron F, Cotte L, et al. Factors associated with the time elapsed between the initial detection of HIV-1 antibodies and a diagnosis of AIDS among patients followed in Lyons University Hospitals. CISIH Collaborators. Sex Transm Infect. 1999;75(6):389-91. Epub 2001/02/07. doi: 10.1136/sti.75.6.389. PubMed PMID: 10754941; PubMed Central PMCID: PMCPMC1758255.
18. Phillips AN, Lee CA, Elford J, Janossy G, Timms A, Bofill M, et al. Serial CD4 lymphocyte counts and development of AIDS. Lancet. 1991;337(8738):389-92. Epub 1991/02/16. doi: 10.1016/0140-6736(91)91166-r. PubMed PMID: 1671424.
19. Gail MH, Tan WY, Pee D, Goedert JJ. Survival after AIDS diagnosis in a cohort of hemophilia patients. Multicenter Hemophilia Cohort Study. J Acquir Immune Defic Syndr Hum Retrovirol. 1997;15(5):363-9. Epub 1997/08/15. doi: 10.1097/00042560-199708150-00006. PubMed PMID: 9342256.
20. Fischl MA, Richman DD, Hansen N, Collier AC, Carey JT, Para MF, et al. The safety and efficacy of zidovudine (AZT) in the treatment of subjects with mildly symptomatic human immunodeficiency virus type 1 (HIV) infection. A double-blind, placebo-controlled trial. The AIDS Clinical Trials Group. Ann Intern Med. 1990;112(10):727-37. Epub 1990/05/15. doi: 10.7326/0003-4819-112-10-727. PubMed PMID: 1970466.
21. Larder BA, Darby G, Richman DD. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science. 1989;243(4899):1731-4. Epub 1989/03/31. doi: 10.1126/science.2467383. PubMed PMID: 2467383.
22. Larder BA, Kemp SD. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). Science. 1989;246(4934):1155-8. Epub 1989/12/01. doi: 10.1126/science.2479983. PubMed PMID: 2479983.
23. Gallant JE. Protease-inhibitor boosting in the treatment-experienced patient. AIDS Rev. 2004;6(4):226-33. Epub 2005/02/11. PubMed PMID: 15700621.
24. Gallant JE, Rodriguez AE, Weinberg WG, Young B, Berger DS, Lim ML, et al. Early virologic nonresponse to tenofovir, abacavir, and lamivudine in HIV-infected antiretroviral-naive subjects. J Infect Dis. 2005;192(11):1921-30. Epub 2005/11/04. doi: 10.1086/498069. PubMed PMID: 16267763.
25. Lalezari JP, Henry K, O'Hearn M, Montaner JS, Piliero PJ, Trottier B, et al. Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. N Engl J Med. 2003;348(22):2175-85. Epub 2003/03/15. doi: 10.1056/NEJMoa035026. PubMed PMID: 12637625.
26. Lennox JL, DeJesus E, Lazzarin A, Pollard RB, Madruga JV, Berger DS, et al. Safety and efficacy of raltegravir-based versus efavirenz-based combination therapy in treatment-naive patients with HIV-1 infection: a multicentre, double-blind randomised controlled

trial. Lancet. 2009;374(9692):796-806. Epub 2009/08/04. doi: 10.1016/S0140-6736(09)60918-1. PubMed PMID: 19647866.
27. Lennox JL, Dejesus E, Berger DS, Lazzarin A, Pollard RB, Ramalho Madruga JV, et al. Raltegravir versus Efavirenz regimens in treatment-naive HIV-1-infected patients: 96-week efficacy, durability, subgroup, safety, and metabolic analyses. J Acquir Immune Defic Syndr. 2010;55(1):39-48. Epub 2010/04/21. doi: 10.1097/QAI.0b013e3181da1287. PubMed PMID: 20404738; PubMed Central PMCID: PMCPMC6065510.
28. Autran B, Carcelain G, Li TS, Blanc C, Mathez D, Tubiana R, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science. 1997;277(5322):112-6. Epub 1997/07/04. doi: 10.1126/science.277.5322.112. PubMed PMID: 9204894.
29. Gallant JE. The ideal nucleoside/nucleotide backbone. J Acquir Immune Defic Syndr. 2004;37 Suppl 1:S44-51. Epub 2004/08/21. doi: 10.1097/01.qai.0000137006.70999.d3. PubMed PMID: 15319669.
30. Arribas JR, Pozniak AL, Gallant JE, Dejesus E, Gazzard B, Campo RE, et al. Tenofovir disoproxil fumarate, emtricitabine, and efavirenz compared with zidovudine/lamivudine and efavirenz in treatment-naive patients: 144-week analysis. J Acquir Immune Defic Syndr. 2008;47(1):74-8. Epub 2007/11/01. doi: 10.1097/QAI.0b013e31815acab8. PubMed PMID: 17971715.
31. Arribas JR, Pialoux G, Gathe J, Di Perri G, Reynes J, Tebas P, et al. Simplification to coformulated elvitegravir, cobicistat, emtricitabine, and tenofovir versus continuation of ritonavir-boosted protease inhibitor with emtricitabine and tenofovir in adults with virologically suppressed HIV (STRATEGY-PI): 48 week results of a randomised, open-label, phase 3b, non-inferiority trial. Lancet Infect Dis. 2014;14(7):581-9. Epub 2014/06/09. doi: 10.1016/S1473-3099(14)70782-0. PubMed PMID: 24908551.
32. Surve DH, Jindal AB. Recent advances in long-acting nanoformulations for delivery of antiretroviral drugs. J Control Release. 2020;324:379-404. Epub 2020/05/29. doi: 10.1016/j.jconrel.2020.05.022. PubMed PMID: 32461114.
33. Howe ZW, Norman S, Lueken AF, Huesgen E, Farmer EK, Jarrell K, et al. Therapeutic review of cabotegravir/rilpivirine long-acting antiretroviral injectable and implementation considerations at an HIV specialty clinic. Pharmacotherapy. 2021. Epub 2021/06/16. doi: 10.1002/phar.2605. PubMed PMID: 34130357.
34. Vella S, Schwartlander B, Sow SP, Eholie SP, Murphy RL. The history of antiretroviral therapy and of its implementation in resource-limited areas of the world. AIDS. 2012;26(10):1231-41. Epub 2012/06/19. doi: 10.1097/QAD.0b013e32835521a3. PubMed PMID: 22706009.
35. AIDS A-LCoIDtE, Keiser O, Anastos K, Schechter M, Balestre E, Myer L, et al. Antiretroviral therapy in resource-limited settings 1996 to 2006: patient characteristics, treatment regimens and monitoring in sub-Saharan Africa, Asia and Latin America. Trop Med Int Health. 2008;13(7):870-9. Epub 2008/04/01. doi: 10.1111/j.1365-3156.2008.02078.x. PubMed PMID: 18373510; PubMed Central PMCID: PMCPMC3722496.
36. Anthony KB, Yoder C, Metcalf JA, DerSimonian R, Orenstein JM, Stevens RA, et al. Incomplete CD4 T cell recovery in HIV-1 infection after 12 months of highly active antiretroviral therapy is associated with ongoing increased CD4 T cell activation and turnover. J Acquir Immune Defic Syndr. 2003;33(2):125-33. Epub 2003/06/10. doi: 10.1097/00126334-200306010-00002. PubMed PMID: 12794543.
37. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12(12):1365-71. Epub 2006/11/23. doi: 10.1038/nm1511. PubMed PMID: 17115046.

38. Cassol E, Malfeld S, Mahasha P, van der Merwe S, Cassol S, Seebregts C, et al. Persistent microbial translocation and immune activation in HIV-1-infected South Africans receiving combination antiretroviral therapy. J Infect Dis. 2010;202(5):723-33. Epub 2010/07/16. doi: 10.1086/655229. PubMed PMID: 20629534.
39. French MA, King MS, Tschampa JM, da Silva BA, Landay AL. Serum immune activation markers are persistently increased in patients with HIV infection after 6 years of antiretroviral therapy despite suppression of viral replication and reconstitution of CD4+ T cells. J Infect Dis. 2009;200(8):1212-5. Epub 2009/09/05. doi: 10.1086/605890. PubMed PMID: 19728788.
40. Hazenberg MD, Stuart JW, Otto SA, Borleffs JC, Boucher CA, de Boer RJ, et al. T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART). Blood. 2000;95(1):249-55. Epub 1999/12/23. PubMed PMID: 10607709.
41. Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B, et al. Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. J Infect Dis. 2009;199(8):1177-85. Epub 2009/03/07. doi: 10.1086/597476. PubMed PMID: 19265479; PubMed Central PMCID: PMCPMC2728622.
42. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci U S A. 1997;94(24):13193-7. Epub 1997/12/16. doi: 10.1073/pnas.94.24.13193. PubMed PMID: 9371822; PubMed Central PMCID: PMCPMC24285.
43. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science. 1997;278(5341):1295-300. Epub 1997/11/21. doi: 10.1126/science.278.5341.1295. PubMed PMID: 9360927.
44. Pierson T, McArthur J, Siliciano RF. Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. Annu Rev Immunol. 2000;18:665-708. Epub 2000/06/03. doi: 10.1146/annurev.immunol.18.1.665. PubMed PMID: 10837072.
45. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nat Med. 2003;9(6):727-8. Epub 2003/05/20. doi: 10.1038/nm880. PubMed PMID: 12754504.
46. Chun TW, Justement JS, Murray D, Hallahan CW, Maenza J, Collier AC, et al. Rebound of plasma viremia following cessation of antiretroviral therapy despite profoundly low levels of HIV reservoir: implications for eradication. AIDS. 2010;24(18):2803-8. Epub 2010/10/22. doi: 10.1097/QAD.0b013e328340a239. PubMed PMID: 20962613; PubMed Central PMCID: PMCPMC3154092.
47. Hirsch VM, Olmsted RA, Murphey-Corb M, Purcell RH, Johnson PR. An African primate lentivirus (SIVsm) closely related to HIV-2. Nature. 1989;339(6223):389-92. Epub 1989/06/01. doi: 10.1038/339389a0. PubMed PMID: 2786147.
48. Huet T, Cheynier R, Meyerhans A, Roelants G, Wain-Hobson S. Genetic organization of a chimpanzee lentivirus related to HIV-1. Nature. 1990;345(6273):356-9. Epub 1990/05/24. doi: 10.1038/345356a0. PubMed PMID: 2188136.
49. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med. 2011;1(1):a006841. Epub 2012/01/10. doi: 10.1101/cshperspect.a006841. PubMed PMID: 22229120; PubMed Central PMCID: PMCPMC3234451.
50. Rey-Cuille MA, Berthier JL, Bomsel-Demontoy MC, Chaduc Y, Montagnier L, Hovanessian AG, et al. Simian immunodeficiency virus replicates to high levels in sooty mangabeys

without inducing disease. J Virol. 1998;72(5):3872-86. Epub 1998/04/29. doi: 10.1128/JVI.72.5.3872-3886.1998. PubMed PMID: 9557672; PubMed Central PMCID: PMCPMC109612.
51. Norley SG, Kraus G, Ennen J, Bonilla J, Konig H, Kurth R. Immunological studies of the basis for the apathogenicity of simian immunodeficiency virus from African green monkeys. Proc Natl Acad Sci U S A. 1990;87(22):9067-71. Epub 1990/11/01. doi: 10.1073/pnas.87.22.9067. PubMed PMID: 2247482; PubMed Central PMCID: PMCPMC55102.
52. Reimann KA, Tenner-Racz K, Racz P, Montefiori DC, Yasutomi Y, Lin W, et al. Immunopathogenic events in acute infection of rhesus monkeys with simian immunodeficiency virus of macaques. J Virol. 1994;68(4):2362-70. Epub 1994/04/01. doi: 10.1128/JVI.68.4.2362-2370.1994. PubMed PMID: 8139022; PubMed Central PMCID: PMCPMC236713.
53. Watson A, Ranchalis J, Travis B, McClure J, Sutton W, Johnson PR, et al. Plasma viremia in macaques infected with simian immunodeficiency virus: plasma viral load early in infection predicts survival. J Virol. 1997;71(1):284-90. Epub 1997/01/01. doi: 10.1128/JVI.71.1.284-290.1997. PubMed PMID: 8985348; PubMed Central PMCID: PMCPMC191049.
54. Mothe BR, Weinfurter J, Wang C, Rehrauer W, Wilson N, Allen TM, et al. Expression of the major histocompatibility complex class I molecule Mamu-A*01 is associated with control of simian immunodeficiency virus SIVmac239 replication. J Virol. 2003;77(4):2736-40. Epub 2003/01/29. doi: 10.1128/jvi.77.4.2736-2740.2003. PubMed PMID: 12552014; PubMed Central PMCID: PMCPMC141082.
55. Yant LJ, Friedrich TC, Johnson RC, May GE, Maness NJ, Enz AM, et al. The high-frequency major histocompatibility complex class I allele Mamu-B*17 is associated with control of simian immunodeficiency virus SIVmac239 replication. J Virol. 2006;80(10):5074-7. Epub 2006/04/28. doi: 10.1128/JVI.80.10.5074-5077.2006. PubMed PMID: 16641299; PubMed Central PMCID: PMCPMC1472056.
56. Loffredo JT, Maxwell J, Qi Y, Glidden CE, Borchardt GJ, Soma T, et al. Mamu-B*08-positive macaques control simian immunodeficiency virus replication. J Virol. 2007;81(16):8827-32. Epub 2007/06/01. doi: 10.1128/JVI.00895-07. PubMed PMID: 17537848; PubMed Central PMCID: PMCPMC1951344.
57. Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. Nature. 2004;427(6977):848-53. Epub 2004/02/27. doi: 10.1038/nature02343. PubMed PMID: 14985764.
58. Lim SY, Rogers T, Chan T, Whitney JB, Kim J, Sodroski J, et al. TRIM5alpha Modulates Immunodeficiency Virus Control in Rhesus Monkeys. PLoS Pathog. 2010;6(1):e1000738. Epub 2010/01/29. doi: 10.1371/journal.ppat.1000738. PubMed PMID: 20107597; PubMed Central PMCID: PMCPMC2809762.
59. Wilson SJ, Webb BL, Maplanka C, Newman RM, Verschoor EJ, Heeney JL, et al. Rhesus macaque TRIM5 alleles have divergent antiretroviral specificities. J Virol. 2008;82(14):7243-7. Epub 2008/05/16. doi: 10.1128/JVI.00307-08. PubMed PMID: 18480454; PubMed Central PMCID: PMCPMC2446970.
60. Reynolds MR, Sacha JB, Weiler AM, Borchardt GJ, Glidden CE, Sheppard NC, et al. The TRIM5{alpha} genotype of rhesus macaques affects acquisition of simian immunodeficiency virus SIVsmE660 infection after repeated limiting-dose intrarectal challenge. J Virol. 2011;85(18):9637-40. Epub 2011/07/08. doi: 10.1128/JVI.05074-11. PubMed PMID: 21734037; PubMed Central PMCID: PMCPMC3165772.
61. Naidu YM, Kestler HW, 3rd, Li Y, Butler CV, Silva DP, Schmidt DK, et al. Characterization of infectious molecular clones of simian immunodeficiency virus (SIVmac) and human

immunodeficiency virus type 2: persistent infection of rhesus monkeys with molecularly cloned SIVmac. J Virol. 1988;62(12):4691-6. Epub 1988/12/01. doi: 10.1128/JVI.62.12.4691-4696.1988. PubMed PMID: 2846880; PubMed Central PMCID: PMCPMC253583.
62. Lewis MG, Bellah S, McKinnon K, Yalley-Ogunro J, Zack PM, Elkins WR, et al. Titration and characterization of two rhesus-derived SIVmac challenge stocks. AIDS Res Hum Retroviruses. 1994;10(2):213-20. Epub 1994/02/01. doi: 10.1089/aid.1994.10.213. PubMed PMID: 8198874.
63. Hirsch VM, Zack PM, Vogel AP, Johnson PR. Simian immunodeficiency virus infection of macaques: end-stage disease is characterized by widespread distribution of proviral DNA in tissues. J Infect Dis. 1991;163(5):976-88. Epub 1991/05/01. doi: 10.1093/infdis/163.5.976. PubMed PMID: 2019773.
64. Baba TW, Trichel AM, An L, Liska V, Martin LN, Murphey-Corb M, et al. Infection and AIDS in adult macaques after nontraumatic oral exposure to cell-free SIV. Science. 1996;272(5267):1486-9. Epub 1996/06/07. doi: 10.1126/science.272.5267.1486. PubMed PMID: 8633242.
65. Reimann KA, Li JT, Veazey R, Halloran M, Park IW, Karlsson GB, et al. A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate env causes an AIDS-like disease after in vivo passage in rhesus monkeys. J Virol. 1996;70(10):6922-8. Epub 1996/10/01. doi: 10.1128/JVI.70.10.6922-6928.1996. PubMed PMID: 8794335; PubMed Central PMCID: PMCPMC190741.
66. Bar KJ, Coronado E, Hensley-McBain T, O'Connor MA, Osborn JM, Miller C, et al. Simian-Human Immunodeficiency Virus SHIV.CH505 Infection of Rhesus Macaques Results in Persistent Viral Replication and Induces Intestinal Immunopathology. J Virol. 2019;93(18). Epub 2019/06/21. doi: 10.1128/JVI.00372-19. PubMed PMID: 31217249; PubMed Central PMCID: PMCPMC6714786.
67. Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, et al. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science. 1998;280(5362):427-31. Epub 1998/05/09. doi: 10.1126/science.280.5362.427. PubMed PMID: 9545219.
68. Guadalupe M, Reay E, Sankaran S, Prindiville T, Flamm J, McNeil A, et al. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol. 2003;77(21):11708-17. Epub 2003/10/15. doi: 10.1128/jvi.77.21.11708-11717.2003. PubMed PMID: 14557656; PubMed Central PMCID: PMCPMC229357.
69. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med. 2004;200(6):749-59. Epub 2004/09/15. doi: 10.1084/jem.20040874. PubMed PMID: 15365096; PubMed Central PMCID: PMCPMC2211962.
70. Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med. 2004;200(6):761-70. Epub 2004/09/15. doi: 10.1084/jem.20041196. PubMed PMID: 15365095; PubMed Central PMCID: PMCPMC2211967.
71. Brenchley JM, Paiardini M, Knox KS, Asher AI, Cervasi B, Asher TE, et al. Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. Blood. 2008;112(7):2826-35. Epub 2008/07/31. doi: 10.1182/blood-2008-05-159301. PubMed PMID: 18664624; PubMed Central PMCID: PMCPMC2556618.
72. Douek D. HIV disease progression: immune activation, microbes, and a leaky gut. Top HIV Med. 2007;15(4):114-7. Epub 2007/08/28. PubMed PMID: 17720995.

73. Walker CM, Moody DJ, Stites DP, Levy JA. CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. Science. 1986;234(4783):1563-6. Epub 1986/12/19. doi: 10.1126/science.2431484. PubMed PMID: 2431484.
74. Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J Virol. 1994;68(9):6103-10. Epub 1994/09/01. doi: 10.1128/JVI.68.9.6103-6110.1994. PubMed PMID: 8057491; PubMed Central PMCID: PMCPMC237022.
75. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol. 1994;68(7):4650-5. Epub 1994/07/01. doi: 10.1128/JVI.68.7.4650-4655.1994. PubMed PMID: 8207839; PubMed Central PMCID: PMCPMC236393.
76. Matano T, Shibata R, Siemon C, Connors M, Lane HC, Martin MA. Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. J Virol. 1998;72(1):164-9. Epub 1998/01/07. doi: 10.1128/JVI.72.1.164-169.1998. PubMed PMID: 9420212; PubMed Central PMCID: PMCPMC109361.
77. Jin X, Bauer DE, Tuttleton SE, Lewin S, Gettie A, Blanchard J, et al. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. J Exp Med. 1999;189(6):991-8. Epub 1999/03/17. doi: 10.1084/jem.189.6.991. PubMed PMID: 10075982; PubMed Central PMCID: PMCPMC2193038.
78. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science. 1999;283(5403):857-60. Epub 1999/02/05. doi: 10.1126/science.283.5403.857. PubMed PMID: 9933172.
79. Kiepiela P, Ngumbela K, Thobakgale C, Ramduth D, Honeyborne I, Moodley E, et al. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. Nat Med. 2007;13(1):46-53. Epub 2006/12/19. doi: 10.1038/nm1520. PubMed PMID: 17173051.
80. Fuller DH, Rajakumar P, Che JW, Narendran A, Nyaundi J, Michael H, et al. Therapeutic DNA vaccine induces broad T cell responses in the gut and sustained protection from viral rebound and AIDS in SIV-infected rhesus macaques. PLoS One. 2012;7(3):e33715. Epub 2012/03/24. doi: 10.1371/journal.pone.0033715. PubMed PMID: 22442716; PubMed Central PMCID: PMCPMC3307760.
81. Chouquet C, Autran B, Gomard E, Bouley JM, Calvez V, Katlama C, et al. Correlation between breadth of memory HIV-specific cytotoxic T cells, viral load and disease progression in HIV infection. AIDS. 2002;16(18):2399-407. Epub 2002/12/04. doi: 10.1097/00002030-200212060-00004. PubMed PMID: 12461413.
82. Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, Abraham J, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood. 2006;107(12):4781-9. Epub 2006/02/10. doi: 10.1182/blood-2005-12-4818. PubMed PMID: 16467198; PubMed Central PMCID: PMCPMC1895811.
83. Hersperger AR, Pereyra F, Nason M, Demers K, Sheth P, Shin LY, et al. Perforin expression directly ex vivo by HIV-specific CD8 T-cells is a correlate of HIV elite control. PLoS Pathog. 2010;6(5):e1000917. Epub 2010/06/05. doi: 10.1371/journal.ppat.1000917. PubMed PMID: 20523897; PubMed Central PMCID: PMCPMC2877741.

84. Douek DC, Brenchley JM, Betts MR, Ambrozak DR, Hill BJ, Okamoto Y, et al. HIV preferentially infects HIV-specific CD4+ T cells. Nature. 2002;417(6884):95-8. Epub 2002/05/03. doi: 10.1038/417095a. PubMed PMID: 11986671.
85. Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science. 1997;278(5342):1447-50. Epub 1997/12/31. doi: 10.1126/science.278.5342.1447. PubMed PMID: 9367954.
86. Soghoian DZ, Jessen H, Flanders M, Sierra-Davidson K, Cutler S, Pertel T, et al. HIV-specific cytolytic CD4 T cell responses during acute HIV infection predict disease outcome. Sci Transl Med. 2012;4(123):123ra25. Epub 2012/03/02. doi: 10.1126/scitranslmed.3003165. PubMed PMID: 22378925; PubMed Central PMCID: PMCPMC3918726.
87. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature. 2006;443(7109):350-4. Epub 2006/08/22. doi: 10.1038/nature05115. PubMed PMID: 16921384.
88. Wherry EJ, Ha SJ, Kaech SM, Haining WN, Sarkar S, Kalia V, et al. Molecular signature of CD8+ T cell exhaustion during chronic viral infection. Immunity. 2007;27(4):670-84. Epub 2007/10/24. doi: 10.1016/j.immuni.2007.09.006. PubMed PMID: 17950003.
89. Kaufmann DE, Kavanagh DG, Pereyra F, Zaunders JJ, Mackey EW, Miura T, et al. Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction. Nat Immunol. 2007;8(11):1246-54. Epub 2007/10/02. doi: 10.1038/ni1515. PubMed PMID: 17906628.
90. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11(11):3887-95. Epub 1992/11/01. PubMed PMID: 1396582; PubMed Central PMCID: PMCPMC556898.
91. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439(7077):682-7. Epub 2005/12/31. doi: 10.1038/nature04444. PubMed PMID: 16382236.
92. Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, et al. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. Nat Med. 2006;12(10):1198-202. Epub 2006/08/19. doi: 10.1038/nm1482. PubMed PMID: 16917489.
93. Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, et al. PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. J Exp Med. 2006;203(10):2281-92. Epub 2006/09/07. doi: 10.1084/jem.20061496. PubMed PMID: 16954372; PubMed Central PMCID: PMCPMC2118095.
94. Zhang JY, Zhang Z, Wang X, Fu JL, Yao J, Jiao Y, et al. PD-1 up-regulation is correlated with HIV-specific memory CD8+ T-cell exhaustion in typical progressors but not in long-term nonprogressors. Blood. 2007;109(11):4671-8. Epub 2007/02/03. doi: 10.1182/blood-2006-09-044826. PubMed PMID: 17272504.
95. Velu V, Titanji K, Zhu B, Husain S, Pladevega A, Lai L, et al. Enhancing SIV-specific immunity in vivo by PD-1 blockade. Nature. 2009;458(7235):206-10. Epub 2008/12/17. doi: 10.1038/nature07662. PubMed PMID: 19078956; PubMed Central PMCID: PMCPMC2753387.
96. Hokey DA, Johnson FB, Smith J, Weber JL, Yan J, Hirao L, et al. Activation drives PD-1 expression during vaccine-specific proliferation and following lentiviral infection in macaques. Eur J Immunol. 2008;38(5):1435-45. Epub 2008/04/05. doi: 10.1002/eji.200737857. PubMed PMID: 18389475; PubMed Central PMCID: PMCPMC2996615.

97. Dyavar Shetty R, Velu V, Titanji K, Bosinger SE, Freeman GJ, Silvestri G, et al. PD-1 blockade during chronic SIV infection reduces hyperimmune activation and microbial translocation in rhesus macaques. J Clin Invest. 2012;122(5):1712-6. Epub 2012/04/24. doi: 10.1172/JCI60612. PubMed PMID: 22523065; PubMed Central PMCID: PMCPMC3336983.
98. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity. 1995;3(5):541-7. Epub 1995/11/01. doi: 10.1016/1074-7613(95)90125-6. PubMed PMID: 7584144.
99. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science. 1995;270(5238):985-8. Epub 1995/11/10. doi: 10.1126/science.270.5238.985. PubMed PMID: 7481803.
100. Chambers CA, Kuhns MS, Egen JG, Allison JP. CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol. 2001;19:565-94. Epub 2001/03/13. doi: 10.1146/annurev.immunol.19.1.565. PubMed PMID: 11244047.
101. Teft WA, Kirchhof MG, Madrenas J. A molecular perspective of CTLA-4 function. Annu Rev Immunol. 2006;24:65-97. Epub 2006/03/23. doi: 10.1146/annurev.immunol.24.021605.090535. PubMed PMID: 16551244.
102. Deng K, Pertea M, Rongvaux A, Wang L, Durand CM, Ghiaur G, et al. Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. Nature. 2015;517(7534):381-5. Epub 2015/01/07. doi: 10.1038/nature14053. PubMed PMID: 25561180; PubMed Central PMCID: PMCPMC4406054.
103. Fukazawa Y, Park H, Cameron MJ, Lefebvre F, Lum R, Coombes N, et al. Lymph node T cell responses predict the efficacy of live attenuated SIV vaccines. Nat Med. 2012;18(11):1673-81. Epub 2012/09/11. doi: 10.1038/nm.2934. PubMed PMID: 22961108; PubMed Central PMCID: PMCPMC3493820.
104. Zolla-Pazner S, Alvarez R, Kong XP, Weiss S. Vaccine-induced V1V2-specific antibodies control and or protect against infection with HIV, SIV and SHIV. Curr Opin HIV AIDS. 2019;14(4):309-17. Epub 2019/04/18. doi: 10.1097/COH.0000000000000551. PubMed PMID: 30994501; PubMed Central PMCID: PMCPMC6542703.
105. Forthal DN, Finzi A. Antibody-dependent cellular cytotoxicity in HIV infection. AIDS. 2018;32(17):2439-51. Epub 2018/09/21. doi: 10.1097/QAD.0000000000002011. PubMed PMID: 30234611; PubMed Central PMCID: PMCPMC6497078.
106. Autran B, Descours B, Avettand-Fenoel V, Rouzioux C. Elite controllers as a model of functional cure. Curr Opin HIV AIDS. 2011;6(3):181-7. Epub 2011/04/05. doi: 10.1097/COH.0b013e328345a328. PubMed PMID: 21460722.
107. Baker BM, Block BL, Rothchild AC, Walker BD. Elite control of HIV infection: implications for vaccine design. Expert Opin Biol Ther. 2009;9(1):55-69. Epub 2008/12/10. doi: 10.1517/14712590802571928. PubMed PMID: 19063693; PubMed Central PMCID: PMCPMC3731764.
108. Baba TW, Liska V, Khimani AH, Ray NB, Dailey PJ, Penninck D, et al. Live attenuated, multiply deleted simian immunodeficiency virus causes AIDS in infant and adult macaques. Nat Med. 1999;5(2):194-203. Epub 1999/02/04. doi: 10.1038/5557. PubMed PMID: 9930868.
109. Chakrabarti LA, Metzner KJ, Ivanovic T, Cheng H, Louis-Virelizier J, Connor RI, et al. A truncated form of Nef selected during pathogenic reversion of simian immunodeficiency virus SIVmac239Deltanef increases viral replication. J Virol. 2003;77(2):1245-56. Epub 2002/12/28. doi: 10.1128/jvi.77.2.1245-1256.2003. PubMed PMID: 12502842; PubMed Central PMCID: PMCPMC140812.

110. HIV vaccine--immune response. AG 1661, IR-8101, IR8101, REMUNE, RG 83894. Drugs R D. 2002;3(6):411-20. Epub 2003/01/09. doi: 10.2165/00126839-200203060-00011. PubMed PMID: 12516948.
111. Kinloch-de Loes S, Hoen B, Smith DE, Autran B, Lampe FC, Phillips AN, et al. Impact of therapeutic immunization on HIV-1 viremia after discontinuation of antiretroviral therapy initiated during acute infection. J Infect Dis. 2005;192(4):607-17. Epub 2005/07/20. doi: 10.1086/432002. PubMed PMID: 16028129.
112. Turner JL, Kostman JR, Aquino A, Wright D, Szabo S, Bidwell R, et al. The effects of an HIV-1 immunogen (Remune) on viral load, CD4 cell counts and HIV-specific immunity in a double-blind, randomized, adjuvant-controlled subset study in HIV infected subjects regardless of concomitant antiviral drugs. HIV Med. 2001;2(2):68-77. Epub 2001/12/12. doi: 10.1046/j.1468-1293.2001.00051.x. PubMed PMID: 11737381.
113. Kahn JO, Cherng DW, Mayer K, Murray H, Lagakos S. Evaluation of HIV-1 immunogen, an immunologic modifier, administered to patients infected with HIV having 300 to 549 x 10(6)/L CD4 cell counts: A randomized controlled trial. JAMA. 2000;284(17):2193-202. Epub 2000/11/01. doi: 10.1001/jama.284.17.2193. PubMed PMID: 11056590.
114. Trauger RJ, Daigle AE, Giermakowska W, Moss RB, Jensen F, Carlo DJ. Safety and immunogenicity of a gp120-depleted, inactivated HIV-1 immunogen: results of a double-blind, adjuvant controlled trial. J Acquir Immune Defic Syndr Hum Retrovirol. 1995;10 Suppl 2:S74-82. Epub 1995/01/01. PubMed PMID: 7552517.
115. Moss RB, Ferre F, Trauger R, Jensen F, Daigle A, Richieri SP, et al. Inactivated HIV-1 Immunogen: impact on markers of disease progression. J Acquir Immune Defic Syndr (1988). 1994;7 Suppl 1:S21-7. Epub 1994/01/01. PubMed PMID: 7910207.
116. Valor L, Navarro J, Carbone J, Rodriguez-Sainz C, Gil J, Lopez F, et al. Immunization with an HIV-1 immunogen induces CD4+ and CD8+ HIV-1-specific polyfunctional responses in patients with chronic HIV-1 infection receiving antiretroviral therapy. Vaccine. 2008;26(22):2738-45. Epub 2008/04/25. doi: 10.1016/j.vaccine.2008.03.019. PubMed PMID: 18433946.
117. Fernandez-Cruz E, Moreno S, Navarro J, Clotet B, Bouza E, Carbone J, et al. Therapeutic immunization with an inactivated HIV-1 Immunogen plus antiretrovirals versus antiretroviral therapy alone in asymptomatic HIV-infected subjects. Vaccine. 2004;22(23-24):2966-73. Epub 2004/08/07. doi: 10.1016/j.vaccine.2004.03.040. PubMed PMID: 15297045.
118. Chantratita W, Sukeepaisarncharoen W, Chandeying V, Kulpradist S, Israngkura Na Ayudhtaya B, Rugpao S, et al. Delayed progression to AIDS in volunteers treated with long-term HIV-1 Immunogen (REMUNE) therapy in Thailand. HIV Med. 2004;5(5):317-25. Epub 2004/09/17. doi: 10.1111/j.1468-1293.2004.00230.x. PubMed PMID: 15369506.
119. Sukeepaisarncharoen W, Churdboonchart V, Kulpradist S, Isarangkura Na Ayudthya B, Rugpao S, Chandeying V, et al. Long-term follow-up of HIV-1-infected Thai patients immunized with Remune monotherapy. HIV Clin Trials. 2001;2(5):391-8. Epub 2001/10/24. doi: 10.1310/Q5XX-A5CH-XTB9-FN33. PubMed PMID: 11673813.
120. Churdboonchart V, Sakondhavat C, Kulpradist S, Na Ayudthya BI, Chandeying V, Rugpao S, et al. A double-blind, adjuvant-controlled trial of human immunodeficiency virus type 1 (HIV-1) immunogen (Remune) monotherapy in asymptomatic, HIV-1-infected thai subjects with CD4-cell counts of >300. Clin Diagn Lab Immunol. 2000;7(5):728-33. Epub 2000/09/06. doi: 10.1128/cdli.7.5.728-733.2000. PubMed PMID: 10973445; PubMed Central PMCID: PMCPMC95946.
121. Costiniuk CT, Kovacs C, Routy JP, Singer J, Gurunathan S, Sekaly RP, et al. Short communication: human immunodeficiency virus rebound in blood and seminal plasma following discontinuation of antiretroviral therapy. AIDS Res Hum Retroviruses.

2013;29(2):266-9. Epub 2012/08/23. doi: 10.1089/AID.2011.0343. PubMed PMID: 22908887; PubMed Central PMCID: PMCPMC3552161.
122. Angel JB, Routy JP, Tremblay C, Ayers D, Woods R, Singer J, et al. A randomized controlled trial of HIV therapeutic vaccination using ALVAC with or without Remune. AIDS. 2011;25(6):731-9. Epub 2011/02/19. doi: 10.1097/QAD.0b013e328344cea5. PubMed PMID: 21330911.
123. Glidden D, Kim S, Lagakos S. Effectiveness of remune. Clin Diagn Lab Immunol. 2001;8(2):468-9. Epub 2001/04/28. doi: 10.1128/cdli.8.2.468-469.2001. PubMed PMID: 11324587; PubMed Central PMCID: PMCPMC96084.
124. Lurie P, Wolfe SM. Outcomes of a trial of HIV-1 immunogen in patients with HIV infection. JAMA. 2001;285(17):2193-5. Epub 2001/05/18. PubMed PMID: 11325316.
125. Rossio JL, Esser MT, Suryanarayana K, Schneider DK, Bess JW, Jr., Vasquez GM, et al. Inactivation of human immunodeficiency virus type 1 infectivity with preservation of conformational and functional integrity of virion surface proteins. J Virol. 1998;72(10):7992-8001. Epub 1998/09/12. doi: 10.1128/JVI.72.10.7992-8001.1998. PubMed PMID: 9733838; PubMed Central PMCID: PMCPMC110135.
126. Vagenas P, Williams VG, Piatak M, Jr., Bess JW, Jr., Lifson JD, Blanchard JL, et al. Tonsillar application of AT-2 SIV affords partial protection against rectal challenge with SIVmac239. J Acquir Immune Defic Syndr. 2009;52(4):433-42. Epub 2009/09/26. doi: 10.1097/QAI.0b013e3181b880f3. PubMed PMID: 19779309; PubMed Central PMCID: PMCPMC2783539.
127. Vagenas P, Aravantinou M, Williams VG, Jasny E, Piatak M, Jr., Lifson JD, et al. A tonsillar PolyICLC/AT-2 SIV therapeutic vaccine maintains low viremia following antiretroviral therapy cessation. PLoS One. 2010;5(9):e12891. Epub 2010/09/30. doi: 10.1371/journal.pone.0012891. PubMed PMID: 20877632; PubMed Central PMCID: PMCPMC2943484.
128. Tung FY, Tung JK, Pallikkuth S, Pahwa S, Fischl MA. A therapeutic HIV-1 vaccine enhances anti-HIV-1 immune responses in patients under highly active antiretroviral therapy. Vaccine. 2016;34(19):2225-32. Epub 2016/03/24. doi: 10.1016/j.vaccine.2016.03.021. PubMed PMID: 27002500.
129. Pallikkuth S, Bolivar H, Fletcher MA, Babic DZ, De Armas LR, Gupta S, et al. A therapeutic HIV-1 vaccine reduces markers of systemic immune activation and latent infection in patients under highly active antiretroviral therapy. Vaccine. 2020;38(27):4336-45. Epub 2020/05/11. doi: 10.1016/j.vaccine.2020.04.015. PubMed PMID: 32387010.
130. Choi E, Michalski CJ, Choo SH, Kim GN, Banasikowska E, Lee S, et al. First Phase I human clinical trial of a killed whole-HIV-1 vaccine: demonstration of its safety and enhancement of anti-HIV antibody responses. Retrovirology. 2016;13(1):82. Epub 2016/11/30. doi: 10.1186/s12977-016-0317-2. PubMed PMID: 27894306; PubMed Central PMCID: PMCPMC5126836.
131. Clark E, Nava B, Caputi M. Tat is a multifunctional viral protein that modulates cellular gene expression and functions. Oncotarget. 2017;8(16):27569-81. Epub 2017/02/12. doi: 10.18632/oncotarget.15174. PubMed PMID: 28187438; PubMed Central PMCID: PMCPMC5432358.
132. Butto S, Fiorelli V, Tripiciano A, Ruiz-Alvarez MJ, Scoglio A, Ensoli F, et al. Sequence conservation and antibody cross-recognition of clade B human immunodeficiency virus (HIV) type 1 Tat protein in HIV-1-infected Italians, Ugandans, and South Africans. J Infect Dis. 2003;188(8):1171-80. Epub 2003/10/11. doi: 10.1086/378412. PubMed PMID: 14551888.
133. Reiss P, Lange JM, de Ronde A, de Wolf F, Dekker J, Debouck C, et al. Speed of progression to AIDS and degree of antibody response to accessory gene products of

HIV-1. J Med Virol. 1990;30(3):163-8. Epub 1990/03/01. doi: 10.1002/jmv.1890300303. PubMed PMID: 2341832.
134. Rezza G, Fiorelli V, Dorrucci M, Ciccozzi M, Tripiciano A, Scoglio A, et al. The presence of anti-Tat antibodies is predictive of long-term nonprogression to AIDS or severe immunodeficiency: findings in a cohort of HIV-1 seroconverters. J Infect Dis. 2005;191(8):1321-4. Epub 2005/03/19. doi: 10.1086/428909. PubMed PMID: 15776379.
135. Zagury JF, Sill A, Blattner W, Lachgar A, Le Buanec H, Richardson M, et al. Antibodies to the HIV-1 Tat protein correlated with nonprogression to AIDS: a rationale for the use of Tat toxoid as an HIV-1 vaccine. J Hum Virol. 1998;1(4):282-92. Epub 1999/04/09. PubMed PMID: 10195253.
136. Cafaro A, Caputo A, Fracasso C, Maggiorella MT, Goletti D, Baroncelli S, et al. Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine. Nat Med. 1999;5(6):643-50. Epub 1999/06/17. doi: 10.1038/9488. PubMed PMID: 10371502.
137. Maggiorella MT, Baroncelli S, Michelini Z, Fanales-Belasio E, Moretti S, Sernicola L, et al. Long-term protection against SHIV89.6P replication in HIV-1 Tat vaccinated cynomolgus monkeys. Vaccine. 2004;22(25-26):3258-69. Epub 2004/08/17. doi: 10.1016/j.vaccine.2004.03.009. PubMed PMID: 15308348.
138. Watkins JD, Lancelot S, Campbell GR, Esquieu D, de Mareuil J, Opi S, et al. Reservoir cells no longer detectable after a heterologous SHIV challenge with the synthetic HIV-1 Tat Oyi vaccine. Retrovirology. 2006;3:8. Epub 2006/01/31. doi: 10.1186/1742-4690-3-8. PubMed PMID: 16441880; PubMed Central PMCID: PMCPMC1434768.
139. Pauza CD, Trivedi P, Wallace M, Ruckwardt TJ, Le Buanec H, Lu W, et al. Vaccination with tat toxoid attenuates disease in simian/HIV-challenged macaques. Proc Natl Acad Sci U S A. 2000;97(7):3515-9. Epub 2000/03/22. doi: 10.1073/pnas.070049797. PubMed PMID: 10725402; PubMed Central PMCID: PMCPMC16271.
140. Liang X, Casimiro DR, Schleif WA, Wang F, Davies ME, Zhang ZQ, et al. Vectored Gag and Env but not Tat show efficacy against simian-human immunodeficiency virus 89.6P challenge in Mamu-A*01-negative rhesus monkeys. J Virol. 2005;79(19):12321-31. Epub 2005/09/15. doi: 10.1128/JVI.79.19.12321-12331.2005. PubMed PMID: 16160159; PubMed Central PMCID: PMCPMC1211517.
141. Ensoli B, Bellino S, Tripiciano A, Longo O, Francavilla V, Marcotullio S, et al. Therapeutic immunization with HIV-1 Tat reduces immune activation and loss of regulatory T-cells and improves immune function in subjects on HAART. PLoS One. 2010;5(11):e13540. Epub 2010/11/19. doi: 10.1371/journal.pone.0013540. PubMed PMID: 21085635; PubMed Central PMCID: PMCPMC2978690.
142. Ensoli B, Nchabeleng M, Ensoli F, Tripiciano A, Bellino S, Picconi O, et al. HIV-Tat immunization induces cross-clade neutralizing antibodies and CD4(+) T cell increases in antiretroviral-treated South African volunteers: a randomized phase II clinical trial. Retrovirology. 2016;13(1):34. Epub 2016/06/10. doi: 10.1186/s12977-016-0261-1. PubMed PMID: 27277839; PubMed Central PMCID: PMCPMC4899930.
143. Loret EP, Darque A, Jouve E, Loret EA, Nicolino-Brunet C, Morange S, et al. Intradermal injection of a Tat Oyi-based therapeutic HIV vaccine reduces of 1.5 log copies/mL the HIV RNA rebound median and no HIV DNA rebound following cART interruption in a phase I/II randomized controlled clinical trial. Retrovirology. 2016;13:21. Epub 2016/04/03. doi: 10.1186/s12977-016-0251-3. PubMed PMID: 27036656; PubMed Central PMCID: PMCPMC4818470.
144. Sgadari C, Monini P, Tripiciano A, Picconi O, Casabianca A, Orlandi C, et al. Continued Decay of HIV Proviral DNA Upon Vaccination With HIV-1 Tat of Subjects on Long-Term ART: An 8-Year Follow-Up Study. Front Immunol. 2019;10:233. Epub 2019/03/01. doi: 10.3389/fimmu.2019.00233. PubMed PMID: 30815001; PubMed Central PMCID: PMCPMC6381398.

145. Asjo B, Stavang H, Sorensen B, Baksaas I, Nyhus J, Langeland N. Phase I trial of a therapeutic HIV type 1 vaccine, Vacc-4x, in HIV type 1-infected individuals with or without antiretroviral therapy. AIDS Res Hum Retroviruses. 2002;18(18):1357-65. Epub 2002/12/19. doi: 10.1089/088922202320935438. PubMed PMID: 12487807.
146. Kran AM, Sorensen B, Nyhus J, Sommerfelt MA, Baksaas I, Bruun JN, et al. HLA- and dose-dependent immunogenicity of a peptide-based HIV-1 immunotherapy candidate (Vacc-4x). AIDS. 2004;18(14):1875-83. Epub 2004/09/09. doi: 10.1097/00002030-200409240-00003. PubMed PMID: 15353973.
147. Kran AM, Sommerfelt MA, Sorensen B, Nyhus J, Baksaas I, Bruun JN, et al. Reduced viral burden amongst high responder patients following HIV-1 p24 peptide-based therapeutic immunization. Vaccine. 2005;23(31):4011-5. Epub 2005/06/21. doi: 10.1016/j.vaccine.2005.03.010. PubMed PMID: 15963359.
148. Kran AM, Sorensen B, Sommerfelt MA, Nyhus J, Baksaas I, Kvale D. Long-term HIV-specific responses and delayed resumption of antiretroviral therapy after peptide immunization targeting dendritic cells. AIDS. 2006;20(4):627-30. Epub 2006/02/14. doi: 10.1097/01.aids.0000210620.75707.ac. PubMed PMID: 16470131.
149. Pollard RB, Rockstroh JK, Pantaleo G, Asmuth DM, Peters B, Lazzarin A, et al. Safety and efficacy of the peptide-based therapeutic vaccine for HIV-1, Vacc-4x: a phase 2 randomised, double-blind, placebo-controlled trial. Lancet Infect Dis. 2014;14(4):291-300. Epub 2014/02/15. doi: 10.1016/S1473-3099(13)70343-8. PubMed PMID: 24525316.
150. De Rose R, Fernandez CS, Smith MZ, Batten CJ, Alcantara S, Peut V, et al. Control of viremia and prevention of AIDS following immunotherapy of SIV-infected macaques with peptide-pulsed blood. PLoS Pathog. 2008;4(5):e1000055. Epub 2008/05/03. doi: 10.1371/journal.ppat.1000055. PubMed PMID: 18451982; PubMed Central PMCID: PMCPMC2323103.
151. Kloverpris HN, Jackson A, Handley A, Hayes P, Gilmour J, Riddell L, et al. Non-immunogenicity of overlapping gag peptides pulsed on autologous cells after vaccination of HIV infected individuals. PLoS One. 2013;8(10):e74389. Epub 2013/10/15. doi: 10.1371/journal.pone.0074389. PubMed PMID: 24124451; PubMed Central PMCID: PMCPMC3790804.
152. Jackson A, Kloverpris HN, Boffito M, Handley A, Atkins M, Hayes P, et al. A randomised, placebo-controlled, first-in-human study of a novel clade C therapeutic peptide vaccine administered ex vivo to autologous white blood cells in HIV infected individuals. PLoS One. 2013;8(9):e73765. Epub 2013/09/27. doi: 10.1371/journal.pone.0073765. PubMed PMID: 24069230; PubMed Central PMCID: PMCPMC3775760.
153. Martin-Gayo E, Yu XG. Role of Dendritic Cells in Natural Immune Control of HIV-1 Infection. Front Immunol. 2019;10:1306. Epub 2019/06/28. doi: 10.3389/fimmu.2019.01306. PubMed PMID: 31244850; PubMed Central PMCID: PMCPMC6563724.
154. da Silva LT, Santillo BT, de Almeida A, Duarte A, Oshiro TM. Using Dendritic Cell-Based Immunotherapy to Treat HIV: How Can This Strategy be Improved? Front Immunol. 2018;9:2993. Epub 2019/01/09. doi: 10.3389/fimmu.2018.02993. PubMed PMID: 30619346; PubMed Central PMCID: PMCPMC6305438.
155. Lu W, Arraes LC, Ferreira WT, Andrieu JM. Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. Nat Med. 2004;10(12):1359-65. Epub 2004/11/30. doi: 10.1038/nm1147. PubMed PMID: 15568033.
156. Garcia F, Lejeune M, Climent N, Gil C, Alcami J, Morente V, et al. Therapeutic immunization with dendritic cells loaded with heat-inactivated autologous HIV-1 in patients with chronic HIV-1 infection. J Infect Dis. 2005;191(10):1680-5. Epub 2005/04/20. doi: 10.1086/429340. PubMed PMID: 15838795.

157. Garcia F, Climent N, Assoumou L, Gil C, Gonzalez N, Alcami J, et al. A therapeutic dendritic cell-based vaccine for HIV-1 infection. J Infect Dis. 2011;203(4):473-8. Epub 2011/01/15. doi: 10.1093/infdis/jiq077. PubMed PMID: 21233310; PubMed Central PMCID: PMCPMC3071229.
158. Garcia F, Climent N, Guardo AC, Gil C, Leon A, Autran B, et al. A dendritic cell-based vaccine elicits T cell responses associated with control of HIV-1 replication. Sci Transl Med. 2013;5(166):166ra2. Epub 2013/01/04. doi: 10.1126/scitranslmed.3004682. PubMed PMID: 23283367.
159. Macatangay BJ, Riddler SA, Wheeler ND, Spindler J, Lawani M, Hong F, et al. Therapeutic Vaccination With Dendritic Cells Loaded With Autologous HIV Type 1-Infected Apoptotic Cells. J Infect Dis. 2016;213(9):1400-9. Epub 2015/12/10. doi: 10.1093/infdis/jiv582. PubMed PMID: 26647281; PubMed Central PMCID: PMCPMC4813736.
160. Ide F, Nakamura T, Tomizawa M, Kawana-Tachikawa A, Odawara T, Hosoya N, et al. Peptide-loaded dendritic-cell vaccination followed by treatment interruption for chronic HIV-1 infection: a phase 1 trial. J Med Virol. 2006;78(6):711-8. Epub 2006/04/22. doi: 10.1002/jmv.20612. PubMed PMID: 16628588.
161. Kloverpris H, Karlsson I, Bonde J, Thorn M, Vinner L, Pedersen AE, et al. Induction of novel CD8+ T-cell responses during chronic untreated HIV-1 infection by immunization with subdominant cytotoxic T-lymphocyte epitopes. AIDS. 2009;23(11):1329-40. Epub 2009/06/17. doi: 10.1097/QAD.0b013e32832d9b00. PubMed PMID: 19528789.
162. Levy Y, Thiebaut R, Montes M, Lacabaratz C, Sloan L, King B, et al. Dendritic cell-based therapeutic vaccine elicits polyfunctional HIV-specific T-cell immunity associated with control of viral load. Eur J Immunol. 2014;44(9):2802-10. Epub 2014/07/22. doi: 10.1002/eji.201344433. PubMed PMID: 25042008.
163. Routy JP, Boulassel MR, Yassine-Diab B, Nicolette C, Healey D, Jain R, et al. Immunologic activity and safety of autologous HIV RNA-electroporated dendritic cells in HIV-1 infected patients receiving antiretroviral therapy. Clin Immunol. 2010;134(2):140-7. Epub 2009/11/06. doi: 10.1016/j.clim.2009.09.009. PubMed PMID: 19889582; PubMed Central PMCID: PMCPMC2818410.
164. Allard SD, De Keersmaecker B, de Goede AL, Verschuren EJ, Koetsveld J, Reedijk ML, et al. A phase I/IIa immunotherapy trial of HIV-1-infected patients with Tat, Rev and Nef expressing dendritic cells followed by treatment interruption. Clin Immunol. 2012;142(3):252-68. Epub 2011/12/20. doi: 10.1016/j.clim.2011.10.010. PubMed PMID: 22177848.
165. Gandhi RT, O'Neill D, Bosch RJ, Chan ES, Bucy RP, Shopis J, et al. A randomized therapeutic vaccine trial of canarypox-HIV-pulsed dendritic cells vs. canarypox-HIV alone in HIV-1-infected patients on antiretroviral therapy. Vaccine. 2009;27(43):6088-94. Epub 2009/05/20. doi: 10.1016/j.vaccine.2009.05.016. PubMed PMID: 19450647; PubMed Central PMCID: PMCPMC2820102.
166. Jacobson JM, Routy JP, Welles S, DeBenedette M, Tcherepanova I, Angel JB, et al. Dendritic Cell Immunotherapy for HIV-1 Infection Using Autologous HIV-1 RNA: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. J Acquir Immune Defic Syndr. 2016;72(1):31-8. Epub 2016/01/12. doi: 10.1097/QAI.0000000000000926. PubMed PMID: 26751016; PubMed Central PMCID: PMCPMC4836975.
167. Gay CL, DeBenedette MA, Tcherepanova IY, Gamble A, Lewis WE, Cope AB, et al. Immunogenicity of AGS-004 Dendritic Cell Therapy in Patients Treated During Acute HIV Infection. AIDS Res Hum Retroviruses. 2018;34(1):111-22. Epub 2017/06/22. doi: 10.1089/aid.2017.0071. PubMed PMID: 28636433; PubMed Central PMCID: PMCPMC5771540.
168. Gay CL, Kuruc JD, Falcinelli SD, Warren JA, Reifeis SA, Kirchherr JL, et al. Assessing the impact of AGS-004, a dendritic cell-based immunotherapy, and vorinostat on persistent

HIV-1 Infection. Sci Rep. 2020;10(1):5134. Epub 2020/03/22. doi: 10.1038/s41598-020-61878-3. PubMed PMID: 32198428; PubMed Central PMCID: PMCPMC7083965.
169. Engelmayer J, Larsson M, Lee A, Lee M, Cox WI, Steinman RM, et al. Mature dendritic cells infected with canarypox virus elicit strong anti-human immunodeficiency virus CD8+ and CD4+ T-cell responses from chronically infected individuals. J Virol. 2001;75(5):2142-53. Epub 2001/02/13. doi: 10.1128/JVI.75.5.2142-2153.2001. PubMed PMID: 11160718; PubMed Central PMCID: PMCPMC114798.
170. Taylor J, Meignier B, Tartaglia J, Languet B, VanderHoeven J, Franchini G, et al. Biological and immunogenic properties of a canarypox-rabies recombinant, ALVAC-RG (vCP65) in non-avian species. Vaccine. 1995;13(6):539-49. Epub 1995/04/01. doi: 10.1016/0264-410x(94)00028-l. PubMed PMID: 7483774.
171. Karasavvas N, Billings E, Rao M, Williams C, Zolla-Pazner S, Bailer RT, et al. The Thai Phase III HIV Type 1 Vaccine trial (RV144) regimen induces antibodies that target conserved regions within the V2 loop of gp120. AIDS Res Hum Retroviruses. 2012;28(11):1444-57. Epub 2012/10/06. doi: 10.1089/aid.2012.0103. PubMed PMID: 23035746; PubMed Central PMCID: PMCPMC3484815.
172. Angel JB, Routy JP, Graziani GM, Tremblay CL. The Effect of Therapeutic HIV Vaccination With ALVAC-HIV With or Without Remune on the Size of the Viral Reservoir (A CTN 173 Substudy). J Acquir Immune Defic Syndr. 2015;70(2):122-8. Epub 2015/09/17. doi: 10.1097/QAI.0000000000000734. PubMed PMID: 26375464; PubMed Central PMCID: PMCPMC4577611.
173. Kilby JM, Bucy RP, Mildvan D, Fischl M, Santana-Bagur J, Lennox J, et al. A randomized, partially blinded phase 2 trial of antiretroviral therapy, HIV-specific immunizations, and interleukin-2 cycles to promote efficient control of viral replication (ACTG A5024). J Infect Dis. 2006;194(12):1672-6. Epub 2006/11/17. doi: 10.1086/509508. PubMed PMID: 17109338.
174. Autran B, Murphy RL, Costagliola D, Tubiana R, Clotet B, Gatell J, et al. Greater viral rebound and reduced time to resume antiretroviral therapy after therapeutic immunization with the ALVAC-HIV vaccine (vCP1452). AIDS. 2008;22(11):1313-22. Epub 2008/06/27. doi: 10.1097/QAD.0b013e3282fdce94. PubMed PMID: 18580611.
175. Papagno L, Alter G, Assoumou L, Murphy RL, Garcia F, Clotet B, et al. Comprehensive analysis of virus-specific T-cells provides clues for the failure of therapeutic immunization with ALVAC-HIV vaccine. AIDS. 2011;25(1):27-36. Epub 2010/11/16. doi: 10.1097/QAD.0b013e328340fe55. PubMed PMID: 21076273.
176. Tubiana R, Carcelain G, Vray M, Gourlain K, Dalban C, Chermak A, et al. Therapeutic immunization with a human immunodeficiency virus (HIV) type 1-recombinant canarypox vaccine in chronically HIV-infected patients: The Vacciter Study (ANRS 094). Vaccine. 2005;23(34):4292-301. Epub 2005/06/02. doi: 10.1016/j.vaccine.2005.04.013. PubMed PMID: 15927325.
177. Levy Y, Gahery-Segard H, Durier C, Lascaux AS, Goujard C, Meiffredy V, et al. Immunological and virological efficacy of a therapeutic immunization combined with interleukin-2 in chronically HIV-1 infected patients. AIDS. 2005;19(3):279-86. Epub 2005/02/19. PubMed PMID: 15718838.
178. Brezar V, Hani L, Surenaud M, Hubert A, Lacabaratz C, Lelievre JD, et al. Negative modulation of suppressive HIV-specific regulatory T cells by IL-2 adjuvanted therapeutic vaccine. PLoS Pathog. 2017;13(7):e1006489. Epub 2017/07/15. doi: 10.1371/journal.ppat.1006489. PubMed PMID: 28708863; PubMed Central PMCID: PMCPMC5529021.
179. Levy Y, Durier C, Lascaux AS, Meiffredy V, Gahery-Segard H, Goujard C, et al. Sustained control of viremia following therapeutic immunization in chronically HIV-1-infected

individuals. AIDS. 2006;20(3):405-13. Epub 2006/01/28. doi: 10.1097/01.aids.0000206504.09159.d3. PubMed PMID: 16439874.
180. Goujard C, Marcellin F, Hendel-Chavez H, Burgard M, Meiffredy V, Venet A, et al. Interruption of antiretroviral therapy initiated during primary HIV-1 infection: impact of a therapeutic vaccination strategy combined with interleukin (IL)-2 compared with IL-2 alone in the ANRS 095 Randomized Study. AIDS Res Hum Retroviruses. 2007;23(9):1105-13. Epub 2007/10/09. doi: 10.1089/aid.2007.0047. PubMed PMID: 17919105.
181. Volz A, Sutter G. Modified Vaccinia Virus Ankara: History, Value in Basic Research, and Current Perspectives for Vaccine Development. Adv Virus Res. 2017;97:187-243. Epub 2017/01/07. doi: 10.1016/bs.aivir.2016.07.001. PubMed PMID: 28057259; PubMed Central PMCID: PMCPMC7112317.
182. Harrer E, Bauerle M, Ferstl B, Chaplin P, Petzold B, Mateo L, et al. Therapeutic vaccination of HIV-1-infected patients on HAART with a recombinant HIV-1 nef-expressing MVA: safety, immunogenicity and influence on viral load during treatment interruption. Antivir Ther. 2005;10(2):285-300. Epub 2005/05/04. PubMed PMID: 15865223.
183. Mothe B, Climent N, Plana M, Rosas M, Jimenez JL, Munoz-Fernandez MA, et al. Safety and immunogenicity of a modified vaccinia Ankara-based HIV-1 vaccine (MVA-B) in HIV-1-infected patients alone or in combination with a drug to reactivate latent HIV-1. J Antimicrob Chemother. 2015;70(6):1833-42. Epub 2015/03/01. doi: 10.1093/jac/dkv046. PubMed PMID: 25724985.
184. Rosas-Umbert M, Mothe B, Noguera-Julian M, Bellido R, Puertas MC, Carrillo J, et al. Virological and immunological outcome of treatment interruption in HIV-1-infected subjects vaccinated with MVA-B. PLoS One. 2017;12(9):e0184929. Epub 2017/09/28. doi: 10.1371/journal.pone.0184929. PubMed PMID: 28953921; PubMed Central PMCID: PMCPMC5617163.
185. Hancock G, Moron-Lopez S, Kopycinski J, Puertas MC, Giannoulatou E, Rose A, et al. Evaluation of the immunogenicity and impact on the latent HIV-1 reservoir of a conserved region vaccine, MVA.HIVconsv, in antiretroviral therapy-treated subjects. J Int AIDS Soc. 2017;20(1):21171. Epub 2017/05/26. doi: 10.7448/IAS.20.1.21171. PubMed PMID: 28537062; PubMed Central PMCID: PMCPMC5515041.
186. Letourneau S, Im EJ, Mashishi T, Brereton C, Bridgeman A, Yang H, et al. Design and pre-clinical evaluation of a universal HIV-1 vaccine. PLoS One. 2007;2(10):e984. Epub 2007/10/04. doi: 10.1371/journal.pone.0000984. PubMed PMID: 17912361; PubMed Central PMCID: PMCPMC1991584.
187. Rosas-Umbert M, Ruiz-Riol M, Fernandez MA, Marszalek M, Coll P, Manzardo C, et al. In vivo Effects of Romidepsin on T-Cell Activation, Apoptosis and Function in the BCN02 HIV-1 Kick&Kill Clinical Trial. Front Immunol. 2020;11:418. Epub 2020/04/09. doi: 10.3389/fimmu.2020.00418. PubMed PMID: 32265913; PubMed Central PMCID: PMCPMC7100631.
188. Mothe B, Rosas-Umbert M, Coll P, Manzardo C, Puertas MC, Moron-Lopez S, et al. HIVconsv Vaccines and Romidepsin in Early-Treated HIV-1-Infected Individuals: Safety, Immunogenicity and Effect on the Viral Reservoir (Study BCN02). Front Immunol. 2020;11:823. Epub 2020/05/22. doi: 10.3389/fimmu.2020.00823. PubMed PMID: 32435247; PubMed Central PMCID: PMCPMC7218169.
189. Robert-Guroff M. Replicating and non-replicating viral vectors for vaccine development. Curr Opin Biotechnol. 2007;18(6):546-56. Epub 2007/12/08. doi: 10.1016/j.copbio.2007.10.010. PubMed PMID: 18063357; PubMed Central PMCID: PMCPMC2245896.

190. Patterson LJ, Robert-Guroff M. Replicating adenovirus vector prime/protein boost strategies for HIV vaccine development. Expert Opin Biol Ther. 2008;8(9):1347-63. Epub 2008/08/13. doi: 10.1517/14712598.8.9.1347. PubMed PMID: 18694354; PubMed Central PMCID: PMCPMC2538611.
191. Colloca S, Barnes E, Folgori A, Ammendola V, Capone S, Cirillo A, et al. Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. Sci Transl Med. 2012;4(115):115ra2. Epub 2012/01/06. doi: 10.1126/scitranslmed.3002925. PubMed PMID: 22218691; PubMed Central PMCID: PMCPMC3627206.
192. Schooley RT, Spritzler J, Wang H, Lederman MM, Havlir D, Kuritzkes DR, et al. AIDS clinical trials group 5197: a placebo-controlled trial of immunization of HIV-1-infected persons with a replication-deficient adenovirus type 5 vaccine expressing the HIV-1 core protein. J Infect Dis. 2010;202(5):705-16. Epub 2010/07/29. doi: 10.1086/655468. PubMed PMID: 20662716; PubMed Central PMCID: PMCPMC2916952.
193. Li JZ, Brumme ZL, Brumme CJ, Wang H, Spritzler J, Robertson MN, et al. Factors associated with viral rebound in HIV-1-infected individuals enrolled in a therapeutic HIV-1 gag vaccine trial. J Infect Dis. 2011;203(7):976-83. Epub 2011/03/16. doi: 10.1093/infdis/jiq143. PubMed PMID: 21402549; PubMed Central PMCID: PMCPMC3068025.
194. Mothe B, Manzardo C, Sanchez-Bernabeu A, Coll P, Moron-Lopez S, Puertas MC, et al. Therapeutic Vaccination Refocuses T-cell Responses Towards Conserved Regions of HIV-1 in Early Treated Individuals (BCN 01 study). EClinicalMedicine. 2019;11:65-80. Epub 2019/07/18. doi: 10.1016/j.eclinm.2019.05.009. PubMed PMID: 31312806; PubMed Central PMCID: PMCPMC6610778 research program for an HIV vaccine and Fundacio Gloria Soler. BM holds a post-doctoral fellowship grant from ISCIII (JR 13/00024) from 2014 to 2016 during the conduct of the study and is a consultant for AELIX THERAPEUTICS, S.L., outside the submitted work. SML holds a PhD grant from DGR (2013FI_B 00275) from 2013 to 2016 during the conduct of the study. JMM received a personal 80:20 research grant from the Institut d'Investigacions Biomediques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, during 2017-19 and reports grants and personal fees from Abbvie, Angelini, Contrafect, Genentech, Gilead, Jansen, Medtronic, MSD, Pfizer, ViiV Healthcare, outside the submitted work. LD reports being employer of Immunocore. TH reports grants from Medical Research Council UK, during the conduct of the study, and has a patent US 7981430B2 issued. CB is founder, CSO and shareholder of AELIX THERAPEUTIC, S.L. BC is founder, consultant and shareholder of AELIX THERAPEUTICS, S.L. TH reports grants from Medical Research Council UK, during the conduct of the study, and has a patent US 7981430B2 issued. SML hold a PhD grant from DGR (2013FI_B 00275) from 2013 to 2016 during the conduct of the study. CM, ASB, PCo, RE, NP, IR, CR, MM, AC, NB, EW, HY have nothing to disclose.
195. Fidler S, Stohr W, Pace M, Dorrell L, Lever A, Pett S, et al. Antiretroviral therapy alone versus antiretroviral therapy with a kick and kill approach, on measures of the HIV reservoir in participants with recent HIV infection (the RIVER trial): a phase 2, randomised trial. Lancet. 2020;395(10227):888-98. Epub 2020/02/23. doi: 10.1016/S0140-6736(19)32990-3. PubMed PMID: 32085823.
196. Barouch DH, O'Brien KL, Simmons NL, King SL, Abbink P, Maxfield LF, et al. Mosaic HIV-1 vaccines expand the breadth and depth of cellular immune responses in rhesus monkeys. Nat Med. 2010;16(3):319-23. Epub 2010/02/23. doi: 10.1038/nm.2089. PubMed PMID: 20173752; PubMed Central PMCID: PMCPMC2834868.
197. Colby DJ, Sarnecki M, Barouch DH, Tipsuk S, Stieh DJ, Kroon E, et al. Safety and immunogenicity of Ad26 and MVA vaccines in acutely treated HIV and effect on viral

rebound after antiretroviral therapy interruption. Nat Med. 2020;26(4):498-501. Epub 2020/04/03. doi: 10.1038/s41591-020-0774-y. PubMed PMID: 32235883.
198. Boyer JD, Ugen KE, Chattergoon M, Wang B, Shah A, Agadjanyan M, et al. DNA vaccination as anti-human immunodeficiency virus immunotherapy in infected chimpanzees. J Infect Dis. 1997;176(6):1501-9. Epub 1997/12/12. doi: 10.1086/514148. PubMed PMID: 9395361.
199. MacGregor RR, Boyer JD, Ugen KE, Lacy KE, Gluckman SJ, Bagarazzi ML, et al. First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: safety and host response. J Infect Dis. 1998;178(1):92-100. Epub 1998/07/04. doi: 10.1086/515613. PubMed PMID: 9652427.
200. Boyer JD, Chattergoon MA, Ugen KE, Shah A, Bennett M, Cohen A, et al. Enhancement of cellular immune response in HIV-1 seropositive individuals: A DNA-based trial. Clin Immunol. 1999;90(1):100-7. Epub 1999/01/13. doi: 10.1006/clim.1998.4616. PubMed PMID: 9884357.
201. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. Science. 1995;270(5243):1811-5. Epub 1995/12/15. doi: 10.1126/science.270.5243.1811. PubMed PMID: 8525373.
202. Calarota S, Bratt G, Nordlund S, Hinkula J, Leandersson AC, Sandstrom E, et al. Cellular cytotoxic response induced by DNA vaccination in HIV-1-infected patients. Lancet. 1998;351(9112):1320-5. Epub 1998/06/27. doi: 10.1016/S0140-6736(97)09440-3. PubMed PMID: 9643795.
203. Calarota SA, Leandersson AC, Bratt G, Hinkula J, Klinman DM, Weinhold KJ, et al. Immune responses in asymptomatic HIV-1-infected patients after HIV-DNA immunization followed by highly active antiretroviral treatment. J Immunol. 1999;163(4):2330-8. Epub 1999/08/10. PubMed PMID: 10438897.
204. Bostrom AC, Hejdeman B, Matsuda R, Fredriksson M, Fredriksson EL, Bratt G, et al. Long-term persistence of vaccination and HAART to human immunodeficiency virus (HIV). Vaccine. 2004;22(13-14):1683-91. Epub 2004/04/08. doi: 10.1016/j.vaccine.2003.09.049. PubMed PMID: 15068851.
205. Hejdeman B, Bostrom AC, Matsuda R, Calarota S, Lenkei R, Fredriksson EL, et al. DNA immunization with HIV early genes in HIV type 1-infected patients on highly active antiretroviral therapy. AIDS Res Hum Retroviruses. 2004;20(8):860-70. Epub 2004/08/24. doi: 10.1089/0889222041725190. PubMed PMID: 15320990.
206. Dorrell L, Yang H, Iversen AK, Conlon C, Suttill A, Lancaster M, et al. Therapeutic immunization of highly active antiretroviral therapy-treated HIV-1-infected patients: safety and immunogenicity of an HIV-1 gag/poly-epitope DNA vaccine. AIDS. 2005;19(12):1321-3. Epub 2005/07/30. doi: 10.1097/01.aids.0000180104.65640.16. PubMed PMID: 16052088.
207. MacGregor RR, Boyer JD, Ugen KE, Tebas P, Higgins TJ, Baine Y, et al. Plasmid vaccination of stable HIV-positive subjects on antiviral treatment results in enhanced CD8 T-cell immunity and increased control of viral "blips". Vaccine. 2005;23(17-18):2066-73. Epub 2005/03/10. doi: 10.1016/j.vaccine.2005.01.010. PubMed PMID: 15755572.
208. Aguiar JC, Hedstrom RC, Rogers WO, Charoenvit Y, Sacci JB, Jr., Lanar DE, et al. Enhancement of the immune response in rabbits to a malaria DNA vaccine by immunization with a needle-free jet device. Vaccine. 2001;20(1-2):275-80. Epub 2001/09/25. doi: 10.1016/s0264-410x(01)00273-0. PubMed PMID: 11567774.
209. Graham BS, Enama ME, Nason MC, Gordon IJ, Peel SA, Ledgerwood JE, et al. DNA vaccine delivered by a needle-free injection device improves potency of priming for antibody and CD8+ T-cell responses after rAd5 boost in a randomized clinical trial. PLoS

One. 2013;8(4):e59340. Epub 2013/04/12. doi: 10.1371/journal.pone.0059340. PubMed PMID: 23577062; PubMed Central PMCID: PMCPMC3620125.
210. Harris M, Joy R, Larsen G, Valyi M, Walker E, Frick LW, et al. Enfuvirtide plasma levels and injection site reactions using a needle-free gas-powered injection system (Biojector). AIDS. 2006;20(5):719-23. Epub 2006/03/04. doi: 10.1097/01.aids.0000216372.53819.db. PubMed PMID: 16514302.
211. Rosenberg ES, Graham BS, Chan ES, Bosch RJ, Stocker V, Maenza J, et al. Safety and immunogenicity of therapeutic DNA vaccination in individuals treated with antiretroviral therapy during acute/early HIV-1 infection. PLoS One. 2010;5(5):e10555. Epub 2010/05/19. doi: 10.1371/journal.pone.0010555. PubMed PMID: 20479938; PubMed Central PMCID: PMCPMC2866663.
212. Kashem SW, Haniffa M, Kaplan DH. Antigen-Presenting Cells in the Skin. Annu Rev Immunol. 2017;35:469-99. Epub 2017/02/23. doi: 10.1146/annurev-immunol-051116-052215. PubMed PMID: 28226228.
213. Mathers AR, Larregina AT. Professional antigen-presenting cells of the skin. Immunol Res. 2006;36(1-3):127-36. Epub 2007/03/06. doi: 10.1385/IR:36:1:127. PubMed PMID: 17337773.
214. Fehres CM, Garcia-Vallejo JJ, Unger WW, van Kooyk Y. Skin-resident antigen-presenting cells: instruction manual for vaccine development. Front Immunol. 2013;4:157. Epub 2013/06/27. doi: 10.3389/fimmu.2013.00157. PubMed PMID: 23801994; PubMed Central PMCID: PMCPMC3687254.
215. Lori F, Trocio J, Bakare N, Kelly LM, Lisziewicz J. DermaVir, a novel HIV immunisation technology. Vaccine. 2005;23(17-18):2030-4. Epub 2005/03/10. doi: 10.1016/j.vaccine.2005.01.004. PubMed PMID: 15755566.
216. Lisziewicz J, Trocio J, Xu J, Whitman L, Ryder A, Bakare N, et al. Control of viral rebound through therapeutic immunization with DermaVir. AIDS. 2005;19(1):35-43. Epub 2005/01/01. doi: 10.1097/00002030-200501030-00004. PubMed PMID: 15627031.
217. Lisziewicz J, Bakare N, Calarota SA, Banhegyi D, Szlavik J, Ujhelyi E, et al. Single DermaVir immunization: dose-dependent expansion of precursor/memory T cells against all HIV antigens in HIV-1 infected individuals. PLoS One. 2012;7(5):e35416. Epub 2012/05/17. doi: 10.1371/journal.pone.0035416. PubMed PMID: 22590502; PubMed Central PMCID: PMCPMC3348904.
218. Rodriguez B, Asmuth DM, Matining RM, Spritzler J, Jacobson JM, Mailliard RB, et al. Safety, tolerability, and immunogenicity of repeated doses of dermavir, a candidate therapeutic HIV vaccine, in HIV-infected patients receiving combination antiretroviral therapy: results of the ACTG 5176 trial. J Acquir Immune Defic Syndr. 2013;64(4):351-9. Epub 2013/10/31. doi: 10.1097/QAI.0b013e3182a99590. PubMed PMID: 24169120; PubMed Central PMCID: PMCPMC3858388.
219. Gudmundsdotter L, Wahren B, Haller BK, Boberg A, Edback U, Bernasconi D, et al. Amplified antigen-specific immune responses in HIV-1 infected individuals in a double blind DNA immunization and therapy interruption trial. Vaccine. 2011;29(33):5558-66. Epub 2011/02/09. doi: 10.1016/j.vaccine.2011.01.064. PubMed PMID: 21300092.
220. Williams RS, Johnston SA, Riedy M, DeVit MJ, McElligott SG, Sanford JC. Introduction of foreign genes into tissues of living mice by DNA-coated microprojectiles. Proc Natl Acad Sci U S A. 1991;88(7):2726-30. Epub 1991/04/01. doi: 10.1073/pnas.88.7.2726. PubMed PMID: 2011582; PubMed Central PMCID: PMCPMC51311.
221. Trimble C, Lin CT, Hung CF, Pai S, Juang J, He L, et al. Comparison of the CD8+ T cell responses and antitumor effects generated by DNA vaccine administered through gene gun, biojector, and syringe. Vaccine. 2003;21(25-26):4036-42. Epub 2003/08/19. doi: 10.1016/s0264-410x(03)00275-5. PubMed PMID: 12922140.

222. Tang DC, DeVit M, Johnston SA. Genetic immunization is a simple method for eliciting an immune response. Nature. 1992;356(6365):152-4. Epub 1992/03/12. doi: 10.1038/356152a0. PubMed PMID: 1545867.
223. Eisenbraun MD, Fuller DH, Haynes JR. Examination of parameters affecting the elicitation of humoral immune responses by particle bombardment-mediated genetic immunization. DNA Cell Biol. 1993;12(9):791-7. Epub 1993/11/01. doi: 10.1089/dna.1993.12.791. PubMed PMID: 8216850.
224. Fuller DH, Murphey-Corb M, Clements J, Barnett S, Haynes JR. Induction of immunodeficiency virus-specific immune responses in rhesus monkeys following gene gun-mediated DNA vaccination. J Med Primatol. 1996;25(3):236-41. Epub 1996/06/01. doi: 10.1111/j.1600-0684.1996.tb00021.x. PubMed PMID: 8892045.
225. Fuller DH, Corb MM, Barnett S, Steimer K, Haynes JR. Enhancement of immunodeficiency virus-specific immune responses in DNA-immunized rhesus macaques. Vaccine. 1997;15(8):924-6. Epub 1997/06/01. doi: 10.1016/s0264-410x(96)00271-x. PubMed PMID: 9234549.
226. Loudon PT, Yager EJ, Lynch DT, Narendran A, Stagnar C, Franchini AM, et al. GM-CSF increases mucosal and systemic immunogenicity of an H1N1 influenza DNA vaccine administered into the epidermis of non-human primates. PLoS One. 2010;5(6):e11021. Epub 2010/06/15. doi: 10.1371/journal.pone.0011021. PubMed PMID: 20544035; PubMed Central PMCID: PMCPMC2882341.
227. McCluskie MJ, Brazolot Millan CL, Gramzinski RA, Robinson HL, Santoro JC, Fuller JT, et al. Route and method of delivery of DNA vaccine influence immune responses in mice and non-human primates. Mol Med. 1999;5(5):287-300. Epub 1999/07/03. PubMed PMID: 10390545; PubMed Central PMCID: PMCPMC2230426.
228. Roy MJ, Wu MS, Barr LJ, Fuller JT, Tussey LG, Speller S, et al. Induction of antigen-specific CD8+ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. Vaccine. 2000;19(7-8):764-78. Epub 2000/12/15. doi: 10.1016/s0264-410x(00)00302-9. PubMed PMID: 11115698.
229. Fuller DH, Rajakumar PA, Wu MS, McMahon CW, Shipley T, Fuller JT, et al. DNA immunization in combination with effective antiretroviral drug therapy controls viral rebound and prevents simian AIDS after treatment is discontinued. Virology. 2006;348(1):200-15. Epub 2006/01/28. doi: 10.1016/j.virol.2005.12.008. PubMed PMID: 16439000.
230. Widera G, Austin M, Rabussay D, Goldbeck C, Barnett SW, Chen M, et al. Increased DNA vaccine delivery and immunogenicity by electroporation in vivo. J Immunol. 2000;164(9):4635-40. Epub 2000/04/26. doi: 10.4049/jimmunol.164.9.4635. PubMed PMID: 10779767.
231. Otten G, Schaefer M, Doe B, Liu H, Srivastava I, zur Megede J, et al. Enhancement of DNA vaccine potency in rhesus macaques by electroporation. Vaccine. 2004;22(19):2489-93. Epub 2004/06/15. doi: 10.1016/j.vaccine.2003.11.073. PubMed PMID: 15193413.
232. Otten GR, Schaefer M, Doe B, Liu H, Megede JZ, Donnelly J, et al. Potent immunogenicity of an HIV-1 gag-pol fusion DNA vaccine delivered by in vivo electroporation. Vaccine. 2006;24(21):4503-9. Epub 2005/09/27. doi: 10.1016/j.vaccine.2005.08.017. PubMed PMID: 16181711.
233. Luckay A, Sidhu MK, Kjeken R, Megati S, Chong SY, Roopchand V, et al. Effect of plasmid DNA vaccine design and in vivo electroporation on the resulting vaccine-specific immune responses in rhesus macaques. J Virol. 2007;81(10):5257-69. Epub 2007/03/03. doi: 10.1128/JVI.00055-07. PubMed PMID: 17329330; PubMed Central PMCID: PMCPMC1900241.

234. Cristillo AD, Weiss D, Hudacik L, Restrepo S, Galmin L, Suschak J, et al. Persistent antibody and T cell responses induced by HIV-1 DNA vaccine delivered by electroporation. Biochem Biophys Res Commun. 2008;366(1):29-35. Epub 2007/11/27. doi: 10.1016/j.bbrc.2007.11.052. PubMed PMID: 18036339.
235. Vasan S, Hurley A, Schlesinger SJ, Hannaman D, Gardiner DF, Dugin DP, et al. In vivo electroporation enhances the immunogenicity of an HIV-1 DNA vaccine candidate in healthy volunteers. PLoS One. 2011;6(5):e19252. Epub 2011/05/24. doi: 10.1371/journal.pone.0019252. PubMed PMID: 21603651; PubMed Central PMCID: PMCPMC3095594.
236. Morrow MP, Tebas P, Yan J, Ramirez L, Slager A, Kraynyak K, et al. Synthetic consensus HIV-1 DNA induces potent cellular immune responses and synthesis of granzyme B, perforin in HIV infected individuals. Mol Ther. 2015;23(3):591-601. Epub 2014/12/23. doi: 10.1038/mt.2014.245. PubMed PMID: 25531694; PubMed Central PMCID: PMCPMC4351468.
237. Hirao LA, Wu L, Khan AS, Satishchandran A, Draghia-Akli R, Weiner DB. Intradermal/subcutaneous immunization by electroporation improves plasmid vaccine delivery and potency in pigs and rhesus macaques. Vaccine. 2008;26(3):440-8. Epub 2007/12/18. doi: 10.1016/j.vaccine.2007.10.041. PubMed PMID: 18082294.
238. De Paoli P, Zanussi S, Simonelli C, Bortolin MT, D'Andrea M, Crepaldi C, et al. Effects of subcutaneous interleukin-2 therapy on CD4 subsets and in vitro cytokine production in HIV+ subjects. J Clin Invest. 1997;100(11):2737-43. Epub 1998/02/12. doi: 10.1172/JCI119819. PubMed PMID: 9389737; PubMed Central PMCID: PMCPMC508477.
239. Barouch DH, Santra S, Steenbeke TD, Zheng XX, Perry HC, Davies ME, et al. Augmentation and suppression of immune responses to an HIV-1 DNA vaccine by plasmid cytokine/Ig administration. J Immunol. 1998;161(4):1875-82. Epub 1998/08/26. PubMed PMID: 9712056.
240. Kim JJ, Simbiri KA, Sin JI, Dang K, Oh J, Dentchev T, et al. Cytokine molecular adjuvants modulate immune responses induced by DNA vaccine constructs for HIV-1 and SIV. J Interferon Cytokine Res. 1999;19(1):77-84. Epub 1999/02/27. doi: 10.1089/107999099314441. PubMed PMID: 10048771.
241. Kim JJ, Yang JS, Manson KH, Weiner DB. Modulation of antigen-specific cellular immune responses to DNA vaccination in rhesus macaques through the use of IL-2, IFN-gamma, or IL-4 gene adjuvants. Vaccine. 2001;19(17-19):2496-505. Epub 2001/03/21. doi: 10.1016/s0264-410x(00)00479-5. PubMed PMID: 11257383.
242. Pido-Lopez J, Burton C, Hardy G, Pires A, Sullivan A, Gazzard B, et al. Thymic output during initial highly active antiretroviral therapy (HAART) and during HAART supplementation with interleukin 2 and/or with HIV type 1 immunogen (Remune). AIDS Res Hum Retroviruses. 2003;19(2):103-9. Epub 2003/03/18. doi: 10.1089/088922203762688603. PubMed PMID: 12639245.
243. Hardy GA, Imami N, Nelson MR, Sullivan AK, Moss R, Aasa-Chapman MM, et al. A phase I, randomized study of combined IL-2 and therapeutic immunisation with antiretroviral therapy. J Immune Based Ther Vaccines. 2007;5:6. Epub 2007/04/13. doi: 10.1186/1476-8518-5-6. PubMed PMID: 17428345; PubMed Central PMCID: PMCPMC1864986.
244. Smith KA, Andjelic S, Popmihajlov Z, Kelly-Rossini L, Sass A, Lesser M, et al. Immunotherapy with canarypox vaccine and interleukin-2 for HIV-1 infection: termination of a randomized trial. PLoS Clin Trials. 2007;2(1):e5. Epub 2007/01/30. doi: 10.1371/journal.pctr.0020005. PubMed PMID: 17260026; PubMed Central PMCID: PMCPMC1783674.

245. Group I-ES, Committee SS, Abrams D, Levy Y, Losso MH, Babiker A, et al. Interleukin-2 therapy in patients with HIV infection. N Engl J Med. 2009;361(16):1548-59. Epub 2009/10/16. doi: 10.1056/NEJMoa0903175. PubMed PMID: 19828532; PubMed Central PMCID: PMCPMC2869083.
246. Boyer JD, Cohen AD, Ugen KE, Edgeworth RL, Bennett M, Shah A, et al. Therapeutic immunization of HIV-infected chimpanzees using HIV-1 plasmid antigens and interleukin-12 expressing plasmids. AIDS. 2000;14(11):1515-22. Epub 2000/09/13. doi: 10.1097/00002030-200007280-00007. PubMed PMID: 10983638.
247. Halwani R, Boyer JD, Yassine-Diab B, Haddad EK, Robinson TM, Kumar S, et al. Therapeutic vaccination with simian immunodeficiency virus (SIV)-DNA + IL-12 or IL-15 induces distinct CD8 memory subsets in SIV-infected macaques. J Immunol. 2008;180(12):7969-79. Epub 2008/06/05. doi: 10.4049/jimmunol.180.12.7969. PubMed PMID: 18523260.
248. Jacobson JM, Zheng L, Wilson CC, Tebas P, Matining RM, Egan MA, et al. The Safety and Immunogenicity of an Interleukin-12-Enhanced Multiantigen DNA Vaccine Delivered by Electroporation for the Treatment of HIV-1 Infection. J Acquir Immune Defic Syndr. 2016;71(2):163-71. Epub 2016/01/14. doi: 10.1097/QAI.0000000000000830. PubMed PMID: 26761518; PubMed Central PMCID: PMCPMC4712745.
249. Valentin A, von Gegerfelt A, Rosati M, Miteloudis G, Alicea C, Bergamaschi C, et al. Repeated DNA therapeutic vaccination of chronically SIV-infected macaques provides additional virological benefit. Vaccine. 2010;28(8):1962-74. Epub 2010/03/02. doi: 10.1016/j.vaccine.2009.10.099. PubMed PMID: 20188252; PubMed Central PMCID: PMCPMC2830913.
250. Kalams SA, Parker S, Jin X, Elizaga M, Metch B, Wang M, et al. Safety and immunogenicity of an HIV-1 gag DNA vaccine with or without IL-12 and/or IL-15 plasmid cytokine adjuvant in healthy, HIV-1 uninfected adults. PLoS One. 2012;7(1):e29231. Epub 2012/01/14. doi: 10.1371/journal.pone.0029231. PubMed PMID: 22242162; PubMed Central PMCID: PMCPMC3252307.
251. Wu X, Gu Z, Chen Y, Chen B, Chen W, Weng L, et al. Application of PD-1 Blockade in Cancer Immunotherapy. Comput Struct Biotechnol J. 2019;17:661-74. Epub 2019/06/18. doi: 10.1016/j.csbj.2019.03.006. PubMed PMID: 31205619; PubMed Central PMCID: PMCPMC6558092.
252. Rotte A. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. J Exp Clin Cancer Res. 2019;38(1):255. Epub 2019/06/15. doi: 10.1186/s13046-019-1259-z. PubMed PMID: 31196207; PubMed Central PMCID: PMCPMC6567914.
253. Harper J, Gordon S, Chan CN, Wang H, Lindemuth E, Galardi C, et al. CTLA-4 and PD-1 dual blockade induces SIV reactivation without control of rebound after antiretroviral therapy interruption. Nat Med. 2020;26(4):519-28. Epub 2020/04/15. doi: 10.1038/s41591-020-0782-y. PubMed PMID: 32284611; PubMed Central PMCID: PMCPMC7790171.
254. Villarreal DO, Svoronos N, Wise MC, Shedlock DJ, Morrow MP, Conejo-Garcia JR, et al. Molecular adjuvant IL-33 enhances the potency of a DNA vaccine in a lethal challenge model. Vaccine. 2015;33(35):4313-20. Epub 2015/04/19. doi: 10.1016/j.vaccine.2015.03.086. PubMed PMID: 25887087; PubMed Central PMCID: PMCPMC4546882.
255. Sarkar S, Piepenbrink MS, Basu M, Thakar J, Keefer MC, Hessell AJ, et al. IL-33 enhances the kinetics and quality of the antibody response to a DNA and protein-based HIV-1 Env vaccine. Vaccine. 2019;37(17):2322-30. Epub 2019/03/31. doi: 10.1016/j.vaccine.2019.03.044. PubMed PMID: 30926296; PubMed Central PMCID: PMCPMC6506229.

256. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005;23(5):479-90. Epub 2005/11/16. doi: 10.1016/j.immuni.2005.09.015. PubMed PMID: 16286016.
257. Bonilla WV, Frohlich A, Senn K, Kallert S, Fernandez M, Johnson S, et al. The alarmin interleukin-33 drives protective antiviral CD8(+) T cell responses. Science. 2012;335(6071):984-9. Epub 2012/02/11. doi: 10.1126/science.1215418. PubMed PMID: 22323740.
258. Pau AK, George JM. Antiretroviral therapy: current drugs. Infect Dis Clin North Am. 2014;28(3):371-402. Epub 2014/08/26. doi: 10.1016/j.idc.2014.06.001. PubMed PMID: 25151562; PubMed Central PMCID: PMCPMC4143801.
259. Ho YC, Shan L, Hosmane NN, Wang J, Laskey SB, Rosenbloom DI, et al. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. Cell. 2013;155(3):540-51. Epub 2013/11/19. doi: 10.1016/j.cell.2013.09.020. PubMed PMID: 24243014; PubMed Central PMCID: PMCPMC3896327.
260. Saez-Cirion A, Bacchus C, Hocqueloux L, Avettand-Fenoel V, Girault I, Lecuroux C, et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. PLoS Pathog. 2013;9(3):e1003211. Epub 2013/03/22. doi: 10.1371/journal.ppat.1003211. PubMed PMID: 23516360; PubMed Central PMCID: PMCPMC3597518.
261. Schackman BR, Gebo KA, Walensky RP, Losina E, Muccio T, Sax PE, et al. The lifetime cost of current human immunodeficiency virus care in the United States. Med Care. 2006;44(11):990-7. Epub 2006/10/26. doi: 10.1097/01.mlr.0000228021.89490.2a. PubMed PMID: 17063130.
262. Ammassari A, Murri R, Pezzotti P, Trotta MP, Ravasio L, De Longis P, et al. Self-reported symptoms and medication side effects influence adherence to highly active antiretroviral therapy in persons with HIV infection. J Acquir Immune Defic Syndr. 2001;28(5):445-9. Epub 2001/12/18. doi: 10.1097/00042560-200112150-00006. PubMed PMID: 11744832.
263. Neuhaus J, Jacobs DR, Jr., Baker JV, Calmy A, Duprez D, La Rosa A, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J Infect Dis. 2010;201(12):1788-95. Epub 2010/05/08. doi: 10.1086/652749. PubMed PMID: 20446848; PubMed Central PMCID: PMCPMC2872049.
264. Zicari S, Sessa L, Cotugno N, Ruggiero A, Morrocchi E, Concato C, et al. Immune Activation, Inflammation, and Non-AIDS Co-Morbidities in HIV-Infected Patients under Long-Term ART. Viruses. 2019;11(3). Epub 2019/03/02. doi: 10.3390/v11030200. PubMed PMID: 30818749; PubMed Central PMCID: PMCPMC6466530.
265. Borducchi EN, Cabral C, Stephenson KE, Liu J, Abbink P, Ng'ang'a D, et al. Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. Nature. 2016;540(7632):284-7. Epub 2016/11/15. doi: 10.1038/nature20583. PubMed PMID: 27841870; PubMed Central PMCID: PMCPMC5145754.
266. Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. Nature. 2011;473(7348):523-7. Epub 2011/05/13. doi: 10.1038/nature10003. PubMed PMID: 21562493; PubMed Central PMCID: PMCPMC3102768.
267. Sneller MC, Justement JS, Gittens KR, Petrone ME, Clarridge KE, Proschan MA, et al. A randomized controlled safety/efficacy trial of therapeutic vaccination in HIV-infected individuals who initiated antiretroviral therapy early in infection. Sci Transl Med. 2017;9(419). Epub 2017/12/08. doi: 10.1126/scitranslmed.aan8848. PubMed PMID: 29212716.

268. Larijani MS, Ramezani A, Sadat SM. Updated Studies on the Development of HIV Therapeutic Vaccine. Curr HIV Res. 2019;17(2):75-84. Epub 2019/06/19. doi: 10.2174/1570162X17666190618160608. PubMed PMID: 31210114.
269. Ensoli B, Cafaro A, Monini P, Marcotullio S, Ensoli F. Challenges in HIV Vaccine Research for Treatment and Prevention. Front Immunol. 2014;5:417. Epub 2014/09/25. doi: 10.3389/fimmu.2014.00417. PubMed PMID: 25250026; PubMed Central PMCID: PMCPMC4157563.
270. Seddiki N, Levy Y. Therapeutic HIV-1 vaccine: time for immunomodulation and combinatorial strategies. Curr Opin HIV AIDS. 2018;13(2):119-27. Epub 2018/01/13. doi: 10.1097/COH.0000000000000444. PubMed PMID: 29329113.
271. Bagley KC, Abdelwahab SF, Tuskan RG, Fouts TR, Lewis GK. Cholera toxin and heat-labile enterotoxin activate human monocyte-derived dendritic cells and dominantly inhibit cytokine production through a cyclic AMP-dependent pathway. Infect Immun. 2002;70(10):5533-9. Epub 2002/09/14. doi: 10.1128/iai.70.10.5533-5539.2002. PubMed PMID: 12228279; PubMed Central PMCID: PMCPMC128358.
272. Bagley K, Xu R, Ota-Setlik A, Egan M, Schwartz J, Fouts T. The catalytic A1 domains of cholera toxin and heat-labile enterotoxin are potent DNA adjuvants that evoke mixed Th1/Th17 cellular immune responses. Hum Vaccin Immunother. 2015;11(9):2228-40. Epub 2015/06/05. doi: 10.1080/21645515.2015.1026498. PubMed PMID: 26042527; PubMed Central PMCID: PMCPMC4635876.
273. Jalah R, Patel V, Kulkarni V, Rosati M, Alicea C, Ganneru B, et al. IL-12 DNA as molecular vaccine adjuvant increases the cytotoxic T cell responses and breadth of humoral immune responses in SIV DNA vaccinated macaques. Hum Vaccin Immunother. 2012;8(11):1620-9. Epub 2012/08/17. doi: 10.4161/hv.21407. PubMed PMID: 22894956; PubMed Central PMCID: PMCPMC3601136.
274. Kalams SA, Parker SD, Elizaga M, Metch B, Edupuganti S, Hural J, et al. Safety and comparative immunogenicity of an HIV-1 DNA vaccine in combination with plasmid interleukin 12 and impact of intramuscular electroporation for delivery. J Infect Dis. 2013;208(5):818-29. Epub 2013/07/11. doi: 10.1093/infdis/jit236. PubMed PMID: 23840043; PubMed Central PMCID: PMCPMC3733506.
275. McLaren JE, Clement M, Marsden M, Miners KL, Llewellyn-Lacey S, Grant EJ, et al. IL-33 Augments Virus-Specific Memory T Cell Inflation and Potentiates the Efficacy of an Attenuated Cytomegalovirus-Based Vaccine. J Immunol. 2019;202(3):943-55. Epub 2019/01/13. doi: 10.4049/jimmunol.1701757. PubMed PMID: 30635396; PubMed Central PMCID: PMCPMC6341181.
276. Yang Q, Li G, Zhu Y, Liu L, Chen E, Turnquist H, et al. IL-33 synergizes with TCR and IL-12 signaling to promote the effector function of CD8+ T cells. Eur J Immunol. 2011;41(11):3351-60. Epub 2011/09/03. doi: 10.1002/eji.201141629. PubMed PMID: 21887788; PubMed Central PMCID: PMCPMC3332117.
277. Holechek SA, McAfee MS, Nieves LM, Guzman VP, Manhas K, Fouts T, et al. Retinaldehyde dehydrogenase 2 as a molecular adjuvant for enhancement of mucosal immunity during DNA vaccination. Vaccine. 2016;34(46):5629-35. Epub 2016/10/25. doi: 10.1016/j.vaccine.2016.09.013. PubMed PMID: 27670072; PubMed Central PMCID: PMCPMC5080981.
278. Cassani B, Villablanca EJ, De Calisto J, Wang S, Mora JR. Vitamin A and immune regulation: role of retinoic acid in gut-associated dendritic cell education, immune protection and tolerance. Mol Aspects Med. 2012;33(1):63-76. Epub 2011/11/29. doi: 10.1016/j.mam.2011.11.001. PubMed PMID: 22120429; PubMed Central PMCID: PMCPMC3246074.
279. Hryniewicz A, Boasso A, Edghill-Smith Y, Vaccari M, Fuchs D, Venzon D, et al. CTLA-4 blockade decreases TGF-beta, IDO, and viral RNA expression in tissues of SIVmac251-

infected macaques. Blood. 2006;108(12):3834-42. Epub 2006/08/10. doi: 10.1182/blood-2006-04-010637. PubMed PMID: 16896154; PubMed Central PMCID: PMCPMC1895471.
280. Amedee AM, Lacour N, Gierman JL, Martin LN, Clements JE, Bohm R, Jr., et al. Genotypic selection of simian immunodeficiency virus in macaque infants infected transplacentally. J Virol. 1995;69(12):7982-90. Epub 1995/12/01. doi: 10.1128/JVI.69.12.7982-7990.1995. PubMed PMID: 7494311; PubMed Central PMCID: PMCPMC189743.
281. Fuller DH, Rajakumar PA, Wilson LA, Trichel AM, Fuller JT, Shipley T, et al. Induction of mucosal protection against primary, heterologous simian immunodeficiency virus by a DNA vaccine. J Virol. 2002;76(7):3309-17. Epub 2002/03/09. doi: 10.1128/jvi.76.7.3309-3317.2002. PubMed PMID: 11884556; PubMed Central PMCID: PMCPMC136011.
282. Blasi M, Wescott EC, Baker EJ, Mildenberg B, LaBranche C, Rountree W, et al. Therapeutic vaccination with IDLV-SIV-Gag results in durable viremia control in chronically SHIV-infected macaques. NPJ Vaccines. 2020;5(1):36. Epub 2021/02/14. doi: 10.1038/s41541-020-0186-5. PubMed PMID: 33580065.
283. Nakamura-Hoshi M, Takahara Y, Matsuoka S, Ishii H, Seki S, Nomura T, et al. Therapeutic vaccine-mediated Gag-specific CD8(+) T-cell induction under anti-retroviral therapy augments anti-virus efficacy of CD8(+) cells in simian immunodeficiency virus-infected macaques. Sci Rep. 2020;10(1):11394. Epub 2020/07/11. doi: 10.1038/s41598-020-68267-w. PubMed PMID: 32647227; PubMed Central PMCID: PMCPMC7347614.
284. Turk G, Ghiglione Y, Falivene J, Socias ME, Laufer N, Coloccini RS, et al. Early Gag immunodominance of the HIV-specific T-cell response during acute/early infection is associated with higher CD8+ T-cell antiviral activity and correlates with preservation of the CD4+ T-cell compartment. J Virol. 2013;87(13):7445-62. Epub 2013/04/26. doi: 10.1128/JVI.00865-13. PubMed PMID: 23616666; PubMed Central PMCID: PMCPMC3700299.
285. Ferrando-Martinez S, Casazza JP, Leal M, Machmach K, Munoz-Fernandez MA, Viciana P, et al. Differential Gag-specific polyfunctional T cell maturation patterns in HIV-1 elite controllers. J Virol. 2012;86(7):3667-74. Epub 2012/01/27. doi: 10.1128/JVI.07034-11. PubMed PMID: 22278254; PubMed Central PMCID: PMCPMC3302538.
286. Ferre AL, Lemongello D, Hunt PW, Morris MM, Garcia JC, Pollard RB, et al. Immunodominant HIV-specific CD8+ T-cell responses are common to blood and gastrointestinal mucosa, and Gag-specific responses dominate in rectal mucosa of HIV controllers. J Virol. 2010;84(19):10354-65. Epub 2010/07/30. doi: 10.1128/JVI.00803-10. PubMed PMID: 20668079; PubMed Central PMCID: PMCPMC2937770.
287. Peterson CW, Younan P, Polacino PS, Maurice NJ, Miller HW, Prlic M, et al. Robust suppression of env-SHIV viremia in Macaca nemestrina by 3-drug ART is independent of timing of initiation during chronic infection. J Med Primatol. 2013;42(5):237-46. Epub 2013/09/13. doi: 10.1111/jmp.12060. PubMed PMID: 24025078; PubMed Central PMCID: PMCPMC3772551.
288. Tryniszewska E, Nacsa J, Lewis MG, Silvera P, Montefiori D, Venzon D, et al. Vaccination of macaques with long-standing SIVmac251 infection lowers the viral set point after cessation of antiretroviral therapy. J Immunol. 2002;169(9):5347-57. Epub 2002/10/23. doi: 10.4049/jimmunol.169.9.5347. PubMed PMID: 12391256.
289. Lewis MG, Norelli S, Collins M, Barreca ML, Iraci N, Chirullo B, et al. Response of a simian immunodeficiency virus (SIVmac251) to raltegravir: a basis for a new treatment for simian AIDS and an animal model for studying lentiviral persistence during antiretroviral therapy. Retrovirology. 2010;7:21. Epub 2010/03/18. doi: 10.1186/1742-4690-7-21. PubMed PMID: 20233398; PubMed Central PMCID: PMCPMC2853490.
290. Hong JJ, Silveira E, Amancha PK, Byrareddy SN, Gumber S, Chang KT, et al. Early initiation of antiretroviral treatment postSIV infection does not resolve lymphoid tissue

activation. AIDS. 2017;31(13):1819-24. Epub 2017/07/12. doi: 10.1097/QAD.0000000000001576. PubMed PMID: 28692537; PubMed Central PMCID: PMCPMC5557084.
291. zur Megede J, Sanders-Beer B, Silvera P, Golightly D, Bowlsbey A, Hebblewaite D, et al. A therapeutic SIV DNA vaccine elicits T-cell immune responses, but no sustained control of viremia in SIVmac239-infected rhesus macaques. AIDS Res Hum Retroviruses. 2008;24(8):1103-16. Epub 2008/07/16. doi: 10.1089/aid.2008.0055. PubMed PMID: 18620495; PubMed Central PMCID: PMCPMC3139522.
292. Letvin NL, Rao SS, Montefiori DC, Seaman MS, Sun Y, Lim SY, et al. Immune and Genetic Correlates of Vaccine Protection Against Mucosal Infection by SIV in Monkeys. Sci Transl Med. 2011;3(81):81ra36. Epub 2011/05/06. doi: 10.1126/scitranslmed.3002351. PubMed PMID: 21543722; PubMed Central PMCID: PMCPMC3718279.
293. Lim SY, Chan T, Gelman RS, Whitney JB, O'Brien KL, Barouch DH, et al. Contributions of Mamu-A*01 status and TRIM5 allele expression, but not CCL3L copy number variation, to the control of SIVmac251 replication in Indian-origin rhesus monkeys. PLoS Genet. 2010;6(6):e1000997. Epub 2010/06/30. doi: 10.1371/journal.pgen.1000997. PubMed PMID: 20585621; PubMed Central PMCID: PMCPMC2891712.
294. Makedonas G, Betts MR. Polyfunctional analysis of human t cell responses: importance in vaccine immunogenicity and natural infection. Springer Semin Immunopathol. 2006;28(3):209-19. Epub 2006/08/26. doi: 10.1007/s00281-006-0025-4. PubMed PMID: 16932955.
295. McBrien JB, Kumar NA, Silvestri G. Mechanisms of CD8(+) T cell-mediated suppression of HIV/SIV replication. Eur J Immunol. 2018;48(6):898-914. Epub 2018/02/11. doi: 10.1002/eji.201747172. PubMed PMID: 29427516; PubMed Central PMCID: PMCPMC6531861.
296. Cecchinato V, Trindade CJ, Laurence A, Heraud JM, Brenchley JM, Ferrari MG, et al. Altered balance between Th17 and Th1 cells at mucosal sites predicts AIDS progression in simian immunodeficiency virus-infected macaques. Mucosal Immunol. 2008;1(4):279-88. doi: 10.1038/mi.2008.14. PubMed PMID: WOS:000260470900005.
297. Pallikkuth S, Micci L, Ende ZS, Iriele RI, Cervasi B, Lawson B, et al. Maintenance of intestinal Th17 cells and reduced microbial translocation in SIV-infected rhesus macaques treated with interleukin (IL)-21. PLoS Pathog. 2013;9(7):e1003471. Epub 2013/07/16. doi: 10.1371/journal.ppat.1003471. PubMed PMID: 23853592; PubMed Central PMCID: PMCPMC3701718.
298. Kanwar B, Favre D, McCune JM. Th17 and regulatory T cells: implications for AIDS pathogenesis. Curr Opin HIV AIDS. 2010;5(2):151-7. Epub 2010/06/15. doi: 10.1097/COH.0b013e328335c0c1. PubMed PMID: 20543593; PubMed Central PMCID: PMCPMC2999911.
299. Chevalier MF, Weiss L. The split personality of regulatory T cells in HIV infection. Blood. 2013;121(1):29-37. Epub 2012/10/09. doi: 10.1182/blood-2012-07-409755. PubMed PMID: 23043072.
300. Winstone N, Wilson AJ, Morrow G, Boggiano C, Chiuchiolo MJ, Lopez M, et al. Enhanced control of pathogenic Simian immunodeficiency virus SIVmac239 replication in macaques immunized with an interleukin-12 plasmid and a DNA prime-viral vector boost vaccine regimen. J Virol. 2011;85(18):9578-87. Epub 2011/07/08. doi: 10.1128/JVI.05060-11. PubMed PMID: 21734035; PubMed Central PMCID: PMCPMC3165762.
301. Starke CE, Vinton CL, Ladell K, McLaren JE, Ortiz AM, Mudd JC, et al. SIV-specific CD8+ T cells are clonotypically distinct across lymphoid and mucosal tissues. J Clin Invest. 2020;130(2):789-98. Epub 2019/10/30. doi: 10.1172/JCI129161. PubMed PMID: 31661461; PubMed Central PMCID: PMCPMC6994127.

302. Buggert M, Nguyen S, Salgado-Montes de Oca G, Bengsch B, Darko S, Ransier A, et al. Identification and characterization of HIV-specific resident memory CD8(+) T cells in human lymphoid tissue. Sci Immunol. 2018;3(24). Epub 2018/06/03. doi: 10.1126/sciimmunol.aar4526. PubMed PMID: 29858286; PubMed Central PMCID: PMCPMC6357781.
303. Strongin Z, Micci L, Fromentin R, Harper J, McBrien J, Ryan E, et al. Virologic and Immunologic Features of Simian Immunodeficiency Virus Control Post-ART Interruption in Rhesus Macaques. J Virol. 2020;94(14). Epub 2020/05/01. doi: 10.1128/JVI.00338-20. PubMed PMID: 32350073; PubMed Central PMCID: PMCPMC7343203.
304. Whitney JB, Hill AL, Sanisetty S, Penaloza-MacMaster P, Liu J, Shetty M, et al. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. Nature. 2014;512(7512):74-7. Epub 2014/07/22. doi: 10.1038/nature13594. PubMed PMID: 25042999; PubMed Central PMCID: PMCPMC4126858.
305. Ziani W, Shao J, Wang X, Russell-Lodrigue K, Liu YZ, Montaner LJ, et al. Increased Proviral DNA in Circulating Cells Correlates with Plasma Viral Rebound in Simian Immunodeficiency Virus-Infected Rhesus Macaques after Antiretroviral Therapy Interruption. J Virol. 2021;95(6). Epub 2021/01/08. doi: 10.1128/JVI.02064-20. PubMed PMID: 33408173.
306. Frater J, Ewings F, Hurst J, Brown H, Robinson N, Fidler S, et al. HIV-1-specific CD4(+) responses in primary HIV-1 infection predict disease progression. AIDS. 2014;28(5):699-708. Epub 2014/02/20. doi: 10.1097/QAD.0000000000000130. PubMed PMID: 24549145.
307. O'Connor MA, Munson PV, Tunggal HC, Hajari N, Lewis TB, Bratt D, et al. Mucosal T Helper 17 and T Regulatory Cell Homeostasis Correlate with Acute Simian Immunodeficiency Virus Viremia and Responsiveness to Antiretroviral Therapy in Macaques. AIDS Res Hum Retroviruses. 2019;35(3):295-305. Epub 2018/11/07. doi: 10.1089/AID.2018.0184. PubMed PMID: 30398361; PubMed Central PMCID: PMCPMC6434588.
308. Munson P, Liu Y, Bratt D, Fuller JT, Hu X, Pavlakis GN, et al. Therapeutic conserved elements (CE) DNA vaccine induces strong T-cell responses against highly conserved viral sequences during simian-human immunodeficiency virus infection. Hum Vaccin Immunother. 2018;14(7):1820-31. Epub 2018/04/13. doi: 10.1080/21645515.2018.1448328. PubMed PMID: 29648490; PubMed Central PMCID: PMCPMC6067903.
309. Klatt NR, Canary LA, Sun X, Vinton CL, Funderburg NT, Morcock DR, et al. Probiotic/prebiotic supplementation of antiretrovirals improves gastrointestinal immunity in SIV-infected macaques. J Clin Invest. 2013;123(2):903-7. Epub 2013/01/17. doi: 10.1172/JCI66227. PubMed PMID: 23321668; PubMed Central PMCID: PMCPMC3561826.
310. Vujkovic-Cvijin I, Swainson LA, Chu SN, Ortiz AM, Santee CA, Petriello A, et al. Gut-Resident Lactobacillus Abundance Associates with IDO1 Inhibition and Th17 Dynamics in SIV-Infected Macaques. Cell Rep. 2015;13(8):1589-97. Epub 2015/11/21. doi: 10.1016/j.celrep.2015.10.026. PubMed PMID: 26586432; PubMed Central PMCID: PMCPMC4782968.
311. Ortiz AM, Klase ZA, DiNapoli SR, Vujkovic-Cvijin I, Carmack K, Perkins MR, et al. IL-21 and probiotic therapy improve Th17 frequencies, microbial translocation, and microbiome in ARV-treated, SIV-infected macaques. Mucosal Immunol. 2016;9(2):458-67. Epub 2015/08/20. doi: 10.1038/mi.2015.75. PubMed PMID: 26286233; PubMed Central PMCID: PMCPMC4760912.
312. Logerot S, Figueiredo-Morgado S, Charmeteau-de-Muylder B, Sandouk A, Drillet-Dangeard AS, Bomsel M, et al. IL-7-Adjuvanted Vaginal Vaccine Elicits Strong Mucosal

Immune Responses in Non-Human Primates. Front Immunol. 2021;12:614115. Epub 2021/03/16. doi: 10.3389/fimmu.2021.614115. PubMed PMID: 33717097; PubMed Central PMCID: PMCPMC7947860.
313. Kim AR, Park J, Kim JH, Kwak JE, Cho Y, Lee H, et al. Herpes Zoster DNA Vaccines with IL-7 and IL-33 Molecular Adjuvants Elicit Protective T Cell Immunity. Immune Netw. 2018;18(5):e38. Epub 2018/11/08. doi: 10.4110/in.2018.18.e38. PubMed PMID: 30402333; PubMed Central PMCID: PMCPMC6215899.
314. Stenstad H, Ericsson A, Johansson-Lindbom B, Svensson M, Marsal J, Mack M, et al. Gut-associated lymphoid tissue-primed CD4+ T cells display CCR9-dependent and -independent homing to the small intestine. Blood. 2006;107(9):3447-54. Epub 2006/01/05. doi: 10.1182/blood-2005-07-2860. PubMed PMID: 16391017.
315. Kutzler MA, Wise MC, Hutnick NA, Moldoveanu Z, Hunter M, Reuter M, et al. Chemokine-adjuvanted electroporated DNA vaccine induces substantial protection from simian immunodeficiency virus vaginal challenge. Mucosal Immunol. 2016;9(1):13-23. Epub 2015/05/07. doi: 10.1038/mi.2015.31. PubMed PMID: 25943275; PubMed Central PMCID: PMCPMC4636490.