Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Lynette Siv Chea Date

Novel Vaccines and Therapeutic Approaches Against HIV-1

By

Lynette Siv Chea
Doctor of Philosophy

Graduate Division of Biological and Biomedical Science
Immunology and Molecular Pathogenesis

_____

Rama Rao Amara, Ph.D.

Advisor

_____

Lawrence Boise, Ph.D.

Committee Member

_____

Eric Hunter, Ph.D.

Committee Member

_____

Bernard Moss, M.D., Ph.D.

Committee Member

_____

Paul Spearman, M.D.

Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____

Date

Novel Vaccines and Therapeutic Approaches Against HIV-1

By

Lynette Siv Chea

B.S., University of California, Los Angeles, 2010

Advisor: Rama Rao Amara, Ph.D.

An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

Graduate Division of Biological and Biomedical Science
Immunology and Molecular Pathogenesis
2019

Abstract

Novel Vaccines and Therapeutic Approaches Against HIV-1

By Lynette S. Chea

Currently, approximately 37 million people are infected with Human Immunodeficiency Virus-1 (HIV) world-wide and there is a great need for developing both preventative and functional cure approaches to control the epidemic. Advancements toward a preventive vaccine and functional cure for HIV have made significant progress in the past decade. A successful vaccine aims to achieve antibody and T cell responses of high magnitude and durability to prevent infection. Thus, the development of vaccine delivery vectors to generate potent immune responses is critical. Modified vaccinia Ankara (MVA) has been used as a promising viral vaccine vector for inducing vaccine-specific humoral and cellular responses against multiple infectious diseases including HIV. A primary goal of this dissertation was to further enhance the immunogenicity of MVA by targeting the apoptotic pathway induced after MVA infection of cells. We provide evidence that delaying apoptosis during MVA vaccination improves antigen-specific humoral responses. We generated an MVA expressing an anti-apoptotic gene B13R, MVA-B13R, and showed that MVA-B13R infection markedly delays apoptosis of infected cells. We further demonstrate that MVA-B13R expressing Simian Immunodeficiency Virus (SIV) and HIV antigens develop greater vaccine-induced humoral responses with enhanced durability. Interestingly, MVA-B13R immunization resulted in a delayed interferon response. These findings report a novel MVA that can be used as a vaccine vector for enhancing humoral immunity against multiple infectious diseases.

HIV cure research aims to allow individuals to control viremia in the absence of anti-retroviral therapy (ART). Dysfunctional anti-viral immunity and persistence of the latent HIV reservoir are the main barriers to HIV cure. This dissertation additionally aimed to investigate the ability of PD-1 checkpoint blockade therapy combined with ART to restore and improve anti-viral immunity and reduce the HIV viral reservoir. Our findings demonstrate that this regimen enhanced anti-viral CD8+ T cell functionality and destabilized the viral reservoir leading to improved control of viral rebound after ART interruption. This study provides evidence that PD-1 blockade co-administered with ART can effectively enhance immune functionality during chronic SIV infection and establishes the foundation for identifying optimal HIV cure therapies.

Novel Vaccines and Therapeutic Approaches Against HIV-1

By

Lynette Siv Chea

B.S., University of California, Los Angeles, 2010

Advisor: Rama Rao Amara, Ph.D.

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

Graduate Division of Biological and Biomedical Science
Immunology and Molecular Pathogenesis

2019

ACKNOWLEDGEMENTS

I would first like to thank my mentor, Rama Amara, for all the support and guidance he has imparted on me over the years as a graduate student in his lab. I am grateful for all the opportunities to learn not only skills in the lab but the skills that has made me a better scientist. You provided me a supportive environment to make mistakes, learn, and grow in the process. I leave your lab better prepared to think critically about scientific questions which I will take with me any where I go. I also would like to thank all current and past lab members of the Amara lab and the community of Yerkes as a whole for creating an atmosphere that has made me want to come to work every day. Everyone has made the last six and a half years an enjoyable experience filled with laughs, food, and random coffee breaks which I will always cherish. A special thanks to Dr. Sailaja Gangadhara for teaching me all she knows about virology and being a constant source of advice and help whenever I needed it. Additionally, a special thank you to Dr. Geetha Mylvaganam for allowing me to work with her and being an amazing friend and collaborator in the process.

I would next like to acknowledge and thank all of the friends I have made throughout my years in Atlanta. These relationships I developed mainly through Emory are among the closest I have had in my life and they are too numerous to list here. Their support and love throughout this journey of graduate school cannot be understated. In each of their own ways, everyone has helped me to retain calm and focus when I needed it, work through frustrations, and supported me when I needed it the most. Through all the dinners, the road trips, and the festivals, it was all of my friends that has made the adventure of being at Emory and in Atlanta worthwhile.

Finally, I would like to thank my family for their unwavering support of me throughout the years and in particularly during graduate school when I was away from home the longest. My parents Vinson and Aimee worked hard for me to be able to pursue my scholarly aspirations without burdens and I hope to

have made them proud with my accomplishments over the years and in the future. My dad helped to develop my scientific curiosity by always wanting to better understand how things worked and how things can be fixed. I believe this helped mold me into the scientist and person I am today and for that I will always be grateful. I also want to thank my baby brother, sister-in-law, grandparents, baby cousins, aunts, and uncles who also may not have always understood what I was trying to do with a Ph.D. but were always there for me with encouraging words and love which I will never forget.

Thank you all.

TABLE OF CONTENTS

FIGURE INDEX

CHAPTER I

INTRODUCTION

Chapter adapted from:

Chea, LS & Amara, RR. (2017). Immunogenicity and efficacy of DNA/MVA HIV vaccines in rhesus macaque models. *Expert Rev Vaccines*, 16(10), 973-985. doi:10.1080/14760584.2017.1371594

HIV EPIDEMIOLOGY AND VIROLOGY

HIV ORIGIN AND EPIDEMIOLOGY

The global health community has been faced with the problem of human immunodeficiency virus (HIV) for over 30 years now, with nearly 37 million people in the world living with HIV in 2017 (1, 2). The first cases of the devastating epidemic of Acquired Immunodeficiency Syndrome (AIDS) were reported in the United States in 1981 (3). This disease was initially associated with men who have sex with men, followed by injection drug users, people who had received blood transfusions, and finally the population at large (4). The scale and progression of this epidemic prompted world-wide efforts into identifying the cause and further understanding of the disease gripping the international community. In 1983, researchers at the Institute Pasteur, Paris, France isolated a new retrovirus from a lymph node biopsy sample of a patient with generalized lymphadenopathy which was then termed lymphadenopathy-associated virus (LAV) (5). Similar viruses were being isolated from patients with AIDS and it was this retrovirus that came to be known as HIV-1 (6, 7)

Development of anti-retroviral therapy (ART) and educating individuals on HIV prevention strategies have contributed significantly to the drop in the number of new infections from 3.4 million in 1996 to 1.8 million in 2017 (2). According to the World Health Organization (WHO), 59% of adults and 52% of children with HIV were receiving ART in 2017. Key populations who are at increased risk of HIV acquisition are men who have sex with men, injection drug users, people in prisons, sex workers and transgender people (1). Despite these advances in therapeutic treatment, 940,000 people died from HIV-related causes globally in 2017.

The WHO African Region is the most affected region with 25.7 million people living with HIV and accounting for over two thirds of the global total of new HIV infections in 2017 (1). In sub-Saharan Africa, three in four new infections in adolescents aged 15 to 19 are girls and young women 15 to 24 years of age are twice as likely to be living with HIV than men (2). In the United States, the Center for

Disease Control and Prevention (CDC) estimated 38,500 new HIV infections in 2015 with the Southern states accounting for more than half of these new infections (8, 9). While people receiving an HIV diagnosis live in urban areas in most of the United States, 23% and 21% of diagnoses in the South and Midwest, respectively, are in the suburban and rural areas (9).

Our global HIV epidemic is considered most likely to have arose from zoonotic infections of simian immunodeficiency virus (SIV) from African primates (10). This transmission likely occurred via cutaneous or mucous membrane exposure to blood or bodily fluids from an infected primate. Bushmeat hunters are likely the first group to be infected with HIV (11). Sequence comparisons indicate that HIV-1 is the result of cross-species transmissions of simian immunodeficiency virus (SIV) from chimpanzees while HIV-2 resulted from transmissions of SIV from sooty mangabeys (12). HIV-1 is more prevalent and pathogenic than HIV-2 and constitutes the majority of the HIV infections world-wide. HIV-1 is comprised of 4 lineages resulting from independent cross-species transmission events. The four groups are M (majority), O (outlier), N (non-M/non-O) and P with M being the pandemic form present across the globe (10). Groups N, O, and P differ in their distribution and represent a much lower frequency of all HIV-1 infections world-wide. Group M has at least 9 different subtypes (A, B, C, D, F, G, H, J, and K) in addition to circulating recombinant forms (CRFs) which are hybrid viruses with genes derived from different subtypes (13).

HIV-1 VIROLOGY

HIV-1 is lentivirus from the family of retroviruses which characteristically has an RNA genome within a capsid and lipid bilayer envelope (14). The HIV-1 genome is approximately 9 kb RNA and is encoded as two genomic-length RNA molecules packaged within the viral particle (15). The genome of HIV-1 encodes nine open reading frames which include the Gag, Pol, and Env polypeptides that become subsequently proteolyzed into individual proteins. Gag is processed to yield four structure proteins that

comprise the HIV core and a small C-terminal protein: MA (p17), CA (p24), NC (p9), and p6 (16). CA is the capsid protein that participates in Gag assembly, formation of the conical capsid core, and regulation of the nuclear import of viral DNA (17). MA is the matrix protein that is involved in Gag targeting and binding to the plasma membrane in addition to incorporation of the envelope glycoprotein (Env) into new, immature virions. NC is the nucleocapsid protein that non-covalently attaches to the viral RNA for RNA encapsidation and also participates in Gag assembly. NC recruits the viral RNA genome into virions and facilitates assembly of virions (17). P6 is involved in Vpr incorporation and recruitment of the endosomal sorting complex required for transport (ESCRT) components which leads to release of immature virions. Pol encodes three enzymatic proteins that are integral to the viral life cycle and are also encapsulated within the particle: protease, reverse transcriptase, and integrase. The Env polypeptide, gp160, becomes cleaved to produce gp120 and gp41, the surface and transmembrane region of the main viral glycoproteins that are integral for viral entry. HIV additionally encodes non-structural or accessory proteins such as the transcription regulators Tat and Rev as well as Vif, Vpr, and Nef that are found within the viral particle. Vpu indirectly assists with virion assembly.

The HIV-1 replication cycle begins with the virion binding to its receptor, CD4, and co-receptors on the cell surface. The marker CD4 was identified as the main receptor for HIV infection (18-20) and the required co-receptors, CCR5 and CXCR4, were identified by researchers nearly a decade later (21, 22). CD4 is expressed on T lymphocytes, monocytes, macrophages, and dendritic cells (DCs) (12). CCR5 is highly expressed on memory T cells, but not naïve T cells while CXCR4 is expressed on both subsets. CCR5 is also expressed on macrophages and DCs although at lower levels. The importance of CCR5 as a co-receptor is illustrated by the observation that patients that are homozygous for CCR5 deletion were resistant to HIV infection (23-25). The preferred target cell types for HIV infection are activated T cells with intracellular environments that appear to be more permissive to infection than resting cells (12). DCs are more difficult to be directly infected with HIV though have been shown to capture virus to facilitate trans-infection of neighboring T cells (26).

After receptor binding, Env undergoes conformational changes that allows viral fusion to the host cell membrane and begins the process of uncoating the viral capsid (4). Reverse transcriptase transcribes HIV RNA to double-stranded DNA, most likely occurring prior to uncoating and may in fact be responsible for capsid disassembly. Reverse transcriptase is an error-prone transcriptase which incorporates an incorrect nucleotide every 1500 to 4000 bases resulting in the high mutation rate observed with HIV (14). The pre-integration complex is formed and is transported to the nucleus of the cell. Here, viral integrase integrates the viral DNA into the host genome which allows for transcription of the viral genome. The integration of the viral genome into the host genome makes complete eradication of the virus in an infected individual incredibly difficult (12). Following translocation of RNA from nucleus, new viral proteins can be produced, which are transported to the cell surface for assembly of immature virions. Once released from the cell surface, protease cleaves the structural Gag precursor to form the mature, individual Gag proteins (MA, CA, NC, and p6) leading to morphological changes to the virion structure that results in a mature, infectious virion.

The HIV gene expression pattern is complex and is regulated by the Tat and Rev viral proteins (27). For retroviruses, the long terminal repeat (LTR) serves as an extremely efficient viral promoter. The HIV-1 LTR includes several upstream regulatory elements that serve as binding sites for cellular transcription initiation factors (28). Tat serves as a transactivator of viral transcription by stimulating elongation from LTR of the viral genome (29-31). Unlike typical transcriptional activators that bind DNA, Tat binds to the RNA hairpin trans-activating response element (TAR) at the 5’ end of a nascent viral transcript and promotes further elongation of the transcript (15).

To yield the full complement of mRNAs required for viral protein synthesis, HIV primary transcripts undergo extensive and complex alternative splicing in the nucleus (27). More than 40 different spliced mRNA species can be detected within infected cells including incompletely and completely spliced mRNAs. As these unspliced or incompletely spliced mRNAs are typically degraded in the nucleus and

prevented from transport into the cytoplasm, retroviruses express regulatory factors such as Rev to avoid detection of intron-containing RNA which allows for their release from the nucleus. Rev interacts with a structured RNA element in the *env* gene, the Rev-responsive element (RRE) to transport unspliced and incompletely spliced mRNAs that encode structural proteins from the nucleus into the cytoplasm (32, 33).

HIV encodes the accessory proteins Vif, Vpu, Vpr, and Nef to counteract host antiviral defenses. APOBEC3 (apolipoprotein B mRNA-editing catalytic polypeptide-like 3) proteins are cellular cytidine deaminases that restrict retrovirus replication by hypermutating the viral cDNA and inhibiting reverse transcriptase. Vif has been shown to inhibit and reduce the amount of APOBEC3 via proteasome-mediated degradation and other mechanisms (34-36). Cellular tetherin normally prevents the release of budding viruses by tethering viral particles to the membranes and HIV encoded Vpu inhibits this effect (37, 38). Vpr is an auxiliary protein that is specifically incorporated into viral particles via direct interactions with the Gag precursor, so it is present upon viral entry of a cell (39-42). Vpr has also been shown to block proliferation of infected T cells (43-45) and mediates nuclear import of the viral pre-integration complex which is critical for viral replication (46, 47). Vpx is a viral protein that is similar to Vpr that is encoded by HIV-2 but not HIV-1. Vpx and Vpr are small, 100 amino acid proteins with 20-25% sequence similarity and are predicted to have similar structure (48, 49). Vpx inhibits cellular SAMHD1 (SAM and HD domain-containing protein 1) which serves to deplete pools of nucleoside triphosphates that is required for reverse transcription (50). HIV Nef serves as an viral adaptor protein that diverts cellular proteins in a manner that enhances viral replication (51). Nef has been shown to downregulate cell surface expression of CD4 (52-56) and major histocompatibility class I (MHC I) molecules (57-61), mediate cell signaling and activation (62-65), as well as enhance viral infectivity by CD4 independent mechanisms (66-71). Recently, SERINC3 and SERINC5 were established as novel HIV-1 restriction factors and Nef targeted these proteins to the endosome, thereby preventing their incorporation into budding virions (72, 73).

HIV PATHOGENESIS

Studies of HIV pathogenesis since the early 1980s have revealed extensive details regarding the immune dysfunction HIV infection can induce. Rapid depletion of host CD4+ T cells as well as chronic systemic immune activation and dysregulation are hallmarks of HIV infection. Sexual transmission of HIV in semen or mucosal surfaces reports the most common route of HIV transmission globally where typically one founder virus is transmitted (74, 75). During this acute phase of infection, HIV rapidly replicates and leads to induction of inflammatory cytokines and chemokines (76). Though difficult to identify individuals during the early acute phase of infection, SIV non-human primate models of infection have indicated that partially activated CD4+ T cells of the genital mucosa are the first targets of infection and propagation of SIV continues in susceptible activated CD4+ T cells (77). The virus will then migrate to the gut-associated lymphoid tissue (GALT) where it results in massive depletion of memory CD4+ T cells which has also been shown to occur to HIV+ individuals (78-82).

For approximately two to four weeks following infection, infected individuals experience a symptomatic phase that includes fever, lymphadenopathy, rash, and/or malaise (12, 83). During this phase, the virus disseminates systemically leading to seeding of lymphoid tissues and establishment of HIV reservoirs. Detectable viremia in patient plasma typically peak at during this time which can range from 1 to 10 million copies of HIV virus per milliliter (84, 85). HIV-specific adaptive immune responses are generated during this time which lead to the drop in plasma viremia levels of patients leading to a steady set point viral load which results in approximately 100-fold decrease (12). The set point levels of patients in the absence of anti-retroviral therapy (ART) are important as set point levels correlate with clinical outcome wherein patients with higher viral loads typically progress to acquired immune deficiency syndrome (AIDS) more rapidly (86).

CELLULAR IMMUNE RESPONSE TO HIV

The role of HIV-specific cellular responses during HIV pathogenesis have been under intense study with the goal of understanding what types of responses could contribute to protection or control of the virus. Below, the role of HIV-specific CD4+ cells during pathogenesis and control of HIV is outlined. HIV-specific CD8+ T cells are one arm of the adaptive immune response that is believed to contribute to reduction of infected cells and plasma viremia (75). The rise of these virus-specific cells will often place immune pressure on the virus leading to selection of viruses with escape mutations to avoid CD8+ T cell-mediated killing. Here, the role of CD8+ T cells during HIV infection and their dynamics in the lymphoid and mucosal tissues will be reviewed.

ROLE OF CD4+ T CELLS

The contributions of CD4+ T cell responses to disease progression of HIV are not as well studied as CD8+ T cell responses are (discussed below). Preferential infection of CD4+ T cells as target cells and the fear of generating vaccine-induced CD4+ T cells that may subsequently serve as target cells likely contributed to research efforts primarily focusing on CD8+ T cell responses. The early depletion of bulk memory CD4+ T cells results from productive infection, bystander activation-induced death along with abortive viral infection leading to pyroptosis (79, 87, 88). Despite this however, Gag-specific CD4+ T cell responses are detectable in patients with active and progressive infection with non-progressive patients experiencing higher frequencies (89). The early disruption seen in CD4+ T cell levels affects the immune system in a wide-ranging manner. CD4+ T cells provide helper cell functionality to help in the induction and maintenance of CD8+ T cells. Additionally, CD4+ T cells, particularly in the form of Tfh cells, are critical for the induction of B cell proliferation, antibody production and maturation. These Tfh cells are however a double-edge sword as they contribute largely to the latent reservoir as discussed in the Reservoir section below.

More recently, there have been studies to understand the role of HIV-specific CD4+ T cell responses during acute infection and their evolution into chronic infection. In a cohort of acutely infected individuals that spontaneously controlled their viremia, these individuals had significant expansion of HIV-specific CD4+ T cell responses compared to typical viremic progressors (90). The expansion of these cells was characterized by robust cytolytic activity and expression of a distinct perforin and granzyme profile. The levels of granzyme A+ HIV-specific CD4+ T cells observed at baseline proved to be predictive of slower disease progression indicating a role for these cells in contribution to control of viremia. Another study showed that the breadth and magnitude of HIV-specific CD4+ T cell responses remained fairly stable from the acute to chronic phases of infection (91). Epitopes for CD4+ T cell responses are present in all HIV viral proteins, but many of them are for the Gag and Nef proteins (92). The level of Gag-specific CD4+ T cell responses inversely correlated with set point viremia during chronic infection in multiple studies while the opposite was observed with Env-specific CD4+ T cell responses (91, 93, 94). Analysis of HLA class II-restricted CD4+ T cell responses led to the discovery that HLA-DRB1*15:02 and HLA-DRB1*1303 are significant HLA-DRB1 alleles that associate with low viremia (95, 96). Early ART treatment in infected individuals have been shown to lead to better maintenance and proliferative capacity of CD4+ T cells (97, 98). These HIV-specific responses are considered to be beneficial during the acute phase and does not necessarily promote disease progression.

ROLE OF CD8+ T CELLS

While HIV is characterized by the significant loss of CD4+ T cells, CD8+ T cell immune responses been shown to have a role in control of HIV and disease progression (99, 100). Early studies demonstrated the ability of CD8+ T cells from HIV infected individuals to inhibit HIV replication *in vitro* though these individuals still progressed to AIDS (101). Additional assays confirmed that functionality and presence of virus-specific CD8+ T cell responses through ELISpots for interferon-γ (102) and intracellular cytokine stimulation assays (103). The development of peptide MHC tetramers that allowed for detection of

epitope-specific CD8+ T cells also documented the significant presence of HIV-specific CD8+ T cell responses (104).

The role of these virus-specific CD8+ T cells during the course of HIV infection and how they shape disease progression has been implicated through viral evolution studies, analyses of host genetics, and experimental studies. The emergence of virus-specific CD8+ T cells during acute infection in individuals has been associated with the initial control of viremia resulting in set point viral load levels (105, 106). The role of these cytotoxic T lymphocytes (CTLs) in the course of disease progression is also demonstrated by analysis of viral sequences from infected individuals which showed immune selection of HIV that was CTL mediated (107). As mentioned above, typically a single transmitted founder virus establishes infection in an individual. Diversification of this single genetic variant is observed at peak infection time points as inferred by single genome amplification and sequencing of plasma virion RNA (108, 109). The single genome amplification technique also allowed for detection of viral evolution at sites of CTL pressure during peak viremia which leads to viral diversification and generation of viral quasispecies (110). These observations in viral evolution, particularly at CTL epitopes sites of the virus, are signs of CD8+ T cell directed immune pressure on HIV.

It is difficult to study the early phase of immune responses after HIV infection as individuals are not typically aware of their infection status during the acute infection phase. However, there have been studies that have shown that early events during this acute phase of infection predict disease progression in individuals (86, 111). The virus-specific T cell responses that initially emerge during this period are shown to be very narrowly directed with responses to autologous virus focused on only one to three viral epitopes (110). Studies have shown that these acute responses are predominantly generated against Env and Nef epitopes (110, 112, 113). These focused responses then begin to diversity after viremia levels reach set point (102, 114). This increased breadth of response is also evidenced by the increase in number of HLA alleles involved in recognizing HIV-infected cells (115, 116). The presence of high avidity HIV-

specific CTLs were shown to be higher during early infection than in the later chronic phase and that maintenance of these high avidity cells was associated with low level set point viral loads (117). Studies using large cohorts of patients to determine host genetic factors that contribute to disease progression implicate the role of CTLs in driving disease progression. The strongest association with disease outcome and progression to AIDS found in these studies was expression of certain HLA class I alleles, in particular HLA-B*35 and HLA-B*57 (118-120). Individuals with the HLA-B*57 allele are associated with low set point viral loads and long-term non-progressive chronic HIV infections (119-131). These long-term non-progressors (LTNPs) are associated with slower disease progression, have HLA-B*57-restricted CTL responses that dominate early responses, and have reduced symptoms during the acute phase. Together, these studies highly suggest a role for HIV-specific CD8+ T cells in the control of viremia and delaying the onset of AIDS.

To experimentally determine the role of CD8+ T cells during HIV infection, scientists have used models of HIV pathogenesis in non-human primates. Rhesus macaques infected with SIV exhibit hallmark characteristics of HIV infection in humans. In SIV-infected rhesus macaques that had reached a steady set point level of viremia, depletion of CD8+ T cells via antibody administration resulted in large increases in plasma viremia which waned as CD8+ T cell levels reemerged demonstrating the importance of CD8+ T cells during chronic infection (132, 133). A role for CD8+ T cells during suppressive ART therapy has also been demonstrated. ART regimens prevent infection of new target cells as well as prevent production of new, infectious virions and when ART regimens are interrupted, rapid viral rebound occurs. The role of CD8+ T cells in maintaining suppression of viremia under ART was demonstrated when SIV-infected rhesus macaques treated with ART were depleted of CD8+ T cells. These animals exhibited significant increases in plasma viremia in the absence of their CD8+ lymphocytes which came under control when CD8+ T cells reemerged (134). Together, these experimental studies indicate a function of CD8+ T cells in maintaining control of viremia in infected individuals.

The mechanisms underlying the importance of CD8+ T cells during acute and chronic HIV infection are still debated as precise correlates of protection are still being determined. The ability of HIV-specific CD8+ T cells to lyse virally infected cells (CTLs) as well as produce cytokines and chemokines such as IFNγ, IL-2, TNFα, MIP-1α (CCL3), MIP-1β (CCL4), and RANTES (CCL5) are important anti-viral properties though the relative contributions of these functions to viral control are still debated (135, 136). The role of β-chemokines (CCL3, CCL4, CCL5) during an immune response is to recruit leukocytes to the site of injury or infection by binding to the CCR5 receptor expressed on cells (100). Production of these β-chemokines can help prevent infection of new target cells by directly competing for CCR5 binding by HIV virions as well as reducing CCR5 expression on target cells *in vitro* though the role of these chemokines *in vivo* is not well defined (137, 138). There are indications that early during infection the cytolytic functionalities of CD8+ T cells are more important than cytokine production. CD8+ T cells expressing high levels of perforin were shown to correlate with control and have a role in selection of early virus escape mutants (139). During chronic infection, the lytic potential of CD8+ T cells contribute to control but other anti-viral functions of these cells may become more important (140). Overall, it is not abundantly clear to the field exactly what CD8+ T cell functionalities are necessarily important for the long-term control of HIV and prevention of viral dissemination.

CELLULAR DYNAMICS IN THE MUCOSAE

Most studies on HIV pathogenesis in humans utilize peripheral blood samples to understand cellular and humoral dynamics during infection though the majority of HIV replication is known to occur in the mucosal and lymphoid issues. It is not entirely clear if the responses detected in the blood are necessarily representative of responses found at these sites of replication (100). Studies utilizing SIV pathogenesis models sought to answer elucidate the dynamics of cellular responses at these sites. The gastrointestinal tract is one of the most important organs in the pathogenesis of HIV/SIV (141). The GALT is not only one of the largest, but also one of the most important sites of viral persistence in SIV-infected rhesus

macaques (142). Here, activated, memory CD4+ CCR5+ T cells undergo massive depletion as observed in SIV+ rhesus macaques (143, 144) as well as in humans (79, 81, 82). These cells of the lamina propria are considered the major targets for infection (79, 82, 145, 146). Depletion of specific CD4+ T cell subsets that contribute to disease pathogenesis have additionally been observed. CD4+ T helper cells that express IL-17 (Th17 cells) in the lamina propria are important mediators of the immune response in the gut and are induced by the microbiota (147). Th17 cells produce IL-17 and IL-22 which help promote neutrophil infiltration during a bacterial infection, help to maintain mucosal epithelial integrity, and induce production of antibacterial defensins (148). These Th17 cells are observed to be rapidly depleted in the intestinal tract during infection (148-152). The loss of this cell subset is associated with the loss of intestinal mucosal integrity resulting in microbial translocation. This disruption of the mucosal epithelial barrier and subsequent release of bacterial products such as LPS, flagellin, or CpG-containing DNA from the intestine to the circulation likely contributes to the chronic systemic immune activation observed during disease progression (148, 153, 154). Immunosuppressive regulatory CD4+ T cells, Tregs, are abundant in the intestinal tract and are also disrupted during HIV infection (155). In SIV infected rhesus macaques, the increased frequency of Tregs detected in the gastrointestinal tract of animals were correlated with their viral loads indicating a role for Tregs in suppressing anti-viral responses (156). With regards to CD8+ T cell responses, virus-specific CTLs were observed to have emerged in the intestine at the same time as the blood (157). During the chronic phase, the frequency of CTLs detected in the blood were higher than that observed in the mucosal tissue indicating the importance of CTLs at a major site of viral replication (158). In dissecting the contributions of cytokine producing HIV-specific CD8+ T cells of the gut mucosa of humans, significant associations were found between disease status and the polyfunctionality of CD8+ T cells producing IFNγ, IL-2, TNFα, MIP-1β, and CD107a (159, 160).

CELLULAR DYNAMICS IN LYMPHOID TISSUES

The lymph nodes (LNs) are secondary lymphoid organs that are important sites for generating antigen-specific cellular and humoral adaptive immune responses in germinal centers (GCs) and possess a

regulated cellular architecture (161). As a site of constant local inflammation and altered tissue architecture, the lymphoid tissues hold a key role in HIV pathogenesis as an environment ripe for viral evolution and persistence of the viral reservoir, particularly in T follicular helper cells (Tfh) (162). Tfhs interact with B cells to promote B cell activation, affinity maturation, somatic hypermutation, and aid in their differentiation into memory B cells and plasma cells. Tfhs have been observed to aberrantly accumulate in GCs during chronic HIV (163, 164) and SIV infection (165-168) and very likely contribute to systemic immune activation as well as hypergammaglobulinemia and activation of GC B cells (166).

Given the persistence of virus in the LNs, the presence of CD8+ T cells is to be expected though their ability to localize, detect, and eliminate virally-infected cells currently under debate. Early studies have shown that HIV-specific CD8+ T cells were found at a greater frequency in the LNs than was detected in peripheral blood of infected individuals (169). Subsequent studies demonstrated that these anti-viral CTLs had limited ability to access the follicles of LNs where HIV replication is thought to occur (170, 171). These effector CD8+ T cells were shown to lack the receptors such as CXCR5 required for B cell follicle entry thereby preventing detection of latently infected Tfh cells (170, 172-175). This is also indicated through the observation that HIV- and SIV-specific CTL frequencies are lower within follicles compared to extrafollicular regions (170, 171, 175-178).

It is under debate whether the CD8+ T cells that can enter the follicle also have effective cytolytic functionalities. *In situ* studies from SIV-infected rhesus macaques show follicular CD8+ T cells in contact with Tregs and were shown to express PD-1 indicating potentially less cytotoxic potential (179). Other studies indicate that follicular CXCR5 expressing CD8+ T cells are potently cytolytic against HIV RNA+ cells, have better IFNγ production capabilities than CXCR5- cells, and express less markers of exhaustion (180). In HIV+ patients, the study was able to show that virus-specific CXCR5+ CD8+ T cell numbers were inversely correlated with patient viral load and that these cells had higher levels of perforin than CXCR5- cells. Though follicular CXCR5+ CD8+ T cells may be a rarer population than CXCR5- cells,

they may also be more cytolytic as well. Another study showed that follicular CD8+ T cells from the lymph nodes exhibited higher cytolytic activity via granzyme B and perforin expression than extrafollicular CD8+ T cells though they also expressed a lower polyfunctional cytokine profile and higher PD-1 expression (181). In a rhesus macaque model, the expansion of SIV-specific CXCR5+ CD8+ T cells in the lymph nodes, which demonstrated enhanced polyfunctionality and were able to restrict proliferation of antigen-pulsed Tfh cells *in vitro*, was associated with greater control of viremia (182). Meanwhile, there are other groups that contend that these cells are a new subset of CD8+ follicular Treg cells due to their low expression of perforin, increased expression of the inhibitory molecule Tim-3 and IL-10, and ability to modestly limit Tfh IL-21 production (183). These cells were also shown to inhibit Tfh expansion, antibody production, and autoimmunity implicating their role in suppression of the immune response (184, 185). It is yet unclear the role of follicular CD8+ T cells in HIV infection and elucidation of their contributions to pathogenesis will be important to future vaccine strategy efforts.

HIV CONTROLLERS

There is a subset of HIV-infected individuals known as HIV controllers who are able to control viremia to extremely low levels without the use of ART. This highly effective control of viremia is seen in approximately 7% of infected patients, but the control is typically transitory and does not last beyond 12 months (186). If the control is observed past 12 months, the chances of maintain undetectable viremia for years is significantly improved. Controllers are able to maintain plasma viral load levels below 50 copies/ml, the typical threshold of detection, and they account for less than 0.5% of infected patients (187-190). The viremia of HIV controllers reaches similar levels to that of ART treated patients with a median viremia of 2 copies/ml in one cohort studied (191-194). Many studies indicate that host factors of these individuals help to control the virus as these patients have non-symptomatic primary infections, maintain high CD4+ T cell levels, control viral replication, and have lower levels of viral reservoir in the blood (120, 186, 187, 190, 195, 196). The latent reservoirs in these patients are also observed to be

smaller than that of ART treated patients (195, 197, 198). These observations indicate that factors related to control are occurring early after infection (187).

The focus of research on HIV controllers aims to understand the mechanism of viral control in these individuals in order to inform vaccine study designs, so much analysis has been performed on their cellular responses (199). In controllers, HIV-specific CD8+ T cells had a greater ability to proliferate compared to viremic progressors and these controllers were associated with the protective HLA-B*57 allele (120, 200, 201). These virus-specific cells were shown to be able to effectively suppress viral replication in CD4+ T cells by killing without exogenous stimulation which was not observed in progressors or ART treated patients (201). This was also shown to be associated with effective loading of perforin and granzyme B in CD8+ T cells in controllers (202, 203). Studies also have shown that HIV controllers had increased frequencies and functionality of Gag-specific CD8+ T cells in the rectal mucosae and this was more strongly associated with controller status than responses in the periphery would indicate (160, 204). Strong CD8+ T cell responses at the intestinal mucosae suggest that these cellular responses contribute to reduce CD4+ T cell depletion and preserve rectal mucosal integrity. CD4+ T cell responses in controllers were also observed to be well maintained (93). Controller CD4+ T cells also express less of the negative immunoregulatory molecule CTLA-4 (205). This coincides with the observation that these CD4+ T cells maintain their ability to secrete IL-2 and proliferate after Gag stimulation as well as have better preservation of their central memory cell population (206, 207). These observations are consistent with functional CD4+ T cells helping to drive and sustain the anti-viral CD8+ T cell response observed.

HUMORAL IMMUNE RESPONSE TO HIV

HIV-specific humoral responses are induced early during acute infection though these responses do not lead to control of viremia. As Env gp120 and gp41 are required for viral entry and their conserved regions are functionally required, it is assumed that antibodies targeting the correct regions on Env would be

effective at blocking infection (208, 209). Virus-specific IgM and IgG complexed to HIV are detectable in plasma of patients at approximately 8 days after transmission (210). Free plasma antibody targeting Env gp41 appeared by approximately 13 days after detection of plasma viremia while Env gp120-specific antibodies appeared two weeks later (210). This initial humoral response is directed against non-native, non-neutralizing Env epitopes which is in line with their inability to control viremia during acute infection. During chronic infection, the Env-specific antibody responses are observed to be short-lived with their levels increasing and decreasing particularly in the presence of ART where viremia and thus antigen is reduced (211, 212). Some of the conserved regions on Env where targets for antibody binding reside are poorly immunogenic (213).

Neutralizing antibodies (NAb) specifically bind to pathogens to abrogate the pathogen's ability to infect host cells and in HIV infected individuals. NAbs, which can neutralize 70-80% of HIV strains with a degree of potency, take months to develop against the autologous virus. NAbs against a patient's transmitted founder virus does not typically develop until approximately 3 months after transmission has occurred (214, 215). HIV Env is such that regions that are susceptible to neutralization are shielded from the reach of antibodies (216-220). Glycan shields make up more than half the molecular weight of the Env protein thereby masking and hindering a NAb from reaching conserved susceptible epitopes. Variable loops on Env also aid in making important neutralizing epitopes. Other Env epitopes are recessed from the surface, are only transiently exposed, or otherwise are spatially inaccessible to these antibodies. The eventual development of NAb responses are considered too late to prevent establishment of infection and latency (221).

Broadly neutralizing antibodies (bNAbs) are only developed by a small proportion of infected individuals and these typically take years to develop. Approximately 20% of chronically infected individuals make antibodies with some NAb breadth and only 2% of those chronically infected develop bNAbs which are NAbs that can broadly and potently neutralize most HIV strains tested (222-225). BNAbs are difficult to

develop as they possess unusual antibody characteristics in regard to having a high degree of somatic hypermutation, long heavy chain complementary determining region 3 (CDR3), and are typically polyreactive (222, 226, 227). For infected individuals, development of these bNAbs occur too late during infection to help with disease progression, but they have become a field of intense study for use in novel preventative and therapeutic treatments (228).

Non-neutralizing antibodies (non-NAb) are pathogen-specific antibodies that do not inhibit infectivity but serve to tag pathogens or infected cells for destruction by innate effector cells. Binding of the non-NAb Fc (crystallizable fragment) region to Fc receptors (FcR) on innate cells signals the cell to clear the opsonized target. IgG-specific FcR are differentially expressed on innate cells and lead to different pathogen clearance mechanisms. Phagocytes mediate antibody-dependent cellular phagocytosis, while natural killer cells perform antibody-dependent cell-mediated cytotoxicity (ADCC) by releasing granules and utilizing Fas/FasL interactions to induce cell death. (229, 230). ADCVI, or antibody-dependent cell-mediated virus inhibition, is a metric of antibody-mediated antiviral activity of FcR-bearing cells that measures ADCC, beta chemokine production, and phagocytosis. The role of non-NAb in HIV still remains to be completely elucidated, though some investigators have demonstrated the potential for non-NAb to contribute to protection and control of SIV (231, 232). Antibodies that perform ADCC functions arise during acute infection and these responses are seen to be cross-reactive, can target diverse strains of HIV, but also appear to develop too late during infection to help mediate viral control (222).

ANTI-RETROVIRAL THERAPY

The advent of anti-retroviral therapy (ART) that targets the main steps of the viral life cycle is considered one of the greatest achievements in modern medicine. When the regimen is properly adhered to, viremia in patients is reduced by multiple orders of magnitude in a matter of weeks (12, 233). The degree of suppression that can be achieved with combination ART is such that viral evolution and emergence of drug-resistant mutations are prevented. ART also dramatically reduces the systemic levels of

inflammation and immune activation present during chronic infections (234). In the absence of viral replication, the immune system is able to recover much of the functions it had lost, and AIDS is prevented. However, even with durable long-term suppression by ART, many still fail to restore optimal immune functionality even after years of treatment.

There are five therapeutic classes of ART drugs that target specific steps of the viral life cycle: I) nucleoside reverse transcriptase inhibitors (NRTI), II) integrase strand transfer inhibitors (INSTI), III) non-nucleoside reverse transcriptase inhibitors (NNRTI), IV) protease inhibitors, and V) entry inhibitors (12). NRTI are analogues of natural nucleosides or nucleotides and are preferentially incorporated into the HIV DNA leading to termination of DNA synthesis thereby inhibiting reverse transcriptase activity. Combination therapies comprising three drugs typically consist of at least two NRTIs plus an additional inhibitor. First line regimens with two NRTIs include tenofovir and emtricitabine or abacavir and lamivudine at a fixed dose given once daily. Zidovudine (AZT) and stavudine are not as widely used in developed countries due to associations w anemia, neuropathy, hepatic steatosis, lactic acidosis, and lipoatrophy causing them to be phased out due to these toxicity effects. INSTIs such as dolutegravir and raltegravir prevent the HIV genome from being integrated into the host genome. These drugs are considered potent, well-tolerated, and safe for use (235-237). NNRTIs inhibit reverse transcriptase in a different manner than NRTIs do. These drugs bind a pocket near the active site of reverse transcriptase that causes a conformational change in the enzyme which inhibits its activity. These drugs are considered potent, safe, and easy to produce. Protease-mediated enzymatic cleavage of Gag into individual functional proteins is required for the virion maturation and budding process of the viral life cycle. Protease inhibitors block this step and when administered in combination with two nucleoside analogues is considered a highly effective combination therapy. Darunavir and atazanavir are two of the most widely used protease inhibitors. Entry inhibitors such as maraviroc prevent HIV from entering the cell. Maraviroc binds CCR5 on cells to prevent CCR5-mediated HIV entry of the target cell. Enfuvirtide is an inhibitor that binds HIV directly. The development of these drugs that target specific steps of the HIV life

cycle allow for decades of viral suppression and improvement in life expectancy of HIV infected individuals.

ART alone however cannot eliminate HIV infection due to the persistence of the viral reservoir. In patients undergoing ART treatment, studies have shown that the decay in reservoir levels have a half-life of 44 months, implying that reservoir eradication with ART alone would require over 73 years (238). Another issue related to combination ART is the emergence of drug-resistant viral mutants. Incomplete adherence to the treatment regimen will lead to suboptimal suppression and allows for viral replication. This allows for a greater possibility that mutations will occur and selection of viral variants that are resistant to ART. Mutations emerge rapidly for NNRTIs and some NRTIs, but emerge more slowly for most NRTIs and INSTIs (12). It is rarer for mutations against protease inhibitors to occur. The prevalence of drug resistance in transmitted HIV variants is in the rage of 5-15% in high income countries (239-242).

HIV RESERVOIR

One of the central challenges to HIV eradication is the persistence of quiescent, replication-competent proviral genomes present in resting CD4+ T cells despite highly suppressive ART (106, 238, 243-247). These cells represent the latently infected HIV viral reservoir that serves as the source of viral rebound that occurs after ART interruption. The latent reservoirs are undetectable by the immune system as they do not express viral antigens and thus do not appear infected. Additionally, they are impervious to the actions of ART drugs that target various steps of the viral life cycle (248, 249). In patients under suppressive ART, the levels of the latent reservoir have been shown to be stable and decays slowly (238, 250). Despite efforts in vaccine development and other preventative strategies, it is also important to help the millions of individuals that are currently infected. HIV eradication efforts (sterilizing cure) or cure research which aims to allow HIV-infected individuals to control viremia in the absence of ART

(functional cure) are currently areas of intense study. Development in these areas would help alleviate the burden of continuous medical care and lifelong treatment with ART in affected individuals.

The establishment of the latent reservoir has been shown to be established within days of infection and established in a small fraction of the first subset of cells to become infected (251, 252). As mentioned previously, in patients under highly suppressive ART, the decay in the reservoir levels has been shown to have a half-life of approximately 44 months which implies that eradication of the reservoir solely with ART would require over 73 years of therapy making eradication efforts with ART alone unlikely (238). Early administration of ART in infected patients has been shown to limit HIV replication, been associated with lower levels of integrated HIV DNA and lower frequencies of latently infected cells (253-255). It is of note that the majority of HIV DNA genomes detected in cells by quantitative PCR methods contain lethal mutations or deletions of the integrated viral DNA implying these infected cells are unlikely to produce replication-competent virus (256, 257). Other assays such as the quantitative viral outgrowth assay (QVOA) measured the replication-competent HIV reservoir though it is time consuming, expensive, and requires a large number of cells.

Detection of replication-competent HIV indicates that the central memory subset of CD4+ T cells are the main pool of the latent reservoir (258). Specifically, it has been identified that central memory CXCR5 expressing T follicular helper cells (Tfh) preferentially harbor HIV DNA in ART-suppressed patients and constitute a major proportion of the HIV viral reservoir (163, 164, 166, 259, 260). In addition, these cells that contain HIV DNA and can express HIV RNA were found to preferentially express the immune checkpoint markers LAG-3, PD-1, and TIGIT (261). The intracellular environment of the reservoir cells and the molecular mechanisms that allow for establishment of latent infection are not yet completely understood. There are likely multiple pathways which lead to latent infection though it is thought that resting T cell infection occurs as an activated T cell is in the process of reverting to a resting state. In this reversion process, the T cells could support the early phases of viral infection such as reverse

transcription and integration but the later steps of the viral life cycle are halted as cells reach a resting state (262). The mechanism of persistence of reservoir cells are still to be fully elucidated though cells with proviral DNA has been shown to undergo proliferation thus likely contributing to ongoing persistence (263-266).

LATENCY REVERSAL

There has been much work in recent years to target the latent reservoir in order to disrupt or reverse HIV latency. The attempts aim to induce viral reactivation in the reservoir, cause expression of viral antigens in reactivated cells, and lead to eradication of these cells through immune clearance mechanisms (248, 249). These latency reversing agents (LRAs) target host cell HIV latency promoting pathways to lead to HIV provirus expression. Various *in vivo* attempts have been shown to reverse latency as measured by increased expression of cell-associated HIV RNA, but this was not concomitantly seen with reduction in replication-competent HIV or persistent infection (267-270). Another approach to generating a functional cure includes combining LRAs with treatments to improve immune-mediated clearance of infected cells termed the shock and kill approach. The goal of this is to improve elimination of reactivated cells through reversing latency in reservoir cells ("shock") and generation of virus-specific immune responses that will target and clear the reservoir ("kill"). The immune-based strategies include vaccines, antibody treatment, and immunotherapy to enhance T cell functionality (271, 272).

One of the best-validated targets for clinical development of LRAs include epigenetic silencing of HIV transcription (273, 274). Histone deacetylases (HDACs) play a role in the establishment and maintenance of latency. Drugs that target and inhibit HDAC activities have been demonstrated to induce HIV transcription in latently infected cells. The HDAC inhibitors that have been tested most in the clinic for HIV latency reversal include vorinostat, romidepsin and panobinostat (267-270, 275, 276). Development of molecules that alter metabolism and activation status of reservoir cells is another approach. Resting cells express low levels of the co-activating factors NF-κB and NFAT and increasing their expression

may lead cells to induce transcription of the proviral genome (277-279). Agonists for toll-like receptors (TLRs) are another attempt to reverse *in vivo* latency via modulating the intracellular environment of reservoir cells (280-285). Many of these small molecules have been tested now in clinical studies and have demonstrated increased RNA transcription though not necessarily in the number of infected cells. Further development of approaches that lead to the shock and killing of the latent reservoir are required.

T CELL EXHAUSTION

PD-1: IMMUNE CHECKPOINT MARKER

T cell activation is a highly regulated process beginning with initial co-stimulatory signals that need to accompany peptide-MHC complexes binding to cognate T cell receptors (TCR) in addition to the co-inhibitory signals required to aid in the contraction of an immune response to avoid autoimmunity. The co-inhibitory checkpoint receptors programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) are two of the most well studied as to their role in balancing immunity and immunopathology. During activation, all naïve T cells express PD-1 thus serving as a marker for effector T cells (286-288). PD-1 is also expressed on other cell subsets such as regulatory T cells, Tfh cells, memory T cells as well as B cells and NK cells. PD-1 expression on effector T cells serves as a brake on the immune response, becoming an immune checkpoint that cells must pass to exert their functionalities. In PD-1 knockout mice, development of autoimmune pathologies becomes accelerated highlighting the important role of PD-1 in regulating activation of T cells (289-292). In scenarios of acute infection or inflammation, PD-1 expression levels on activated T cells decrease as the immune response resolves (293, 294).

PD-1 SIGNALING

In order for PD-1 to exert its inhibitory effects on the T cell, the PD-1 ligands must be expressed on the same cell that is presenting the cognate peptide-MHC complex to the TCR (286). PD-1 signaling through binding of its ligands programmed cell death 1 ligand 1 (PD-L1) or programmed cell death 1 ligand 2

(PD-L2) during T cell activation counteracts the TCR and CD28 co-stimulatory signaling (295-299). Receptor-ligand binding of PD-1 recruits phosphatases like SHP2 to the immunoreceptor tyrosine-based switch motif (ITSM) of the cytoplasmic tail of PD-1 (286). This pathway leads to the decreased activation of transcription factors such as AP-1, NF-kB, and NFAT which are necessary for T cell activation, proliferation, survival, and effector functions. Overall, the PD-1 signaling pathway has been shown to regulate the initial T cell activation, fine tune T cell functionalities, aid in the development of T cell tolerance, and play a role in returning the immune response to homeostasis (286, 297, 300-302).

CELLULAR EXHAUSTION DURING CHRONIC INFECTIONS

While PD-1 signaling during acute conditions have defined roles in aiding the activation and contraction of responses, persistent signaling of PD-1 on a T cell can drive effector T cells into a state of exhaustion (303, 304). An exhausted T cell with sustained PD-1 expression has reduced effector functionalities, poor recall responses, and a distinct transcriptional state from either effector or memory T cells (304). In conditions of cancer or chronic infections such as with HIV or hepatitis B virus (HBV) where antigen is not cleared and persists at high levels, high expression levels of PD-1 and its ligands occur thereby limiting the overall immune response exerted against the cancerous cells or infected cells. This was first described in chronically lymphocytic choriomeningitis virus (LCMV) in mice and with people infected with HIV, HBV, or hepatitis C virus (HCV) (293, 294, 305). PD-1 signaling is not required for the induction of the T cell exhaustion state but does have a role in maintaining exhaustion (306). Of incredible interest was the observation that blocking the PD-1/PD-L1 signaling axis with a monoclonal antibody restored function to exhausted T cells during chronic infections. PD-1 blockade led to increased virus-specific CD8+ T cell proliferation, cytokine production, improved killing potential and decreased viral loads in LCMV, SIV, and HCV infections (293, 307-309).

In HIV infected individuals, the increased and persistent expression of PD-1 is predominantly on central memory T cells (303, 310). In addition to PD-1 in untreated infected people, we observe an upregulation

of other immune inhibitory markers such as CTLA-4, TIM-3, TIGIT, and LAG-3 on CD4+ and CD8+ T cells (205, 311, 312). When patients are under ART treatment, the expression of inhibitory markers declines though is still considered elevated when compared to healthy control individuals (312). PD-1 expression is high on cytotoxic CD8+ T cells that are found to migrate to lymphoid follicles e.g. expressing CXCR5 and are low in other immune checkpoint proteins (177). There is an observed inverse association between the frequency of cytotoxic CD8+ T cells and HIV infected cells found in lymphoid follicles (177, 179, 182). The increase in PD-1 expression is observed on both total and HIV-specific CD8+ T cells in untreated HIV individuals (311, 313, 314). The expression level of PD-1 on CD4+ or CD8+ T cells are associated with clinical outcome and for individuals not on ART treatment, increased PD-1 expression is associated with faster decline in CD4+ T cell numbers (311, 315). Even for individuals on ART treatment, the PD-1 expression on CD8+ T cells is associated with impaired CD4+ T cell immune reconstitution as well as faster viral rebound after ART interruption (316, 317). When expressed on HIV-specific CD4+ T cells, PD-1 expression is associated with exacerbating CD8+ T cell exhaustion and viral immune escape (318).

In addition to how PD-1 expression impacts cellular immune responses and disease outcome, there are correlations observed between the frequency of PD-1 on CD4+ and CD8+ T cells with various HIV persistence markers for individuals on ART. This has been characterized in the peripheral blood, lymph nodes, and gastrointestinal tract (260, 319-322). Additionally, enrichment of HIV has been observed in PD-1 high expressing CD4+ T cells compared to PD-1 low expressing cells (319). The HIV enrichment seen in PD-1 high cells may be a result of the inhibitory effects PD-1 exerts on the T cell which could limit HIV transcription and RNA export and translation thereby favoring latent infection over productive infection (303).

PD-1 BLOCKADE DURING HIV INFECTION

To date, disrupting the signaling between PD-1 and its ligand to abrogate the inhibitory signal PD-1 imparts on T cells has been performed in a variety of chronic infections and cancer settings. Use of monoclonal antibodies to serve as a PD-1 checkpoint blockade has led to improvement and reinvigoration of T cell functionality and reduction of viral loads or tumor burdens (293, 305, 311, 313, 323-327). In a mouse model of chronic LCMV infection, *in vivo* blockade using a blocking monoclonal antibody (mAb) against PD-L1 (αPD-L1) restored virus-specific CD8+ T cell proliferative capacity, cytokine production, cytotoxic activity, and led to reduced viral loads (293). In HIV, *ex vivo* blockade of PD-1 with αPD-L1 mAbs has repeatedly been shown to improve HIV-specific CD8+ T cell functionality and ability to kill infected cells. These early studies include the observation that PD-1 is highly up-regulated on HIV-specific CD8+ T cells and is correlated with viral load as well as reduced capacity of the T cell to produce cytokines and proliferate (311). Trautmann *et al*. was also one of the first to show that *ex vivo* blockade with a αPD-L1 mAb enhanced HIV-specific CD8+ T cell survival, proliferative capacity, and cytokine and cytotoxic molecule production in response to antigen. Using cells from ART-naïve, HIV-infected individuals, Day *et al*. similarly showed that blockade with αPD-L1 mAb augmented HIV-specific CD8+ and CD4+ T cell functionality (313). Petrovas *et al*. at the same time showed that PD-1 blockade improved HIV-specific CD8+ T cells survival as PD-1 served as an important regulator of survival during HIV infection (314). These studies were the first to demonstrate the role of PD-1 expression during chronic HIV infection and showed that blockade of the inhibitory PD-1 signaling pathway was capable of reversing the immune exhaustion that had for so long been considered a hallmark of chronic HIV infection.

Though PD-1 blockade has been tested in clinical studies and licensed for a wide range of cancers, studies evaluating PD-1 blockade in HIV infected individuals have not been extensively performed as safety concerns persist. Studies in non-human primates to establish safety and efficacy will be required first. Our

lab has previously published the effect of PD-1 blockade using a mAb against PD-1 (αPD-1) in chronically SIVmac251 infected rhesus macaques (308). PD-1 blockade in these animals were well-tolerated and resulted in rapid expansion of SIV-specific CD8+ T cells with improved effector functions, enhanced survival, and lower plasma viremia. Additionally, rhesus macaques treated with αPD-1 mAb showed significant reduction in interferon-related signaling and thus lowered hyperimmune activation observed during chronic SIV infection as well as improved gut barrier functionality (328).

The next steps in testing efficacy of PD-1 blockade during HIV infection is administration of blockade in conjunction with ART therapy as most HIV+ individuals are currently on therapy after diagnosis. Recent preliminary work on the administration of αPD-L1 mAb to a small cohort of chronically SIV-infected rhesus macaques under suppressive ART was shown to be safe and well-tolerated in the animals and also demonstrated transient viral control after ART treatment interruption (329). In a phase I dose escalation clinical study using αPD-L1 mAb for blockade in six HIV+ individuals under suppressive ART treatment, severe adverse events were not observed and two of the volunteers showed increased Gag-specific CD4+ and CD8+ T cells after administration of the lowest dose (303, 330). The study was stopped however due to retinal toxicity observed in a concurrent rhesus macaque study (303). This is currently the only immune checkpoint inhibitor trial performed in HIV infected individuals without malignancies and more safety data will be required before more extensive studies in HIV+ individuals can be performed. The potential effects of PD-1 blockade during HIV infection to monitor for are I) enhancement of HIV-specific CD4+ and CD8+ T cell responses in the blood and lymphoid tissues, II) reversal of HIV latency, and III) depletion of the newly reactivated latently infected cells, possibly through enhanced T cell mediated killing (303).

HIV VACCINES IN THE CLINIC

While there is work in progress on development of a functional cure, it will be essential to have an efficacious vaccine as a long-term solution to control the HIV epidemic. A vaccine should prevent new infections and/or help to control virus replication in infected individuals to limit disease progression. There are many challenges facing the development of an HIV vaccine including an incomplete knowledge of the immunological correlates of protection, the high diversity of HIV within an infected individual (2-6% (13, 331)) as well as in the population (about 15-20% within a clade; 25-35% between clades (332-334)), the ability of HIV to rapidly mutate in an infected host, and a complex glycan structure that helps protect the virus against neutralization. It is clear that overcoming these challenges for an effective HIV vaccine will require the induction of both humoral and cellular immunity against conserved viral targets. NAbs to prevent infection and spread of the virus will be critical and non-neutralizing antibodies (non-NAb) to assist in antibody-dependent cellular cytotoxicity (ADCC) and other antibody-mediated clearance mechanisms also play a significant role (335, 336). Cytolytic $CD8^+$ T cells will be crucial in the elimination of HIV-infected cells and $CD4^+$ helper T cells will be required for the proper development and maturation of antibody and $CD8^+$ T cell responses; in particular, $CD4^+$ T follicular helper (Tfh) cells are needed for long-lived and high avidity antibodies (166, 337). Development of protective immune responses by vaccination will likely require a multi-faceted approach since other regimens have shown to be ineffectual. Although there are many vaccines under preclinical development, to date only six have been tested for efficacy in humans and will be reviewed here.

AIDSVAX TRIALS

The AIDSVAX trials were the first two HIV vaccine efficacy studies (338-340). These trials sought to engage humoral responses alone to protect from infection by utilizing bivalent Envelope gp120 protein immunogens derived from two subtype B isolates (AIDSVAX B/B) in one study or subtype B and E isolates (AIDSVAX B/E) in the second study. AIDSVAX B/B was used in participants in North America and the Netherlands who were at risk for sexual exposure to HIV while AIDSVAX B/E was studied in

Thailand in injection drug users. In the first study, HIV infection rates were 6.7% in 3598 vaccinees and 7.0% in 1805 placebo participants with an estimated vaccine efficacy of 6% thus showing no reduction of infection in vaccinated participants (338). Vaccine efficacy in the second trial was considered to be 0%. Though all participants produced neutralizing and CD4-blocking antibody responses, the vaccine failed to provide any protection from HIV acquisition or influencing post-infection disease progression (338-340). Lessons learned from these studies indicate that recombinant gp120 protein is insufficient on its own to protect against a diverse population of primary HIV-1 isolates and new vaccines approaches will be required in the form of improved protein design, new adjuvants to enhance appropriate helper T cell responses, or combination vaccines that additionally promote cellular immunity against HIV (338).

STEP AND PHAMBILI TRIALS

The third and fourth efficacy studies were the phase IIB Step (HVTN 502) and Phambili (HVTN 503) studies which employed Merck recombinant adenovirus 5 (Ad5) vectors expressing HIV Gag, Pol, and Nef that aimed to generate cell-mediated immunity to protect against HIV infection or change early plasma virus levels (341-343). Rationale for this model came from understanding the role of cellular immunity in viral control and disease progression in elite controllers and long-term non-progressors and from non-human primate challenge models as discussed in the HIV Controllers section above. Ad5 vectors are considered highly immunogenic regarding induction of antigen-specific T cell responses. The studies enrolled individuals considered at high risk of HIV acquisition and the regimen consisted of 1:1:1 mixture of replication-defective Ad5 vectors expressing either the HIV-1 Gag, Pol, or Nef gene and was given at day 1, week 4, and week 26. The trial did not include an HIV Envelope immunogen and thus did not generate antibody responses against HIV Envelope. In the Step trial, the regimen was shown to be immunogenic for inducing HIV-specific $CD8^+$ T cells, however did not confer protection against HIV infection or aid in control of HIV replication as determined by viral load setpoints (341). Surprisingly, risk of HIV acquisition was increased in vaccinated Ad5-seropositive men as well as in uncircumcised men, whereas this increase was not observed in Ad5-seronegative or circumcised men. Risk assessment of

study participants based on both Ad5 and circumcision status revealed that risk of HIV acquisition in participants receiving the vaccine compared to those receiving placebo was highest in uncircumcised and Ad5 seropositive men. Risk was intermediate in men with either one of those two factors. The Phambili study conducted in the Republic of South Africa was stopped shortly after the Step study revealed lack of efficacy and more infections among vaccinees than placebo individuals (343, 344). The study revealed no evidence of vaccine efficacy and was not dependent on Ad5 serostatus or circumcision. Changes to setpoint viral loads were also not observed though halting and unblinding of the study early may have impacted the ability to determine those effects. Though the trials were unable to achieve the primary endpoints by using a vaccine inducing cell-mediated responses and were stopped for futility, the studies provided important data to the field and significantly influenced subsequent vaccine design strategies against HIV.

HVTN 505

HVTN 505 was a Phase IIb trial that utilized a heterologous DNA prime/Ad5 boost regimen aimed at reducing HIV viral loads as well as preventing HIV acquisition (345). Here, heterologous regimens refer to the use of two or more different vehicles for delivery of the antigens of interest such as DNA and Ad5. In contrast to Step trial, the HVTN 505 trial included HIV Env gp140 immunogens of multiple clades in addition to Gag, Pol, and Nef and the Ad5 vector used here differed from that used in the Step trial in that there were more deletions present in the Ad5 genome. The study was conducted in participants that were circumcised with low to no Ad5-specific antibodies. The multigene, multiclade DNA prime/Ad5 boost regimen was designed to induce HIV-specific, multifunctional $CD4^+$ and $CD8^+$ T cells and antibodies to envelopes of the major circulating strains. Vaccinations however were eventually halted for lack of efficacy when it was determined that the vaccine did not reduce the rate of HIV acquisition nor viral load set points compared to the placebo arm.

RV144 THAI TRIAL

In 2009, the RV144 trial performed in Thailand was the first phase III HIV vaccine trial to achieve a modest vaccine efficacy of 31.2% (50-60% within the first year of vaccination) and was the first human trial to demonstrate that it is possible to prevent HIV infection by vaccination (346, 347). This was achieved by utilizing a heterologous prime-boost regimen, combining recombinant canarypox vector (ALVAC) immunizations with the AIDSVAX B/E protein immunogen to induce both arms of the adaptive immune system. Volunteers received ALVAC immunizations at 0, 1, 3, and 6 months with AIDSVAX B/E additionally administered at months 3 and 6. Vaccinations however did not affect disease course as determined by viral setpoint or $CD4^+$ T cell count. Extensive analysis was performed to determine correlates of infection risk in participants in this study. Haynes *et al*. was able to determine that for vaccinated individuals, IgG antibodies specific to the variable regions 1 and 2 (V1V2) of HIV Envelope proteins inversely correlated with the rate of HIV infection while plasma Env-specific plasma IgA antibodies directly correlated with the rate of infection (348). The authors were able to show that neither low V1V2 antibodies nor high Env-specific IgA antibodies in vaccinated individuals were associated with higher rates of infection compared to the placebo group indicating that the vaccine-induced IgA titers did not confer added risk of infection relative to placebo. Though the IgA antibodies were not considered infection-enhancing antibodies, the antibodies abrogated the protective effects of vaccination e.g. IgG antibody avidity, antibody-dependent cellular cytotoxicity, neutralizing antibodies, and $CD4^+$ T cells, (348, 349). Thus, when Env-specific IgA levels were low, antibody-dependent cellular cytotoxicity and neutralizing antibody levels reduced the risk of infection (350, 351).

Though the trial using AIDSVAX B/E alone was able to induce high Envelope-specific IgG responses compared to those observed in RV144, Env- and V2-specific IgG3 subclass antibodies were significantly greater in RV144 participants (349, 352). IgG3 subclass antibodies have a higher affinity for the Fcγ receptor, can fix complement, and has been shown to associate with protection in other settings of infection (353, 354). The RV144-induced IgG3 antibodies showed multifunctional effector functions that

likely resulted from the addition of the ALVAC prime which contributed to the vaccine efficacy observed. These results provided a ray of hope to the HIV vaccine field and highlighted the contribution of anti-Env antibody responses as an important immune correlate of reduced risk of infection.

POXVIRUS

The RV144 trial highlighted the potential of poxvirus vectors as candidate HIV vaccines. The poxviridae family of viruses are linear double-stranded DNA viruses with covalently closed hairpin loops at each termini (355). Poxviruses are categorized into two subfamilies, *Choropoxvirinae* and *Entomopoxvirinae*, that are further subdivided into multiple genera. The *Orthopoxviruses* of the *Chordopoxvirinae* family encompass vaccinia, variola, cowpox, ectromelia, and monkeypox with vaccinia virus considered the prototypic member. Poxviruses are enveloped and are large for animal viruses with vaccinia virions measuring approximately 350 nm x 270 nm by cryoelectron microscopy. Poxvirus genomes can range from 135,000 to 289,000 base pairs in length leading to 136 to 260 open reading frames (356, 357). Among all poxviruses, genes located within the center of the genome are most heavily conserved, typically encoding for common viral machinery such as proteins for replication or virion assembly. Genes found at the terminal ends of the poxvirus genomes are more variable and involved in host range restrictions and immune modulation.

VIRIONS

Poxvirus virions contain a nucleoprotein core which contains the double stranded DNA genome and virally encoded enzymes and proteins that allow for transcription of an early set of genes as well as replication which occurs solely in the cytoplasm at sites referred to as factories (355). Two infectious forms of virions are typically produced: a mature virion (MV) or extracellular enveloped virion (EEV) (358). MVs have a single lipoprotein bilayer membrane and can remain in the cytoplasm of the cell until release by cell lysis (359-364). Some MVs can be further wrapped in a modified trans-Golgi or endosomal double membrane to be a triple-membrane virion and transported to the cell plasma membrane

where the outer membrane will fuse to exocytose an EEV which appears as an MV with an additional membrane layer (double membraned) (358, 365, 366).

VIRAL ENTRY

A receptor for viral entry for poxviruses that mediates binding and internalization has not been identified though viral binding sites which allow for viral attachment but not necessarily entry have been characterized. MV binding to cell surfaces are known to be mediated in part by four viral proteins: D8 binding to chondroitin (367), A27 and H3 binding to heparan (368-371), and A26 binding to laminin (372). Interestingly, inactivating any one of these attachment proteins does not prevent viral entry and these proteins have additional roles beyond cell binding. Eleven more proteins are utilized by poxviruses for membrane fusion and core entry and are conserved for all poxviruses (373). These proteins form an entry fusion complex embedded into the membrane of the mature virion and are all required for core entry. Upon entry, endocytosed MVs are trafficked to early endosomes and recycling endosome compartments prior to membrane fusion (374). Fusion is activated by low pH which serves to dissociate a fusion suppressor protein from the entry fusion complex (373).

POXVIRUS IMMUNE EVASION TACTICS

Common amongst most poxviruses is the presence of viral genes that target immune signaling including interferon and other chemokine signaling pathways (357). Viral modulation of the immune responses occurs by both preventing secreted molecules from binding to their respective receptors as well as by inhibiting intracellular signaling after receptor-ligand interactions. Conservation of these viral immune evasion genes that target host immune responses highlight the importance of these responses in clearance of the virus. Some of the extracellular and intracellular modulation of the immune response by these viral genes will be briefly reviewed here.

MODULATION OF COMPLEMENT

Complement is an important component of the innate immune response that upon activation leads to release of proinflammatory anaphylatoxins, generation of opsonins that target pathogens or cells for degradation, and formation of the membrane attack complex that serves to directly lyse targeted pathogens or damaged cells (375). Vaccinia is known to encode and secrete vaccinia complement-control protein (VCP) that inhibits both the classical and alternative complement activation pathways by binding and inhibiting C4b and C2b (376). Expression of VCP has been show to contribute to pathogenesis of vaccinia (377). Smallpox inhibitor of complement enzymes (SPICE) encoded by variola virus is even more potent than VCP at inactivating C3b and C4b (378). In cowpox, the inflammation modulatory protein (IMP) is a complement control protein with a role in limiting immune cell infiltration and reducing tissue destruction (379-381). Expression of complement control proteins by multiple poxvirus family members indicate the important role the complement pathway has in limiting pathogenesis.

INHIBITION OF INTERFERONS

Interferons (IFNs) are integral to combating pathogens as secretion of IFNs and signaling through their cognate receptors leads to an antiviral state among cells of the immune system rendering cells more capable of combating viruses, bacteria, and parasites. IFN receptor intracellular signaling is mediated by the Janus kinase and signal transducer and activator of transcription (JAK-STAT) pathway which leads to translation of interferon-stimulated genes (ISGs). Type I and type II IFNs have distinctive properties in regards to the ISGs induced as well as the antiviral activities they possess (382). IFNs act to control infections by targeting pathways and functions necessary to the life cycle of a pathogen, therefore poxvirus immune modulatory genes targeting IFNs are integral to viral pathogenesis.

Sequestering of secreted IFNs inhibits IFN binding to IFN receptors and abrogates activation of the immune system. B18R is a type I IFN binding protein encoded by the Western Reserve (WR) strain of vaccinia and is the best characterized of poxvirus type I IFN inhibitors. B18R competitively binds

IFNα and IFNβ thus preventing IFN receptor activation and signaling (383-385). A B18R deletion mutant of vaccinia led to viral attenuation in an intranasal mouse model highlighting the importance of type I IFN binding proteins in vaccinia pathogenesis (383). IFNγ, a type II IFN, is important in coordinating the antiviral innate and adaptive immune responses against poxviruses. Transgenic mice without the capability to produce IFNγ or express IFNγ receptor are more susceptible to infection indicating the role IFNγ has in providing protection (386-388). Poxviruses express IFNγ receptor homologs which are binding proteins with a high sequence homology to host IFNγ receptors. These homologs are competitive antagonists for host IFNγ and can have either species-specific efficacy or broad specificity (389-391). Deletion of an IFNγ receptor homolog B8R in vaccinia resulted in an attenuated virus in a rabbit model of infection (392).

Poxviruses have evolved to not only target IFN molecules specifically, but also the signaling pathways induced after viral infection. The vaccinia H1L gene is a phosphatase that acts to prevent activation of STAT1, a transcription factor important for type I and II IFN receptor signal transduction (393). Pattern recognition receptors such as protein kinase R (PKR) and 2',5'-oligoadenylate synthetase (OAS) sense viral RNA in the cytoplasm. PKR and OAS activation leads to significant reduction in host translation and synthesis of proteins often leading to apoptotic cell death (394). PKR signaling in addition leads to activation of the NF-κB signaling pathway (ref Schneider 2014). E3L encoded by vaccinia is a double-stranded RNA binding protein which acts to prevent activation of PKR and OAS (395, 396). E3L can also directly bind and inhibit PKR leading to less phosphorylation of eukaryotic initiation factor 2a (eIF2α) (397), IFN response factor 3 (IRF3) (398), and IRF7 (399). The K3L gene from vaccinia prevents PKR activity by acting as a nonphosphorylatable pseudosubstrate of PKR and thereby competitively inhibits phosphorylation of eIF2α to inhibit protein synthesis (395, 400, 401). An E3L deletion mutant of vaccinia was demonstrated to be sensitive to host IFN responses and showed a more limited host range as well as reduced pathogenicity (402-404). Interestingly, expression of both E3L and K3L in a canarypox vaccine

vector led to improved antigen expression and inhibition of apoptosis indicating that modifying the kinetics of cell death and sensitivity to IFNs could be beneficial for vaccine efforts (405).

INHIBITION OF INFLAMMATORY CYTOKINES

In addition to the role of antiviral IFNs, cytokines that lead to immune system activation and inflammation are targets of poxvirus immune modulatory genes. Interleukin-1β (IL-1β) of the IL-1 family of cytokines is a prominent pro-inflammatory cytokine and poxviruses have developed multiple approaches to target the IL-1β pathway. The pro-IL-1β precursor requires cleavage by caspase 1 to be converted into its mature form (406). Poxviruses have developed inhibitors to caspase 1 activity such as cytokine response modifier A (CrmA) by cowpox to prevent maturation of IL-1b and its secretion into the microenvironment (407). Vaccinia and cowpox viruses also encode B15R, a secreted decoy IL-1β receptor, to bind and sequester IL-1β thereby reducing inflammation and fever (408, 409). In an intranasal infection model in mice, B15R is reported to prevent the febrile response during infection and smallpox strains lacking this IL-1β receptor induced fever in mice (410).

IL-18 is pleiotropic cytokine of the IL-1 family that helps regulate T helper 1 (Th1) responses, activates natural killer (NK) cells, and possesses inflammatory activities similar to other members of the IL-1 family (406). Like IL-1β, IL-18 requires cleavage by caspase 1 to reach its mature, active form for secretion implicating that CrmA inhibition of caspase 1 not only targets IL-1β maturation but IL-18 as well. IL-18 is also targeted by IL-18 binding proteins that block its binding to the IL-18 receptor leading to a dampened Th1 response, inhibition of early induction of IFNγ, and reduced NK cell functionality (411). To target the signaling pathways of IL-1β and IL-18, the vaccinia A52R gene contains a Toll/IL-1 receptor (TLR) domain motif on its cytoplasmic tail that sequesters adapter molecules from IL-1 receptor, IL-18 receptor, and TLR4 signaling and thereby preventing NF-kB activation (412).

INHIBITION OF APOPTOSIS

Tumor necrosis factor alpha (TNFα) is another potent inflammatory cytokine that promotes an antiviral state as well as mediate apoptosis or cell death of infected cells. *Orthopoxvirus* TNF receptor (TNFR) homologs act to inhibit TNFα activity (413, 414). Cytokine response modifier (Crm) orthologs are among the best studied of TNFR homologs. Cowpox expresses CrmB (415), CrmC (416), CrmD (417), and CrmE (418) which vary in ligand specificity and expression patterns. These TNF decoy receptors act as a cell extrinsic anti-apoptotic factor to prevent infected cell death.

Poxviruses also have factors to prevent apoptosis in an intracellular manner. Apoptosis is a form of programmed cell death mediated by host caspases (cysteine proteases) with characteristic physiological features such as cell shrinking, nuclear condensation and fragmentation, membrane blebbing, and budding of apoptotic bodies (419). Inhibition of particular caspases by viral proteins can lead to inhibition of apoptosis. Viral FLICE/caspase 8 inhibitory proteins (vFLIPs) encoded by molluscum contagiosum virus, MC159 and MC160, contain a death effector domain (DED) motif and bind to the FADD motif and pro-caspase 8 to inhibit autocleavage and activation of the apoptotic signaling pathway (420-422). CrmA by cowpox as mentioned above is an inhibitor of caspase 1 and disruption of CrmA leads to a modest attenuation of disease in mice (423). CrmA has also been demonstrated to inhibit apoptosis induced by NK cell or cytotoxic T lymphocyte-derived granzyme B (424-426). The SPI-2 family of serine protease inhibitors (serpins) like B13R in vaccinia is able to protect infected cells from Fas and TNF receptor-mediated apoptosis but is generally less potent than CrmA (427, 428). Knockout of B13R in vaccinia does not noticeably attenuate the virus (428, 429). The myxoma virus gene M11L targets the mitochondria and prevents the activation of the intrinsic apoptotic pathway which would result from the loss of inner mitochondrial membrane potential (430). Impeding apoptosis of an infected cell is important for poxviruses which is highlighted by the variety of ways poxviruses modulate this pathway.

MODIFIED VACCINIA ANKARA, MVA

Poxviruses, specifically vaccinia virus, have been utilized as expression vectors for foreign DNA for over 30 years now (431-434). One of the major advantages of poxviruses for vaccine development is that they can stably accommodate at least 25 kb of foreign DNA without loss of infectivity, allowing for insertion of large genes or an array of genes (435). In addition, preexisting immunity to vaccinia in the population is low since its discontinued use in the smallpox vaccination campaign that ended in 1980. Unfortunately, the use of live virus as a vaccine for smallpox possessed some safety risks with 1–2 deaths and hundreds of cases with complications severe enough to require hospitalization for every million vaccine recipients (436). As a result, development of next generation poxvirus vectors sought an increased safety profile leading to the development of modified vaccinia Ankara (MVA).

DEVELOPMENT OF MVA

MVA is derived from the parent vaccinia virus Ankara strain that was originally propagated on the skin of calves and donkeys at the Turkish Vaccine Institute in Ankara for smallpox vaccine production (437). In 1953, the team of Herrlich and Mayr cultivated the virus in Munich on chorioallantois membranes of embryonated chicken eggs which led to the development of chorioallantois vaccinia virus Ankara (CVA) (438). CVA was grown on calf skin for manufacturing of the smallpox vaccine used in the Munich vaccination campaigns of 1954-5. Anton Mayr continued the serial passaging of CVA in chicken embryo fibroblasts and in 1968, after the 516th passage, renamed the virus MVA (439).

The MVA genome has six major deletions relative to the parental CVA strain. Deletions sites I, V, and II are in the left terminal region, VI and III in the central conserved region, and site IV in the right terminal region (440). Additionally, mutations were observed to affect 124 open reading frames (441). This is a loss of approximately 12% of its genome with the deletions and mutations. Many of the genes impacted by this attenuation are important regulators of vaccinia-host interactions resulting in part in severe host range restrictions. The deletions in host-range genes of MVA resulted in MVA being replication-

incompetent in human and other mammalian cells. MVA is unable to disseminate in the host and cause pathology, even in immune-compromised hosts.

In addition, MVA demonstrated a reduction in virulence when compared to its parental vaccinia strains. *In vitro*, MVA did not cause prominent cytopathic effects or form plaques in chicken embryo fibroblast or HeLa cells (437). Intradermal or cutaneous infection via scarification with vaccinia typically leads to skin lesions but this was significantly absent with MVA inoculations. Furthermore, intracranial inoculations of MVA into macaques did not lead to obvious adverse effects while macaques receiving CVA developed severe systemic disease. With regards to vaccine immunogenicity, macaques intradermally or intramuscularly vaccinated with MVA were protected from disease after challenge with vaccinia indicating the efficacy of MVA as a vaccine candidate despite its reduced virulence. The first licensed MVA vaccine was approved in Germany in 1977 as a preventative vaccine to smallpox. Until 1980, the vaccine was administered to over 120,000 individuals without development of severe adverse effects that would not have otherwise been observed with conventional vaccinia-based vaccines (442).

VACCINE VECTOR

To further development of MVA for use as a vaccine candidate against other diseases and pathogens, research towards genome mapping and sequencing began using the MVA isolate from the 572nd *in vitro* passage (440, 443). This work also led to the discovery that MVA did not express many virulence factors such as important immunomodulatory genes encoded by vaccinia (443). Sutter *et al*. was one of the first to demonstrate that heterologous gene expression by MVA could occur unperturbed (444). Despite initial concerns regarding the ability of MVA to express recombinant genes at high levels, this work demonstrated that the β-galactosidase and guanine-phosphoribosyl-transferase genes inserted into the MVA deletion III site did not impair early and late viral gene expression in infected human cells. The replication defect in MVA occurs at the late stage of virion assembly therefore allowing for uncompromised late gene and immunogen expression (438). Further work in development of recombinant

MVA vectors utilized the existing major deletion sites in MVA in an attempt to avoid unnecessary changes to the MVA genome or its phenotype. The thymidine kinase locus however had been previously heavily utilized as an insertion site in vaccinia virus vectors and also proved to be a successful insertion site for MVA immunogens (445, 446).

Genetic instability of the inserted recombinant gene, such as HIV Env, in MVA has been reported to lead to truncation of the gene or exertion of negative selection pressure on viral growth thereby favoring growth of rare mutants with reduced insert expression (447-449). In generation of a novel recombinant MVA, it is likely advisable to confirm the stability of the inserted recombinant gene over ten serial passages in the relevant cell line before the large-scale manufacturing process. The lab of B. Moss showed frame shift mutations due to consecutive C or G bases and large genetic deletions flanking the insertion site (the deletion III locus of MVA) of the recombinant gene contribute to the genetic instability in MVA (450). These effects can be mitigated through introduction of silent mutations and insertion of the recombinant gene into a site between essential, conserved MVA genes leading to enhanced stability of the insert.

A unique aspect of MVA among poxviruses that likely contributes to its immunogenicity despite high attenuation is the ability to induce type I interferon responses in mice and bone marrow-derived plasmacytoid dendritic cells (451). This is due to the loss of viral proteins that function to antagonize the host anti-viral interferon response. Additionally, MVA is capable of causing activation of the NF-kB pathway via PKR which makes it distinct amongst other vaccinia virus strains (452). Despite the loss in host-range MVA experienced due to its genetic attenuation, the simultaneous loss of genes that suppress the host anti-viral response significantly improved its immunogenicity as a viral vaccine vector. Research described in the following section demonstrate an important strategy in attempts to further improve MVA-based vaccine-induced responses with targeted disruption of immunomodulatory genes that remain functional in the MVA genome (Figure 1).

MVA has proven to be effective at inducing strong $CD4^+$ and moderate $CD8^+$ T cell responses and durable antibody responses. Vaccine regimens utilizing MVA have undergone numerous preclinical and phase I/II clinical trials for infectious diseases including HIV, Ebola, and tuberculosis as well as for a variety of cancers (Table 1) (453-466). These studies have demonstrated that MVA is capable of inducing antigen-specific cellular and humoral responses in participants. Additionally, the safety profile and responses generated led MVA to be a leading candidate as a third-generation smallpox vaccine (467). However, the immunogenicity of MVA is limited in a homologous prime/boost approach due to the induction of strong anti-MVA immunity that will limit transgene expression. To avoid the problem of anti-vector immunity, a number of studies used MVA as a booster immunization in conjunction with multiple vectors (heterologous prime/boost approach) and observed a profound boost of both cellular and humoral immune responses. These priming vectors include DNA, protein, bacteria, adenoviruses, or other poxviruses. Additionally, there still remains the need to induce better responses from MVA immunizations as not all participants induce antigen-specific immune responses even after multiple immunizations (456, 462, 464). A vaccine capable of eliciting a more robust response has the potential to be used as a prophylactic vaccine preventing infections or as a therapeutic one to help against disease progression.

GENETIC MODIFICATION OF MVA

Poxviruses express a wide variety of immune modulatory genes such as cytokine binding proteins and inhibitors of interferon activity as described above. Though MVA has lost a large portion of its genome resulting in lost in host-range and immunomodulatory genes, many of these genes remain functional. The directed disruption of these genes that antagonize host-specific immune responses aims to enhance the virally stimulated, pro-inflammatory conditions required for a robust adaptive response (468-474).

This strategy includes deletion of the anti-apoptotic F1L gene in MVA in order to increase the rate of apoptosis induction via the intrinsic pathway (468). F1L in the vaccinia virus Copenhagen strain encodes

a protein that localizes to the outer mitochondrial membrane where it inhibits the inner mitochondrial membrane potential and release of cytochrome c thereby inhibiting mitochondrial-associated apoptosis induction. The F1 protein has also been shown to interact with the pro-apoptotic Bak protein which prevents this activation pathway of apoptosis. F1 additionally interacts with Bim which indirectly inhibits the pro-apoptotic Bax activity. F1 orthologs in in orthopoxviruses are considered to be the only Bcl-2-like protein to directly inhibit the Bak/Bax checkpoint. Separately, F1 has been shown to bind and thereby inhibit caspase 9 activity which would lead to the induction of the intrinsic apoptotic pathway so F1 has the ability to inhibit apoptosis at different points of the cell death pathway. Using an MVA-ΔF1L-deletion mutant, the authors demonstrated enhancement in apoptosis induction and induction of type I interferon and pro-inflammatory cytokine gene expression of infected cells. The immunogenicity of this deletion mutant expressing HIV antigens, with a DNA priming immunization, was tested in BALB/c mice and revealed high, broad, and poly-functional HIV-specific CD8+ T cell responses with better duration of responses. The levels of vaccine-induced gp120-specific antibodies however were not improved.

Vaccinia C6 protein is expressed early during infection and based on bioinformatics analyses, believed to possibly possess the ability to inhibit the Toll-like receptor signaling pathway (469). An epitope of C6 also has proved to be highly immunogenic in BALB/c mice. Directed deletion of the viral C6L gene in MVA led to the up-regulation of IFNβ and interferon stimulatory genes implicating its role in inhibiting the IFNβ signaling pathway in some manner. In a DNA prime, MVA boost immunization regimen, the C6L deletion mutant significantly improved the magnitude and polyfunctionality of HIV-specific CD4+ and CD8+ T cell memory responses. The mutant also generated enhanced Env-specific antibody titers. Falivene *et al.* targeted the IL-18 binding protein gene C12L in MVA for deletion (470). The IL-18 binding protein as described above is a soluble inhibitor that binds to and neutralizes host-derived IL-18. IL-18 is a pro-inflammatory cytokine with important roles in regulating both the innate and adaptive immune response. IL-18 is also a key mediator in Th1 responses as well as enhances T and NK cell

cytokine production and cytotoxicity. MVA with a deletion of the C12L gene demonstrated enhanced immunogenicity with regards to higher magnitude of IFNγ, IL-2, and cytotoxic potential for CD8+ T cells. A DNA prime, MVA boost regimen in mice showed enhanced frequencies of HIV-specific IFNγ responses as well indicating that removal of C12L from the MVA backbone is an effective approach towards improving MVA immunogenicity. Vaccinia virus N2L is part of the vaccinia Bcl-2 family of genes which are intracellular proteins important for inhibiting the TLR signaling pathway at various points (471). N2L is a virulence factor transcribed early during infection that locates to the nucleus to inhibit the activation of IRF3 in some manner. MVA with a deletion of the N2L gene resulted in increased IFNβ and proinflammatory cytokine and chemokine expression. In mice, this vector showed improved magnitude and polyfunctionality of HIV-specific $CD4^+$ and $CD8^+$ T cells and enhanced Env-specific antibodies after the peak of response.

Disruption of an immunosuppressive gene was also demonstrated with deletion of 146R gene from MVA which is termed A35 in the vaccinia virus Copenhagen strain(472). A35 is conserved across most mammalian-tropic poxviruses such as MVA and is an important virulence factor. A35 in the Western Reserve strain impedes MHC class II-restricted antigen presentation and significantly reduces induction of virus-specific antibodies and cytotoxic T cells. Deletion of the A35 gene in MVA increased vaccinia virus-specific immunoglobulin production and class switching to IgG isotypes after a single immunization. The deletion mutant also enhanced the level of virus-specific IFNγ-secreting cells from splenocytes of immunized mice supporting the deletion of A35 from the MVA backbone to improve immunogenicity of vaccine-based responses.

With as many studies performed regarding how disruption of a single MVA gene may improve vaccine-generated responses, some groups went a step further and performed directed deletions of two or more immunomodulatory genes to determine potential synergistic effects on immunogenicity. Garcia-Arriaza *et*

al. performed deletions of two such genes in MVA: A41L and B16R (473). B16R is a viral IL-1B binding protein and A41L is a secreted protein that binds CC-chemokines (CCL21, CCL25, CCL26, CCL28) and sequesters them from performing their activities. These CC-chemokines play a role in the development of T cell responses such as by priming T cells or inducing Th1 cells. Using a DNA prime/MVA boost protocol in mice, the authors saw significant improvement in HIV-specific CD4+ and CD8+ T cell responses in the double MVA mutant vector though antibody responses against HIV Env did not differ between the mutant and wild-type vectors.

Another group developed an MVA vector that possessed four simultaneous deletions of immune-modulatory genes. Garber *et al*. deleted a secreted IL-18 binding protein (MVA008L), a soluble IL-1β receptor (MVA184R), a CC-chemokine binding protein (MVA153L), and a dominant negative Toll/IL-1 signaling adapter (MVA159R) (474). Previous work had been shown that MVA vectors lacking MVA184R and/or MVA153L generated improved CD8+ T cell responses in mice. This study also included a mutant with a deletion in the udg gene which is an essential viral replication gene whose deletion would abrogate late gene expression in order to lower the MVA antigen profile that could compete with the recombinant immunogen of interest. The MVA vectors with either four or five simultaneous deletions elicited a 6-fold increase in HIV-specific cellular responses and 25-fold higher titers of HIV Env gp120-specific antibodies compared to the parental vaccine strain in a prime/boost model in rhesus macaques.

Targeted modifications of the MVA genome has been shown to significantly influence the innate as well as adaptive responses in the host and provides a potential way forward for further enhancement of MVA immunogenicity. Despite the significant genomic deletions and mutations of the MVA genome, there still remain genes that contribute to suppression in host inflammatory responses and signaling (Figure 1). Disruption of these genes singularly or in combination with other viral genes has the potential to provide a synergistic improvement on vaccine-induced responses. Additionally, disruption of genes that affect

host tropism has the potential to improve responses. Host range genes interfere with intracellular host defenses such as through viral sensing, signal transduction, the cell cycle, or the induction of apoptosis. Disruption of these genes may augment the anti-viral signaling pathways resulting in enhanced immunogen-specific responses.

THESIS OVERVIEW

Development of an effective HIV vaccine and steps towards a functional cure have made great progress in the past decade (Figure 2). Use of MVA as a viral vaccine vector has been shown to be capable of inducing antigen-specific humoral and cellular responses in pre-clinical and clinical studies though there still remains a need to generate greater response as some individuals do not induce these responses even after multiple immunizations. In this dissertation, we provide evidence that delaying apoptosis induction during MVA vaccination improves antigen-specific humoral responses (Chapter II). The greater understanding of HIV pathogenesis and the latent reservoir that serves as a persistent source of virus has informed the development of regimens that aim to both reverse latency of the viral reservoir as well as induce immune-mediated clearance of HIV infected cells. Here, we investigated the effect of PD-1 blockade administered in conjunction with ART treatment on reinvigoration of exhausted T cells during infection as well as the effect on reducing the latent viral reservoir (Chapter III). The findings described in this dissertation illustrate further progress in emerging preventative and therapeutic treatments against HIV and will inform future efforts in their development.

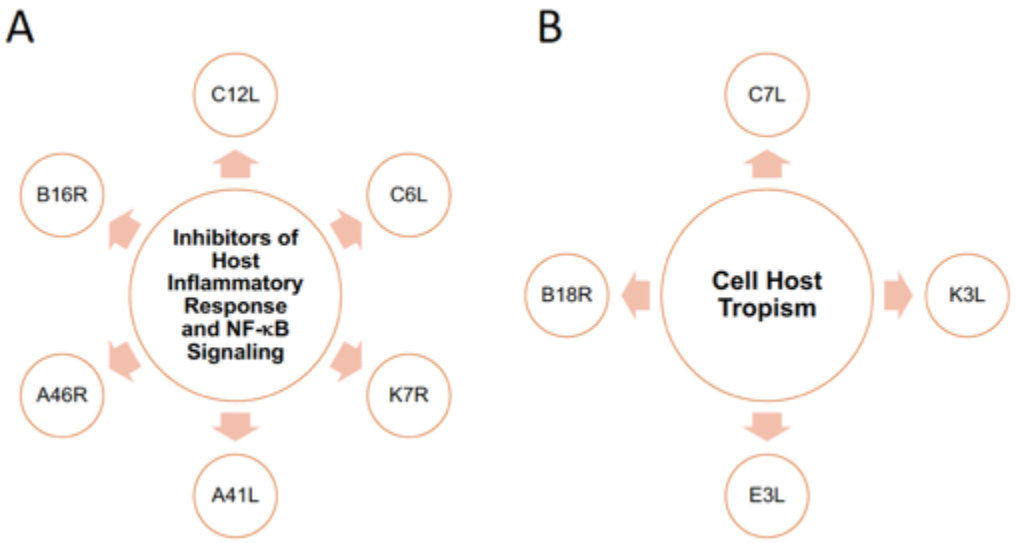


Figure 1. Potential Target Genes of the MVA Genome to Improve Immunogenicity. A) Viral inhibitor genes in MVA that act to inhibit the host inflammatory response and NF-kB signaling cascade. Disruption of these immunosuppressive genes could enhance the anti-viral immune response and improve the adaptive response. B) Deletion of host range genes has the potential to abrogate MVA interference of the anti-viral response.

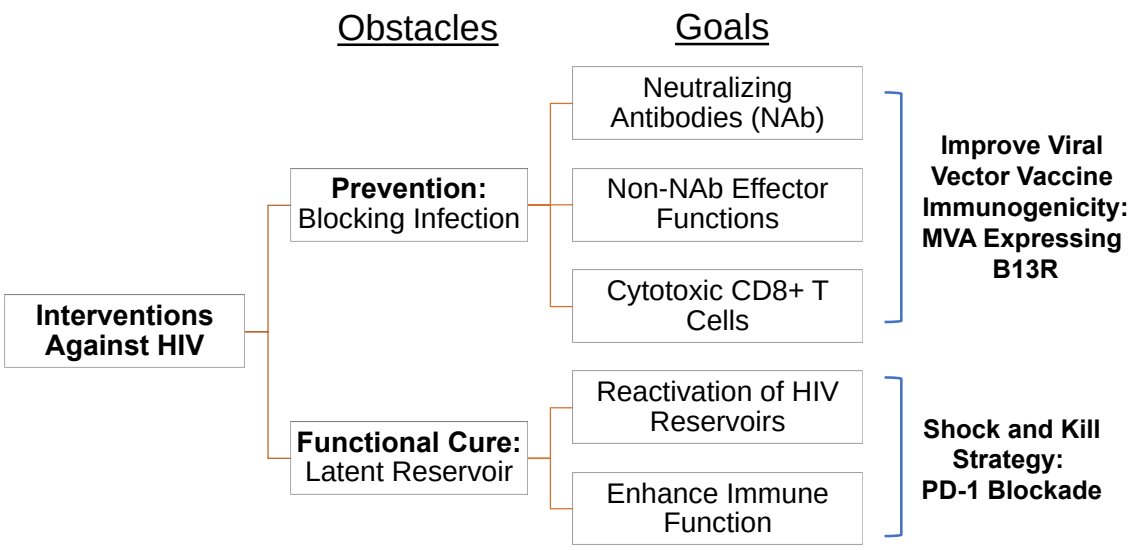


Figure 2. Overview of Dissertation Aims. HIV intervention approaches include development of a preventative HIV vaccine and an HIV functional cure. Developing a vaccine to block HIV infection will require induction of neutralizing antibodies (NAbs), non-NAbs, and cytotoxic CD8+ T cells. The functionality, magnitude, and duration of these responses will be influenced by the vaccine vector utilized. We continue the improvement of the immunogenicity of the viral vector Modified vaccinia Ankara (MVA) as an HIV candidate vaccine by construction of MVA expressing an anti-apoptotic gene B13R. As a functional cure strategy, the shock and kill immune-based approach aims to reactivate the latent HIV reservoir and simultaneously develop enhanced immune responses to be able to detect and eliminate the reservoir. We aim to test this approach using PD-1 immune checkpoint blockade therapy.

Pathogen	Antigen	Reference
HIV/AIDS	2 mosaic HIV-1 Gag/Pol/Env inserts	(475)
	HIV-1 clade A Gag sequences and a string of CD8 and T-cell epitopes	(454)
	HIV-1 Gag, PR, RT, and Env (clade B)	(456)
	HIV-1 Env, Gag, Tat-Rev and Nef-RT (clade C)	(476)
	HIV-1 Env (clade E), Gag-Pol (clade A)	(477)
	HIV-1 Env, Gag-Pol, Nef-Tat (clades B/C)	(478)
	HIV-1 Env/Gag, Tat/Rev/Nef-RT (clade B)	(479)
	T cell epitopes from HIV-1 Gag, Pol, Vpr, Nef, Rev and Env	(480)
Influenza A	NP + M1	(481)
	H5N1 HA	(463)
Hepatitis B	HBs	
Hepatitis C	NS3, NS4 and NS5B (genotype 1b)	(482)
Tuberculosis	Antigen 85A	(466)
Malaria	ME-TRAP	(483)
	AMA1	(484)
	MSP1	(485)
	CS	(486)
CMV	UL83 (pp65), UL123 (IE1-exon4), and UL122 (IE2-exon5)	(458)
Epithelial Tumors	Transcription Factor Brachyury, B7.1, ICAM-1, and LFA-3,	(461)
EBV+ Cancer	EBNA1 and LMP2	(462)
Gastrointestinal Cancers	p53	(464)

Table 1. Clinical Trials Using MVA-based Vector as a Vaccine Candidate.

CHAPTER II

Novel MVA Vector Expressing Anti-apoptotic Gene B13R Delays Apoptosis and Enhances Humoral Responses

Lynette S. Chea[1,2], Linda S. Wyatt[3], Sailaja Gangadhara[1,2], Bernard Moss[3], and Rama R. Amara[1,2]

[1]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA, USA; [2]Division of Microbiology and Immunology, Emory Vaccine Center, Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA; [3]Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.

*Currently in press at *Journal of Virology*.

Chea *et al*. Novel MVA Vector Expressing Anti-apoptotic Gene B13R Delays Apoptosis and Enhances Humoral Responses. *J. Virol*. 2018; Epub ahead of print.

DOI: 10.1128/JVI.01648-18

Available at: https://jvi.asm.org/content/early/2018/12/06/JVI.01648-18

Abstract

Modified vaccinia Ankara (MVA), an attenuated poxvirus, has been developed as a potential vaccine vector for use against cancer and multiple infectious diseases including HIV. MVA is highly immunogenic and elicits strong cellular and humoral responses in pre-clinical models and humans. However, there is potential to further enhance the immunogenicity of MVA as MVA-infected cells undergo rapid apoptosis leading to faster clearance of recombinant antigens and potentially blunting a greater response. Here, we generated MVA-B13R by replacing the fragmented 181R/182R genes of MVA with a functional anti-apoptotic gene B13R and confirmed its anti-apoptotic function against chemically induced apoptosis *in vitro*. In addition, MVA-B13R showed a significant delay in induction of apoptosis in muscle cells derived from mice and humans as well as pDCs and CD141+ DCs from rhesus macaques compared to MVA infected cells. MVA-B13R expressing SIV Gag, Pol, and HIV envelope (SHIV) produced higher levels of envelope in the supernatants compared to MVA/SHIV infected DF-1 cells *in vitro*. Immunization of BALB/c mice showed induction of higher Envelope-specific antibody secreting cells, IgG antibody titers, memory B cells, and better persistence of antibody titers by MVA-B13R/SHIV compared to MVA/SHIV. Gene set enrichment analysis of draining lymph nodes cells from day 1 after immunization showed a negative enrichment for interferon responses in MVA-B13R/SHIV mice compared to MVA/SHIV mice. Taken together, these results demonstrate that restoring B13R functionality in MVA significantly delays MVA-induced apoptosis in muscle and antigen-presenting cells *in vitro* and augments vaccine-induced humoral immunity in mice.

Importance

MVA is an attractive viral vector for vaccine development due to its safety and immunogenicity in multiple species and humans even under conditions of immunodeficiency. Here, to further improve the immunogenicity of MVA, we developed a novel vector MVA-B13R by replacing the fragmented anti-apoptotic genes 181R/182R with a functional version derived from vaccinia virus, B13R. Our results

show that MVA-B13R significantly delays apoptosis in antigen-presenting cells and muscle cells *in vitro* and augments vaccine-induced humoral immunity in mice leading to the development of a novel vector for vaccine development against infectious diseases and cancer.

Introduction

The goals of an effective vaccine are to induce robust and durable humoral and cellular immunity to prevent and control pathogenic infection. To date, attenuated poxviruses such as ALVAC, NYVAC, and modified vaccinia Ankara (MVA) have been explored as vaccine vectors for use against multiple infectious diseases and even cancers. MVA is a highly attenuated derivative of vaccinia virus with an excellent safety record as well as proven sustained immunogenicity in humans and most mammals despite being replication-incompetent (437). The genetic attenuation achieved by MVA was due to over 570 passages of vaccinia in chicken embryo fibroblast cells that led to approximately 12% of its genome being deleted or fragmented, many of which were host-range and host immune-modulatory genes. For use as a vector, the MVA genome can stably accommodate recombinant genes as large as 25 kb and due to the defect at the late stage of virion assembly, late gene and recombinant gene expression can occur undeterred (435, 444).

These characteristics make MVA a promising vector for use in vaccinations. Vaccine regimens utilizing MVA have undergone numerous preclinical and phase I/II clinical trials for infectious diseases including HIV, Ebola, and tuberculosis as well as for a variety of cancers (453-466). These studies have demonstrated that MVA is capable of inducing antigen-specific cellular and humoral responses in participants. However, there still remains the need to induce better responses from MVA immunizations as not all participants induced antigen-specific immune responses even after multiple immunizations (456, 462, 464). A vaccine capable of eliciting a more robust response has the potential to be used as a prophylactic vaccine for preventing infections or as a therapeutic one to help against disease progression.

Improving the immunogenicity of MVA by modulating the viral genome has been used by many groups, namely through the deletion of immunomodulatory genes (468-471, 473, 474, 487). This strategy includes deletion of the anti-apoptotic F1L gene in MVA in order to increase the rate of apoptosis induction via the intrinsic pathway (468). Apoptosis is a form of programmed cell death mediated by

host caspase enzymes with characteristic physiological features such as cell shrinking, nuclear condensation and fragmentation, membrane blebbing, and budding of apoptotic bodies (488, 489). The immunogenicity of apoptotic cells may stem from prolonged storage of the cell-associated antigens within dendritic cells (DCs) that capture and present the antigens (490). The persistence of antigen by apoptotic cells, and thus its immunogenicity, could in part be attributed to its targeted delivery to recycling endosomes and ability to recruit and stimulate immune cells.

While it is clear that apoptotic cells are immunogenic, the kinetics of their generation may play an important role in improving antigen-specific responses. Using a canarypox vector for HIV vaccination, Fang *et al.* expressed two vaccinia genes that reduced the level of apoptosis, leading to enhanced HIV-1 pseudovirion production *in vitro* (405). Additionally, an *in vivo* study using DNA-delivered mutated caspase 2 or 3 showed delayed apoptosis induction and increased immunogen expression before the generation of apoptotic bodies (491). The mutated caspases performed similarly to an adjuvant as the authors observed enhanced CD4+ and CD8+ T cell responses compared to delivery of wild-type caspases. Modifying the MVA vector to delay the induction of apoptosis during vaccination has the potential to improve antigen-specific immune responses by allowing for more immunogen production before apoptosis and enhancing the ability of DCs to generate memory B and T cells.

Here, we aimed to enhance the immunogenicity of the MVA vector by delaying apoptosis induction through the expression of a vaccinia virus derived anti-apoptotic gene. B13R is a gene in the Western Reserve (WR) strain of vaccinia that has been shown to protect against apoptosis via the extrinsic pathway in cells infected with vaccinia virus (427). The gene product, serpin-2 (SPI-2), is expressed early during infection and is related to the serine protease inhibitor family of genes (428, 492). SPI-2 and its ortholog in cowpox, cytokine response modifier A (CrmA), are the best studied of the poxvirus serpins. CrmA inhibits IL-1β converting enzyme (caspase 1) and prevents apoptosis (428). It has also been shown to block apoptosis initiated by the Fas and TNF receptors by potently inhibiting caspase 8-mediated

apoptosis (493). Additionally, CrmA has the ability to abrogate the effects of granzyme B released from cytotoxic T lymphocytes (CTLs) *in vitro* (424). CrmA thus has the ability to prevent apoptosis initiated by the extrinsic and CTL-mediated pathways. The ortholog of WR B13R in MVA however is fragmented (181R/182R) and rendered nonfunctional (440). In this study, we have replaced the fragmented genes in MVA with a functional copy of B13R from the WR strain to delay the onset of apoptosis, increase antigen load and persistence, as well as improve the adaptive immune response. Our data show that MVA expressing the full-length B13R, MVA-B13R, is capable of delaying apoptosis of infected cells compared to MVA and this leads to enhanced humoral responses in mice following vaccination.

Results

MVA induced cell death is mediated by caspases

To determine the degree of apoptosis induced by MVA, we infected Hela cells at an MOI of 3 and measured caspase 3 activation in MVA-infected cells by staining for the early vaccinia virus E3 protein to assess MVA infection (MVA+ cells) and for active caspase 3 (Fig. 1A). We used active caspase 3 to detect cells undergoing apoptosis as it is a main downstream effector caspase in the apoptosis cascade that is activated upon cleavage from its inactive pro-caspase 3 form by initiator caspases 8 or 9. In addition, we stained cells with an amine reactive dye, Live/Dead near-IR dead cell stain, to detect membrane permeability which denotes cells undergoing later stages of apoptosis (also active caspase 3+) or necrosis (active caspase 3-). Through 88 hours after infection, MVA infection significantly increased caspase 3 activation indicating caspase-dependent cell death induced by MVA (Fig. 1B). To further confirm the role of cellular caspases during MVA induced cell death, we added inhibitors specific for individual caspases or for all caspases during MVA infection and determined caspase 3 activation levels at 50 hours post infection. Inhibition by a pan-caspase inhibitor as well as individual caspases such as 3, 4, 6, and 8 led to reduced activation of caspase 3 during MVA infection compared to the vehicle treated MVA infected cells indicating a role for multiple caspases in MVA-induced cell death (Fig. 1C).

MVA induced apoptosis is delayed by B13R expression

After confirming the role of caspases in MVA-mediated cell death, we expressed the B13R gene derived from WR vaccinia to determine if B13R expression during MVA infection would lead to a delay in cell death (Fig. 1D). The MVA genes 181R/182R in MVA comprise the non-functional disrupted orthologs of the WR B13R gene and we initially removed them via homologous recombination with a GFP-encoding PCR product to generate MVA-GFP□181R/182R. The GFP gene was then replaced with the full-length WR B13R gene generating MVA-B13R. A Western blot of MVA-B13R infected cell lysates showed a band of expected size of 38.5 kDa for MVA-B13R confirming its expression (Fig. 1E). WR vaccinia infected cells served as the positive control and the absence of a band in MVA and mock infected DF-1

cells (cell line permissive to MVA replication) confirmed B13R expression by our novel construct, MVA-B13R.

To confirm functionality of the B13R gene, we infected Hela cells with MVA-B13R and subsequently treated the infected cells with TNFα and cycloheximide to chemically induce apoptosis. As expected, TNFα and cycloheximide treatment induced strong caspase 3 activation in cells as early as 24 hours. However, infection with MVA-B13R significantly reduced caspase 3 activation by as much as 30% at 24 hours post-treatment (Fig. 1F). These data indicate that B13R expression is capable of delaying activation of the apoptotic process. To address whether expression of B13R would lead to enhanced replication of the virus, we determined the growth curves of MVA or MVA-B13R by infecting DF-1 cells at an MOI of 0.05 and viral titers were measured from cell lysates. The growth curves for the viruses did not differ over time indicating that B13R expression did not affect the replication and spread of MVA in DF-1 cells (Fig. 1G). Additionally, EM imaging of infected cells showed no structural differences in budding, mature virions produced by the two viruses at 24 hours after infection (Fig. 1H). Thus, B13R expression during the course of MVA infection is able to delay cell death induction without interfering with the growth rate or structure of the virus.

MVA-B13R delays death of infected cells compared to MVA

After confirming that B13R expression is capable of delaying chemically induced cell death, we compared the kinetics of cell death between MVA-B13R and MVA. MVA infection resulted in strong activation of caspase 3 in Hela, RD, and C2C12 cell lines. Kinetic analysis of Hela cells infected at MOI of 2 demonstrated significant delay of caspase 3 activation by MVA-B13R compared to MVA beginning at 36 hours after infection (Fig. 2A). Interestingly, MVA-B13R infection significantly increased the frequency of necrotic cells e.g. cells undergoing non-apoptotic cell death (Fig. 2A). We performed the same analysis in RD cells, a human muscle cell line derived from a patient's rhabdomyosarcoma, as

immunizations are typically administered through the intramuscular route. At an MOI of 3 for infections, we again saw a delay in caspase 3 activation by MVA-B13R in the RD cells (Fig. 2B). Additionally, as we aimed to perform immunogenicity studies in a mouse model, we used a mouse myoblast cell line, C2C12, to test the effect of B13R expression during MVA infection. The MOI used for the C2C12 cell line was lowered to 0.3 to achieve approximately 50% infection as greater amounts of virus caused rapid cell death for both virus strains. The reduced level of infectivity allowed us to detect lowered caspase 3 activation as well as increased cell necrosis over time for MVA-B13R compared to MVA, similar to what was observed during infection in Hela cells (Fig. 2C).

As the next ideal preclinical model, we hope to test the immunogenicity of MVA-B13R in is rhesus macaques, we assessed how MVA-B13R would influence viability of immune cells from rhesus macaques. Peripheral blood mononuclear cells (PBMCs) from naïve rhesus macaques were infected *ex vivo* with MVA or MVA-B13R at MOI 3 and stained to assess MVA infection and viability among various cell subsets by flow cytometry (Fig. 2D-G). We first determined the level of MVA infectivity of different immune cell subsets (Fig. 2F). Plasmacytoid DCs (pDCs, Lin- HLA-DR+ CD11c- CD123+), followed by CD141 (BDCA-3)+ DCs (Lin- HLA-DR+ CD11c+ CD141+ CD1c-) and CD1c (BDCA-1)+ DCs (Lin- HLA-DR+ CD11c+ CD1c+ CD141-) were among the more highly infected subsets with 50-95% infectivity. B cells (HLA-DR+ CD20+) as well as CD16+ CD14- (Lin- HLA-DR+) cells were moderately infected, ranging from about 15-35% infectivity. Both CD8+ and CD4+ T cells remained lowly infected with a range of 0.5-10% infection.

Given the important role antigen presenting cells (APCs) play in instigating immune responses and the higher level of infectivity observed in these subsets, we focused on the effect of MVA infection on DCs. pDCs play an important role for anti-viral responses through rapid release of type I interferons (IFNs) upon activation and antigen presentation capabilities through MHC class I and II (494, 495). Here, we observed a decrease in caspase 3 activation for MVA-B13R infected pDCs compared to MVA infected

cells (Fig. 2G). CD141+ DCs highly express type I IFNs, IL-12p70, and TLR3, have the capacity to induce T helper 1 cell responses, and can cross-present efficiently to cytotoxic T cells (496-499). Here, CD141+ DCs that were infected with MVA exhibited a higher frequency of caspase 3 activation compared to MVA-B13R infected cells from the same animal indicating better viability of these cells after MVA-B13R infection, similar to that observed in pDCs (Fig. 2G). As B cells are APCs as well as the source of antigen-specific antibodies, we assessed B cell viability after MVA infection and saw a trend for improved viability after infection with MVA-B13R (Fig. 2G). The remaining APC and T cell subsets did not demonstrate differences in viability after MVA-B13R infection relative to MVA (Fig. 2G). Together, these data indicate the possibility that MVA-B13R based vaccinations *in vivo* can significantly influence the viability of APCs which would substantially shape the adaptive immune response.

Generation of MVA/SHIV and MVA-B13R/SHIV

In our goal to generate a viral vector for immunogenicity studies, we generated recombinant MVA and MVA-B13R viruses that express SIV Gag and Pol, and HIV Envelope (Env) antigens (Fig. 3A). For both viral strains, we inserted the SIVmac239-derived Gag and Pol genes into the deletion III site and the clade C HIV Env 1086C gene between essential genes of the viral genomes resulting in viruses expressing SHIV antigens. We confirmed dual expression of SIV Gag and HIV Env by infecting DF-1 cells and staining with antibodies specific to these proteins for flow cytometry (Fig. 3B). Greater than 85% of the infected cells expressed Gag and Env, and about 90% of the Gag+ cells co-expressed Env (Fig. 3B). In addition, we performed Western blot analysis of infected cell lysates to confirm the sizes of the expressed proteins which revealed the presence of Gag and Env proteins in cell lysates at the expected sizes (Fig. 3C). To test if there was a difference in the level of protein expression between the two recombinant vectors over time, we performed a kinetic analysis over 96 hours after infection for Env expression in cell lysates as well as in the supernatants as these constructs are designed to make SHIV virus-like particles (VLPs). Interestingly, Env production through the course of infection appeared to be higher in the MVA-

B13R/SHIV infected cell lysates and in particular in the supernatant compared to MVA/SHIV (Fig. 3D). Using infected DF-1 cells from the same time points, we determined that the level of caspase 3 activation in the MVA-B13R/SHIV infected cells was significantly lower compared to MVA/SHIV infected cells with a concomitant increase in necrosis (Fig. 3E). This may indicate the ability of MVA-B13R to produce more antigen through its prolonged viability of infected cells.

MVA-B13R/SHIV induces higher and durable Env-specific humoral responses compared to MVA/SHIV

To determine the immunogenicity of MVA-B13R/SHIV *in vivo*, we immunized BALB/c mice intramuscularly with either MVA/SHIV or MVA-B13R/SHIV at a dose of 10^7 pfu on weeks 0 and 4 (Fig. 4A). To determine antigen-specific humoral immunity, we focused our analyses on after the booster immunization and measured the Env-specific antibody secreting cells (ASCs) in the spleen at week 5, Env-specific IgG titers in serum at weeks 5-8, and memory B cells (MBCs) in the spleen at week 8. Impressively, the MVA-B13R/SHIV immunized mice induced approximately 3-fold higher number of Env-specific ASCs compared to MVA/SHIV immunized mice (Fig. 4B). This was associated with significantly higher levels of Env-specific IgG titers detected in the serum of MVA-B13R/SHIV mice at weeks 5 and 6 (Fig. 4C). Serum titers continued to be higher for MVA-B13R/SHIV mice at week 8. Additionally, we saw less contraction of serum titers at week 8 compared to week 5 for MVA-B13R/SHIV mice (Fig. 4D). Impressively, at week 8, we also saw an increased frequency of Env-specific MBCs detected in the spleens of MVA-B13R/SHIV mice compared to MVA/SHIV (Fig. 4E). Taken together, our data demonstrate the ability of MVA-B13R/SHIV to induce greater and more durable Env-specific humoral responses compared to MVA/SHIV in our prime/boost vaccination regimen.

We additionally measured MVA-specific IgG serum titers and observed no difference between the groups at weeks 5 or 6 (Fig. 4F). When we compared the ratio of Env-specific to MVA-specific serum IgG titers, MVA-B13R/SHIV immunized mice had a higher ratio which indicated skewing of humoral responses to

the recombinant antigen of interest in comparison to the viral vector backbone itself (Fig. 4G). Together, our results demonstrate the ability of our MVA-B13R/SHIV construct to generate more antigen-specific humoral responses while avoiding generation of excessive anti-vector responses. We also determined SHIV-specific cellular responses to autologous HIV Env and SIV Gag antigens by stimulating with peptide pools for cytokine detection by intracellular cytokine staining (ICS) and ELISpot assays. These analyses did not reveal significant differences between the groups at weeks 5 demonstrating that MVA-B13R/SHIV did not enhance T cell response (Fig. 4H).

MVA-B13R/SHIV delays anti-viral IFN responses

In an effort to understand if there are any differences between MVA and MVA-B13R on a global level early after vaccination we performed RNA-Seq analysis. We immunized mice intramuscularly with 10^7 pfu of virus and cells from the draining inguinal lymph node were isolated at 1, 2, and 6 days after immunization for RNA-Seq analysis. Inguinal lymph nodes were isolated from naïve mice as a control. Differentially expressed genes (DEGs) between groups were defined as having a $\log_2$ fold change of > 1.0 or < -1.0 and an adjusted p-value of < 0.05. For MVA/SHIV mice compared to naïve mice, the number of DEGs peaked at day 1 with 255 DEGs and then decreased at days 2 and 6 (Fig. 5A). Metacore was used to curate a list of the top 50 enriched gene ontology (GO) processes for DEGs of each day and subsequently, REViGO was used to summarize and display the GO processes in the form of a network (500). Analysis of the GO processes related to day 1 DEGs highlighted processes mainly related to type I and type II IFN responses, anti-viral defense responses, and antigen processing and presentation (Fig. 5B). Analysis of MVA/SHIV day 2 samples indicated mostly type I IFN responses, anti-viral defense responses, and responses to compounds such as corticosterone, cAMP, and calcium ion. The response to molecules considered to be components of signaling cascades indicate a heightening of the global immune response at this point (Fig. 5C). At day 6, we observed a shift from innate immune response to more adaptive immune responses which included B cell activation, leukocyte differentiation, and antigen processing and presentation (Fig. 5D).

The number of DEGs detected for MVA-B13R/SHIV immunizations compared to naïve began with 66 DEGs at day 1, then increased to 226 at day 2, and 318 at day 6 (Fig. 5A). The different number of DEGs detected on each day for each vaccination group when compared to naïve mice indicated the potential differences in the early response generated by both viruses, particularly at day 1 after vaccination. Comparison of MVA-B13R/SHIV versus MVA/SHIV at day 1 did not yield DEGs but there were 45 genes that had an adjusted p-value of < 0.05 regardless of $\log_2$ fold change values and select genes are depicted here (Fig. 5E). These genes are related to anti-viral response (*Bst2, Ifi35, Nmi, Lgals9, Samhd1*), antigen processing and presentation (*Psme2, Psmb9*), and apoptosis (*Casp8, Sp110*). Interestingly, 80% of the genes (36 of 45) have lower expression in the MVA-B13R/SHIV group than the MVA/SHIV (data not shown).

Gene set enrichment analysis (GSEA) was performed to determine gene sets that were differentially enriched between groups. Gene sets with false discovery rates of < 0.05 and normalized enrichment scores of > 1.35 or < -1.35 were considered significantly enriched. GSEA results of day 1 samples for MVA/SHIV mice compared to naïve mice demonstrated enrichment in the IFN Alpha Response gene set, supporting the enhanced type I IFN responses detected in the GO processes analysis (Fig. 5F, gray). MVA-B13R/SHIV day 1 samples also demonstrated enrichment for IFNα responses compared to naïve mice (Fig. 5F, red).

Surprisingly, when we performed GSEA of MVA-B13R/SHIV compared to MVA/SHIV at day 1 of vaccination, the MVA-B13R/SHIV group was negatively enriched for IFNα responses when compared to MVA/SHIV indicating that MVA-B13R/SHIV mice were mounting an IFNα response to a lesser degree than MVA/SHIV mice (Fig. 5G). From the three GSEA comparisons performed (MVA/SHIV vs naïve, MVA-B13R/SHIV vs naïve, and MVA-B13R/SHIV vs MVA/SHIV) with the Hallmark IFN Alpha

Response gene set, we compiled a cumulative list of leading edge genes and depicted the values from individual animals in a heatmap (501). We were able to observe that the MVA/SHIV group had higher expression levels of IFNα signaling related genes compared to the MVA-B13R/SHIV group (Fig. 5H). This pattern of lower expression levels in the MVA-B13R/SHIV group at day 1 was also seen for the Hallmark IFN Gamma Response gene set (data not shown). GSEA comparisons between both groups at days 2 and 6 resulted in no differences in responses indicating MVA-B13R/SHIV immunized mice had delayed anti-viral responses that became comparable by day 2 (data not shown). The data indicate that while both immunizations induced *in vivo* anti-viral immune responses, MVA-B13R/SHIV mice were generating a less robust type I and II IFN response within the first day of immunization which could influence the subsequent adaptive response.

Discussion

Genetic modulation of poxviruses in order to create both safe and immunogenic vectors has been extensively studied and reviewed elsewhere (502-504). Here, we introduced a functional copy of the anti-apoptosis gene B13R from the WR strain of vaccinia into MVA. The expression of B13R during MVA infection demonstrated delayed cell death *in vitro* in muscle cells as well as professional APCs and improved the magnitude and durability of Env-specific antibody titers in addition to memory B cell responses *in vivo*. Interestingly, MVA-B13R/SHIV immunizations were associated with negative enrichment of both type I and II IFN responses at one day after the priming vaccination when compared to the MVA/SHIV group. Collectively, these data show that restoring the function of the 181R/182R genes using WR B13R in MVA significantly delays apoptosis of MVA infected cells and this is associated with enhanced humoral immunity and altered IFN signaling.

The immune mechanisms that led to enhanced humoral immunity needs further investigation. The delay observed in apoptosis after infection with MVA-B13R *in vitro* along with the concomitant increase in necrosis may have been sufficient to influence the microenvironment and enhance the humoral response. Necrosis is a form of regulated cell death that can manifest in a variety of ways including necroptosis and pyroptosis (505, 506). These forms of regulated necrosis are morphologically associated with cell swelling, mild chromatin condensation though nuclei remain intact, and the rapid loss of membrane integrity leading to the release of cellular contents into the microenvironment such as damage-associated molecular patterns (DAMPs) which alert the immune system (507). The delayed apoptosis potentially allowed for a prolonged period of time where Env-loaded apoptotic bodies were being released and phagocytosed by APCs. Additionally, DAMPs and antigen directly released into the microenvironment due to necrosis altogether could have influenced the humoral response. Tam *et. al* demonstrated that administration of exponentially increasing doses of antigen over one week generated greater antibody titers compared to a single bolus administration and that the longer period of time the increasing doses were administered over, the greater the antibody response (508). The relatively greater amounts of Env

detected in cell lysates and supernatants through 96 hours of infection by MVA-B13R/SHIV indicate its potential to sustain Env production and release for a longer period of time than MVA/SHIV which could have contributed to the improved humoral response (Fig. 3D).

Previously, Perdiguero *et al*. modulated MVA induced apoptosis by deleting the anti-apoptotic F1L gene in MVA which led to increased apoptosis and production of type I IFNs from infected cells (468). Immunizations with the F1L deletion mutant resulted in enhanced T cell responses, mainly directed at the Pol antigen, while gp120-specific antibody responses were not significantly different compared to immunizations with the parental MVA strain. Our approach of delaying apoptosis instead led to enhancement in antigen-specific humoral, but not cellular responses. A possible difference between our B13R expressing MVA and the F1L deletion MVA mutant may be the cytokines induced after infection. The F1L deleted MVA enhanced production of type I IFN response *in vitro*. This is in contrast to delayed IFN responses observed with our MVA-B13R/SHIV construct in mice, which could contribute to differential intracellular signaling between the two vectors. Additionally, F1L has been described as a suppressor of the pro-apoptotic Bcl-2 family proteins and an inhibitor of caspase 9, the initiator caspase for the intrinsic apoptotic pathway (509-511), while B13R and its orthologs alternatively have been described as inhibitors of the extrinsic pathway (428, 493). The potential differences in IFN production and the targeting of different points of the apoptotic pathway may explain the differences in adaptive immune responses observed.

Dai *et al.* showed that detection of MVA in conventional DCs in mice is mediated by the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS) which signals through the adaptor protein STING and leads to induction of type I IFNs (512). They also indicated that processing of MVA virions after viral entry in the late endosomal and lysosomal compartments would lead to release of viral DNA into the cytoplasm allowing for detection by cGAS. Our RNA-Seq results from day 1 after immunization indicate that MVA-B13R/SHIV vaccinated mice have significantly lower expression of genes related to immunoproteasome

assembly and antigen processing compared to MVA/SHIV vaccinated mice which is not observed at subsequent timepoints after immunization (Fig. 5E). Together, this indicates the possibility that MVA-B13R is influencing the lysosomal processing pathway of a cell early during infection, hindering the release of viral DNA into the cytoplasm for detection by cGAS, and thereby delaying type I IFN production. STING has also been shown to be an adaptor protein for other cytosolic DNA sensors such as DDX41 (513) which we observed to be expressed lower at day 1 of MVA-B13R/SHIV immunizations, supporting our hypothesis of delayed viral DNA sensing (Fig. 5E). The decreased cell death we observed for MVA-B13R infected pDCs and CD141+ DCs support the idea of slower viral sensing and cell death during MVA-B13R infection (Fig. 2G). The delay in cell death and viral sensing by MVA-B13R in addition to the extended period of time where more antigen could be produced likely are sufficient to influence the adaptive response.

Interestingly, among the DEGs observed at day 1 for MVA-B13R/SHIV vs MVA/SHIV groups, *Casp8* encoding caspase 8 was observed to be more highly expressed in MVA/SHIV group than MVA-B13R/SHIV (Fig. 5E). Caspase 8 is an initiator caspase of the apoptosis cascade that responds to extracellular stimulus for cell death such as TNFα or Fas ligand signaling (514). Caspase 8 activation leads to initiation of downstream effector caspases such as caspase 3, 6, and 7. The increased expression of *Casp8* in MVA/SHIV mice at one day after immunization may indicate an enhanced anti-viral state among the cells of the draining lymph node that are likely responding to the inflammatory microenvironment and presence of virus. The reduced expression of *Casp8* for the MVA-B13R/SHIV group suggest an association with the ability of MVA-B13R to inhibit caspase 8 activation and create a temporarily less inflammatory environment during the early stages of vaccination. It is possible that time points earlier than 24 hours after immunization could have revealed further differences between the anti-viral state induced between our two MVA vectors.

Others have shown the adjuvating properties of type I IFN signaling on humoral responses following acute infection or vaccination (515-518). We showed here that both MVA/SHIV and MVA-B13R/SHIV vaccinations induced significant IFNα and IFNγ responses compared to naïve mice, expected during an anti-viral response, however MVA-B13R administration led to reduced expression levels for IFN signaling genes at the early stages of infection which became comparable by day 2 (Fig. 5F-H, data not shown). The data indicate that there is not an absence of IFN response occurring at this time, but there may be a slower ramping up to the peak of the immune response against MVA-B13R which could be due to less overall cell death initially occurring, delayed viral sensing by infected cells, and greater antigen production for an extended period of time. Altogether, these processes occurring early in the response may be influencing the enhanced Env-specific antibody and memory B cell levels we observed. Future studies to elucidate the differing early responses against these viruses *in vivo* are still required.

In summary, we show that MVA expressing an anti-apoptotic gene, B13R, is effective at delaying cell death of infected cells and when used as a vaccine vector (MVA-B13R/SHIV) is capable of generating strong and durable Env-specific antibody titers and memory B cells. These *in vivo* results are likely due to the increase in antigen levels produced over time in addition to the delay of the anti-viral inflammatory response generated against MVA-B13R. Studies in non-human primate models would be informative to further determine the immunogenicity of the MVA-B13R vector as the NHPs are closer to humans and will better inform the clinical utility of MVA-B13R as a vaccine vector.

Acknowledgments

We thank the veterinary staff at Yerkes for animal care; Emory Electron Microscopy core for imaging services; CFAR Immunology Core for help with flow cytometry; Nirav Patel at the Yerkes NHP Genomics Core for sample processing, library preparation, and sequencing. We thank Gregory Tharp and Lisa Mills for advice and helpful discussions regarding RNA-Seq analysis. We thank Koichi Araki for WR vaccinia infected DF-1 cell lysate and Geoffrey Smith at University of Cambridge for the gift of the rabbit anti-B13R sera. We declare that no conflicts of interest exist.

Funding

This work was supported in part by National Institutes of Health Grants U19AI109633, R01DE02633 (to RRA), Emory University CFAR grant P30 AI050409 and NCRR/NIH base grants P30 RR00165 (to YNPRC).

Methods

Generation of MVA-B13R

Due to high sequence homology between vaccinia virus WR B13R and MVA 181R/182R genes (98.6% homology), we first knocked out MVA 181R/182R from the genome via homologous recombination with PCR product 1 encoding A) the end of the MVA 180R gene and the intergenic region at the end 180R, B) a GFP cassette with a P11 promoter, and C) the intergenic region at the start of MVA 183R and the beginning of the MVA 183R gene. Fragments A, B and C were generated separately and designed to have at least 15bp overhang which allowed for the assembly of each fragment together by overlap extension PCR. Fragment A was amplified from MVA DNA using forward primer LW-540 (5'-GGATATTGCATGATAGAATGGTTCGGTGG-3') and reverse primer LW-541 (5'-AGAAGATATCCATAGTAATCGATATTGGTCG-3'). Fragment B was amplified from the plasmid insertion vector pLAS-1 using forward primer LW-542 (5'-CCAATATCGATTACTATGGATATCTTCTTTTCATTTTGTTTTTTTCTATGCTATAAATGGTGAG CAAGG-3') and reverse primer LW-543 (5'-TTACTTGTACAGCTCGTGCATGCCG-3'). Fragment C was amplified from MVA DNA using forward primer LW-544 (5'-GGCATGCACGAGCTGTACAAGTAACCATTTTTTTTAAAAAAATAGAAAAAACATGTGGTATT AGTGC-3') and reverse primer LW-545 (5'-CACAATCTTTGACATCATCCCACGGC-3'). For overlap extension PCR of fragments A, B, and C, forward primer LW-540 and reverse primer LW-545 were used. Following recombination of the GFP-encoding PCR product into MVA and plaque purification, GFP was subsequently knocked out with PCR product 2 encoding WR B13R flanked by fragments A and C as described above via recombination. PCR product 2 was amplified from WR DNA using forward primer LW-540 and reverse primer LW-545 (can use same primers due to presence of identical sequences in both MVA and WR genomes). Recombinants were plaque purified to generate MVA-B13R. Sequences were confirmed for both PCR products and MVA-B13R viruses (GenBank accession number MK170380). Lysates of virus infected DF-1 cells were purified through a 36% sucrose cushion for virus stocks.

***In vitro* Infections**

DF-1 cells (spontaneously immortalized chicken embryo fibroblast cell line), HeLa cells, C2C12 cells (a mouse myoblast cell line, ATCC, CRL-1772), and RD cells (a human muscle rhabdomyosarcoma, ATCC, CCL-136) were cultured in Dulbecco's modified Eagles medium supplemented (DMEM) with 10% fetal bovine serum (FBS), 100 units penicillin/ml, 0.1 mg/ml streptomycin, and supplemented with 1% L-glutamine. For infections, MVA viruses at indicated MOI were added in complete DMEM media with 2% FBS. After 1.5-2 hours of inoculation, inoculum was removed and fresh 10% FBS complete DMEM media was added. To harvest cells, DMEM media was removed, cells were washed with warm PBS and treated with trypsin to release cells into suspension. For testing the effect of various caspase inhibitors during MVA infection, indicated caspase inhibitors (R&D Systems) were reconstituted in DMSO. Cells were inoculated with virus at MOI 2 for 2 hours. Inoculum was replaced with fresh complete DMEM media containing 100□M of indicated caspase inhibitors and cells were harvested 48 hours post infection to be stained for intracellular active caspase 3 expression. For testing functionality of B13R during MVA infection, Hela cells were first inoculated with virus at MOI of 3 for 1.5-2 hours, inoculum was removed, and fresh DMEM media was added. 16 hours later, media was replaced with DMEM media containing 25ng/ml recombinant human TNFα (Tonbo) and 5μg/ml cycloheximide (Sigma) and cells were harvested for staining at indicated time points post treatment.

Western Blot Analysis

DF-1 cells were infected at indicated MOI of virus and harvested at indicated time points post infection. Supernatant was collected and centrifuged to ensure separation of cells. After infected cells were washed with cold PBS, RIPA buffer (ThermoScientific) with protease inhibitor was added and cells lysates were collected according to manufacture protocol. Lysates or supernatant samples were added to Laemmli buffer containing β-mercaptoethanol, heated to 95°C and run on 4-15% SDS-PAGE gels for Western blots. B13R was detected with polyclonal rabbit anti-B13R serum (gift from Geoffrey Smith, University

of Cambridge; diluted 1:5000), Gag was detected with a mouse anti-Gag p27 monoclonal antibody (2F12; NIH AIDS Reagent Program), and Env was detected with a mouse anti-Env monoclonal antibody (ID6; NIH AIDS Reagent Program). Secondary goat anti-rabbit Ig HRP (Southern Biotech; diluted 1:5000) or goat anti-mouse IgG HRP (Southern Biotech; diluted 1:10,000) was used for detection. ECL substrate (GE Healthcare) was used for chemiluminescence detection. Image Lab V6 (Bio-Rad) was used for densitometry analysis.

Analysis of Virus Growth

DF-1 cells were infected at MOI 0.05 with MVA or MVA-B13R. At indicated time points post infection, cell layers were washed with PBS and trypsinized to harvest cells. Collected cells were freeze/thawed at -80°C/37°C, respectively, and sonicated three times to ensure disruption of cell membranes and release of virus. Viral titers recovered from lysates were performed on DF-1 cells by plaque assay.

Infection of Rhesus Macaque PBMCs

For *ex vivo* infection of rhesus macaque peripheral blood mononuclear cells (PBMCs), blood was collected from naïve rhesus macaques in sodium citrate CPT tubes. Samples were centrifuged at 2800 RPM for 30 minutes at room temperature with no deceleration. Buffy coats containing PBMCs were collected, washed with HBSS without calcium and magnesium, and resuspended in complete RPMI medium (RPMI, 10% FBS, 100 units penicillin/ml, 0.1 mg/ml streptomycin, 10μM HEPES). Isolated PBMCs were infected with virus at MOI 3 for 20 hours at 37°C and then stained for MVA, cell subset markers, and viability markers for flow cytometry analysis.

Generation of Recombinant MVAs Expressing SHIV Antigens

All recombinant SHIV-expressing MVA vectors expressed the SIVmac239 Gag and Pol genes under an independent early/late vaccinia promoter (modified H5, mH5) recombined into the deletion III site of

MVA. The modified clade C HIV Env 1086c gp150 K160N gene (GenBank accession number FJ444399.1) (519) was cloned between two XmaI restriction sites of the plasmid transfer vector pLW-73 and used to recombine into the essential region of the MVA genome (between genes I8R and G1L) under an independent mH5 promoter. Recombinant MVAs were plaque purified and tested for contamination with parental MVAs by PCR using primers in the deletion III flanking regions. Lysates of virus infected DF-1 cells were purified through a 36% sucrose cushion for virus stocks.

Flow Cytometry

For *in vitro* detection of cells undergoing cell death, virally infected cells were harvested and stained with Live/Dead cell viability stain (ThermoFisher), fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences), and stained intracellularly for MVA E3 protein (TW2.3; BEI Resources) and active caspase 3 (C92-605; BD Pharmingen). For detecting expression of SIV Gag and HIV Env by SHIV constructs, virally infected cells were stained with Live/Dead cell viability stain, fixed and permeabilized with Cytofix/Cytoperm, and stained intracellularly with monoclonal antibodies to p27 Gag (2F12; NIH AIDS Reagent Program) and HIV Env (ID6; NIH AIDS Reagent Program). Infected rhesus macaque PBMCs were surface stained with the following: Live/Dead near-IR cell viability stain, CD3 (SP-34-2; BD Biosciences), CD14 (M5E2; BioLegend), CD11c (3.9, BioLegend), CD123 (6H6, BioLegend), CD20 (2H7; BioLegend), CD16 (3G8, Sony Biotechnology), HLA-DR (G46-6, BD Biosciences), and CD141 (1A4; BD Biosciences). Cells were fixed and permeabilized with Cytofix/Cytoperm and stained intracellularly for active caspase 3 and MVA. Cells were acquired on an LSR Fortessa and analyzed using the FlowJo software (Treestar Inc. CA). For intracellular cytokine staining, stimulated mouse splenocytes were stained with Live/Dead cell viability stain, CD4 (RM4-5, Tonbo), and CD8 (53-6.7, BioLegend). Cells were then fixed and permeabilized with Cytofix/Cytoperm and stained intracellularly for IFNγ (XMG1.2, BD Biosciences), IL-2 (JES6-5H4, BD Biosciences), and TNF (MP6-XT22, BD Biosciences).

Mice, Immunizations, and Sample Collections

6- to 8-week-old female BALB/c mice were purchased from Charles River Laboratory. For MVA immunizations, mice were intramuscularly immunized with 10^7 pfu of virus diluted in sterile phosphate-buffered saline. At indicated time points after immunization, blood was collected via tail-vein bleed and serum was isolated and stored at -20°C. Where applicable, cells were isolated from spleens and inguinal lymph nodes by manual disruption through 100μM strainers in complete RPMI medium (RPMI, 10% FBS, 100 units penicillin/ml, 0.1 mg/ml streptomycin, 10μM HEPES, and 55μM β-mercapthoethanol). Red blood cells were lysed with ACK lysing buffer and washed twice with PBS at 1200 RPM for 10 minutes prior to use.

Measuring Antibody Titers

For ELISAs, plates were coated with 0.5μg/ml recombinant HIV gp140 (C.1086 gp140C K160N, NIH Aids Reagent Program) or 10^7 pfu/ml MVA diluted in 0.2M bicarbonate buffer, pH 9.4. 2.0μg/ml of goat anti-mouse IgG (Sigma M2650) was used to coat wells for standards. Plates were blocked for 1 hour at room temperature with 5% non-fat dry milk and 4% whey in PBS with 0.05% Tween20 (PBS-T). Serum samples and standard dilutions were prepared in 4% whey in PBS-T. Purified mouse IgG (Southern Biotech 0107-01) was used as IgG standard. Samples or standards were incubated for 2 hours at room temperature and plates were washed six times with PBS-T. Bound Env-specific IgG was detected using goat anti-mouse IgG HRP (Southern Biotech 1030-05) at 1:40,000 dilution for 1 hour at room temperature, then wells were developed using TMB Peroxidase Substrate (KPL).

ELISpot Assays

For antibody secreting cells, 96-well ELISpot plates (Millipore) were coated with 0.5μg/ml of gp140 or 1.0μg/ml goat anti-mouse Ig (Southern Biotech) in PBS overnight at 4°C. Plates were washed six times with PBS-T and three times with PBS, then blocked with complete RPMI medium for at least 2 hours at

37°C. Cells were resuspended in complete RPMI medium and a series of 1:2 or 1:3 dilutions of cells were added to plates and incubated for 5 hours at 37°C. Goat anti-mouse IgG biotin (Southern Biotech) antibody diluted in PBS-T with 1.0% serum was added for 1 hour at room temperature to detect bound mouse IgG antibody. Streptavidin-alkaline phosphatase (MABTECH) diluted in PBS-T with 1.0% serum was added for 1 hour at room temperature in the dark, followed by 1-Step NBT substrate (ThermoScientific) to detect spots. For memory B cell assays, 10^6 isolated splenocytes were cultured in flat-bottom 24-well plates and stimulated with 1μg/ml resiquimod (Sigma) and 10ng/ml recombinant murine IL-2 (Peprotech) in complete RPMI medium in replicates of 8 or left unstimulated in media. Cells were incubated for 3 days at 37°C. ELISpot plates were prepared as described above. Stimulated cells were washed twice with PBS and resuspended in fresh complete RPMI media. Cells for each replicate were diluted accordingly and divided for plating for detection of either antigen-specific or total IgG antibody production. Cells were incubated on plates for 5 hours at 37°C. Secondary and detection antibody procedures were the same for antibody secreting cell assays as described above.

Intracellular Cytokine Stimulations

10^6 splenocytes were cultured in 200μl complete RPMI medium (RPMI, 10% FBS, 100 units penicillin/ml, 0.1 mg/ml streptomycin, 10μM HEPES, and 55μM β-mercapthoethanol) with HIV Env 1086C peptides or SIVmac239 Gag peptides at 37°C. After 1.5 hours, GolgiStop (BD Biosciences) and brefeldin A (Sigma) were added to each sample and cultured for an additional 4.5 hours followed by antibody staining for flow cytometry.

RNA-Seq

RNA-Seq analyses were conducted at the Yerkes NHP Genomics Core on naïve (n = 5), MVA/SHIV immunized (n = 5/time point, 3 time points), and MVA-B13R/SHIV immunized (n = 5/time point, 3 time points) mice. RNA was extracted from lymph node cells in QIAGEN Buffer RLT by using QIAGEN

RNEasy Micro protocol as described previously (520) and assessed for integrity and quantity using an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). 2 ng of total RNA was used as input for mRNA amplification using 5' template-switch PCR with the SMART-Seq v4 Ultra Low Input RNA kit (Takara Bio USA, Inc.) according to manufacturer's instructions. Amplified mRNA was fragmented and appended with dual indexed bar codes using Illumina NexteraXT DNA Library Prep kits. Amplified libraries were validated using the Agilent 4200 TapeStation and quantified using a Qubit fluorometer. Libraries were normalized, pooled followed by clustering on a HiSeq3000/4000 flow cell using the Illumina cBot. The clustered flow cell was then sequenced on the Illumina HiSeq3000 system employing a single-end 101 cycles run, with multiplexing to achieve approximately 20 million reads per sample.

RNA-Seq Statistical Analyses

RNA-Seq data were aligned to the GRCm38.p3 assembly of the mouse genome from GENCODE (available at ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_mouse/release_M3/). Alignment was performed using STAR v2.5.2b. Transcript abundance was estimated using internally with STAR using the algorithm of htseq-count. One sample (MVA/SHIV mouse, day 1) was discontinued from further analysis as it mapped poorly to the reference sequence. Differential expression analyses were performed between two sample sets using DESeq2 (significance: adjusted p-value < 0.05 and $\log_2$ fold change > 1.0 or < -1.0). Genes with a total count of less than 20 counts across all samples (total n = 35) were excluded from analysis. DEGs from a comparison were analyzed through Metacore from Thomson Reuters for the top 50 enriched gene ontology (GO) processes. REViGO (http://revigo.irb.hr/) and Cytoscape software was used to visualize related enriched GO processes with nodes and links with similar terms linked with edges (500, 521). Color is related to GO process p-values, node size is related to number of related processes, and edge width related to degree of similarity. Attribute values of each network are relative to the maximum and minimum values of that network. Gene set enrichment analysis (GSEA) was performed using the desktop module available from the Broad Institute (https://www.broadinstitute.org/gsea/). GSEA was run for MSigDB curated gene set collections with 1,000 permutations using the gene set

permutation type (normalized enrichment score cutoff > 1.35 or < -1.35 and false-discovery rate < 0.05). Chip platform used was derived from the GRCm38-p3 mouse reference used for mapping and abundance estimation.

Data Availability

RNA-Seq dataset is available at the GEO repository under accession GSE119884.

The data that support the findings of this study are available upon request from the corresponding author.

Statistics

Statistical analysis was performed using repeated measure two-way ANOVA with Tukey test to correct for multiple comparisons (when comparing two or more groups over various time points), two-tailed unpaired Mann Whitney test (when comparing two groups), or two-tailed paired Student's t test (when comparing matched samples) using Prism 7.0d (GraphPad) software. P-values < 0.05 were considered statistically significant.

Study Approval

Mice were maintained and used according to institutional and NIH guidelines in a specific pathogen-free facility. All animal studies were approved by Emory University IACUC (Atlanta, GA).

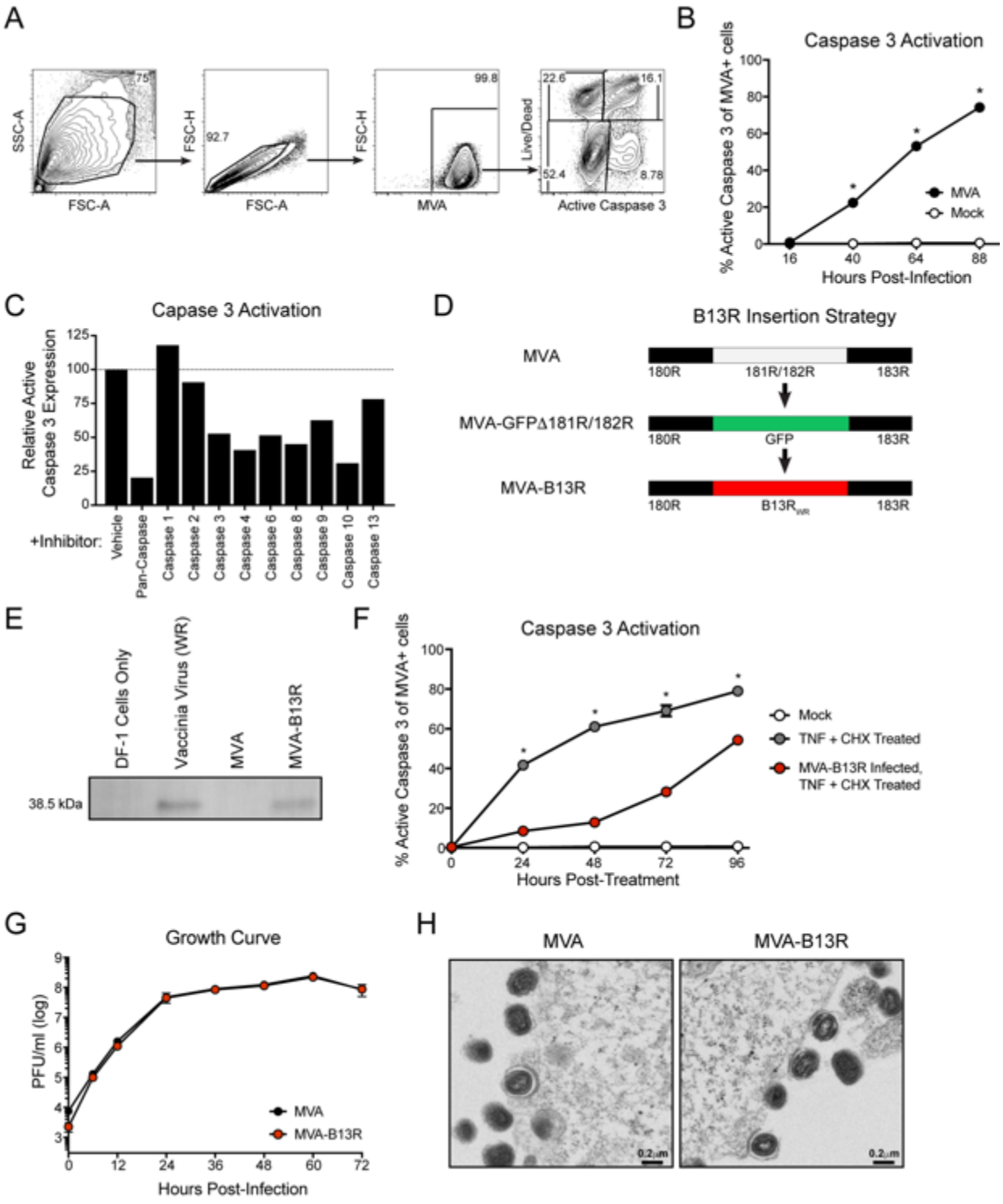

A
SSC-A
FSC-A
75
FSC-H
FSC-A
92.7
FSC-H
MVA
99.8
Live/Dead
Active Caspase 3
22.6
16.1
52.4
8.78
B
Caspase 3 Activation
% Active Caspase 3 of MVA+ cells
Hours Post-Infection
MVA
Mock
C
Capase 3 Activation
Relative Active Caspase 3 Expression
+Inhibitor:
Vehicle
Pan-Caspase
Caspase 1
Caspase 2
Caspase 3
Caspase 4
Caspase 6
Caspase 8
Caspase 9
Caspase 10
Caspase 13
D
B13R Insertion Strategy
MVA
180R
181R/182R
183R
MVA-GFPΔ181R/182R
GFP
MVA-B13R
B13R
E
DF-1 Cells Only
Vaccinia Virus (WR)
MVA
MVA-B13R
38.5 kDa
F
Caspase 3 Activation
% Active Caspase 3 of MVA+ cells
Hours Post-Treatment
Mock
TNF + CHX Treated
MVA-B13R Infected, TNF + CHX Treated
G
Growth Curve
PFU/ml (log)
Hours Post-Infection
MVA
MVA-B13R
H
MVA
MVA-B13R
0.2 μm

Figure 1. B13R expression by MVA during infection delays apoptosis of infected cells. Hela cells were infected with MVA at an MOI of 3 and active caspase 3 expression of MVA infected cells was detected by flow cytometry. (A) Representative flow gating to determine the viability of MVA infected cells: doublet exclusion followed by vaccinia virus E3 protein expression for MVA+ cells and Live/Dead and active caspase 3 staining for viability. (B) Percentages of active caspase 3+ cells of MVA+ cells. (C) Hela cells were infected with MVA at an MOI of 2 for 2 hours and then inoculum was replaced with fresh media containing indicated caspase inhibitors or DMSO (vehicle control) at 100□M. Active caspase 3 expression was detected among MVA+ cells at 48 hours post infection and normalized to vehicle control. (D) Schematic of B13R insertion strategy: 181R/182R gene from the MVA genome was replaced via homologous recombination with GFP. GFP was then replaced with Western Reserve (WR) strain of vaccinia virus-derived B13R to generate MVA-B13R. (E) Western blot analysis of DF-1 cell lysates infected with WR, MVA, or MVA-B13R at an MOI of 0.5 at 24 hours after infection to detect B13R expression. (F) Hela cells were mock infected or infected with MVA-B13R for 16 hours at an MOI of 3. Cells were then treated with 25ng/ml TNF□ and 5□g/ml cycloheximide and caspase 3 activation among MVA+ cells was determined. (G) DF-1 cells were infected at an MOI 0.05 with MVA or MVA-B13R. At indicated times after infection, cell lysates were harvested to determine viral titers by plaque assay. (H) DF-1 cells were infected at an MOI 0.25 with MVA or MVA-B13R and imaged by electron microscopy at 24 hours post infection. For B, C, F, and G, data are mean ± SEM of duplicate samples. Data are representative of two or more independent experiments. *, $p < 0.05$.

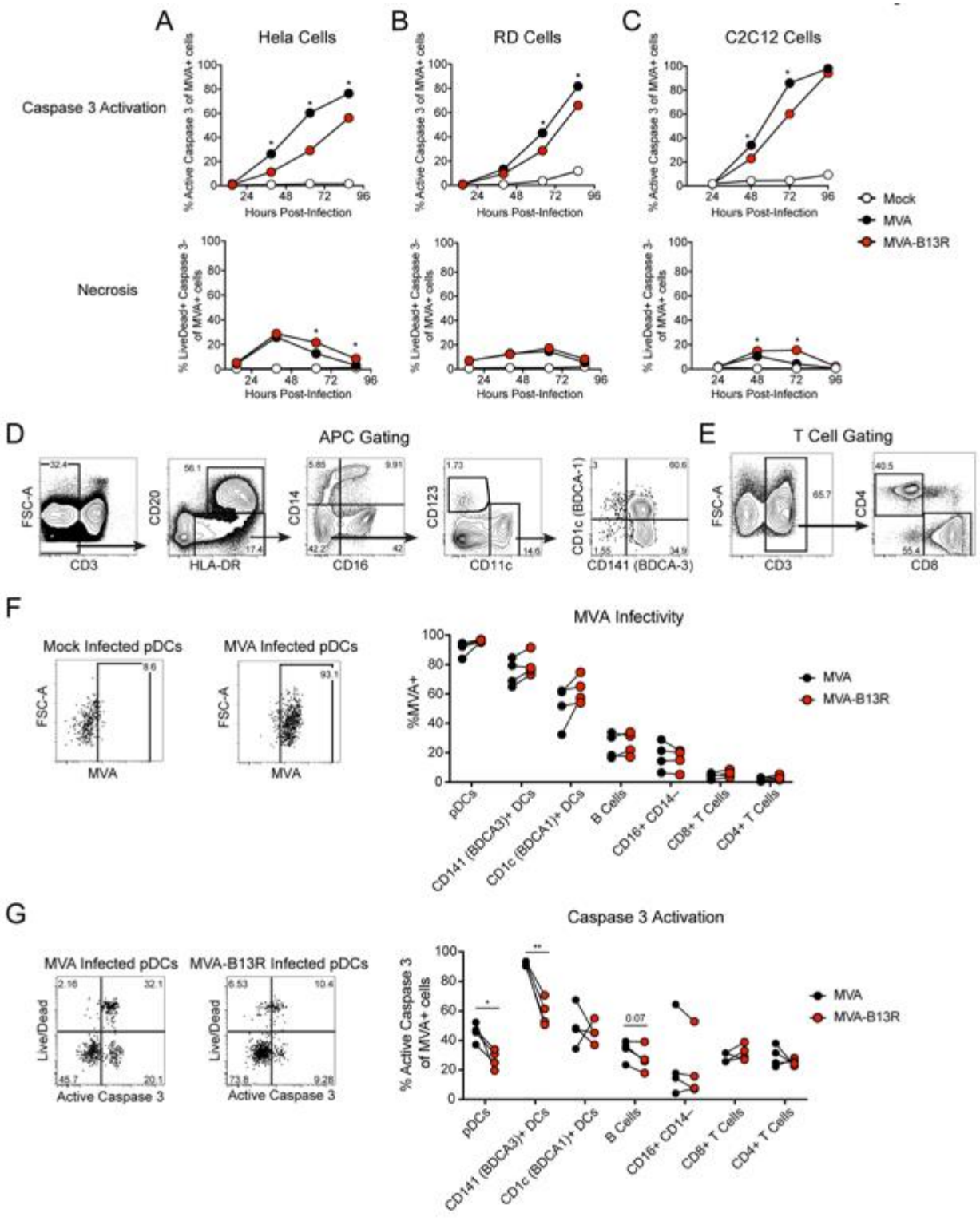

A
Hela Cells
B
RD Cells
C
C2C12 Cells
Caspase 3 Activation
Necrosis
% Active Caspase 3 of MVA+ cells
% LiveDead+ Caspase 3- of MVA+ cells
Hours Post-Infection
Mock
MVA
MVA-B13R
D
APC Gating
FSC-A
CD3
CD20
HLA-DR
CD14
CD16
CD123
CD11c
CD1c (BDCA-1)
CD141 (BDCA-3)
E
T Cell Gating
CD4
CD8
F
Mock Infected pDCs
MVA Infected pDCs
MVA
MVA Infectivity
%MVA+
pDCs
CD141 (BDCA3)+ DCs
CD1c (BDCA1)+ DCs
B Cells
CD16+ CD14-
CD8+ T Cells
CD4+ T Cells
G
MVA Infected pDCs
MVA-B13R Infected pDCs
Live/Dead
Active Caspase 3
Caspase 3 Activation
% Active Caspase 3 of MVA+ cells
0.07

Figure 2. MVA-B13R delays cell death of infected cells compared to MVA. Kinetics of caspase 3 activation and necrosis of mock, MVA, or MVA-B13R infected (A) Hela cells (MOI of 2), (B) RD cells, a human muscle cell line (MOI of 3), and (C) C2C12 cells, a mouse myoblast cell line (MOI of 0.3). (D-G) PBMCs from rhesus macaques were co-incubated with MVA or MVA-B13R (MOI of 3) and analyzed by flow cytometry to detect MVA+ cell subsets and viability. (D) Representative gating strategy of APC subsets: B cells (CD3- CD20+ HLA-DR+), CD16+ CD14- cells, pDCs (Lin- HLA-DR+ CD11c- CD123+), CD141+ DCs (Lin- HLA-DR+ CD11c+ CD141+ CD1c-), CD1c+ DCs (Lin- HLA-DR+ CD11c+ CD1c+ CD141-). (E) Representative gating strategy of CD4+ and CD8+ T cells. (F) Representative MVA staining of mock or MVA infected pDCs and percentages of MVA and MVA-B13R infection for various cell subsets. Lines indicate matched samples. (G) Representative viability staining with active caspase 3 and Live/Dead of MVA or MVA-B13R infected pDCs and percentage of active caspase 3+ cells among MVA+ cell subsets. Lines indicate matched samples. For A-C, data are mean ± SEM of duplicate samples and are representative of two or more independent experiments. Exact p values reported; *, $p < 0.05$; **, $p < 0.01$.

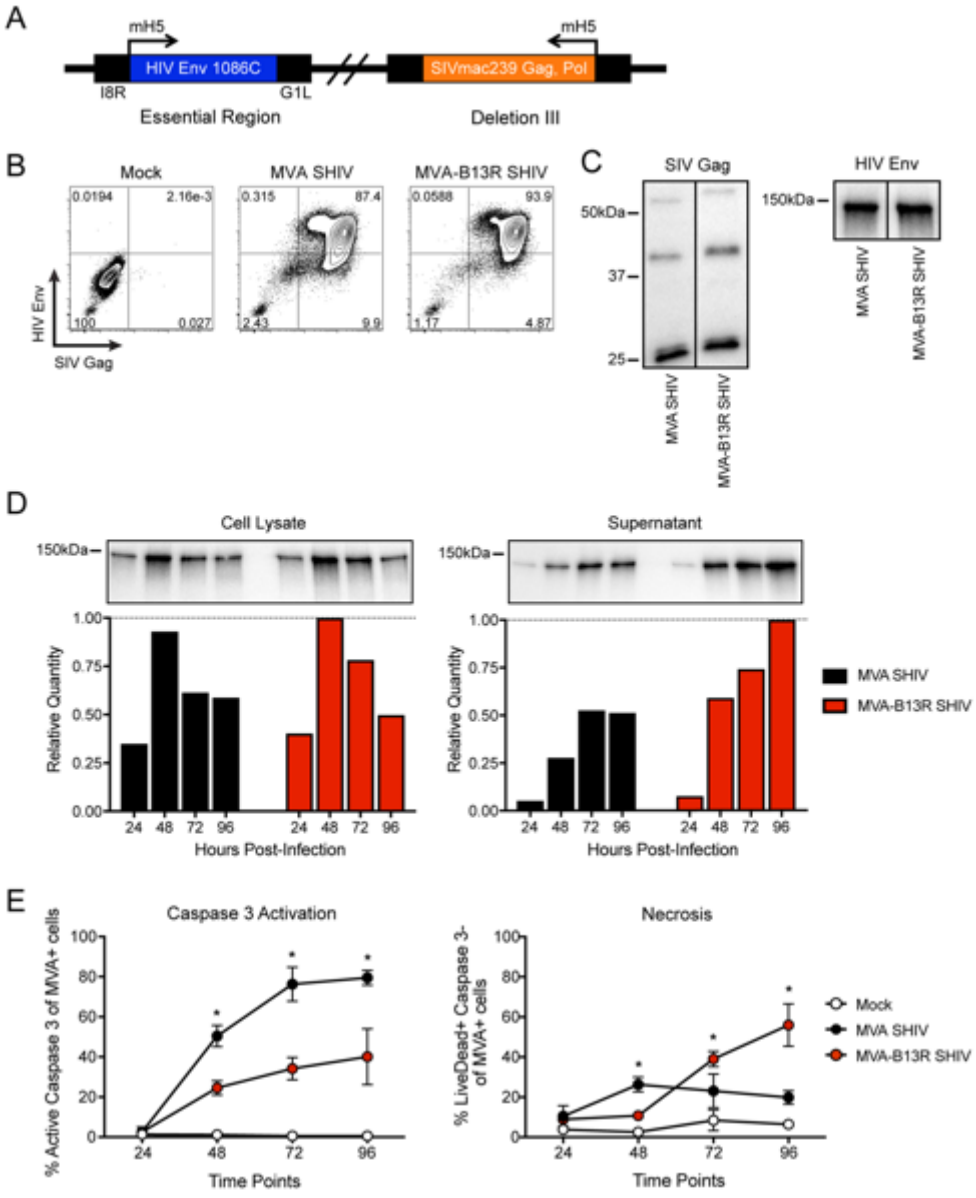


Figure 3. Generation of recombinant MVAs expressing HIV Env 1086C and SIV Gag. (A) Schematic showing insertion sites for HIV Env and SIVmac239 Gag and Pol genes in MVA genomes to generate SHIV constructs. Both gene cassettes are under the control of an independent early/late vaccinia promoter, modified H5 (mH5). (B) Flow cytometry confirmed dual expression of SIV Gag and HIV Env from MVA/SHIV and MVA-B13R/SHIV constructs at 36 hours after infection of DF-1 cells. (C) Western blotting confirmed expression of SIV Gag and HIV Env from MVA/SHIV and MVA-B13R/SHIV infected DF-1 cell lysates at 48hr after infection at MOI of 0.05. (D-E) DF-1 cells were infected with

MVA/SHIV or MVA-B13R/SHIV at MOI 0.05. (D) Cell lysates and supernatant were harvested at indicated time points for detection of HIV Env by Western blot. Relative quantities of each band as determined by densitometry are presented below Western blot images. Representative images from two independent experiments. (E) Cells were harvested at indicated time points and percentages of caspase 3 activation and necrosis were detected for MVA+ cells. For E, data are mean ± SEM of two independent experiments. *, $p < 0.05$

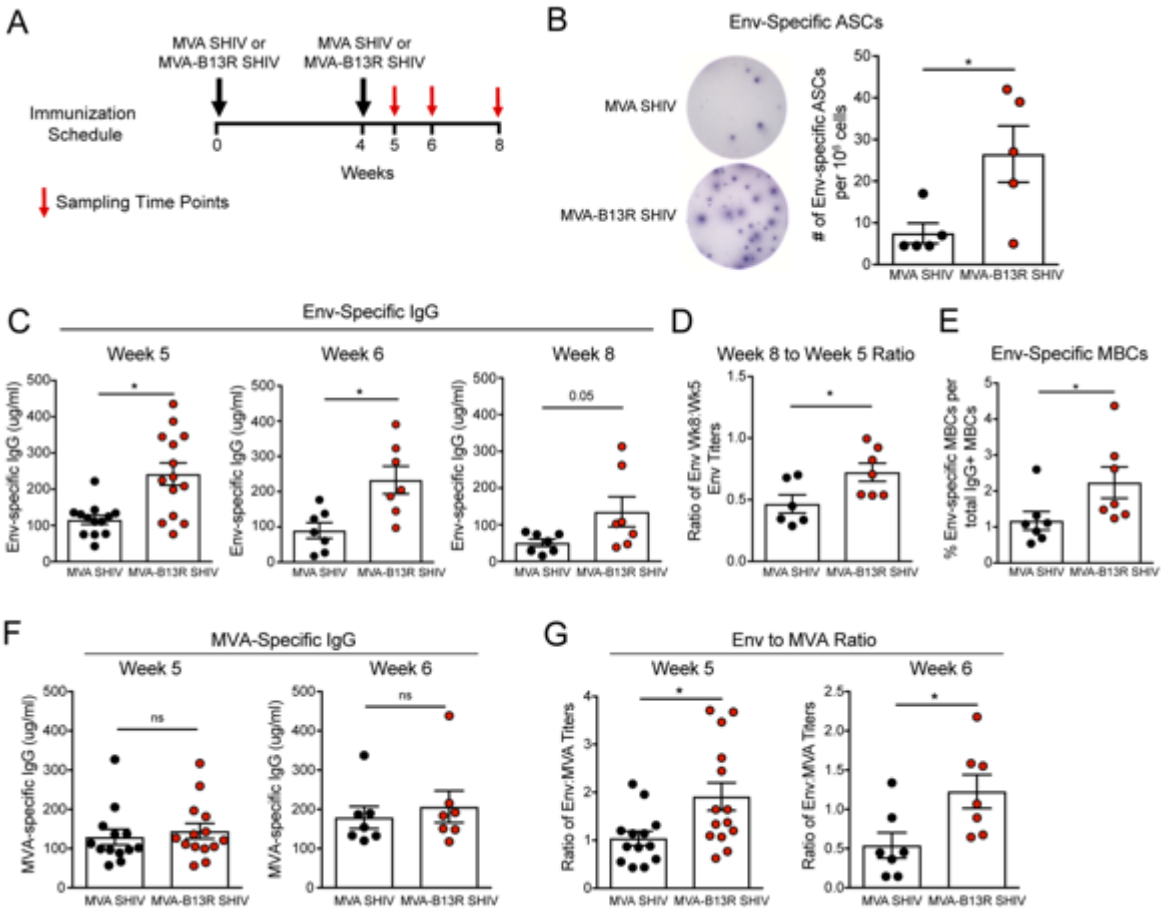


Figure 4. MVA-B13R/SHIV immunization enhances Env-specific humoral responses. BALB/c mice were immunized intramuscularly twice with 10^7 pfu/dose of MVA/SHIV or MVA-B13R/SHIV. (A) Schematic of the prime/boost immunization schedule and sampling time points. (B) Representative ELISPOT of Env-specific IgG antibody secreting cells (ASCs) at week 5 from MVA/SHIV or MVA-B13R/SHIV immunized mice. Env-specific IgG ASCs measured from splenocytes at week 5. (C) Env-specific serum IgG titers were measured at weeks 5, 6, and 8. (D) Ratio of Env-specific serum IgG titers from week 8 to week 5. (E) Env-specific memory B cells (MBCs) measured from splenocytes at week 8. (F) MVA-specific serum IgG titers measured at weeks 5 and 6. (G) Ratio of Env-specific to MVA-specific serum IgG titers at weeks 5 and 6. (H) Frequency of HIV Env-specific and SIV Gag-specific IFN□, IL-2, and TNF producing CD4+ T cells from splenocytes at week 5. Bars are mean ± SEM. Data are representative of two or more independent experiments. Exact p values reported; *, $p < 0.05$; ns, not significant.

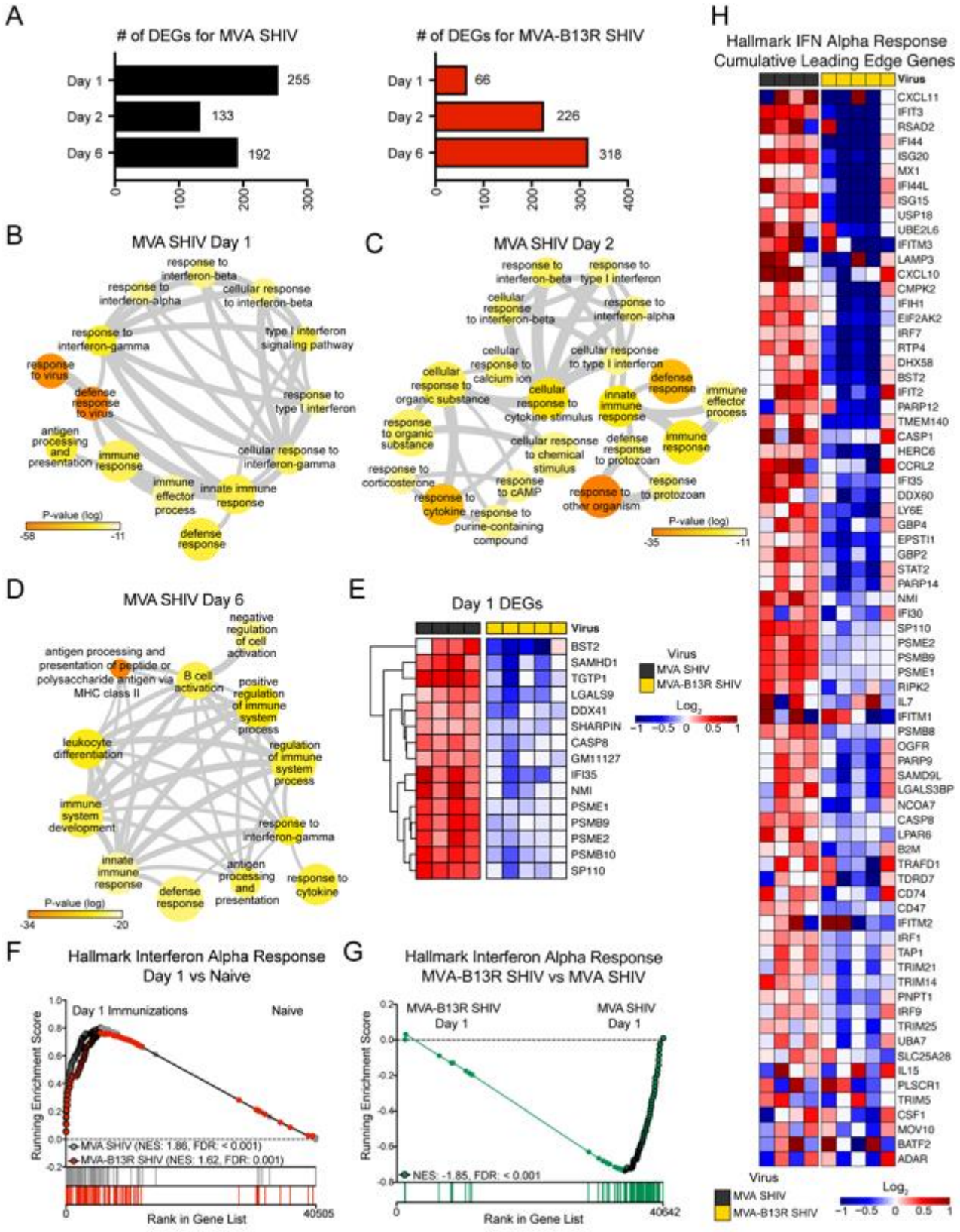

A
# of DEGs for MVA SHIV
Day 1
Day 2
Day 6
255
133
192
0
100
200
300
# of DEGs for MVA-B13R SHIV
Day 1
Day 2
Day 6
66
226
318
0
100
200
300
400
B
MVA SHIV Day 1
response to interferon-beta
response to interferon-alpha
cellular response to interferon-beta
response to interferon-gamma
type I interferon signaling pathway
response to virus
defense response to virus
response to type I interferon
antigen processing and presentation
immune response
cellular response to interferon-gamma
immune effector process
innate immune response
defense response
P-value (log)
-58
-11
C
MVA SHIV Day 2
response to interferon-beta
response to type I interferon
cellular response to interferon-beta
response to interferon-alpha
cellular response to calcium ion
cellular response to type I interferon
cellular response to organic substance
defense response
immune effector process
cellular response to cytokine stimulus
innate immune response
response to organic substance
immune response
cellular response to chemical stimulus
defense response to protozoan
response to corticosterone
response to cAMP
response to cytokine
response to other organism
response to protozoan
response to purine-containing compound
P-value (log)
-35
-11
D
MVA SHIV Day 6
negative regulation of cell activation
antigen processing and presentation of peptide or polysaccharide antigen via MHC class II
B cell activation
positive regulation of immune system process
leukocyte differentiation
regulation of immune system process
immune system development
response to interferon-gamma
innate immune response
antigen processing and presentation
response to cytokine
defense response
P-value (log)
-34
-20
E
Day 1 DEGs
Virus
BST2
SAMHD1
TGTP1
LGALS9
DDX41
SHARPIN
CASP8
GM11127
IFI35
NMI
PSME1
PSMB9
PSME2
PSMB10
SP110
Virus
MVA SHIV
MVA-B13R SHIV
Log2
−1 −0.5 0 0.5 1
F
Hallmark Interferon Alpha Response
Day 1 vs Naive
Day 1 Immunizations
Naive
Running Enrichment Score
MVA SHIV (NES: 1.86, FDR: < 0.001)
MVA-B13R SHIV (NES: 1.62, FDR: 0.001)
0
Rank in Gene List
40505
G
Hallmark Interferon Alpha Response
MVA-B13R SHIV vs MVA SHIV
MVA-B13R SHIV
Day 1
MVA SHIV
Day 1
Running Enrichment Score
NES: -1.85, FDR: < 0.001
0
Rank in Gene List
40642
H
Hallmark IFN Alpha Response
Cumulative Leading Edge Genes
Virus
CXCL11
IFIT3
RSAD2
IFI44
ISG20
MX1
IFI44L
ISG15
USP18
UBE2L6
IFITM3
LAMP3
CXCL10
CMPK2
IFIH1
EIF2AK2
IRF7
RTP4
DHX58
BST2
IFIT2
PARP12
TMEM140
CASP1
HERC6
CCRL2
IFI35
DDX60
LY6E
GBP4
EPSTI1
GBP2
STAT2
PARP14
NMI
IFI30
SP110
PSME2
PSMB9
PSME1
RIPK2
IL7
IFITM1
PSMB8
OGFR
PARP9
SAMD9L
LGALS3BP
NCOA7
CASP8
LPAR6
B2M
TRAFD1
TDRD7
CD74
CD47
IFITM2
IRF1
TAP1
TRIM21
TRIM14
PNPT1
IRF9
TRIM25
UBA7
SLC25A28
IL15
PLSCR1
TRIM5
CSF1
MOV10
BATF2
ADAR
Virus
MVA SHIV
MVA-B13R SHIV
Log2
−1 −0.5 0 0.5 1

Figure 5. MVA-B13R/SHIV induces less robust type I and II interferon responses than MVA/SHIV. BALB/c mice were immunized intramuscularly with 10^7 pfu of MVA/SHIV or MVA-B13R/SHIV and draining inguinal lymph node cells were isolated at days 1, 2, and 6 after immunization (n = 5/group/time point) for RNA-Seq analysis. Lymph nodes from naïve mice (n = 5) isolated as controls. One sample (MVA/SHIV mouse, day 1) was not included in analysis as it mapped poorly to the reference sequence. (A) DEG analysis for mice at days 1, 2, or 6 after immunization compared to naïve mice. DEG criteria: log2 fold change of > 1.0 or < -1.0 and adjusted p-value of < 0.05. Enriched gene ontology (GO) processes associated with DEGs from MVA/SHIV versus naïve mice at (B) day 1, (C) day 2, and (D) day 6 were curated and visualized as a network. Colors are related to GO process p-values, node sizes are related to number of related processes, and edge widths are related to degree of similarity. (E) Heatmap depicting select DEGs from MVA/SHIV compared to MVA-B13R/SHIV mice at day 1 after immunization. DEG criteria: adjusted p-value of < 0.05. (F) Overlaid GSEA comparing MVA/SHIV day 1 mice to naïve mice (gray) and MVA-B13R/SHIV day 1 mice to naïve mice (red). (G) GSEA comparing MVA-B13R/SHIV mice to MVA/SHIV mice at day 1 after vaccination. (H) Heatmap of cumulative leading edge genes from GSEAs of (F) and (G). Heatmap colors represent the $\log_2$ fold change values relative to median value of each gene. NES, normalized enrichment score; FDR, false discovery rate. Leading edge genes are shown as black outlined dots.

CHAPTER III

Combination Anti-PD-1 and Anti-retroviral Therapy Provides Therapeutic Benefit Against SIV

Geetha H. Mylvaganam*[1,2,†], **Lynette S. Chea***[1,2], Gregory Tharp[2], Sakeenah Hicks[1,2], Vijayakumar Velu[1,2], Smita Iyer[1,2,†], Claire Deleage[3], Jacob D. Estes[3†], Steven E. Bosinger[2], Gordon J. Freeman[4], Rafi Ahmed[1,2], & Rama R. Amara[1,2]

[1]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA, USA; [2]Division of Microbiology and Immunology, Emory Vaccine Center, Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA; [3]AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, MD, USA; [4]Department of Medical Oncology and Cancer Vaccine Center, Dana Farber Cancer Institute, Boston, MA, USA;

*These authors contributed equally to this work.

**Originally published in *JCI Insight*.

Mylvaganam & Chea *et al*. Combination Anti-PD-1 and Anti-retroviral Therapy Provides Therapeutic Benefit Against SIV. *JCI Insight* 2018; 3(18):e122940.

DOI: 10.1172/jci.insight.122940

Available at: https://insight.jci.org/articles/view/122940

Abstract

Therapeutic strategies that augment anti-viral immunity and reduce the viral reservoir are critical to achieving durable remission of HIV. The co-inhibitory receptor programmed death-1 (PD-1) regulates CD8+ T cell dysfunction during chronic HIV and SIV infections. We previously demonstrated that *in vivo* blockade of PD-1 during chronic SIV infection improves the function of anti-viral CD8+ T cells and B cells. Here, we tested the immunological and virological effects of PD-1 blockade combined with anti-retroviral therapy (ART) in rhesus macaques. Administration of anti-PD-1 antibody 10 days prior to ART initiation rapidly enhanced anti-viral CD8+ T cell function and diminished interferon stimulated genes. This resulted in faster viral suppression in plasma and better Th17 cell reconstitution in the rectal mucosa following ART initiation. PD-1 blockade during ART resulted in lower levels of cell associated replication-competent virus. Following ART interruption, PD-1 antibody treated animals showed markedly higher expansion of proliferating CXCR5+ Perforin+ Granzyme B+ effector CD8+ T cells and lower regulatory T cells that resulted in better control of viremia. Our results show PD-1 blockade can be administered safely with ART to augment anti-viral CD8+ T cell function and reduce the viral reservoir leading to improved control of viral rebound after ART interruption.

Introduction

Chronic viral infections such as lymphocytic choriomeningitis virus (LCMV), simian immunodeficiency (SIV), and human immunodeficiency virus (HIV) present with a characteristic footprint of immune exhaustion largely due to the presence of persistent antigen and unresolved immune activation. An important signature of immune dysfunction is increased and sustained expression of the co-inhibitory receptor programmed cell death-1 (PD-1) on T cells, B cells, and activated monocytes (308, 311, 313, 522, 523). In chronic LCMV infection, antigen-specific CD8+ T cells maintain high levels of PD-1 expression that is associated with impaired polyfunctionality and proliferation. *In vivo* blockade of the interaction between PD-1 and its cognate ligands, either PD-L1 (B7-H1) or PD-L2 (B7-DC), markedly enhanced T cell responses and viral control (293). In chronically SIV infected rhesus macaques (RMs), we and others demonstrated that *in vivo* blockade of PD-1 augmented the function of anti-viral CD8+ T cells and B cells leading to reduced plasma viremia and prolonged survival (308, 328). In HIV+ individuals, PD-1 expression correlated with impaired HIV-specific CD4+ and CD8+ T cell function, served as a predictor of disease progression, and *in vitro* blockade of PD-1/PD-L1 interactions enhanced T cell function (311, 313). Additionally, *in vitro* PD-1 blockade of cells from HIV+ individuals restored anti-viral CD4+ T cell helper capabilities that enhanced HIV-specific NK cell functionality (524). Thus, augmenting cellular immunity by targeting the PD-1 pathway is of significant interest to HIV cure research as anti-viral CD8+ T cells are critical to control of HIV replication early after primary infection (105, 106, 132, 133, 525, 526) and under suppressive anti-retroviral therapy (ART) (134). In addition, there is considerable value in assessing the therapeutic effects of targeting the PD-1 axis during chronic HIV infection as clinical outcomes in cancer patients have been significant with highly durable anti-tumor responses being observed (527-530). However, anti-PD-1 therapy in HIV+ individuals remain in the early stages and is currently being tested only in patients presenting with HIV and cancer co-morbidities (531, 532) largely due to the lack of data on safety and therapeutic potential in a relevant animal model. It is also important to define the optimal dose and timing of PD-1 blockade with respect to ART in an effort to achieve maximal therapeutic benefit. Thus, there is a critical need for testing the safety and efficacy of

anti-PD-1 therapy for HIV infected individuals receiving ART using a pertinent pre-clinical animal model of SIV infected ART treated macaques.

Chronic HIV/SIV infection also presents with an aberrant increase in PD-1 expression on CD4+ T cells and this has been shown to highly correlate with plasma viremia and progressive disease (313, 320, 533). Furthermore, several studies have demonstrated that PD-1+ CD4+ T cells are highly infected, contribute to ongoing viral replication and production, and harbor a significant fraction of the HIV reservoir in patients under ART (164, 171, 260, 319). This latent viral reservoir is the major obstacle to functional cure studies that aim to establish durable remission of HIV in the absence of ART as the reservoir serves as the immediate source of viral recrudescence post treatment interruption. ART alone is not sufficient to eliminate the reservoir as the half-life is estimated to be about 44 months, indicating that approximately 70 years of ART is required for complete viral eradication (238). Current strategies in the field aim to eliminate the viral reservoir by using latency reversing agents (LRAs) to increase viral transcription in an effort to enhance immune surveillance and targeting of the reservoir, or to promote virus induced cytopathic effects. These intended immunological outcomes are collectively referred to as the "shock and kill" approach (248, 271). Cure strategies also include the use of LRAs in combination with immune-based therapeutics such as vaccinations or check-point blockade to augment immune function and enhance targeting and clearance of infected cells. It is currently not known what impact the check-point inhibitor PD-1 blockade will have on HIV-1 transcription within latently infected CD4+ T cells during ART. It is possible that PD-1 blockade may result in increased transcription of NF-κB, NFAT, and other factors required for T cell activation and consequently reactivation of latent HIV (534-537). If true, PD-1 blockade will be uniquely positioned to simultaneously serve as a latency reversing and immune enhancing agent to significantly improve anti-viral immunity and substantially reduce the viral burden.

In the current study, we aimed to determine the safety and therapeutic potential of *in vivo* PD-1 blockade in combination with ART to enhance anti-viral CD8+ T cell function and immune reconstitution and to

destabilize the latent SIV reservoir in chronically SIV-infected RMs. Our findings demonstrate that PD-1 blockade can be administered safely in combination with ART to augment anti-viral CD8+ T cell function, potentially disrupt the viral reservoir, and limit viral recrudescence post treatment interruption. These results have important implications for developing novel therapeutic interventions to administer in tandem with PD-1 blockade to better enhance anti-viral immunity and viral clearance in an effort to achieve durable remission of HIV.

Results

Study design

RMs enrolled in this study were previously vaccinated with a DNA/MVA SIV vaccine and challenged intra-rectally with SIVmac251 but failed to control infection (Supplemental Table 1). The criteria for enrollment was based on plasma viral load cut-offs of between $1x10^4$ and $5x10^5$ RNA copies/mL of plasma at week 24-post infection (Supplemental Figure 1). Animals were assigned to either the therapeutic or control arm after standardizing for the set point plasma viral loads at 24 weeks after infection, blood CD4+ T cell frequencies, rectal CD4+ T cell frequencies, and GagCM9+ CD8+ T cell frequencies for Mamu-A*01 RMs (Supplemental Figure 2A). Prior to the initiation of the trial, rectal central memory CD4+ T cell and SIV-specific CD4+ and CD8+ T cell frequencies were measured as baseline parameters and served to stratify treatment groups with comparable baseline immune parameters (Supplemental Figure 2B).

To determine the effects of PD-1 blockade in combination with ART and its potential to enhance anti-viral immunity and reduce viral reservoirs, we performed PD-1 blockade in two phases: I) prior to initiation of ART and II) during suppressive ART. The aim for phase I of PD-1 blockade was to restore T cell functionality in the presence of viral antigen, which may consequently aid in the elimination of virus infected cells. ART alone does not clear infected cells, but rather limits HIV/SIV replication by preventing production of new, infectious virions. However, by combining ART with PD-1 blockade, we aimed to simultaneously clear infected cells with functional anti-viral CD8+ T cells as well as potentially diminish the re-seeding of the established viral reservoir. To achieve this, we administered 5 doses of anti-PD1 Ab (3 mg/kg/dose) intravenously on days 0, 3, 7, 10, and 14 to 10 SIVmac251-infected RMs between 24-30 weeks after infection (Figure 1A). As a control, we treated 10 SIVmac251-infected RMs with saline. To allow repeated administrations of PD-1 Ab for longer periods of time, we primatized our anti-PD-1 Ab (clone EH12) by grafting humanized EH12 variable domains onto wild type rhesus

macaque kappa and IgG4 constant regions. We confirmed the activity of the primatized antibody *in vitro* using T cells obtained from chronically SIV-infected RMs (Supplemental Figure 3A).

For phase II of the study, our goal was to determine if PD-1 blockade could cause reactivation of the latent viral reservoir and further expand virus-specific CD8+ T cells while animals were under ART in an effort to detect and clear infected cells. In the lymph nodes (LNs), a major site of the persistent viral reservoirs and where low-level replication of SIV may be occurring, exhausted CD8+ T cells may be unable to clear the infected cells and would benefit from the effects of PD-1 blockade. To determine these effects, the 10 RMs given PD-1 Ab during phase I were again treated with PD-1 Ab (double treated) at 26-30 weeks following ART initiation. Three monthly infusions of PD-1 Ab were administered at 10mg/kg/dose (Figure 1A). To test the influence of PD-1 blockade administered only during suppressive ART, we split the 10 RMs from the saline group into two groups and gave 5 RMs PD-1 Ab (single treated group) and saline to the remaining 5 RMs (saline control group) (Figure 1A).

PD-1 blockade administered prior to ART improves T cell function

At day 3 following initiation of PD-1 blockade during phase I, plasma concentrations of the infused EH12 antibody reached 10-50 μg/mL that persisted until day 14 and declined by day 28 with one animal developing a measurable anti-EH12 response (Supplemental Figure 3B-C). We initiated ART in all animals at day 10 after the initiation of PD-1 blockade. Following administration of PD-1 Ab, we observed a significant induction in the proliferation of circulating CD4+ and CD8+ T cells as measured by Ki-67 expression that peaked around day 7 (Figure 1B). Both central memory (CD28+ CD95+, T_{CM}) and effector memory (CD28- CD95+, T_{EM}) CD4+ and CD8+ T cells showed induction of Ki-67 (Supplemental Figure 3D). Additionally, we observed an increase in the frequency of Ki-67 expressing CD4+ and CD8+ T cells in the rectal mucosa of PD-1 Ab treated RMs (Supplemental Figure 3E). Importantly, at day 10 of PD-1 blockade, we observed a significant increase in the frequency of SIV-specific IFN-γ and TNF-α producing CD4+ and CD8+ T cells (Figure 1C, Supplemental Figure. 3F). A

subset of animals in each group were Mamu-A*01+, which allowed us to assess the effects of PD-1 blockade on the function of SIV-specific CD8+ T cells utilizing the GagCM9 tetramer (Tet+ cells). We found a significant increase in the proportion of Tet+ cells expressing Ki-67, granzyme B, and CXCR5 indicating that these cells are actively proliferating with improved cytolytic and lymphoid follicle homing potential (Figure 1D, Supplemental Figure 3G). We also found an increase in granzyme B expression on CXCR5+ Tet+ cells ($p = 0.02$, data not shown). The increase in CXCR5 expression is consistent with our recent report demonstrating that CXCR5+ CD8+ T cells serve as the predominant CD8+ T cell subset that responds to PD-1 blockade during chronic LCMV infection (538). As expected, following initiation of ART, the frequency of proliferating total and SIV-specific T cells decreased, and this was associated with a decline in the frequency of SIV-specific CD4+ and CD8+ T cells and viral antigen.

PD-1 blockade prior to ART results in global enrichment of effector T cell responses and lowered immune activation

To determine the effects of PD-1 blockade on a global scale, we performed RNASeq to compare gene signatures from the blood of 10 PD-1 Ab treated and 5 saline treated animals at day 0 and day 10 of PD-1 blockade. Gene set enrichment analysis (GSEA) was performed to identify differentially expressed gene sets at day 10 compared to day 0 for each treatment group. We found significant enrichment in numerous gene sets related to immune cell phenotypes and cell signaling pathways that were not enriched in the saline treated group (Figure 1E, Supplemental Table 2-4). Gene signatures of note that were positively enriched in the PD-1 Ab treatment group were signatures associated with effector CD8+ T cell differentiation and E2F transcription factor targets related to cell cycling, indicating an impact of PD-1 blockade on effector CD8+ T cell function and proliferation (Figure 1E-F, Supplemental Figure 4A, B).

PD-1 blockade during chronic infection also led to a downregulation of multiple gene sets associated with interferon stimulated genes (ISGs) and TNF-α signaling (Figure 1E-F, Supplemental Figure 4A, B). The downregulation of ISGs is notable since it has been shown to be associated with slower disease

progression and lower viral reservoirs during chronic HIV/SIV infections (520, 539-542). Moreover, these findings suggest that PD-1 blockade-induced dampening of ISGs may contribute to lower immune activation and better immune reconstitution after the initiation of ART. Reduction in overall immune activation is also indicated by negative enrichment of the TNF-α signaling via NF-kβ gene set in the PD-1 Ab treated RMs compared to saline treated (Figure 1E, Supplemental Figure 4A). Taken together, these gene sets indicate the unique potential of PD-1 blockade to simultaneously enhance effector CD8+ T cell responses while dampening systemic immune activation observed during chronic pathogenic SIV infection (543).

We also sought to compare differences in gene expression of specific T cell related genes (Supplemental Figure 4C) and observed an upregulation of transcripts associated with T follicular helper cells (Tfh) such as *RGS10, CD200, FGF2, ICOS, TNFRSF4,* and *IL21,* T helper 2 cells (Th2) such as *IL4,* and regulatory T cells (Tregs) such as *CTLA4*. Collectively, these results demonstrate that PD-1 blockade during chronic SIV infection resulted in a significant enhancement of genes associated with proliferation and effector CD8+ T cell response and dampening of a type I IFN response.

PD-1 blockade prior to ART improves viral suppression and immune reconstitution in the gut following ART initiation

Impressively, following initiation of ART, we observed an increase in the kinetics of viral suppression in the PD-1 Ab treated RMs compared to saline treated RMs. All animals in the PD-1 Ab group suppressed virus (<100 copies/mL) by 42 days whereas saline treated animals required up to 168 days to achieve the same level of suppression (Figure 2A-C). Remarkably, 60% of RMs in the PD-1 blockade group suppressed viremia by 10 days following ART initiation whereas only 20% of animals in the saline group showed viral suppression at this time. Similar to these results, we observed enhanced viral suppression following PD-1 Ab administration and ART in another study, in which we treated SIVmac251-infected RMs with our humanized version of the PD-1 Ab (Figure 2D). The same time course for PD-1 blockade

and ART regimen was utilized in this study; however, ART was initiated approximately a year after SIV infection. In this study, ART was only partially effective as 3 of the 4 RMs in the ART-only group failed to control viremia (Figure 2D). Impressively however, all 4 animals in the PD-1 Ab group showed profound viral suppression suggesting that PD-1 blockade synergized with ART even in long-term chronically SIV-infected RMs where ART suppression can be challenging. In both the pilot and present trial, we utilized a suboptimal ART regimen when compared to current treatment modalities that include an integrase inhibitor (134, 281, 544). It is however impressive that PD-1 blockade resulted in more rapid viral suppression when ART was initiated either at 26-30 weeks (current trial) or one year post infection (pilot trial). These data demonstrate that PD-1 blockade is effective in augmenting viral suppression when combined with ART.

In addition to improved viral suppression, PD-1 blockade also resulted in early reconstitution of T_{CM} CD4+ T cells (Figure 2E) and better reconstitution of Th17 cells (Figure 2F) in the rectum, both of which have been shown to be critical for the long-term survival of SIV-infected RMs by helping to maintain the GI tract epithelial barrier function and decrease microbial translocation and hyperimmune activation (150, 545-547). Consistent with this, at 36 weeks of ART, PD-1 blockade animals had lower frequencies of myeloperoxidase-expressing neutrophils in the lamina propria than the saline group, indicating PD-1 blockade aided in the restoration of the GI tract epithelial barrier and reduced microbial translocation in the rectal mucosa in the treated animals (Figure 2G). Taken together, these data demonstrate that administration of PD-1 blockade for a short duration just prior to ART initiation improves the functional quality of SIV-specific T cell responses, enhances potency of ART, and better restores mucosal Th17 cells.

PD-1 blockade during ART results in T cell proliferation and higher granzyme B+ CXCR5+ T cells in LN

During phase II, we saw the concentrations of infused EH12 antibody in the serum peak at about 100□g/mL at day 7 after each infusion with 3 double treated animals and 1 single treated animal showing an anti-EH12 response (Supplemental Figure 5A-B). Additionally, we performed complete blood count analysis as well as measured biochemical markers in the blood at day 0, week4, and week 8 of phase II. We observed subtle yet significant changes in some biochemical parameters at week 4 and week 8 in the treated animals though the values largely remained within the normal ranges expected for RMs (Supplemental Figure 6-7).

Following the first PD-1 Ab infusion under suppressive ART, we observed a significant increase in proliferation of CD4+ T cells in the double and single PD-1 treated groups (Figure 3A) and this proliferation was observed for both T_{EM} and T_{CM} CD4+ T cells (Supplemental Figure 5C). CD8+ T cells and Tet+ cells also showed increased proliferation (Figure 3A). However, the proliferation was transient and sporadic following the second and third infusions (Supplemental Figure 5D). Intracellular cytokine analysis revealed an increase in SIV-specific CD8+ T cell responses in some animals after the first infusion (Supplemental Figure 5E). The T cell proliferation observed during phase II was much lower compared to proliferation observed during phase I. However, we think this is significant considering the low burden of SIV antigens during ART suppression. These results demonstrate that PD-1 blockade during suppressive ART can transiently induce proliferation of CD4+ T cells and total and anti-viral CD8+ T cells.

To understand the influence of PD-1 blockade in the lymphoid tissue, we took LN biopsies 2 weeks after the final PD-1 Ab infusion and determined the granzyme B and CXCR5 expression on total CD8+ T cells and Tet+ CD8+ T cells. We observed a higher magnitude of granzyme B+ CD8+ T cells and CXCR5+ Tet+ CD8+ T cells in PD-1 treated RMs compared to saline controls suggesting these virus-specific and

cytolytic cells may be localizing to sites known to harbor the viral reservoir (Figure 3B). We also observed an increased frequency of total CXCR5+ CD4+ T cells within the LN suggesting better preservation of follicular CD4+ T cells in the PD-1 treated animals (Figure 3B). These changes were not observed in the peripheral blood compartment highlighting the tissue specific effects of PD-1 blockade during ART (Supplemental Figure 5F).

PD-1 blockade during ART reduces the inducible viral reservoir and stimulates anti-viral cellular response pathways in blood

To assess if PD-1 blockade impacted viral reservoirs, we isolated CD4+ T cells from the peripheral blood of RMs two weeks following the last PD-1 Ab infusion and co-cultured them with CCR5+ CEM cells to detect viral outgrowth as to indicate the presence of inducible replication-competent virus similar to what has been previously described (548, 549). We found that PD-1 treated animals had measurably lower replication-competent virus assayed by viral RNA in the culture supernatant and p27 staining (Figure 3C-E). Most PD-1 treated animals did not have measurable viral RNA within 500,000 purified CD4+ T cells, therefore we did not perform further dilutions.

To determine if PD-1 blockade was capable of reactivating latent viral reservoirs, we measured plasma viral RNA weekly after each infusion and observed transient increases (blips) in the double and single treated groups (Supplemental Figure 5G-H). Though not significant due to limited power in the study and inability for daily sampling, the frequency of transient increases in plasma viremia observed in the PD-1 treated groups was higher compared to the saline alone, indicating a potential disruption of the viral reservoir. Taken together, these data demonstrate that PD-1 blockade administered under suppressive ART could potentially destabilize the inducible replication-competent viral reservoir and result in a lower viral burden within peripheral blood CD4+ T cells.

To further understand the global transcriptome changes that occur following PD-1 blockade during ART, we again compared gene signatures in the blood of PD-1 Ab treated RMs from day 0 of first PD-1 Ab infusion under ART and 7 days post administration. Due to the limited sample availability for RNASeq during phase II, we combined the double and single treated animals into a single group to determine changes that occurred in response to PD-1 blockade treatment during ART (Figure 4A, Supplemental Table 5-7). Gene sets for effector CD8+ T cells and cell cycling were again positively enriched after PD-1 blockade (Figure 4A-B, Supplemental Figure 8). Interestingly, ISGs were upregulated during this second phase of the PD-1 trial, which is in contrast to being downregulated during phase I (Figure 4A-B, Supplemental Figure 8). In addition, gene sets associated with IL-2/STAT5 signaling, which has been implicated in viral reactivation (550) as well as oxidative phosphorylation also showed significant increases following PD-1 blockade (Figure 4A). Collectively, the transcriptome changes pointed to an active state of cell proliferation and transcription, which could promote reactivation of latent SIV in viral reservoirs resulting in potential increases in plasma viremia observed during phase II (Supplemental Figure 5G-H).

To determine if there was a core set of genes that responded to PD-1 blockade during both phase I and II, we curated a list of positively enriched gene sets that were significant during both phases and performed a leading edge analysis (Supplemental Table 8). Our criterion for a signature gene was presence in at least 7 gene sets (Figure 4C). Many of the genes were related to cell cycling and proliferation, which is in line with the proliferative burst observed in T cells during both phases (Figure 1B, 3A). These genes are considered the main drivers of the gene set enrichment observed for PD-1 blockade both in the absence and presence of ART.

PD-1 blockade enhances viral control in double treated RMs post ART interruption

To better understand the therapeutic benefit of PD-1 blockade, we interrupted ART in all animals two weeks after the 3rd PD-1 Ab infusion. We assessed the kinetics of viral rebound and various immune

parameters until 24 weeks post treatment interruption. The virus rebounded in all animals by 3 weeks; however, consistent with lower viral reservoirs assessed by our viral outgrowth assay, we observed a significant one-week delay in viral rebound in the PD-1 Ab treated compared to the saline treated group (Figure 5A-B). Virus rebounded in 4 of the 5 (80%) saline treated controls by one week, but only 3 of the 15 (20%) PD-1 treated animals showed viral rebound at this time. Additionally, we monitored the time until animals reached a set point viral load similar to pre-ART levels (within 5-fold). PD-1 Ab treated animals demonstrated a delay in approaching their pre-ART viral loads compared to saline control animals, with some PD-1 treated animals never attaining that pre-ART levels (Figure 5C-D). All saline controls reached their pre-ART viral loads by 8 weeks post ART interruption, showing no evidence of improved viral control. At 8 weeks following ART interruption, 5 of the 10 double treated animals and 4 of the 5 single treated animals showed 6-80 fold reduction of viral set points compared to pre-ART levels (Figure 5C). At 24 weeks, 5 out of 10 double treated animals sustained control, but only 1 of the 5 single treated animals did. We believe this level of viral control in PD-1 treated animals is substantial since we initiated ART 6 months after infection by which time it is expected the virus would have accumulated a significant number of escape mutations due to immune pressure.

PD-1 treated animals show enhanced poly-functional CXCR5+ CD8+ T cells and lower Tregs after ART interruption

Analysis of CD8+ and CD4+ T cell responses in the blood early after ART interruption showed a profound increase in the frequency of proliferating total CD8+ T cells for PD-1 Ab treated versus saline groups (Figure 6A, Supplemental Figure 9A). Proliferating CXCR5+ CD8+ T cells, perforin+ CD8+ T cells (Figure 6A, Supplemental Figure 9A), and polyfunctional (IFNγ+ TNFα+) SIV-specific CD8+ T cells (Figure 6B, Supplemental Figure 9B) were found to be significantly increased in the double PD-1 Ab treated compared to saline treated RMs. The double PD-1 Ab treated RMs also demonstrated a higher level of proliferating CXCR5+ CD4+ T cells (Supplemental Figure 9C) and higher T_{CM} CD4+ T cells in the peripheral blood (Figure 6C, Supplemental Figure 9D). Remarkably, we also observed a decrease in

the frequency of Tregs in the double treated group compared to saline controls (Figure 6D). This resulted in 10 times higher levels of perforin+ CD8+ T cells compared to Tregs in the double treated group compared to 5 times higher levels in saline controls (Figure 6E). Taken together these data indicate enhanced immune function in the double treated animals post ART interruption. A Boolean analysis performed on samples acquired at 3 weeks post treatment interruption revealed that the double treated RMs generated higher frequencies of CXCR5+ T_{EM} CD8+ T cells indicating enhanced cytolytic and lymphoid follicle homing potential of anti-viral CD8+ T cells to eliminate virally infected cells within secondary lymphoid organs (Figure 6F, Supplemental Figure 9E). Interestingly, the majority of these immunological benefits were not significantly better in single treated animals compared to controls except for the increase in perforin+ CD8+ T cells (Figure 6A, Supplemental Figure 9A) and the ratio of perforin+ CD8+ T cells to Tregs (Figure 6E). This could also be because of smaller number of animals in this group.

Interestingly, the frequencies of LN granzyme B+ CD8+ T cells on the day of ART interruption correlated with the frequencies of perforin+ CD8+ T cells observed at 4 weeks after ART interruption (Figure 6G). LN granzyme B frequencies also trended toward a significant correlation with the viral load fold change at week 8 of ART interruption (Figure 6H). The data indicate that the anti-viral capacity of CD8+ T cells as detected in the LN could play an important role in viral control after interruption of ART. These associations underscore the importance of understanding the cellular dynamics in the lymphoid tissues during blockade and how it will influence post interruption responses. Taken together, these data demonstrate that PD-1 blockade combined with ART provides therapeutic benefit following ART interruption that includes a profound expansion of CD8+ T cells with increased cytolytic and lymphoid follicle homing potential.

Discussion

It is the goal of HIV cure research to develop therapies that can both improve the anti-viral immune response and simultaneously eliminate the viral reservoir. It is well understood that PD-1 regulates CD8+ T cell dysfunction during chronic HIV and SIV infection and our group has previously shown that blockade of the PD-1 pathway in ART-naïve, chronically SIV-infected RMs is effective at improving the anti-viral function of CD8+ T cells. Additionally, CD4+ T cells that express PD-1 represent a significant fraction of the HIV and SIV reservoir. Therefore, in this current study, we aimed to test the safety and immune potential of PD-1 blockade in conjunction with ART to improve CD8+ T cell function and reduce the viral reservoir that may lead to better control of SIV infection in the absence of ART. Our results show that PD-1 blockade can be safely and effectively combined with ART to enhance the function of anti-viral immunity, improve Th17 cell reconstitution in the rectal mucosa, potentially subside mucosal associated inflammation, and reduce the inducible peripheral blood viral reservoir. The results also demonstrate that the enhanced immune function mediated by PD-1 blockade can lead to higher and faster expansion of polyfunctional CXCR5+ CD8+ T cells after ART interruption and contribute to better control of reemerging viremia. These results reveal important immunological and virological benefits as well as global transcriptome changes following PD-1 blockade and suppressive ART during chronic SIV infection that are important for the design of PD-1 blockade studies in HIV-infected ART treated patients.

The mechanism by which ART suppresses viremia is by preventing the production of new virions and subsequent infection of new target cells. ART however does not eliminate infected cells. Therefore, the faster reduction in plasma viremia observed during phase I in the PD-1 treated animals could be a result of anti-viral CD8+ T cells that emerged following PD-1 blockade with an enhanced ability to detect and clear productively infected cells. This is consistent with other studies demonstrating that CD8+ T cells play a pivotal role in controlling viremia even under conditions of suppressive ART therapy (134). PD-1 blockade could also have resulted in activation induced cytopathic effects of infected CD4+ T cells,

decreasing the overall half-life of an infected CD4+ T cell and resulting in a more rapid decrease in plasma viremia.

Impressively, PD-1 blockade prior to ART initiation resulted in a profound downregulation of ISGs within 10 days while improving T cell function. The downregulation of ISGs is unlikely due to a decrease in viremia since only a small fraction of animals showed a measurable decrease in viremia during this first 10 days following PD-1 blockade. HIV infection has been shown to induce ISGs in monocytes (551-553) and it is possible that PD-1 blockade on monocytes prevented ISG induction leading to our observed downregulation of ISGs (Figure 1E-F). This would suggest that cytolytic anti-viral T cells that were increased during the same period are the main mediators of the rapid suppression of plasma viremia observed. The decrease in ISGs after PD-1 blockade remains notable as lower levels of ISGs is associated with slower disease progression and reduced viral reservoirs during chronic HIV infection (520, 539-541).

PD-1 blockade administered during suppressive ART had a less pronounced impact on T cell proliferation and function when compared to blockade given prior to the initiation of ART. In comparison to phase I, we observed a blunted increase in T cell proliferation for phase II, however the baseline levels of Ki-67+ T cells at day 0 of phase II were already lower than day 0 of phase I. Regardless of this, we still observed a 2-3 fold induction of proliferation after the first PD-1 Ab infusion during phase II. The very low levels of systemic viral antigen present during this phase of treatment, as well as a potential reduction in overall PD-1 expression due to lower level of persistent antigen and continuous ART (320), likely contributed to less significant levels of cell cycling and response to PD-1 blockade. It is possible that at sites enriched in SIV such as the GALT and secondary lymphoid tissues, immune responses could have emerged after blockade that were not captured in the periphery. This is supported in part by the increased frequencies of granzyme B+ CD8+ and CXCR5+ Tet+ CD8+ T cells in the LN suggesting better homing potential of effector cells to the lymphoid tissues (site of viral reservoir) that was not observed systemically.

Our data suggests that PD-1 blockade administered during ART led to a potential destabilization of the viral reservoir. Transcriptional analysis during phase II indicated an upregulation of genes involved in effector CD8+ T cell activation, as expected, as well as cell cycling and type I interferon response genes. It is possible that the increase in cell cycling genes was due to PD-1 blockade inducing reactivation of PD-1+ CD4+ T cells harboring latent virus, thus leading to viral replication, and the observed transient increases in plasma viremia. This is consistent with a recent study demonstrating the PD-1 contributes to the establishment and maintenance of latently infected cells and blocking this pathway in HIV infected individuals resulted in increased cell associated HIV RNA in CD4+ T cells (554). Subsequently, type I interferons genes were upregulated during phase II suggesting that type I IFN could be acting directly on latently infected CD4 T cells, re-activating virus, resulting in the observed plasma 'blips' or PD-1+ CD4 T cells in response to PD-1 blockade have an altered intracellular environment skewing away from latency and towards active viral transcription. Although we observed 'blips' in the saline treated animals, these changes were small and occurred once in 2 of the 4 animals. Furthermore, due to the scheduling of sampling, destabilization of the reservoir and significant increases in plasma viremia may have occurred at earlier time-points post infusion of PD-1 Ab and were therefore not captured by weekly sampling. Taken together, these data suggest that PD-1 blockade contributed to an overall improvement in the anti-viral immune and a potential destabilization of the latent reservoir. Due to the fact that proliferation of T cells was more profound in phase I compared to phase II and that both treatment groups exhibited viral outgrowth during phase II, it is possible that majority of immune related effects of PD-1 blockade on the function of anti-viral CD8 T cells occurred during phase I and PD-1 blockade under suppressive ART largely effected PD-1+ CD4+ T cells.

Disruption of the PD-1 pathway can be achieved through blockade of the PD-1 receptor or its ligands PD-L1 or PD-L2. Anti-PD-L1 therapy is currently in clinical use for reversing immune exhaustion in patients with cancer and has led to similar responses and toxicity profiles as those observed with anti-PD-1 blockade (555). In chronic infection, PD-L1 is highly expressed on antigen presenting cells while PD-1

expression is increased and sustained on dysfunctional T cells. Anti-PD-1 directly inhibits the interaction of PD-1 on T cells with its cognate ligand while anti-PD-L1 inhibits this interaction indirectly, without impacting PD-L2 engagement of PD-1. This would not allow for complete interruption of the PD-1 axis. Furthermore, the clone of antibody used for blockade could likely influence blockade efficacy. As the use of immune checkpoint inhibitors is investigated, it will be important to discern whether PD-1 or PD-L1 blockade results in differential outcomes and toxicities and to identify optimal clones for therapeutic use.

It is important to note that all RMs in the study had been chronically infected with SIVmac251 for 24-30 weeks, well into the chronic and progressive stage of infection before any intervention was administered. This length of chronic infection likely led to significant accumulations of viral mutations and viral escape in the animals. The chronicity and diversity of quasi-species in these animals would have made it difficult for any immune-based intervention to successfully eradicate the viral reservoir. That we were able to see improved anti-viral CD8+ and CD4+ T cell responses, durable control of viremia under suboptimal ART, a delay in viral rebound, and differences in the set-point viral loads post ART interruption is encouraging as individuals infected with HIV are now typically able to begin ART therapy during the acute or early chronic stages, before extensive chronic hyperimmune activation, exhaustion, and viral escape have occurred.

The range of immunological and therapeutic outcomes seen after PD-1 blockade treatment in our RMs is not surprising as resistance to immune checkpoint inhibitor strategies in cancer patients has been well-documented (556). Recently, Routy *et al*. described the influence of gut dysbiosis on PD-1 blockade efficacy (557). In particular, the authors show that use of antibiotics compromised PD-1 blockade efficacy in tumor models and cancer patients and the presence of certain commensal bacterial species improved responsiveness to blockade. In our previous study where PD-1 blockade was administered to chronically infected RMs in the absence of ART, we observed enhanced immune responses against gut-resident pathogenic bacteria (328). It is possible that anti-PD-1 enhanced responses against the microbiota in our

current study and perturbed microbial composition in a way that could influence subsequent immune responses. Future analysis on the influence of the microbiota on PD-1 blockade efficacy in SIV infection would be informative to understand how the microbiota shapes immune dynamics during immune check-point blockade.

The results of this study provide preliminary evidence that PD-1 blockade can significantly augment anti-viral immune responses, synergize with ART increasing the kinetics of viral suppression, and potentially destabilize latent viral reservoirs. Although PD-1 blockade's effects suggest that this treatment may not stand alone as a monotherapy, the development and administration of therapeutic strategies in combination with PD-1 blockade could potentially lead to a significant decrease in the overall viral burden and potential remission of HIV. Combination immunotherapies that target additional check-point inhibitory receptors (CTLA-4, Tim-3, and Lag3) (261, 544) known to contribute to HIV persistence, in tandem with PD-1 blockade and ART would also have the potential to more significantly reverse dysfunction of exhausted cells and impact the stability of the viral reservoir. *In vitro* blockade of PD-1 and IL-10 signaling was shown to restore CD4+ T cell and NK cell functionality thus combination PD-1 and IL-10 blockade to improve immune cell reinvigoration during chronic infection would be of interest (524). Additionally, a recent study utilized a broad caspase inhibitor during acute infection and demonstrated better memory CD4+ T cell preservation and viral control (558). It would be interesting to investigate PD-1 therapy with the caspase inhibitor to assess whether synergy can be achieved. Additionally, co-administration of check-point inhibitors with TLR adjuvants, recently shown to impact post ART interruption viral control (281), or co-stimulatory molecules could further enhance virus-specific immune responses aiding in viral clearance and immune control of HIV.

In conclusion, this study establishes the groundwork for pre-clinical studies to assess the efficacy of PD-1 blockade in ART suppressed HIV infected individuals. PD-1 blockade therapy is currently limited to end-stage cancer patients' due to the risk versus benefits of co-inhibitory blockade in HIV infected patients

tolerating long-term ART. Non-human primate studies such as these helps establish the immunological and virological benefits of PD-1 blockade prior to human clinical trials. Our study demonstrates that PD-1 blockade and ART combination therapy can effectively reshape immune dynamics during chronic SIV infection establishing a path forward in identifying optimal therapeutic strategies for HIV cure research.

Material and Methods

Study group. Twenty-two Indian rhesus macaques (Macaca mulatta) chronically infected with SIVmac251 for 24-30 weeks with stable set point viremia were used for the study. These macaques were previously vaccinated with a DNA/MVA SIV vaccine and challenged intra-rectally with SIVmac251 at a dose of 647 $TCID_{50}$ (1.25 x 10^7 copies of viral RNA) but failed to control infection (Supplemental Table 1). Two animals of the single treated group died due to thrombocytopenia and AIDS related complications, thus only 5 single PD-1 Ab animals were carried through to study completion. Data for the 2 macaques that died during the study were not presented. One saline control RM was interrupted from therapy prior to the initiation of Phase II due to significant weight loss during suppressive ART and thus data was not collected for that time period. RMs with plasma viral loads between $1x10^4$ and $5x10^5$ RNA copies/mL in plasma at week 24-post infection were enrolled in the study. ART was initiated with the following combination of drugs: AZT (5mg/kg, twice daily), PMPA (20mg/kg, daily), and FTC (30mg/kg, daily) were administered subcutaneously, and Kaletra (12+3mg/kg, twice daily) was administered orally.

In vivo antibody treatment. Macaques were infused with 25 mL of primatized anti-PD-1 antibody (clone EH12-2132/2133) in saline or 25 mL of saline alone. The anti-PD-1 monoclonal antibody (mAb) is derived from the mouse anti-human EH12 mAb (559) and has a humanized variable heavy chain domain linked to rhesus macaque IgG4 and a humanized variable light chain domain linked to rhesus macaque Kappa (rhesus macaque constant regions were a kind gift from Rijan Wang and Keith Reimann, Univ Massachusetts Medical School). The EH12 mAb binds to rhesus macaque PD-1 and blocks interaction between PD-1 and its ligands *in vitro* (522). Antibody was produced in CHO cells using DHFR amplification (560), purified from culture supernatants by Protein G affinity chromatography, and verified to have an endotoxin level less than 2 EU/mg. Anti-PD-1 antibody was administered intravenously at 3 mg/kg of body weight on days 0, 3, 7, 10, and 14 during Phase I and at 10 mg/kg monthly for three months during Phase II.

Isolation of mononuclear cells. Mononuclear cells were isolated from the blood, LN, and rectal tissue of RMs, and flow cytometry analysis was performed as previously described (308, 522, 561).

Antibodies. The following antibodies were used: CXCR5 (MU5UBEE; eBioscience), CD3 (SP-34-2; BD Biosciences), Gag-CM9 Tetramer (courtesy of the laboratory of Rafi Ahmed), CD28 (CD28.2; eBioscience), CD95 (DX2; BD Biosciences), CD279 (PD-1; EH12.2H7; BioLegend), CD8 (SK1; BD Bioscience), Live Dead-near IR stain (Invitrogen), Ki-67 (B56; BD Biosciences), CD4 (L200; BD Biosciences), CD25 (BC96; BioLegend), Granzyme B (GB11; BD Biosciences), IFN-γ (B27; BD Biosciences), TNF-α (MAb11; BD Biosciences), Perforin (Pf-80/164; BD Biosciences), IL-17A (ebio64-Dec17; eBioscience), and FoxP3 (206D; BioLegend).

Phenotyping. Mononuclear cells isolated from the blood and rectum as previously described(308, 522). Mononuclear cells were stained with LIVE/DEAD Near-IR Dead Cell stain (Life Technologies) and samples were acquired on an LSR-Fortessa. Cells stained for regulatory T cell phenotyping were stained for surface markers as described above and then fixed and permeabilized with eBioscience FoxP3/Transcription Factor Staining Buffer Set according to manufacturer's protocols.

Intracellular cytokine stimulation and staining. Mononuclear cells isolated from the blood and rectal samples were stimulated and stained as previously described (308, 522). Cells were stimulated with PMA (80ng/mL) and ionomycin (1ug/mL) for 4 hours and then surface stained with Live/Dead near IR, anti-CD8 and anti-CD4 at room temperature for 20 min. Cells were acquired on an LSR-Fortessa and analyzed using the FlowJo software (Treestar Inc. CA).

Viral Quantification

The SIV copy number in the plasma was determined by using a quantitative real-time PCR as previously described (562). All samples were extracted and amplified in duplicate and the mean of the two values were then reported.

RNA-Seq

RNA-Seq analyses were conducted at the Yerkes NHP Genomics Core on PD-1 Ab (n = 10) and saline treated (n = 5) RMs during Phase I and total PD-1 Ab treated RMs (n = 9) during Phase II. RNA was collected and extracted from PAXgene tubes using on- column DNase digestion as described previously (520) and assessed for integrity and quantity using an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA) and a NanoDrop 2000 spectrophotometer (Thermo Scientific Inc., Wilmington, DE). Hemoglobin transcript depletion was performed using Ambion human GLOBINclear kits (Ambion/Thermofisher, Waltham, MA) using 1 ug of RNA as input, according to manufacturer's instructions. Libraries were prepared using the Illumina (Illumina Inc. San Diego, CA, USA) TruSeq™ mRNA stranded kit as per manufacturer's instructions. Briefly, 500-1000 ng of Globin depleted RNA was used for library preparation. ERCC synthetic spike-in controls 1 or 2 (Ambion) were added to each Total RNA sample and processed in parallel. Amplified libraries were validated using the Agilent 4200 TapeStation and quantified using a Qubit flourometer. Libraries were normalized, pooled followed by clustering on a HiSeq3000/4000 flowcell using the Illumina cBot. The clustered flowcell was then sequenced on the Illumina HiSeq3000 system employing a single-end 101 cycles run, with multiplexing to achieve approximately 20 M reads per sample.

RNA-Seq Statistical Analyses

RNA-Seq data were aligned to the MacaM v7.8 assembly of the Indian rhesus macaque genome (available at https://www.unmc.edu/rhesusgenechip/index.htm) Alignment was performed using STAR v2.4.0g1 using the annotation as a splice junction reference. Transcripts were annotated using the MacaM

v7.8.2 annotation. Transcript abundance was estimated using htseq-count v0.6.1p1 and differential expression analyses were performed using DESeq2. Gene set enrichment analysis (GSEA) was performed using the desktop module available from the Broad Institute (https://www.broadinstitute.org/gsea/).

Viral Outgrowth Assays

Replication-competent SIV was cultivated from CD4+ T cells isolated from the peripheral blood of RMs at 14 days after last PD-1 Ab infusion. 5 x 10^5 isolated CD4+ T cells were pre-activated with anti-CD3/CD28 beads (Miltenyi, Non-human primate T cell expansion kit) for 24 hours and subsequently co-cultured with CEM.NK^R CCR5+ Luciferase+ CD4+ cells (CEM cells were a kind gift from David Evans, Univ of Wisconsin) in 24-well plates at a 1:1 ratio. Cells were co-cultured for 25 days and supernatants were harvested at day 9, 17, and 25. SIV RNA in the supernatant was determined using qRT-PCR as previously described(562).

Immunohistochemistry (IHC) and quantitative analysis of Lamina Propria (LP) from the rectal mucosa.

IHC and quantitative analysis for the frequency of myeloperoxidase+ neutrophils in the LP was performed as previously described (563).

ELISA for Anti-PD-1 (EH12) Ab Serum Titers

To measure the levels of infused PD-1 antibody, plates were coated with human PDCD1/PD-1 protein (Sino Biological, catalog number: 10377-H-8H-50) in PBS, blocked and incubated with different dilutions of plasma to capture the infused anti-PD-1 antibody. Bound antibody was detected using anti-rhesus IgG conjugated to HRP. Known amounts of anti-PD-1 antibody captured in the same manner were used to generate a standard curve.

ELISA for Anti-EH12 Ab Response

To measure the level of antibody response generated against the infused EH12 antibody, plates were coated with the EH12 antibody (2.5 μg/mL) in PBS overnight. 1:50 dilutions of plasma samples were incubated followed by mouse anti-rhesus IgG1-Biotin (7H11, NHP Reagent Resource) to detect bound antibody. This detection antibody does not bind to the infused anti-PD-1 antibody due to its rhesus IgG4 heavy chain constant region. Horseradish peroxidase-streptavidin was added, TMB substrate (KPL) was used for development, reaction was stopped with 1N H_3PO_4, and read at 450nm.

Statistical analysis. Statistical analyses were performed using Prism (version 7.0b; GraphPad). Statistical significance was determined using two-tailed paired Student's t-test for comparisons between matched time points for each animal. Two-way repeated measures ANOVA with Dunnett's multiple comparisons test was used to compare longitudinal data to baseline time points or Sidak's multiple comparisons test was used to compare one time point to baseline values. One-way ANOVA with Dunn's multiple comparisons test was used to compare two groups to a control group. Kaplan-Meier curves were compared using log-rank Mantel-Cox tests. Two-tailed unpaired Mann-Whitney test or Student's t-test with Welch's correction were performed for unmatched samples. Statistical analyses of global cytokine profiles were performed by partial permutation tests using SPICE software (NIAID, NIH) as previously described (564). P values < 0.05 was considered significant.

Study Approval

Macaques were housed at the Yerkes National Primate Research Center and were cared for under guidelines established by the Animal Welfare Act and the NIH 'Guide for the Care and Use of Laboratory Animals' using protocols approved by the Emory University IACUC (Atlanta, GA).

Data availability

RNA-Seq dataset is available at the GEO repository under accession # GSE111435**.**

The data that support the findings of this study are available upon request from the corresponding author.

Author Contributions

G.H.M. and L.S.C. performed experiments, analyzed data, and wrote the manuscript. G.H.M. coordinated the study. G.T. and S.E.B. analyzed RNASeq data and discussed data interpretations. S.H. performed sample processing and data analysis. V.V. performed the pilot PD-1 blockade study using the humanized PD-1 Ab and contributed to the design of the current study. S.I. performed initial SIVmac251 infections on RMs prior to treatment initiation. C.D. performed immunohistochemistry analysis. J.D.E. provided helpful discussions regarding immunohistochemistry and data analysis. G.J.F. synthesized primatized PD-1 Ab and provided helpful discussions regarding the study design. G.H.M., R.A., and R.R.A., contributed to the study design and data interpretation throughout the study. R.R.A supervised the study and wrote the manuscript.

Acknowledgements

We thank the veterinary staff at Yerkes for animal care; CFAR virology core for viral RNA and qPCR analysis; and CFAR immunology core for help with flow cytometry. We thank Rijan Wang and Keith Reimann at the University of Massachusetts Medical School in Worcester, MA for the rhesus macaque constant regions of the IgG4 used in EH12 (anti-PD-1) monoclonal antibody. We thank David Evans at the University of Wisconsin in Madison, WI for the CEM.NK^{R} CCR5+ Luciferase+ CD4+ cell line. We thank Nirav Patel at the Yerkes NHP Genomics Core for the sample processing, library preparation, and sequencing. This work was supported by the NIH grants R01 AI074471, R01 AI071852 and RC2 CA149086 to RRA; YNPRC base grant, P51 RR00165; Emory CFAR grant, P30 AI050409.

Author Information

The authors declare the following competing interests: Rafi Ahmed, Gordon Freeman, and Rama Amara are co-inventors of PD-1 technology that has been licensed to Genentech by Emory University.

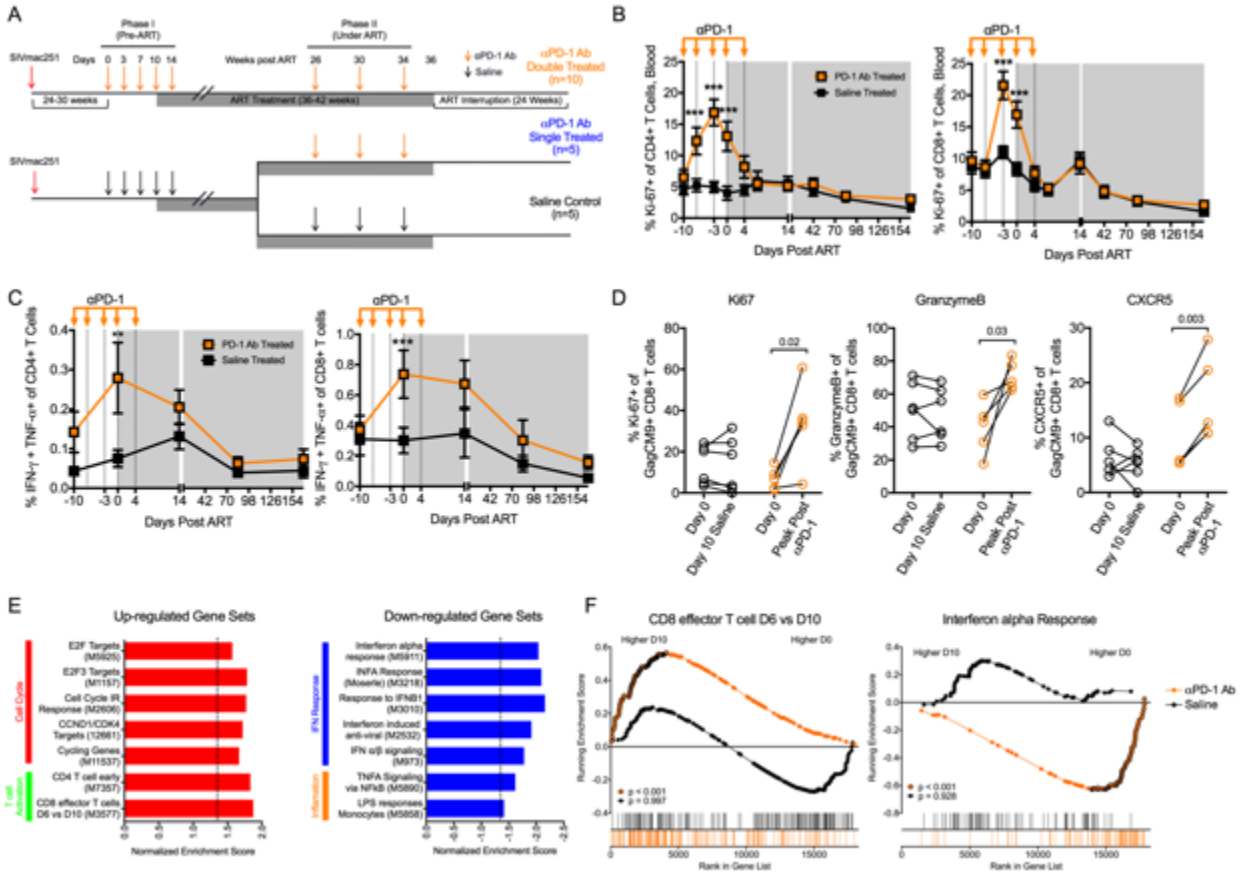


Figure 1. PD-1 blockade administered prior to ART (phase I) results in improved T cell functionality in SIV-infected RMs. (A) Schematic of PD-1 blockade strategy during phase I and II. (B) Frequency of Ki-67+ CD4+ and CD8+ T cells in the blood. (C) Frequency of SIV Gag and Env-specific IFNγ and TNFα producing CD4+ and CD8+ T cells in the blood. (D) Percent of Ki-67+, granzyme B+, and CXCR5+ GagCM9+ CD8+ T cells in the blood (saline, n = 6; PD-1 Ab treated, n = 5). (E) Gene set enrichment analysis (GSEA) of RNASeq data from blood at day 10 compared to day 0 following PD-1 blockade during phase I (PD-1 Ab treated, n = 10). Normalized enrichment scores for select up regulated and down regulated gene sets depicted. Dashed line indicates normalized enrichment score cut-off of > 1.35 for up regulated gene sets and < -1.35 for down regulated gene sets with a false discovery rate of < 0.2. (F) GSEA plots comparing day 10 to day 0 of Phase I for PD-1 Ab and saline treated (n = 5) groups. Leading edge genes from gene sets are shown as black outlined dots. Shaded grey area depicts ART.

Unfilled circles indicate values from Mamu-A*01 RMs. Data in B and C are shown as mean ± SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Two-way ANOVA (B, C) or two-tailed paired Student's t-test (D) were used. N = 10 per group unless otherwise noted.

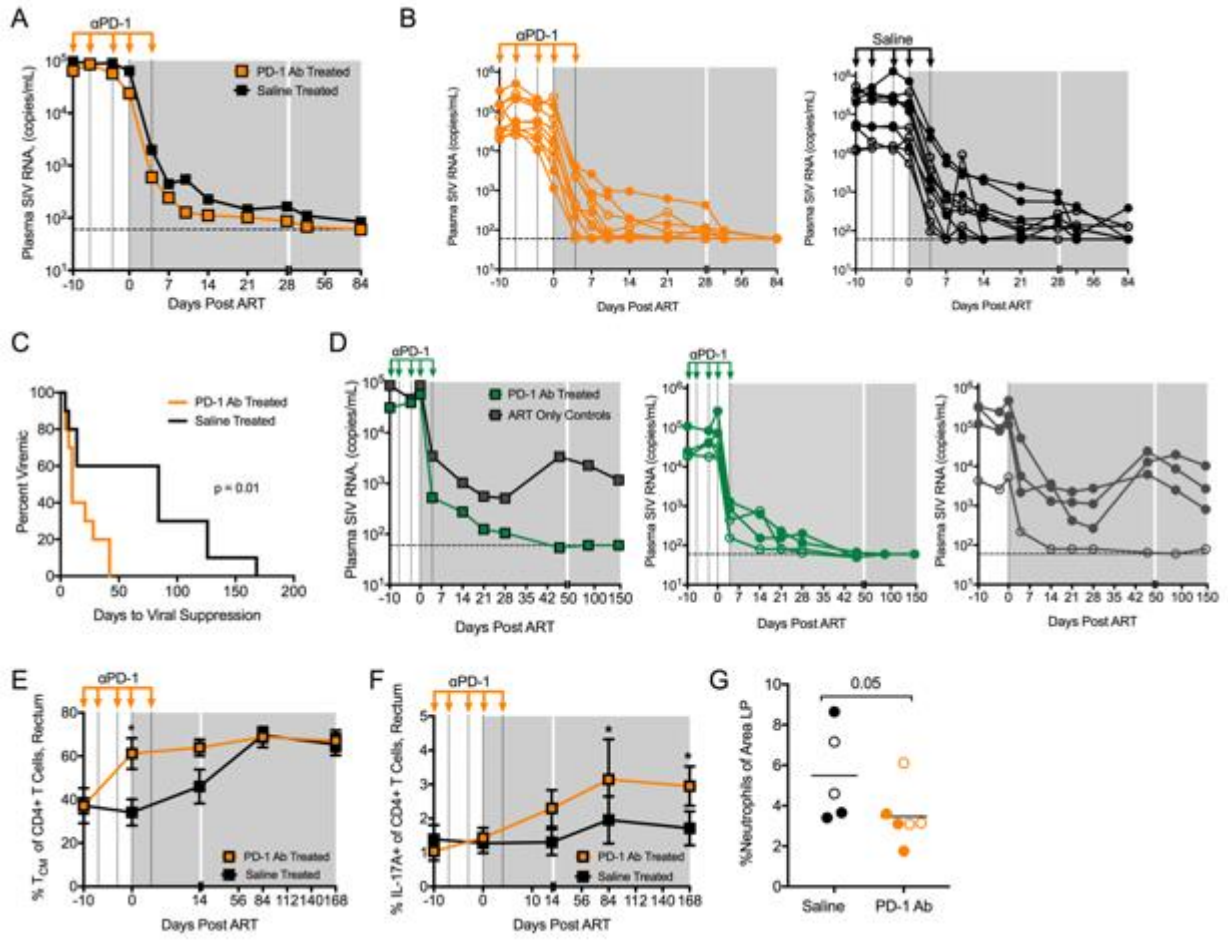


Figure 2. PD-1 blockade administered prior to ART results in improved viral suppression following ART initiation. Plasma SIV RNA viral loads (copies/mL) shown as (**A**) geometric mean for each group and (**B**) for individual RMs at initiation of PD-1 Ab or saline infusion. Limit of detection is 60 copies/mL. (**C**) Kaplan-Meier curve of number of days until viral suppression (<100 copies/mL for at least two or more consecutive time points). (**D**) Plasma SIV RNA viral loads (copies/mL) from chronically SIV-infected RMs administered humanized anti-PD-1 antibody or no antibody during ART initiation in a second study (n = 4 per group). (**E**) Frequency of T_{CM} (CD28+ CD95+) CD4+ T cells in the rectum. (**F**) Frequency of IL-17A producing CD4+ T cells in the rectum after PMA and ionomycin stimulation (saline, n = 9). (**G**) Frequency of neutrophils in lamina propria sections after 36 weeks of ART (saline, n = 5; PD-1 Ab treated, n = 6). Bars indicate mean. Data was not collected for all animals. Shaded grey area

depicts ART. Unfilled symbols indicate values from Mamu-A*01 RMs. Specific T cell frequency data are shown as mean ± SEM. *, $p < 0.05$. Mantel-Cox test (**C**), two-way ANOVA (**E-F**), or two-tailed Mann Whitney test (**G**) were used. N = 10 per group unless otherwise noted.

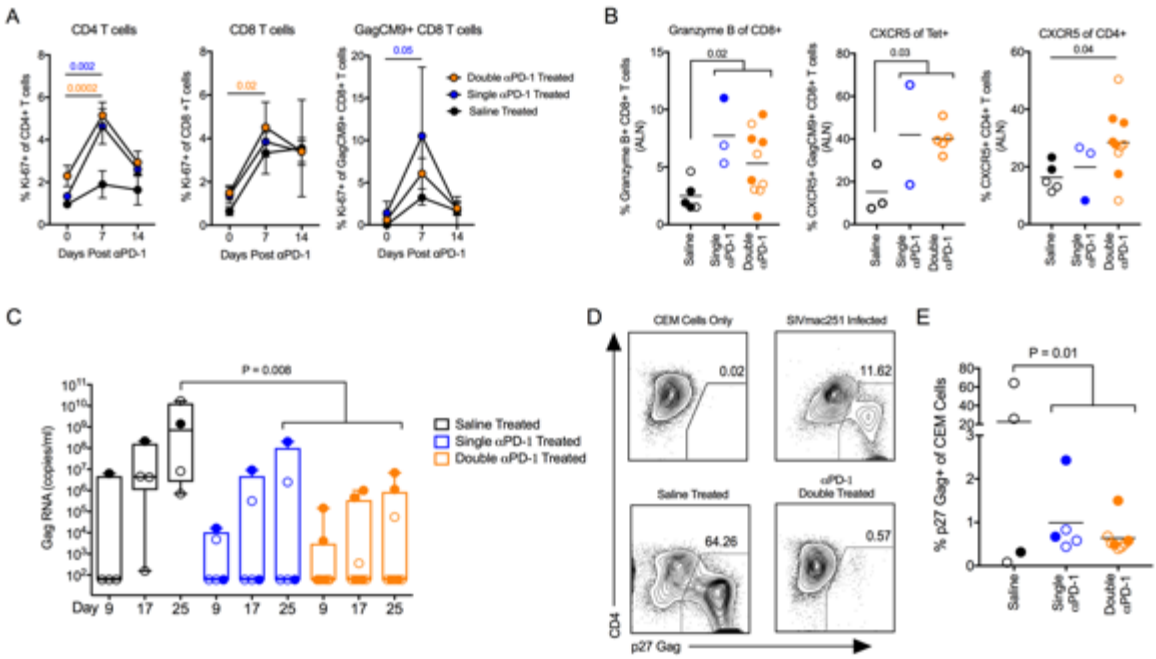


Figure 3. PD-1 blockade during suppressive ART (phase II) results in T cell proliferation and potential destabilization of the viral reservoir. (**A**) Ki-67 expression on CD4+ T cells, CD8+ T cells, and GagCM9+ CD8+ T cells in blood after first PD-1 Ab infusion (right: saline, n = 2; Single αPD-1 Treated (ST), n = 3; Double αPD-1 Treated (DT), n = 5). Data are shown as mean ± SEM. (**B**) Frequency of granzyme B+ CD8+ T cells (saline, n = 5; ST, n = 3; DT, n = 10), CXCR5+ GagCM9+ CD8+ T cells (saline, n = 3; ST, n = 2; DT, n = 5), and CXCR5+ CD4+ T cells (saline, n = 5; ST, n = 3; DT, n = 10) in axillary lymph nodes at two weeks after last PD-1 Ab infusion. Viral outgrowth assay on CD4+ T cells from blood of RMs two weeks after the last PD-1 antibody infusion. (**C**) SIV Gag RNA copies/mL assayed from culture supernatant at days 9, 17, and 25 of culture. (**D**) Representative p27 gag staining and (**E**) frequency of p27 gag+ CEM cells from day 25 of viral outgrowth assay. For viral outgrowth assay, saline, n = 4; ST, n = 5; DT, n = 8. Unfilled circles indicate values from Mamu-A*01 RMs. Bars indicate mean. Exact p values are shown. Two-way ANOVA (**A**), two-tailed Mann-Whitney test (**B, C**), one-way ANOVA with Dunn's multiple comparisons test (**B**, right), or two-tailed Student's t-test (**E**) were used. One saline control animal was interrupted from ART early due to significant weight loss and therefore data is not available for this animal. Unless otherwise noted, saline, n = 4; ST, n = 5; DT, n = 10.

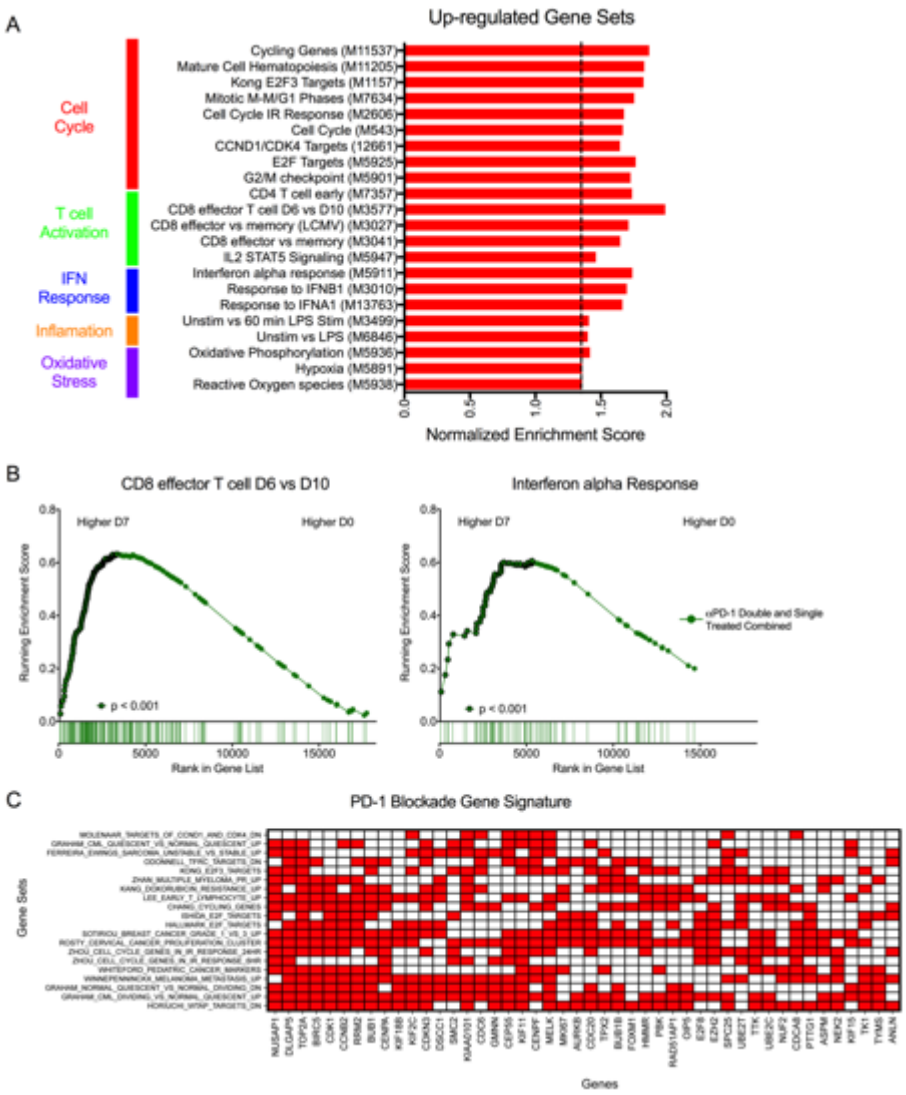


Figure 4. PD-1 blockade during suppressive ART (phase II) stimulates anti-viral cellular response pathways. GSEA of RNASeq data from blood at day 7 compared to day 0 following first PD-1 Ab infusion for all PD-1 Ab treated animals during Phase II. (**A**) Normalized enrichment scores for up regulated gene sets depicted. Dashed line indicates normalized enrichment score cut-off of > 1.35 with a false discovery rate of < 0.2. (**B**) GSEA plots comparing day 7 and day 0 of first PD-1 Ab infusion during Phase II. Leading edge genes from gene sets are shown as black outlined dots. Exact p-values are shown. N = 9. (**C**) Leading edge analysis was performed on positively enriched gene sets that were significant during both phase I and phase II. Genes present in at least 7 gene sets were considered core signature genes. Filled red boxes indicate presence of the gene in the respective gene set.

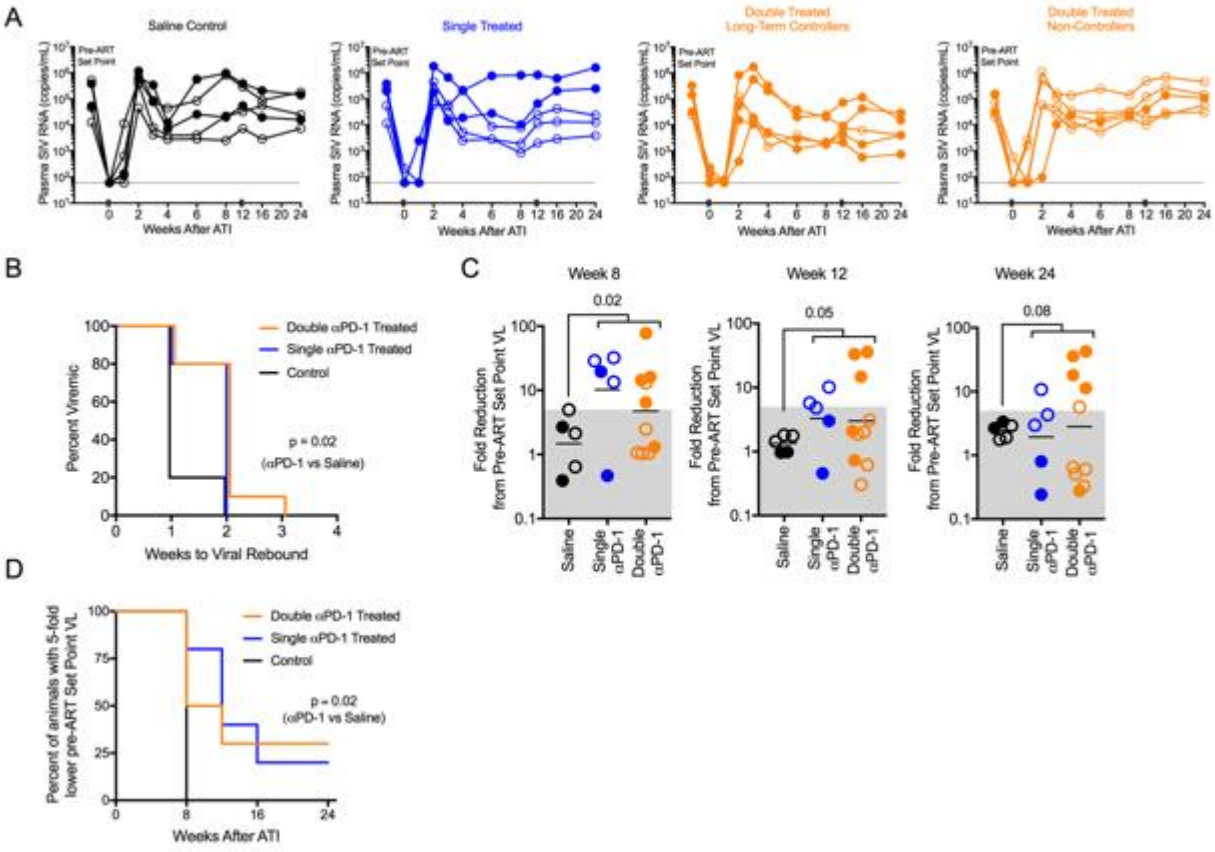


Figure 5. Enhanced viral control in PD-1 Ab treated animals after ART interruption. (**A**) Plasma SIV RNA viral loads (copies/mL) in individual RMs at pre-ART set point and after ART interruption (ATI) (day 0 of phase I and 36-42 weeks of ART treatment, respectively, see Figure 1A). Limit of detection is 60 copies/mL. (**B**) Kaplan-Meier curve showing number of weeks to viral rebound after ATI. Limit of detection is 60 copies/mL. (**C**) Fold reduction of viral loads from pre-ART set point viral loads to weeks 8, 12, and 24 after ATI. Bars indicate geometric mean. (**D**) Kaplan-Meier curve showing number of weeks after ATI until viral loads reach within 5-fold levels of pre-ART viral loads. Unfilled circles indicate values from Mamu-A*01 RMs. Saline, n = 5, ST, n = 5; DT, n = 10. Mantel-Cox test (**B, D**) or two-tailed Student's t-test with Welch's correction (**C**) were used.

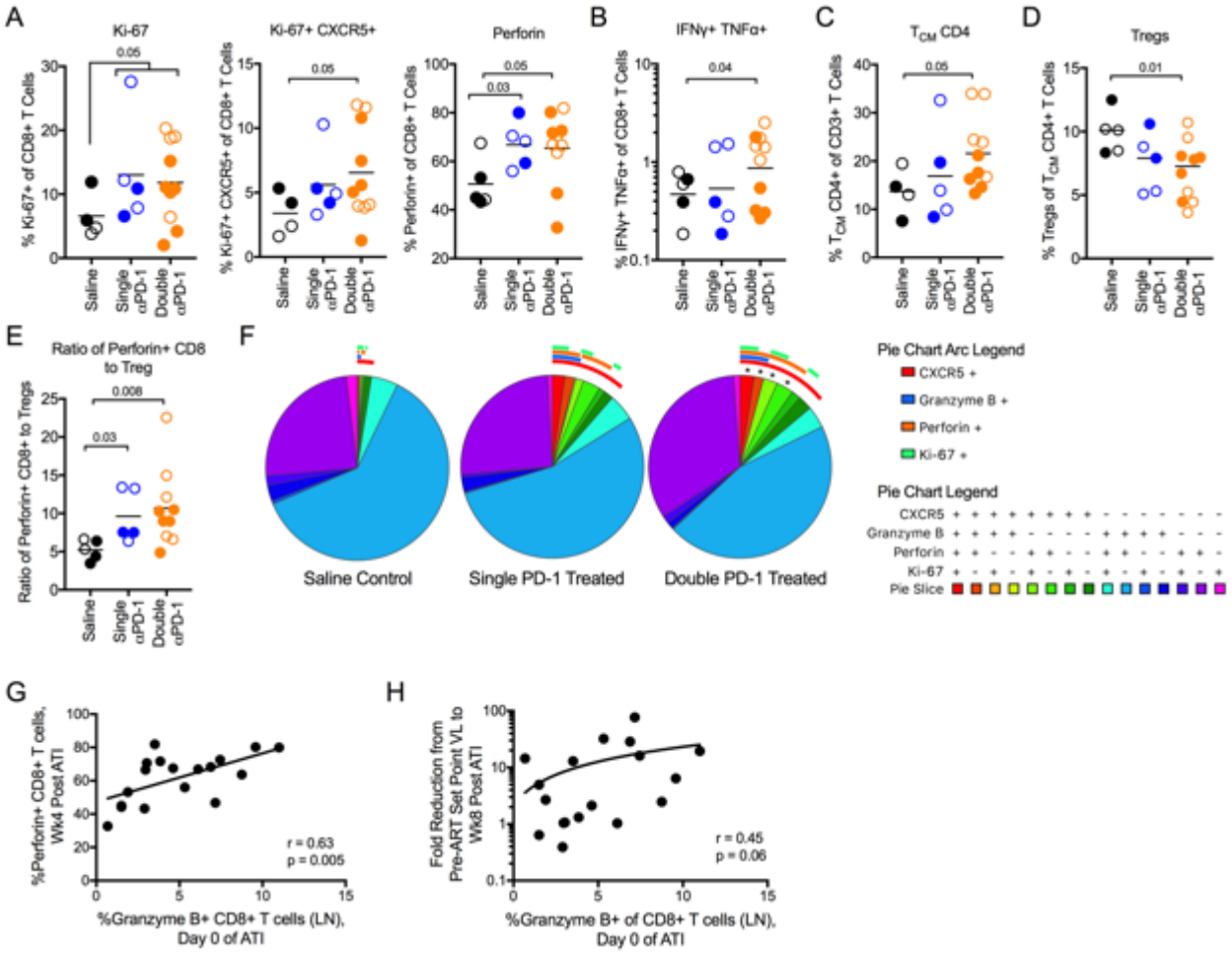
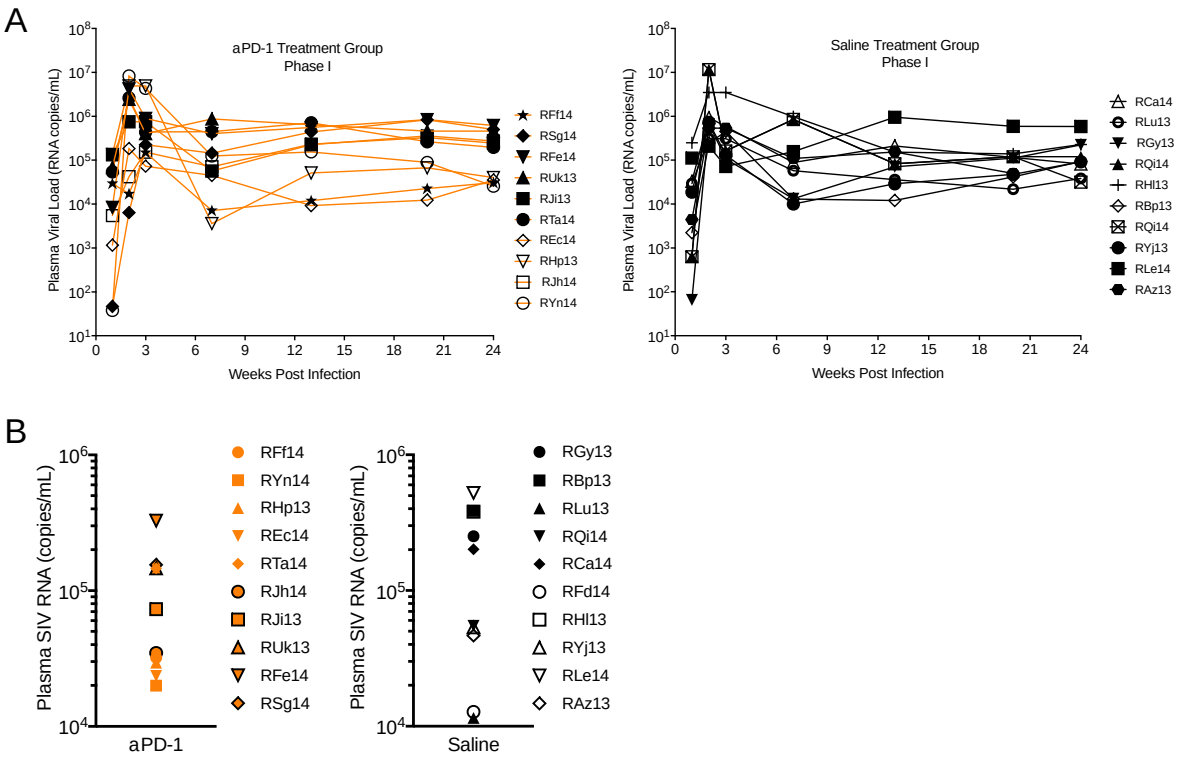
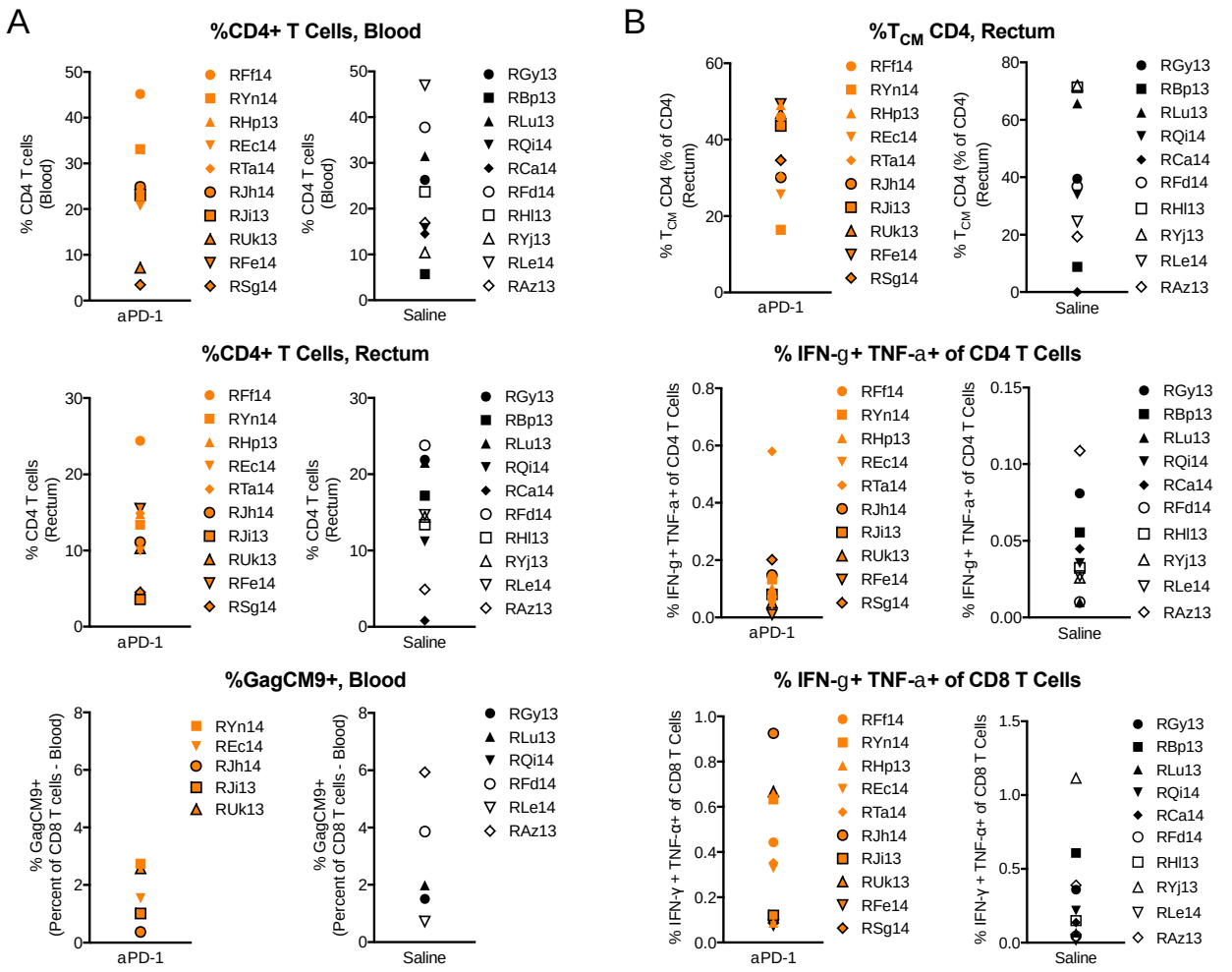


Figure 6. Enhanced immune responses in PD-1 Ab treated animals after ART interruption. (**A**) Frequencies of Ki-67+ (3 weeks after ATI; saline, n = 4), Ki-67+ CXCR5+ (3 weeks after ATI; saline, n = 4), and perforin+ (4 weeks after ATI) CD8+ T cells in the blood. (**B**) Frequencies of SIV Gag- and Env-specific IFNγ and TNFα producing CD8+ T cells in the blood at 4 weeks after ATI. Bars indicate geometric mean. (**C**) Frequency of T_{CM} CD4+ T cells as percentage of CD3+ T cells in the blood at 2 weeks after ATI (saline, n = 4). (**D**) Frequency of Tregs in the blood at 4 weeks after ATI. (**E**) Ratio of perforin+ CD8+ T cells to Tregs at week 4 after ATI. (**F**) Boolean analysis of marker expression of CXCR5, granzyme B, perforin, and Ki-67 on T_{EM} CD8+ T cells at 3 weeks after ATI. Correlations of frequency of granzyme B+ CD8+ T cells from LNs of animals at day 0 of ATI with (**G**) frequency of perforin+ CD8+ T cells at 4 weeks after ATI and (**H**) fold reduction of viral loads from pre-ART set point

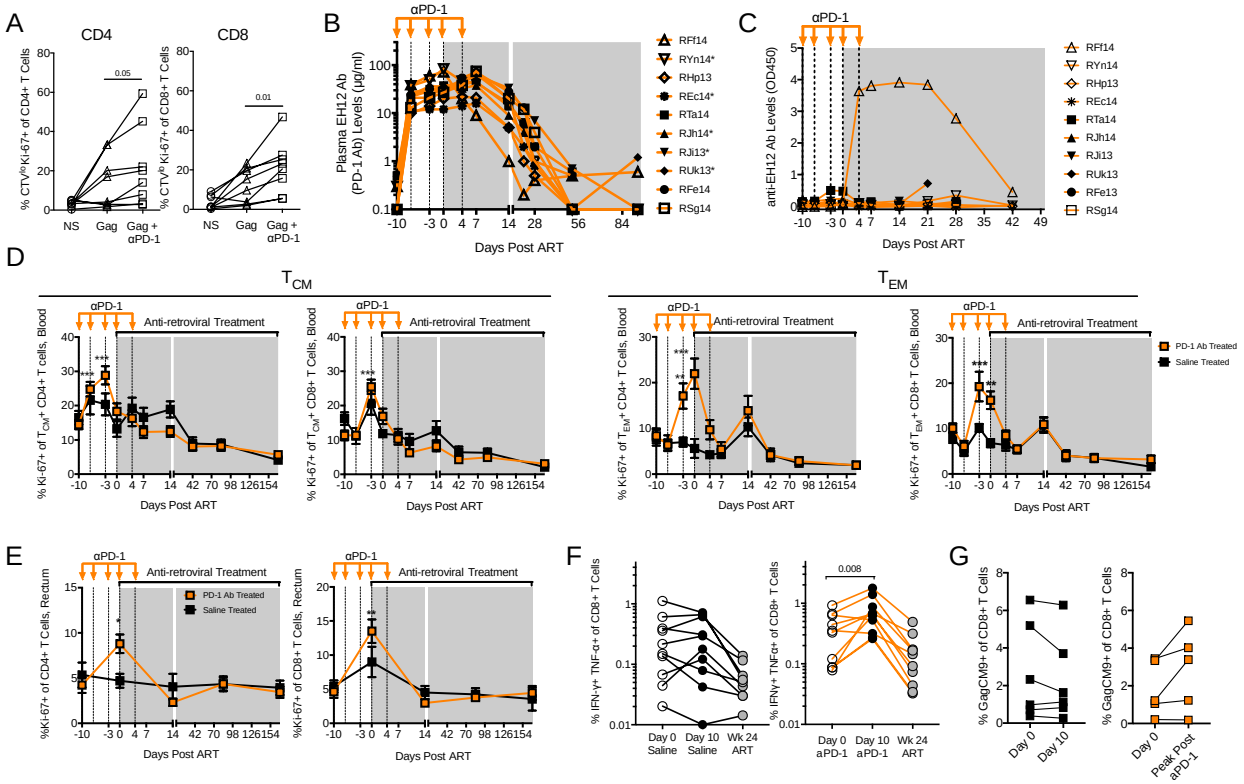
to week 8 after ATI (ST, n = 3). Unfilled circles indicate values from Mamu-A*01 RMs in **A**-**E**. Bars indicate mean unless otherwise noted. *, $p < 0.05$. Two-tailed Student's t-test with Welch's correction (**A-D)**, two-tailed Mann Whitney test (**E, F**), or Spearman's rank-order correlations test (**G, H**) were used. R indicate correlation coefficients. Unless otherwise noted: saline, n = 5; ST, n = 5; DT, n = 10.



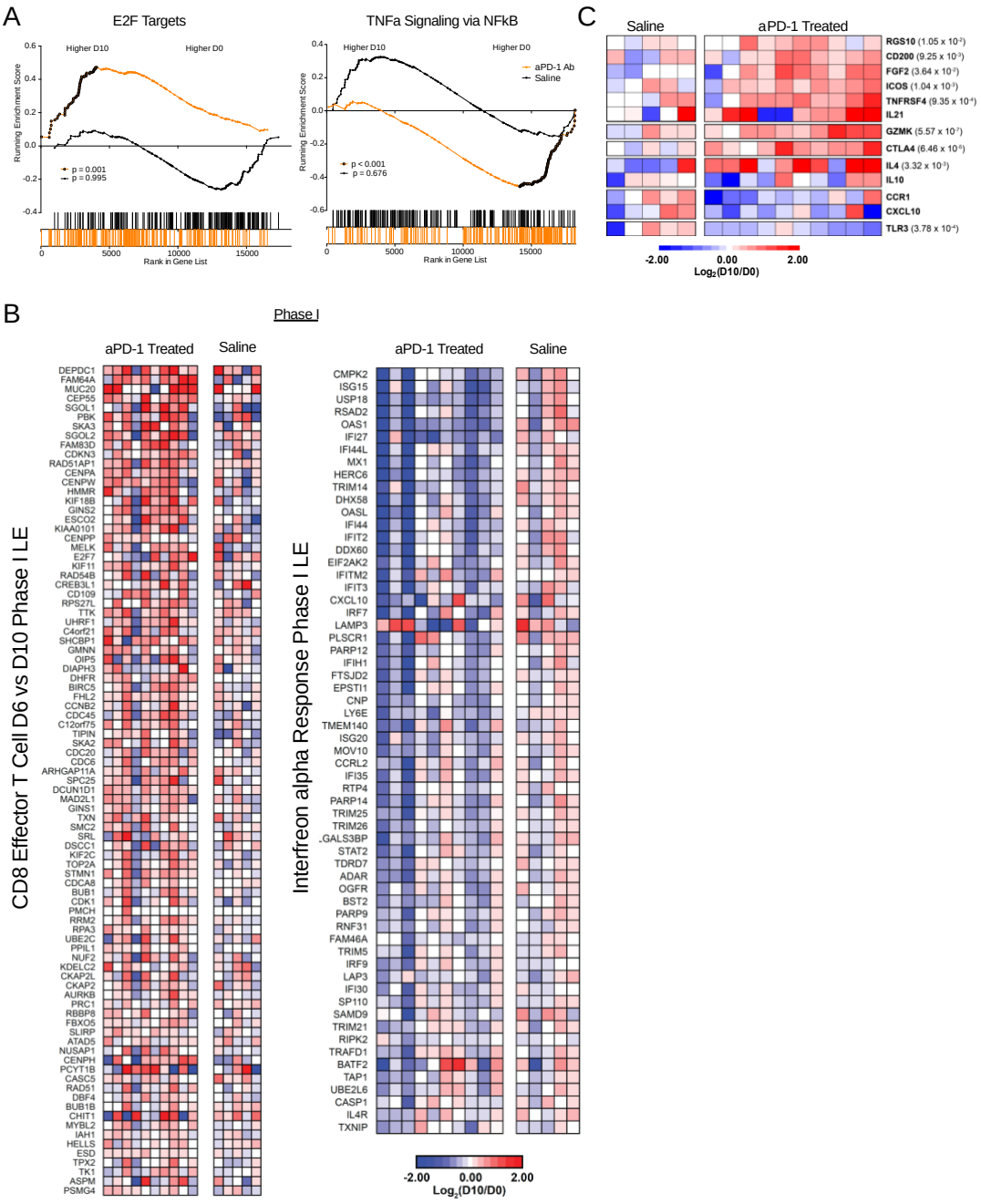
SFigure 1. Plasma SIV RNA viral loads (copies/mL) in individual RMs in PD-1 Ab or saline control groups at (**A**) time of infection through 24 weeks of infection and **(B**) at day -10 of phase I (start of study). Limit of detection is 60 copies/mL.



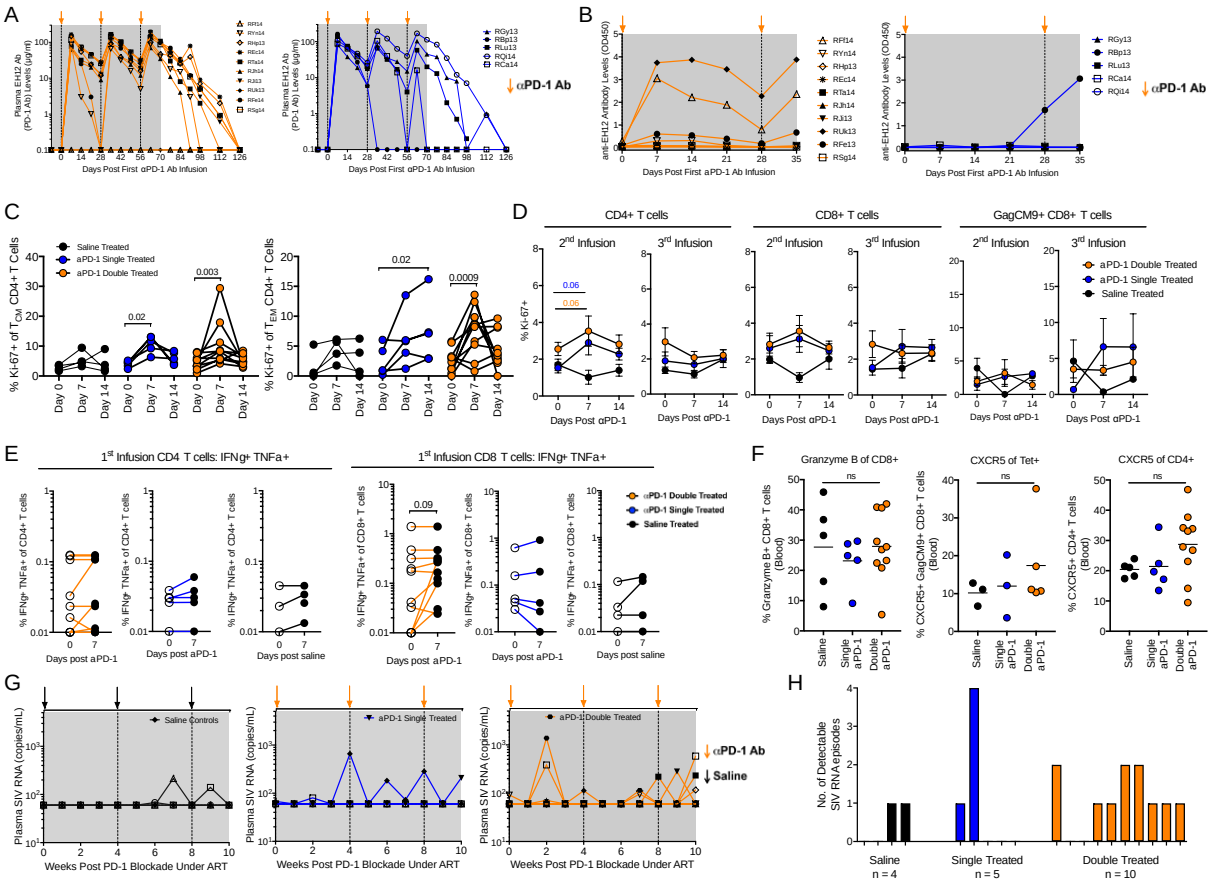
SFigure 2. (**A**) Frequency of CD4+ T cells in the blood, CD4+ T cells in the rectum, and GagCM9+ CD8+ T cells in the blood at 24 weeks of infection for RMs in PD-1 Ab or saline control groups. (**B**) Frequency of Tcm CD4+ T cell in the rectum, and SIV Gag and Env specific IFN-γ and TNF-α producing CD4+ and CD8+ T cells in the blood at day -10 of phase I (start of study) for RMs in PD-1 Ab or saline control groups.



SFigure 3. (**A**) Ki-67 expression on Cell Trace Violet-labeled PBMCs from RMs chronically infected with SIVmac251 for 30 or more weeks that were stimulated with SIV Gag peptides and cultured in the presence or absence of primatized anti-PD-1 antibody (10 ug/ml) for 5 days (n = 8). (**B**) Plasma concentrations of primatized PD-1 Ab (EH12 IgG4) in PD-1 blockade treated RMs as determined by ELISA. (**C**) OD450 values measuring levels of anti-EH12 responses generated in plasma of PD-1 Ab treated RMs. (**D**) Frequency of Ki-67 expressing CD4+ and CD8+ T_{CM} and T_{EM} cells in the blood. (**E**) Frequency of Ki-67+ CD4+ and CD8+ T cells in the rectum. (**F**) Frequency of SIV Gag and Env specific IFN-γ and TNF-α producing CD8+ T cells for individual RMs. (**G**) Frequency of GagCM9+ CD8+ T cells in the blood (saline, n = 6; PD-1 Ab treated, n = 5). Data are shown as mean ± SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Two-tailed paired Student's t test (**A, F**) or two-way ANOVA (**D, E**) were used. N = 10 per group unless otherwise noted. Shaded grey area depicts anti-retroviral treatment.

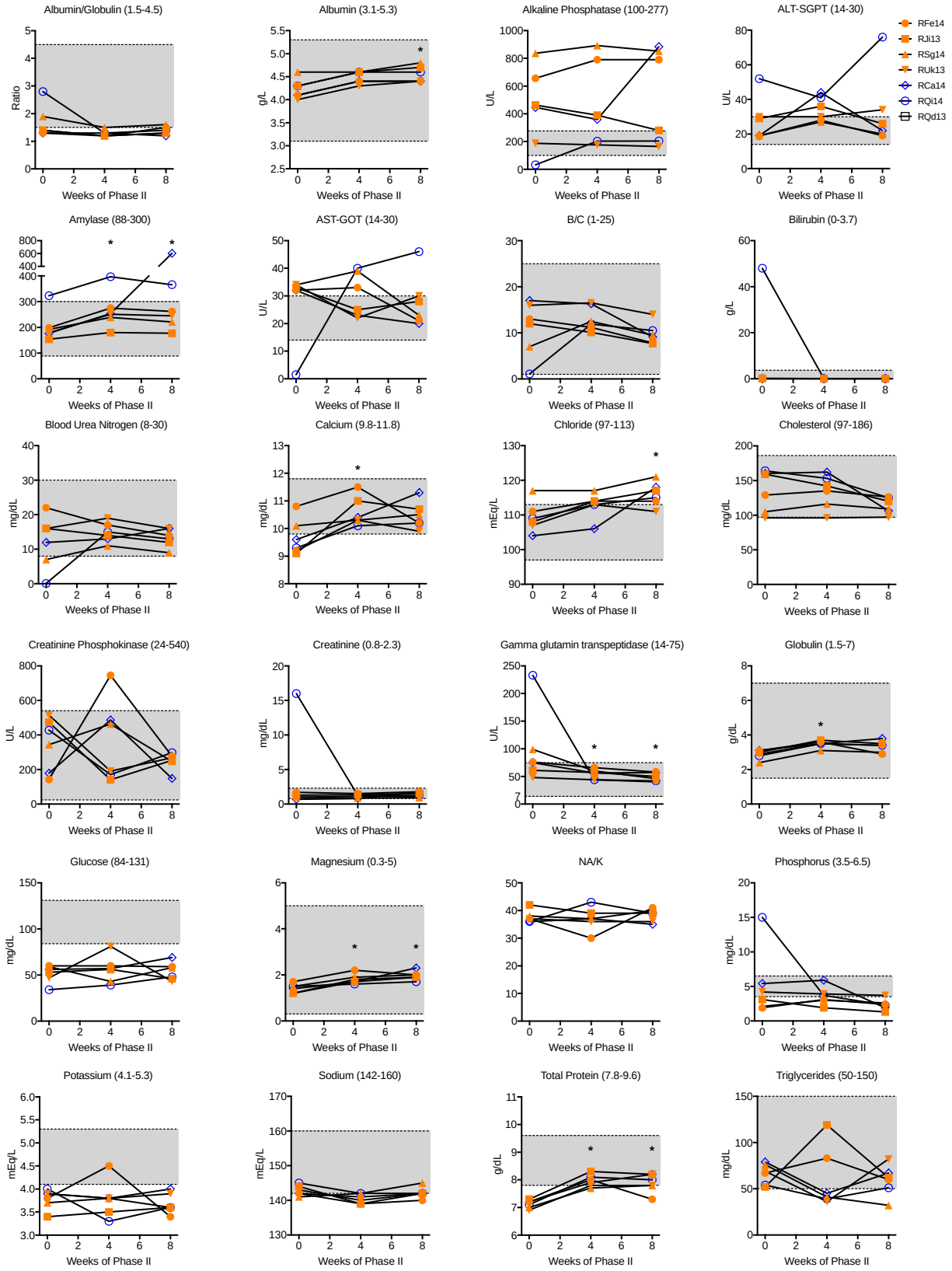

A
E2F Targets
Higher D10
Higher D0
Running Enrichment Score
p = 0.001
p = 0.995
Rank in Gene List
TNFa Signaling via NFkB
aPD-1 Ab
Saline
p < 0.001
p = 0.676
C
Saline
aPD-1 Treated
RGS10 (1.05 x 10-2)
CD200 (9.25 x 10-3)
FGF2 (3.64 x 10-2)
ICOS (1.04 x 10-3)
TNFRSF4 (9.35 x 10-4)
IL21
GZMK (5.57 x 10-7)
CTLA4 (6.46 x 10-6)
IL4 (3.32 x 10-3)
IL10
CCR1
CXCL10
TLR3 (3.78 x 10-4)
-2.00
0.00
2.00
Log2(D10/D0)
B
Phase I
aPD-1 Treated
Saline
CD8 Effector T Cell D6 vs D10 Phase I LE
DEPDC1
FAM64A
MUC20
CEP55
SGOL1
PBK
SKA3
SGOL2
FAM83D
CDKN3
RAD51AP1
CENPA
CENPW
HMMR
KIF18B
GINS2
ESCO2
KIAA0101
CENPP
MELK
E2F7
KIF11
RAD54B
CREB3L1
CD109
RPS27L
TTK
UHRF1
C4orf21
SHCBP1
GMNN
OIP5
DIAPH3
DHFR
BIRC5
FHL2
CCNB2
CDC45
C12orf75
TIPIN
SKA2
CDC20
CDC6
ARHGAP11A
SPC25
DCUN1D1
MAD2L1
GINS1
TXN
SMC2
SRL
DSCC1
KIF2C
TOP2A
STMN1
CDCA8
BUB1
CDK1
PMCH
RRM2
RPA3
UBE2C
PPIL1
NUF2
KDELC2
CKAP2L
CKAP2
AURKB
PRC1
RBBP8
FBXO5
SLIRP
ATAD5
NUSAP1
CENPH
PCYT1B
CASC5
RAD51
DBF4
BUB1B
CHIT1
MYBL2
IAH1
HELLS
ESD
TPX2
TK1
ASPM
PSMG4
aPD-1 Treated
Saline
Interfreon alpha Response Phase I LE
CMPK2
ISG15
USP18
RSAD2
OAS1
IFI27
IFI44L
MX1
HERC6
TRIM14
DHX58
OASL
IFI44
IFIT2
DDX60
EIF2AK2
IFITM2
IFIT3
CXCL10
IRF7
LAMP3
PLSCR1
PARP12
IFIH1
FTSJD2
EPSTI1
CNP
LY6E
TMEM140
ISG20
MOV10
CCRL2
IFI35
RTP4
PARP14
TRIM25
TRIM26
,GALS3BP
STAT2
TDRD7
ADAR
OGFR
BST2
PARP9
RNF31
FAM46A
TRIM5
IRF9
LAP3
IFI30
SP110
SAMD9
TRIM21
RIPK2
TRAFD1
BATF2
TAP1
UBE2L6
CASP1
IL4R
TXNIP
-2.00
0.00
2.00
Log2(D10/D0)

SFigure 4. (**A**) GSEA plots comparing day 10 to day 0 of Phase I for PD-1 Ab and saline treated groups. Leading edge genes from gene sets are shown as black outlined dots. (**B**) Heat map of log_2 fold change of gene expression for leading edge genes of select gene sets comparing day 10 to day 0 during Phase I. (**C**) Heat map of log_2 fold change of gene expression from day 10 of treatment over day 0 during Phase I of selected genes. Numbers indicate nominal p-values for change in gene expression. Exact p values are shown. Saline, n = 5; PD-1 Ab treated, n = 10.

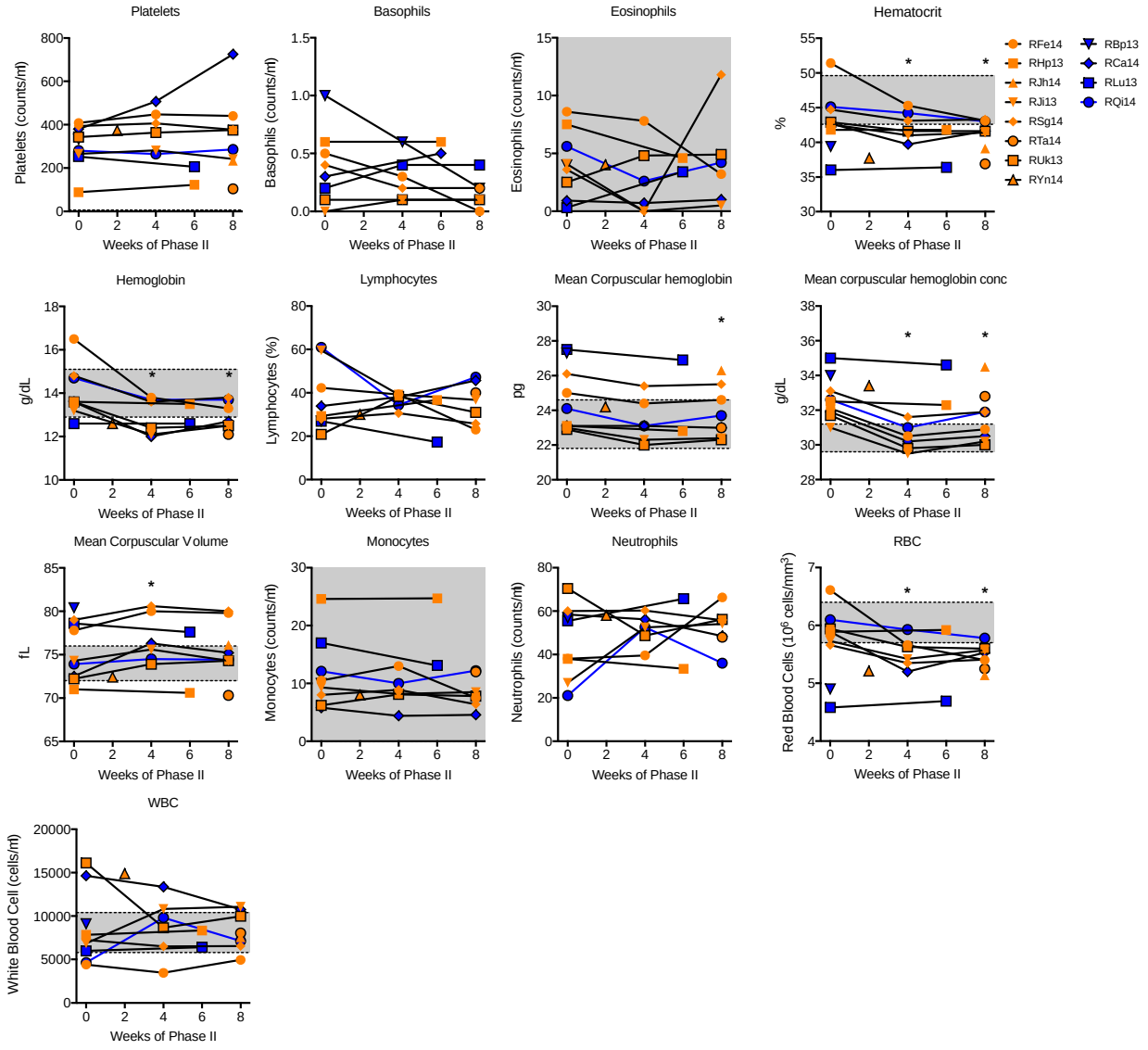


SFigure 5. (**A**) Plasma concentrations of primatized PD-1 Ab (EH12 IgG4) in PD-1 blockade treated RMs as determined by ELISA during Phase II. (**B**) OD450 values measuring levels of anti-EH12 responses generated in plasma of PD-1 Ab treated RMs. (**C**) Ki-67 expression of T_{EM} and T_{CM} CD4+ T cells at day 0, 7, and 14 after first PD-1 Ab infusion under ART. (**D**) Ki-67 expression on CD4+, CD8+, and GagCM9 Tet+ CD8+ T cells from PBMCs after second and third PD-1 Ab infusion (right: saline, n = 2; αPD-1 Single Treated (ST), n = 3; αPD-1 Double Treated (DT), n = 5). Data are shown as mean ± SEM. (**E**) Frequency of SIV Gag and Env-specific IFNγ and TNFα producing CD4+ (left) and CD8+ (right) T cells for individual RMs after first PD-1 Ab infusion. (**F**) Plasma SIV RNA viral loads (copies/mL) during phase II. Limit of detection is 60 copies/mL. (**G**) Number of transient increases in plasma viremia per animal. Exact p values are shown. Two-way ANOVA (**C, D**) or two-tailed paired

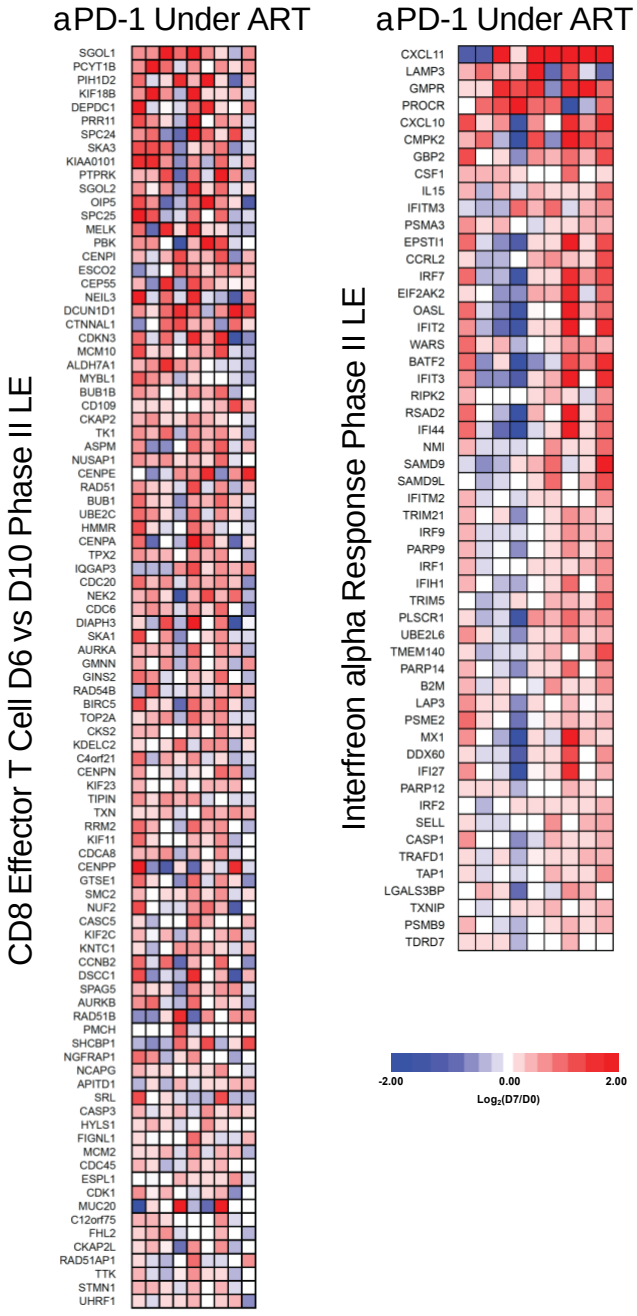
Student's t test (**E**) were used. Saline, n = 4; αPD-1 ST, n = 5; αPD-1 DT, n = 10 unless otherwise noted. Shaded grey area depicts anti-retroviral treatment.


Albumin/Globulin (1.5-4.5)
Albumin (3.1-5.3)
Alkaline Phosphatase (100-277)
ALT-SGPT (14-30)
RFe14
RJi13
RSg14
RUk13
RCa14
RQi14
RQd13
Amylase (88-300)
AST-GOT (14-30)
B/C (1-25)
Bilirubin (0-3.7)
Blood Urea Nitrogen (8-30)
Calcium (9.8-11.8)
Chloride (97-113)
Cholesterol (97-186)
Creatinine Phosphokinase (24-540)
Creatinine (0.8-2.3)
Gamma glutamin transpeptidase (14-75)
Globulin (1.5-7)
Glucose (84-131)
Magnesium (0.3-5)
NA/K
Phosphorus (3.5-6.5)
Potassium (4.1-5.3)
Sodium (142-160)
Total Protein (7.8-9.6)
Triglycerides (50-150)
Weeks of Phase II
Ratio
g/L
U/L
mg/dL
mEq/L
g/dL

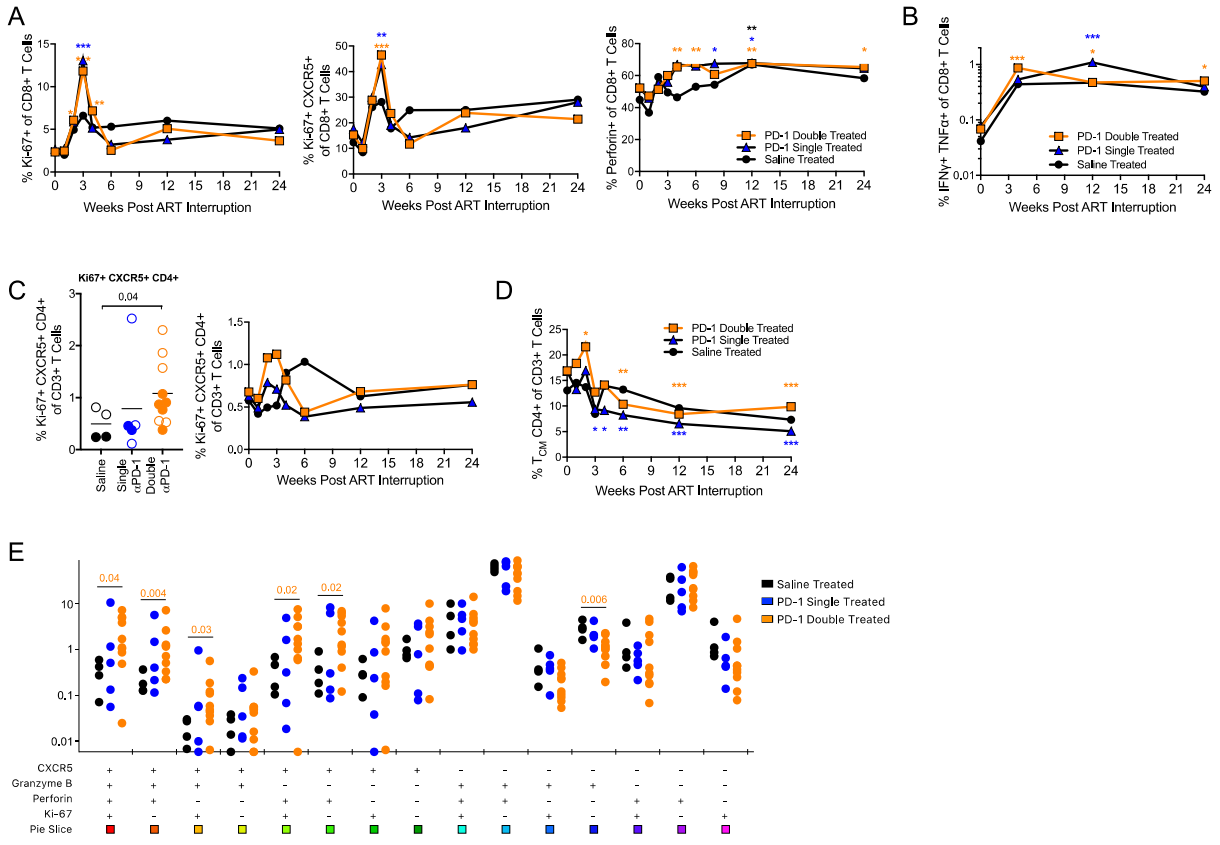
SFigure 6. Biochemical parameters detected in the blood of PD-1 Ab treated animals during Phase II. Orange symbols are αPD-1 double treated and blue symbols are αPD-1 single treated RMs. Shaded grey area depicts normal ranges. *, $p < 0.05$. Two-tailed paired Student's t-test were used to compare week 4 or week 8 of phase II to week 0.



SFigure 7. Complete blood count of PD-1 Ab treated animals during Phase II. Orange symbols are αPD-1 double treated and blue symbols are αPD-1 single treated RMs. Shaded grey area depicts normal ranges. *, $p < 0.05$. Two-tailed paired Student's t-test were used to compare week 4 or week 8 of phase II to week 0.



SFigure 8. Heat map of $\log_2$ fold change of gene expression for leading edge genes of select gene sets comparing day 7 to day 0 during first PD-1 Ab infusion of Phase II. PD-1 Ab double and single treated combined, n = 9.



SFigure 9. (**A**) Frequency of Ki-67+ (left), Ki-67+ CXCR5+ (middle), and perforin+ (right) CD8+ T cells from the blood after ART interruption (ARTi). (**B**) Frequency of SIV Gag and Env-specific IFN-γ and TNF-α producing CD8+ T cells in the blood after ARTi. Data are shown as geometric means. (**C**) Frequency of Ki-67+ CXCR5+ CD4+ T cells as percentage of CD3+ T cells in the blood after ARTi. (**D**) Frequency of T_{CM} CD4+ T cells as percentage of CD3+ T cells in the blood after ARTi. (**E**) Boolean analysis of expression of CXCR5, granzyme B, perforin, and Ki-67 on T_{EM} CD8+ T cells 3 weeks after ARTi. Data and bars are shown as means unless otherwise indicated. * $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Two-tailed unpaired Student's t-test with Welch's correction (**C** left), two-way ANOVA (**A-D**), or two-tailed unpaired Student's t-test (**E**) were used. Saline, n = 4; αPD-1 Single Treated, n = 5; αPD-1 Double Treated, n = 10.

Supplemental Table 1. Rhesus Macaque Characteristics

	Double PD-1 Treated	Previous Vaccinations	A01	B08	B17	Sex	Age (by start of study)
1	RFf14	DMP-CD40L	-	-	-	Male	4
2	RYn14*		+	-	-	Male	3
3	RHp13	DM	-	-	-	Male	5
4	REc14*	DM	+	-	-	Male	4
5	RTa14	DM-CD40L	-	-	-	Male	4
6	RJh14 *	DM	+	-	-	Male	4
7	RJi13*	DM	+	-	-	Male	5
8	RUk13*	$DM_{Rapamycin}$	+	-	-	Male	5
9	RFe14	DM	-	-	-	Male	4
10	RSg14	DM	-	-	-	Male	4
	Single PD-1 Treated						
1	RGy13 *	DM-CD40L	+	-	-	Male	4
2	RBp13	DM	-	-	-	Male	5
3	RLu13*	DM	+	-	-	Male	4
4	RQi14*	$DM_{Rapamycin}$	+	-	-	Male	4
5	RCa14	DM	-	-	-	Male	4
	Saline Treated						
1	RFd14*	DM-CD40L	+	-	-	Male	4
2	RHl13	DM	-	-	-	Male	5
3	RYj13	DM-CD40L	-	-	-	Male	5
4	RLe14*	DMP-CD40L	+	-	-	Male	4
5	RAz13*	DM	+	-	-	Male	4

D: DNA expressing SIVmac239 Gag, Protease, Reverse transcriptase, Tat, Rev and Envelope gp160

M: MVA expressing SIVmac239 Gag, Protease, Reverse transcriptase, Envelope gp150

P: SIVmac239 protein boost in Alum

CD40L – DNA vaccine co-expressed macaque CD40L

Rapamycin – animals received rapamycin daily for 31 days (day -3 to day 28 during DNA or MVA vaccination)

Supplemental Table 2. Significantly Enriched Gene Sets from Phase I Analysis of MSigDB C2 Curated Gene Sets

		PD-1 Ab Treated			Saline Treated		
NAME	SIZE	NES	NOM p-val	FDR q-val	NES	NOM p-val	FDR q-val
GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_UP	174	2.027	0.000	0.001	0.849	0.869	1.000
GRAHAM_NORMAL_QUIESCENT_VS_NORMAL_DIVIDING_DN	87	1.961	0.000	0.003	-0.617	1.000	1.000
ROSTY_CERVICAL_CANCER_PROLIFERATION_CLUSTER	138	1.879	0.000	0.030	0.633	1.000	1.000
WINNEPENNINCKX_MELANOMA_METASTASIS_UP	153	1.843	0.000	0.054	-0.696	1.000	1.000
LEE_EARLY_T_LYMPHOCYTE_UP	100	1.832	0.000	0.052	0.887	0.723	1.000
ISHIDA_E2F_TARGETS	51	1.793	0.000	0.094	-1.193	0.168	1.000
KONG_E2F3_TARGETS	94	1.782	0.000	0.098	0.863	0.729	1.000
STEINER_ERYTHROCYTE_MEMBRANE_GENES	15	1.782	0.000	0.086	-0.994	0.458	1.000
SOTIRIOU_BREAST_CANCER_GRADE_1_VS_3_UP	147	1.777	0.000	0.085	0.765	0.975	1.000
ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_6HR	82	1.770	0.000	0.087	0.619	1.000	1.000
KANG_DOXORUBICIN_RESISTANCE_UP	54	1.766	0.002	0.086	0.664	0.981	1.000
ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_24HR	122	1.743	0.000	0.120	0.827	0.873	1.000
ZHAN_MULTIPLE_MYELOMA_PR_UP	42	1.731	0.002	0.137	0.664	0.975	1.000
MOLENAAR_TARGETS_OF_CCND1_AND_CDK4_DN	55	1.719	0.000	0.157	1.106	0.255	1.000
REACTOME_DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX	27	1.718	0.002	0.148	1.112	0.307	1.000
WHITEFORD_PEDIATRIC_CANCER_MARKERS	115	1.715	0.000	0.146	0.797	0.928	1.000
KEGG_NICOTINATE_AND_NICOTINAMIDE_METABOLISM	23	1.714	0.002	0.141	0.767	0.836	1.000
HORIUCHI_WTAP_TARGETS_DN	299	1.710	0.000	0.141	0.745	0.998	1.000
ZHANG_TLX_TARGETS_36HR_DN	184	1.705	0.000	0.147	0.647	1.000	1.000
SENGUPTA_NASOPHARYNGEAL_CARCINOMA_WITH_LMP1_DN	159	1.689	0.000	0.179	1.048	0.333	1.000
ONDER_CDH1_TARGETS_3_DN	57	1.688	0.002	0.173	0.934	0.566	1.000
GRAHAM_CML_QUIESCENT_VS_NORMAL_QUIESCENT_UP	82	1.682	0.002	0.179	0.866	0.723	1.000

ODONNELL_TFRC_TARGETS_DN	127	1.682	0.000	0.171	0.845	0.863	1.000
SHEDDEN_LUNG_CANCER_POOR_SURVIVAL_A6	435	1.679	0.000	0.172	0.941	0.664	1.000
CHANG_CYCLING_GENES	143	1.668	0.000	0.198	-0.969	0.521	1.000
FERREIRA_EWINGS_SARCOMA_UNSTABLE_VS_STABLE_UP	157	1.666	0.000	0.195	-0.896	0.767	1.000
RADAEVA_RESPONSE_TO_IFNA1_UP	50	-1.714	0.000	0.195	0.781	0.845	1.000
BURTON_ADIPOGENESIS_PEAK_AT_2HR	51	-1.725	0.000	0.198	1.330	0.060	1.000
FARMER_BREAST_CANCER_CLUSTER_1	40	-1.736	0.000	0.177	1.058	0.383	1.000
GRANDVAUX_IRF3_TARGETS_UP	15	-1.745	0.005	0.168	0.456	0.992	1.000
KEGG_PROPANOATE_METABOLISM	32	-1.746	0.000	0.176	0.915	0.608	1.000
KHETCHOUMIAN_TRIM24_TARGETS_UP	46	-1.776	0.000	0.122	0.770	0.853	1.000
TONKS_TARGETS_OF_RUNX1_RUNX1T1_FUSION_SUSTAINDED_IN_ERYTHROCYTE_UP	44	-1.776	0.000	0.132	1.136	0.251	1.000
REACTOME_INTERFERON_ALPHA_BETA_SIGNALING	54	-1.779	0.000	0.137	-0.513	1.000	1.000
UROSEVIC_RESPONSE_TO_IMIQUIMOD	22	-1.785	0.002	0.139	1.111	0.347	1.000
NAGASHIMA_EGF_SIGNALING_UP	56	-1.802	0.000	0.119	0.971	0.510	1.000
FIGUEROA_AML_METHYLATION_CLUSTER_3_DN	36	-1.803	0.003	0.131	-1.196	0.195	1.000
BOWIE_RESPONSE_TO_EXTRACELLULAR_MATRIX	17	-1.817	0.000	0.118	0.763	0.801	1.000
EINAV_INTERFERON_SIGNATURE_IN_CANCER	27	-1.818	0.000	0.132	1.178	0.248	1.000
BROWNE_INTERFERON_RESPONSIVE_GENES	66	-1.888	0.000	0.046	0.718	0.970	1.000
BOSCO_INTERFERON_INDUCED_ANTIVIRAL_MODULE	72	-1.911	0.000	0.036	0.828	0.808	1.000
BOWIE_RESPONSE_TO_TAMOXIFEN	18	-1.913	0.002	0.043	0.871	0.661	1.000
DAUER_STAT3_TARGETS_DN	49	-1.934	0.000	0.037	0.662	0.977	1.000
MOSERLE_IFNA_RESPONSE	31	-2.093	0.000	0.000	1.022	0.418	1.000
HECKER_IFNB1_TARGETS	91	-2.157	0.000	0.000	0.789	0.928	1.000

Supplemental Table 3. Significantly Enriched Gene Sets from Phase I Analysis of MSigDB C7 Immunologic Gene Sets

		PD-1 Ab Treated			Saline Treated		
NAME	SIZE	NES	NOM p-val	FDR q-val	NES	NOM p-val	FDR q-val
GSE15750_DAY6_VS_DAY10_EFF_CD8_TCELL_UP	195	1.873	0.000	0.001	-0.801	0.997	1.000
GSE15750_DAY6_VS_DAY10_TRAF6KO_EFF_CD8_TCELL_UP	192	1.861	0.000	0.000	0.720	1.000	1.000
GSE34205_RSV_VS_FLU_INF_INFANT_PBMC_UP	164	1.770	0.000	0.003	0.852	0.867	1.000
GSE2405_S_AUREUS_VS_UNTREATED_NEUTROPHIL_DN	194	1.732	0.000	0.006	0.756	0.988	1.000
GSE13485_PRE_VS_POST_YF17D_VACCINATION_PBMC_UP	171	1.686	0.000	0.018	1.204	0.097	1.000
GSE41176_WT_VS_TAK1_KO_ANTI_IGM_STIM_BCELL_1H_DN	188	1.676	0.000	0.020	-1.223	0.054	1.000
GSE13547_2H_VS_12_H_ANTI_IGM_STIM_ZFX_KO_BCELL_DN	167	1.665	0.001	0.024	0.915	0.697	1.000
GSE13485_DAY1_VS_DAY21_YF17D_VACCINE_PBMC_DN	181	1.636	0.000	0.038	-0.537	1.000	1.000
GSE29618_PRE_VS_DAY7_POST_TIV_FLU_VACCINE_BCELL_UP	187	1.629	0.000	0.039	1.131	0.154	1.000
GSE3720_LPS_VS_PMA_STIM_VD2_GAMMADELTA_TCELL_UP	130	1.621	0.000	0.043	0.819	0.909	1.000
GSE19941_LPS_VS_LPS_AND_IL10_STIM_IL10_KO_MACROPHAGE_UP	178	1.619	0.000	0.041	0.706	1.000	1.000
GSE21670_UNTREATED_VS_TGFB_IL6_TREATED_CD4_TCELL_UP	180	1.617	0.000	0.038	0.897	0.743	1.000
GSE37532_WT_VS_PPARG_KO_VISCERAL_ADIPOSE_TISSUE_TREG_UP	184	1.614	0.000	0.038	0.750	0.998	1.000
GSE45365_WT_VS_IFNAR_KO_BCELL_MCMV_INFECTION_DN	164	1.610	0.000	0.039	-0.873	0.862	1.000
GSE33292_WT_VS_TCF1_KO_DN3_THYMOCYTE_DN	199	1.605	0.000	0.041	-0.687	1.000	1.000
GSE24634_TEFF_VS_TCONV_DAY7_IN_CULTURE_UP	194	1.604	0.000	0.039	0.743	0.997	1.000
GSE39556_CD8A_DC_VS_NK_CELL_MOUSE_3H_POST_POLYIC_INJ_UP	197	1.591	0.000	0.049	-0.638	1.000	1.000
GSE14415_INDUCED_VS_NATURAL_TREG_DN	176	1.591	0.000	0.047	-0.796	0.990	1.000
GSE34205_HEALTHY_VS_RSV_INF_INFANT_PBMC_DN	192	1.579	0.000	0.056	1.121	0.191	1.000
GSE45365_HEALTHY_VS_MCMV	170	1.563	0.001	0.076	-0.986	0.476	1.000

_INFECTION_CD11B_DC_DN							
GSE13547_CTRL_VS_ANTI_IGM_STIM_BCELL_12H_UP	180	1.553	0.001	0.088	0.747	0.997	1.000
GSE18893_TCONV_VS_TREG_24H_TNF_STIM_UP	193	1.541	0.000	0.105	0.653	1.000	1.000
GSE39110_DAY3_VS_DAY6_POST_IMMUNIZATION_CD8_TCELL_DN	195	1.536	0.000	0.109	-0.863	0.910	1.000
GSE21063_WT_VS_NFATC1_KO_8H_ANTI_IGM_STIM_BCELL_UP	190	1.529	0.001	0.120	-0.750	1.000	1.000
GSE24634_TREG_VS_TCONV_POST_DAY7_IL4_CONVERSION_UP	191	1.528	0.000	0.118	0.842	0.901	1.000
GSE32255_UNSTIM_VS_4H_LPS_STIM_DC_UP	173	1.527	0.001	0.115	-0.587	1.000	1.000
GSE40274_CTRL_VS_FOXP3_TRANSDUCED_ACTIVATED_CD4_TCELL_UP	193	1.521	0.003	0.123	-0.776	0.997	1.000
GSE14415_NATURAL_TREG_VS_TCONV_DN	177	1.519	0.000	0.122	0.761	0.993	1.000
GSE30962_PRIMARY_VS_SECONDARY_ACUTE_LCMV_INF_CD8_TCELL_UP	194	1.519	0.004	0.119	0.870	0.833	1.000
GSE8921_UNSTIM_0H_VS_TLR1_2_STIM_MONOCYTE_24H_UP	186	1.516	0.000	0.121	1.094	0.236	1.000
GSE13522_WT_VS_IFNG_KO_SKIN_UP	117	1.511	0.004	0.128	-0.928	0.634	1.000
GSE5589_WT_VS_IL6_KO_LPS_STIM_MACROPHAGE_180MIN_UP	125	1.505	0.003	0.139	-1.124	0.180	1.000
GSE21379_TFH_VS_NON_TFH_SAP_KO_CD4_TCELL_UP	194	1.491	0.000	0.176	-0.877	0.861	1.000
GSE4748_CYANOBACTERIUM_LPSLIKE_VS_LPS_AND_CYANOBACTERIUM_LPSLIKE_STIM_DC_3H_DN	170	1.489	0.003	0.175	1.255	0.051	1.000
GSE19198_1H_VS_6H_IL21_TREATED_TCELL_DN	193	-1.354	0.010	0.152	0.943	0.604	1.000
GSE16385_IL4_VS_ROSIGLITAZONE_STIM_MACROPHAGE_DN	166	-1.360	0.022	0.141	0.877	0.778	1.000
GSE29615_CTRL_VS_DAY7_LAIV_FLU_VACCINE_PBMC_DN	150	-1.361	0.008	0.141	-1.073	0.264	1.000
GSE19401_UNSTIM_VS_RETINOIC_ACID_STIM_FOLLICULAR_DC_UP	188	-1.368	0.014	0.128	-0.717	0.998	1.000
GSE29618_PDC_VS_MDC_DAY7_FLU_VACCINE_DN	192	-1.368	0.006	0.129	0.814	0.961	1.000
GSE11057_CD4_CENT_MEM_VS_PBMC_DN	182	-1.370	0.013	0.127	-0.621	1.000	1.000
GSE22886_NAIVE_BCELL_VS_DC_DN	196	-1.371	0.015	0.127	0.695	1.000	1.000

GSE2405_HEAT_KILLED_LYSATE_VS_LIVE_A_PHAGOCYTOPHILUM_STIM_NEUTROPHIL_24H_UP	193	-1.372	0.013	0.125	-0.958	0.576	1.000
GSE9316_IL6_KO_VS_IFNG_KO_INVIVO_EXPANDED_CD4_TCELL_DN	194	-1.374	0.000	0.123	0.814	0.945	1.000
GSE19888_CTRL_VS_TCELL_MEMBRANES_ACT_MAST_CELL_PRETREAT_A3R_INH_DN	192	-1.377	0.007	0.119	0.660	1.000	1.000
GSE1432_CTRL_VS_IFNG_24H_MICROGLIA_DN	186	-1.384	0.011	0.108	0.579	1.000	1.000
GSE36826_WT_VS_IL1R_KO_SKIN_STAPH_AUREUS_INF_DN	192	-1.394	0.007	0.093	0.782	0.992	1.000
GSE9988_ANTI_TREM1_AND_LPS_VS_VEHICLE_TREATED_MONOCYTES_DN	190	-1.396	0.007	0.092	0.378	1.000	1.000
GSE42724_NAIVE_VS_B1_BCELL_UP	191	-1.396	0.003	0.092	1.088	0.238	1.000
GSE38681_WT_VS_LYL1_KO_LYMPHOID_PRIMED_MULTIPOTENT_PROGENITOR_DN	192	-1.397	0.003	0.092	0.724	0.995	1.000
GSE1112_OT1_CD8AB_VS_HY_CD8AA_THYMOCYTE_RTOC_CULTURE_UP	141	-1.399	0.007	0.089	0.888	0.757	1.000
GSE21670_IL6_VS_TGFB_AND_IL6_TREATED_STAT3_KO_CD4_TCELL_DN	191	-1.400	0.003	0.089	-1.061	0.276	1.000
GSE40666_STAT1_KO_VS_STAT4_KO_CD8_TCELL_DN	153	-1.402	0.012	0.088	-1.119	0.192	1.000
GSE26343_UNSTIM_VS_LPS_STIM_NFAT5_KO_MACROPHAGE_DN	194	-1.404	0.000	0.086	-0.757	0.997	1.000
GSE9988_ANTI_TREM1_VS_VEHICLE_TREATED_MONOCYTES_DN	189	-1.405	0.007	0.085	-0.461	1.000	1.000
GSE15750_WT_VS_TRAF6KO_DAY6_EFF_CD8_TCELL_DN	192	-1.407	0.003	0.084	1.028	0.372	1.000
GSE45365_HEALTHY_VS_MCMV_INFECTION_CD8A_DC_IFNAR_KO_DN	197	-1.407	0.017	0.084	0.915	0.722	1.000
GSE34006_A2AR_KO_VS_A2AR_AGONIST_TREATED_TREG_UP	193	-1.407	0.000	0.085	0.502	1.000	1.000
GSE19401_PLN_VS_PEYERS_PATCH_FOLLICULAR_DC_DN	195	-1.412	0.009	0.081	1.120	0.187	1.000
GSE9988_LPS_VS_CTRL_TREATED_MONOCYTE_UP	174	-1.414	0.003	0.079	0.883	0.786	1.000
GSE41978_ID2_KO_VS_BIM_KO_KLRG1_LOW_EFFECTOR_CD8_TCELL_UP	189	-1.418	0.010	0.075	1.171	0.126	1.000
GSE6269_FLU_VS_STAPH_AURE	161	-1.421	0.007	0.072	1.133	0.201	1.000

US_INF_PBMC_DN							
GSE2405_HEAT_KILLED_VS_LIVE_A_PHAGOCYTOPHILUM_STIM_NEUTROPHIL_24H_DN	183	-1.421	0.000	0.072	-1.224	0.054	1.000
GSE2706_UNSTIM_VS_2H_LPS_DC_DN	172	-1.423	0.003	0.071	0.983	0.491	1.000
GSE44649_WT_VS_MIR155_KO_ACTIVATED_CD8_TCELL_UP	191	-1.425	0.000	0.070	-0.823	0.970	1.000
GSE21546_UNSTIM_VS_ANTI_CD3_STIM_SAP1A_KO_AND_ELK1_KO_DP_THYMOCYTES_UP	160	-1.435	0.006	0.060	0.863	0.837	1.000
GSE18791_CTRL_VS_NEWCASTLE_VIRUS_DC_16H_DN	169	-1.435	0.000	0.060	-0.723	1.000	1.000
GSE18281_SUBCAPSULAR_VS_CENTRAL_CORTICAL_REGION_OF_THYMUS_DN	192	-1.440	0.000	0.057	-0.656	1.000	1.000
GSE18281_CORTICAL_VS_MEDULLARY_THYMOCYTE_UP	189	-1.442	0.004	0.056	0.863	0.839	1.000
GSE9988_LOW_LPS_VS_VEHICLE_TREATED_MONOCYTE_UP	177	-1.442	0.003	0.056	-0.876	0.888	1.000
GSE11961_MARGINAL_ZONE_BCELL_VS_MEMORY_BCELL_DAY40_UP	192	-1.448	0.000	0.052	-1.084	0.216	1.000
GSE37416_12H_VS_24H_F_TULARENSIS_LVS_NEUTROPHIL_UP	184	-1.455	0.003	0.047	0.810	0.961	1.000
GSE45365_WT_VS_IFNAR_KO_CD8A_DC_DN	194	-1.463	0.003	0.041	0.985	0.494	1.000
GSE45365_WT_VS_IFNAR_KO_CD11B_DC_MCMV_INFECTION_UP	178	-1.468	0.000	0.038	-0.705	1.000	1.000
GSE5589_LPS_AND_IL10_VS_LPS_AND_IL6_STIM_IL6_KO_MACROPHAGE_45MIN_UP	191	-1.469	0.000	0.038	0.852	0.889	1.000
GSE34392_ST2_KO_VS_WT_DAY8_LCMV_EFFECTOR_CD8_TCELL_DN	195	-1.470	0.000	0.038	-0.821	0.957	1.000
GSE19888_ADENOSINE_A3R_INH_VS_TCELL_MEMBRANES_ACT_MAST_CELL_UP	190	-1.472	0.003	0.037	-0.821	0.965	1.000
GSE9006_TYPE_1_DIABETES_AT_DX_VS_1MONTH_POST_DX_PBMC_UP	193	-1.473	0.000	0.037	0.854	0.880	1.000
GSE19888_ADENOSINE_A3R_INH_VS_ACT_WITH_INHIBITOR_PRETREATMENT_IN_MAST_CELL_UP	188	-1.473	0.000	0.037	-0.940	0.652	1.000
GSE9988_LPS_VS_VEHICLE_TREATED_MONOCYTE_UP	177	-1.477	0.000	0.035	1.004	0.442	1.000
GSE18791_CTRL_VS_NEWCASTLE_VIRUS_DC_14H_DN	167	-1.481	0.003	0.034	0.991	0.484	1.000

GSE34205_HEALTHY_VS_FLU_INF_INFANT_PBMC_DN	193	-1.484	0.000	0.032	1.331	0.019	1.000
GSE37605_TREG_VS_TCONV_NOD_FOXP3_FUSION_GFP_UP	133	-1.495	0.003	0.027	-0.886	0.774	1.000
GSE10325_BCELL_VS_MYELOID_DN	190	-1.498	0.000	0.026	1.037	0.345	1.000
GSE21360_NAIVE_VS_QUATERNARY_MEMORY_CD8_TCELL_UP	199	-1.503	0.000	0.024	0.471	1.000	1.000
GSE29618_BCELL_VS_MONOCYTE_DAY7_FLU_VACCINE_DN	189	-1.510	0.000	0.022	1.070	0.267	1.000
GSE9988_LPS_VS_VEHICLE_TREATED_MONOCYTE_DN	188	-1.510	0.000	0.022	0.447	1.000	1.000
GSE11961_FOLLICULAR_BCELL_VS_MEMORY_BCELL_DAY7_UP	189	-1.514	0.000	0.020	1.052	0.333	1.000
GSE34205_RSV_VS_FLU_INF_INFANT_PBMC_DN	176	-1.522	0.000	0.018	0.630	1.000	1.000
GSE9601_NFKB_INHIBITOR_VS_PI3K_INHIBITOR_TREATED_HCMV_INF_MONOCYTE_DN	191	-1.522	0.000	0.018	0.878	0.805	1.000
GSE6269_HEALTHY_VS_STAPH_AUREUS_INF_PBMC_UP	161	-1.524	0.003	0.018	1.011	0.411	1.000
GSE29618_MONOCYTE_VS_MDC_UP	191	-1.527	0.000	0.017	1.192	0.080	1.000
GSE29618_MONOCYTE_VS_PDC_UP	190	-1.529	0.000	0.017	0.973	0.498	1.000
GSE45365_NK_CELL_VS_CD11B_DC_DN	194	-1.532	0.000	0.016	0.985	0.490	1.000
GSE36527_CD69_NEG_VS_POS_TREG_CD62L_LOS_KLRG1_NEG_UP	194	-1.534	0.000	0.015	-0.813	0.981	1.000
GSE369_PRE_VS_POST_IL6_INJECTION_SOCS3_KO_LIVER_DN	192	-1.537	0.000	0.015	0.698	1.000	1.000
GSE22140_HEALTHY_VS_ARTHRITIC_GERMFREE_MOUSE_CD4_TCELL_DN	192	-1.537	0.000	0.015	0.831	0.936	1.000
GSE21546_ELK1_KO_VS_SAP1A_KO_AND_ELK1_KO_DP_THYMOCYTES_UP	169	-1.540	0.000	0.015	0.712	1.000	1.000
GSE34006_WT_VS_A2AR_KO_TREG_DN	188	-1.543	0.000	0.014	-0.700	1.000	1.000
GSE42021_CD24INT_VS_CD24LOW_TREG_THYMUS_DN	188	-1.552	0.000	0.012	0.725	0.998	1.000
GSE29618_BCELL_VS_MONOCYTE_DN	191	-1.555	0.000	0.011	0.822	0.938	1.000
GSE33424_CD161_INT_VS_NEG_CD8_TCELL_UP	192	-1.588	0.000	0.006	-1.012	0.368	1.000
GSE11961_GERMINAL_CENTER_BCELL_DAY7_VS_MEMORY_BCELL_DAY40_DN	194	-1.593	0.000	0.006	-0.958	0.563	1.000

GSE9006_TYPE_1_DIABETES_AT_DX_VS_4MONTH_POST_DX_PBMC_UP	195	-1.593	0.000	0.006	0.665	1.000	1.000
GSE10325_MYELOID_VS_LUPUS_MYELOID_DN	193	-1.594	0.000	0.006	1.092	0.239	1.000
GSE10325_CD4_TCELL_VS_MYELOID_DN	182	-1.596	0.000	0.005	0.876	0.818	1.000
GSE42021_CD24INT_VS_CD24LOW_TCONV_THYMUS_DN	189	-1.601	0.000	0.005	-0.767	0.995	1.000
GSE14000_UNSTIM_VS_4H_LPS_DC_TRANSLATED_RNA_DN	182	-1.610	0.000	0.004	-0.869	0.875	1.000
GSE19401_NAIVE_VS_IMMUNIZED_MOUSE_PLN_FOLLICULAR_DC_UP	187	-1.612	0.000	0.004	0.710	1.000	1.000
GSE18791_CTRL_VS_NEWCASTLE_VIRUS_DC_12H_DN	178	-1.614	0.000	0.004	1.093	0.237	1.000
GSE42021_CD24HI_VS_CD24LOW_TREG_THYMUS_DN	185	-1.617	0.000	0.004	-0.770	0.995	1.000
GSE7548_NAIVE_VS_DAY7_PCC_IMMUNIZATION_CD4_TCELL_DN	198	-1.621	0.000	0.004	1.098	0.218	1.000
GSE17974_IL4_AND_ANTI_IL12_VS_UNTREATED_24H_ACT_CD4_TCELL_DN	165	-1.627	0.000	0.003	1.123	0.214	1.000
GSE21360_PRIMARY_VS_TERTIARY_MEMORY_CD8_TCELL_DN	192	-1.630	0.000	0.003	0.897	0.742	1.000
GSE21360_NAIVE_VS_QUATERNARY_MEMORY_CD8_TCELL_DN	198	-1.643	0.003	0.003	0.660	1.000	1.000
GSE18791_UNSTIM_VS_NEWCATSLE_VIRUS_DC_18H_DN	167	-1.644	0.000	0.003	0.964	0.544	1.000
GSE13485_DAY3_VS_DAY7_YF17D_VACCINE_PBMC_DN	183	-1.651	0.000	0.002	0.980	0.508	1.000
GSE37533_PPARG1_FOXP3_VS_PPARG2_FOXP3_TRANSDUCED_CD4_TCELL_PIOGLITAZONE_TREATED_DN	191	-1.654	0.000	0.002	0.629	1.000	1.000
GSE7509_DC_VS_MONOCYTE_WITH_FCGRIIB_STIM_DN	191	-1.661	0.000	0.002	1.289	0.015	1.000
GSE26030_TH1_VS_TH17_DAY5_POST_POLARIZATION_UP	191	-1.662	0.000	0.002	-0.860	0.896	1.000
GSE22886_NAIVE_CD4_TCELL_VS_MONOCYTE_DN	193	-1.668	0.000	0.002	0.984	0.456	1.000
GSE6259_FLT3L_INDUCED_DEC205_POS_DC_VS_CD8_TCELL_DN	160	-1.673	0.000	0.002	-0.704	1.000	1.000
GSE22886_NAIVE_CD8_TCELL_VS_MONOCYTE_DN	193	-1.685	0.000	0.001	1.085	0.273	1.000
GSE22886_CTRL_VS_LPS_24H_DC_DN	190	-1.690	0.000	0.001	1.011	0.417	1.000
GSE14000_UNSTIM_VS_4H_LPS_DC_DN	186	-1.700	0.000	0.001	-0.714	1.000	1.000

GSE13485_DAY1_VS_DAY7_YF17D_VACCINE_PBMC_DN	187	-1.704	0.000	0.001	0.882	0.782	1.000
GSE22886_NAIVE_TCELL_VS_MONOCYTE_DN	194	-1.711	0.000	0.001	1.211	0.081	1.000
GSE42021_TREG_PLN_VS_CD24INT_TREG_THYMUS_DN	190	-1.715	0.000	0.001	0.550	1.000	1.000
GSE13485_DAY3_VS_DAY21_YF17D_VACCINE_PBMC_UP	181	-1.720	0.000	0.001	0.861	0.864	1.000
GSE10325_LUPUS_BCELL_VS_LUPUS_MYELOID_DN	192	-1.735	0.000	0.000	1.093	0.235	1.000
GSE37533_PPARG1_FOXP3_VS_FOXP3_TRANSDUCED_CD4_TCELL_PIOGLITAZONE_TREATED_UP	193	-1.741	0.000	0.001	1.045	0.345	1.000
GSE22886_NAIVE_BCELL_VS_MONOCYTE_DN	193	-1.742	0.000	0.000	0.701	1.000	1.000
GSE2770_TGFB_AND_IL4_ACT_VS_ACT_CD4_TCELL_2H_DN	191	-1.745	0.000	0.000	1.028	0.388	1.000
GSE18791_CTRL_VS_NEWCASTLE_VIRUS_DC_4H_DN	173	-1.749	0.000	0.000	0.992	0.479	1.000
GSE6269_FLU_VS_STREP_PNEUMO_INF_PBMC_UP	159	-1.758	0.000	0.000	0.461	1.000	1.000
GSE29618_MONOCYTE_VS_PDC_DAY7_FLU_VACCINE_UP	198	-1.760	0.000	0.000	0.993	0.464	1.000
GSE10325_LUPUS_CD4_TCELL_VS_LUPUS_MYELOID_DN	190	-1.776	0.000	0.000	0.943	0.612	1.000
GSE22140_GERMFREE_VS_SPF_MOUSE_CD4_TCELL_UP	193	-1.786	0.000	0.000	0.887	0.790	1.000
GSE42021_TREG_VS_TCONV_PLN_UP	189	-1.791	0.000	0.000	0.865	0.832	1.000
GSE42021_TREG_PLN_VS_TREG_PRECURSORS_THYMUS_DN	189	-1.795	0.000	0.000	0.835	0.911	1.000
GSE21927_SPLEEN_C57BL6_VS_4T1_TUMOR_BALBC_MONOCYTES_DN	194	-1.801	0.000	0.000	0.666	1.000	1.000
GSE6269_HEALTHY_VS_FLU_INF_PBMC_DN	153	-1.820	0.000	0.000	0.894	0.746	1.000
GSE37534_UNTREATED_VS_PIOGLITAZONE_TREATED_CD4_TCELL_PPARG1_AND_FOXP3_TRASDUCED_DN	192	-1.849	0.000	0.000	0.749	0.995	1.000
GSE42021_TREG_PLN_VS_CD24LO_TREG_THYMUS_DN	183	-1.851	0.000	0.000	-0.797	0.987	1.000
GSE6269_FLU_VS_E_COLI_INF_PBMC_UP	152	-1.870	0.000	0.000	0.673	1.000	1.000
GSE18791_CTRL_VS_NEWCASTLE_VIRUS_DC_10H_DN	181	-1.886	0.000	0.000	0.979	0.503	1.000
GSE26890_CXCR1_NEG_VS_POS_EFFECTOR_CD8_TCELL_UP	190	-1.893	0.000	0.000	0.643	1.000	1.000
GSE18791_UNSTIM_VS_NEWCATSLE_VIRUS_DC_10H_DN	179	-1.894	0.000	0.000	1.022	0.393	1.000

GSE37533_PPARG2_FOXP3_VS_FOXP3_TRANSDUCED_CD4_TCELL_DN	189	-1.903	0.000	0.000	-0.802	0.984	1.000
GSE21546_WT_VS_SAP1A_KO_DP_THYMOCYTES_UP	176	-1.915	0.000	0.000	0.977	0.495	1.000
GSE13485_DAY7_VS_DAY21_YF17D_VACCINE_PBMC_UP	187	-1.926	0.000	0.000	0.739	1.000	1.000
GSE18791_UNSTIM_VS_NEWCASTLE_VIRUS_DC_6H_DN	177	-1.955	0.000	0.000	1.049	0.332	1.000
GSE18791_CTRL_VS_NEWCASTLE_VIRUS_DC_6H_DN	186	-1.961	0.000	0.000	1.068	0.292	1.000
GSE42724_NAIVE_BCELL_VS_PLASMABLAST_UP	184	-1.969	0.000	0.000	-0.794	0.997	1.000
GSE18791_CTRL_VS_NEWCASTLE_VIRUS_DC_8H_DN	180	-1.971	0.000	0.000	0.772	0.990	1.000
GSE19888_ADENOSINE_A3R_INH_PRETREAT_AND_ACT_BY_A3R_VS_TCELL_MEMBRANES_ACT_MAST_CELL_UP	191	-1.987	0.000	0.000	-0.831	0.967	1.000
GSE13485_CTRL_VS_DAY7_YF17D_VACCINE_PBMC_DN	194	-1.999	0.000	0.000	0.944	0.603	1.000
GSE13485_PRE_VS_POST_YF17D_VACCINATION_PBMC_DN	192	-2.015	0.000	0.000	0.788	0.983	1.000
GSE13484_UNSTIM_VS_YF17D_VACCINE_STIM_PBMC_DN	190	-2.018	0.000	0.000	-0.628	1.000	1.000
GSE42021_CD24HI_VS_CD24INT_TREG_THYMUS_DN	190	-2.046	0.000	0.000	0.490	1.000	1.000
GSE37533_PPARG1_FOXP3_VS_FOXP3_TRANSDUCED_CD4_TCELL_DN	191	-2.050	0.000	0.000	0.602	1.000	1.000
GSE13485_CTRL_VS_DAY3_YF17D_VACCINE_PBMC_DN	187	-2.058	0.000	0.000	1.123	0.195	1.000

Supplemental Table 4. Significantly Enriched Gene Sets from Phase I Analysis of MSigDB Hallmark Gene Sets

		PD-1 Ab Treated			Saline Treated		
NAME	SIZE	NES	NOM p-val	FDR q-val	NES	NOM p-val	FDR q-val
HALLMARK_INTERFERON_ALPHA_RESPONSE	93	-2.040	0.000	0.000	0.778	0.928	1.000
HALLMARK_HEME_METABOLISM	198	1.858	0.000	0.001	-0.505	1.000	1.000
HALLMARK_INTERFERON_GAMMA_RESPONSE	189	-1.818	0.000	0.002	0.768	0.995	1.000
HALLMARK_TNFA_SIGNALING_VIA_NFKB	200	-1.617	0.000	0.013	0.925	0.676	1.000
HALLMARK_XENOBIOTIC_METABOLISM	197	-1.572	0.000	0.017	-0.930	0.695	1.000
HALLMARK_E2F_TARGETS	199	1.570	0.001	0.022	-0.775	0.995	1.000
HALLMARK_HEDGEHOG_SIGNALING	36	-1.454	0.040	0.051	-0.683	0.971	1.000
HALLMARK_PANCREAS_BETA_CELLS	40	1.386	0.062	0.165	-0.992	0.470	1.000

Supplemental Table 5. Significantly Enriched Gene Sets from Phase II Analysis of MSigDB C2 Curated Gene Sets

		ALL PD-1 Treated		
NAME	SIZE	NES	NOM p-val	FDR q-val
ROSTY_CERVICAL_CANCER_PROLIFERATION_CLUSTER	138	2.066	0.000	0.000
SOTIRIOU_BREAST_CANCER_GRADE_1_VS_3_UP	147	2.061	0.000	0.000
GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_UP	174	2.031	0.000	0.000
GRAHAM_CML_QUIESCENT_VS_NORMAL_QUIESCENT_UP	82	1.969	0.000	0.001
GRAHAM_NORMAL_QUIESCENT_VS_NORMAL_DIVIDING_DN	87	1.945	0.000	0.001
JAATINEN_HEMATOPOIETIC_STEM_CELL_DN	213	1.926	0.000	0.004
ISHIDA_E2F_TARGETS	51	1.898	0.000	0.006
REACTOME_AMYLOIDS	74	1.879	0.000	0.011
VALK_AML_CLUSTER_7	27	1.872	0.000	0.011
CHANG_CYCLING_GENES	143	1.868	0.000	0.010
RUIZ_TNC_TARGETS_DN	137	1.867	0.000	0.009
VALK_AML_CLUSTER_8	25	1.862	0.000	0.010
GAVIN_FOXP3_TARGETS_CLUSTER_P6	87	1.860	0.000	0.010
REACTOME_DEPOSITION_OF_NEW_CENPA_CONTAINING_NUCLEOSOMES_AT_THE_CENTROMERE	60	1.859	0.000	0.009
ZHAN_MULTIPLE_MYELOMA_PR_UP	42	1.857	0.000	0.009
RAGHAVACHARI_PLATELET_SPECIFIC_GENES	68	1.848	0.000	0.010
IVANOVA_HEMATOPOIESIS_MATURE_CELL	276	1.830	0.000	0.016
KONG_E2F3_TARGETS	94	1.825	0.000	0.017
STEINER_ERYTHROCYTE_MEMBRANE_GENES	15	1.819	0.000	0.018
WIERENGA_STAT5A_TARGETS_GROUP2	56	1.815	0.000	0.019
REACTOME_RNA_POL_I_PROMOTER_OPENING	55	1.807	0.002	0.023
KOBAYASHI_EGFR_SIGNALING_24HR_DN	243	1.801	0.000	0.025
HAHTOLA_SEZARY_SYNDROM_UP	92	1.796	0.000	0.026
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	113	1.795	0.000	0.025
REACTOME_FACTORS_INVOLVED_IN_MEGAKARYOCYTE_DEVELOPMENT_AND_PLATELET_PRODUCTION	122	1.792	0.000	0.026
BURTON_ADIPOGENESIS_3	100	1.786	0.000	0.028
MAHADEVAN_RESPONSE_TO_MP470_UP	19	1.766	0.000	0.040
BENPORATH_PROLIFERATION	140	1.758	0.000	0.044
VANTVEER_BREAST_CANCER_METASTASIS_DN	115	1.757	0.000	0.043
REACTOME_MITOTIC_M_M_G1_PHASES	167	1.754	0.000	0.045
KANG_DOXORUBICIN_RESISTANCE_UP	54	1.753	0.000	0.044
REACTOME_RNA_POL_I_TRANSCRIPTION	78	1.753	0.000	0.043
REICHERT_MITOSIS_LIN9_TARGETS	27	1.752	0.002	0.042

WELCH_GATA1_TARGETS	22	1.752	0.000	0.041
REACTOME_MEIOTIC_RECOMBINATION	78	1.751	0.000	0.040
RHODES_UNDIFFERENTIATED_CANCER	69	1.745	0.000	0.043
LEE_EARLY_T_LYMPHOCYTE_UP	100	1.737	0.000	0.049
REACTOME_PACKAGING_OF_TELOMERE_ENDS	46	1.735	0.000	0.050
WINNEPENNINCKX_MELANOMA_METASTASIS_UP	153	1.723	0.000	0.061
REACTOME_CHROMOSOME_MAINTENANCE	116	1.721	0.000	0.061
SENGUPTA_NASOPHARYNGEAL_CARCINOMA_UP	285	1.716	0.000	0.065
REACTOME_DNA_REPLICATION	187	1.715	0.000	0.064
CROONQUIST_IL6_DEPRIVATION_DN	98	1.715	0.002	0.063
KHETCHOUMIAN_TRIM24_TARGETS_UP	46	1.706	0.004	0.072
KUROZUMI_RESPONSE_TO_ONCOCYTIC_VIRUS_AND_CYCLIC_RGD	21	1.704	0.002	0.073
REACTOME_MEIOSIS	106	1.704	0.000	0.072
GAVIN_FOXP3_TARGETS_CLUSTER_T4	91	1.703	0.000	0.071
DUTERTRE_ESTRADIOL_RESPONSE_24HR_UP	314	1.698	0.000	0.076
HECKER_IFNB1_TARGETS	91	1.697	0.000	0.075
WIELAND_UP_BY_HBV_INFECTION	87	1.693	0.000	0.079
GOLDRATH_ANTIGEN_RESPONSE	332	1.692	0.000	0.079
PID_ALPHA_SYNUCLEIN_PATHWAY	33	1.691	0.002	0.078
MISSIAGLIA_REGULATED_BY_METHYLATION_DN	117	1.686	0.000	0.083
REACTOME_REGULATION_OF_IFNA_SIGNALING	21	1.685	0.004	0.084
ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_24HR	122	1.683	0.000	0.084
REACTOME_TELOMERE_MAINTENANCE	73	1.680	0.000	0.088
ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_6HR	82	1.676	0.000	0.091
ODONNELL_TFRC_TARGETS_DN	127	1.671	0.000	0.097
REACTOME_CELL_CYCLE	391	1.667	0.000	0.101
WHITFIELD_CELL_CYCLE_LITERATURE	44	1.665	0.000	0.102
REACTOME_DEFENSINS	42	1.665	0.002	0.100
KAUFFMANN_MELANOMA_RELAPSE_UP	58	1.664	0.000	0.100
RADAEVA_RESPONSE_TO_IFNA1_UP	50	1.664	0.000	0.099
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	53	1.664	0.004	0.098
PID_NFAT_TFPATHWAY	47	1.663	0.000	0.098
CHEMNITZ_RESPONSE_TO_PROSTAGLANDIN_E2_UP	133	1.662	0.000	0.098
REACTOME_MITOTIC_PROMETAPHASE	85	1.659	0.004	0.101
WU_APOPTOSIS_BY_CDKN1A_VIA_TP53	54	1.657	0.000	0.101
TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_10D_UP	178	1.656	0.000	0.101
KEGG_AUTOIMMUNE_THYROID_DISEASE	28	1.656	0.009	0.101
CHIANG_LIVER_CANCER_SUBCLASS_PROLIFERATION_UP	172	1.653	0.000	0.103

GNATENKO_PLATELET_SIGNATURE	40	1.653	0.004	0.102
REACTOME_MEIOTIC_SYNAPSIS	69	1.652	0.002	0.102
REACTOME_ENOS_ACTIVATION_AND_REGULATION	18	1.651	0.004	0.102
SARRIO_EPITHELIAL_MESENCHYMAL_TRANSITION_UP	171	1.651	0.000	0.100
TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_8D_UP	146	1.650	0.000	0.101
LINDGREN_BLADDER_CANCER_CLUSTER_3_UP	311	1.646	0.000	0.106
MOLENAAR_TARGETS_OF_CCND1_AND_CDK4_DN	55	1.645	0.000	0.106
BIOCARTA_EDG1_PATHWAY	27	1.641	0.002	0.110
FERREIRA_EWINGS_SARCOMA_UNSTABLE_VS_STABLE_UP	157	1.640	0.000	0.111
PUIFFE_INVASION_INHIBITED_BY_ASCITES_UP	81	1.640	0.000	0.109
FARMER_BREAST_CANCER_CLUSTER_2	33	1.638	0.004	0.111
WANG_RESPONSE_TO_GSK3_INHIBITOR_SB216763_DN	340	1.637	0.000	0.112
VALK_AML_CLUSTER_4	25	1.637	0.012	0.111
HORIUCHI_WTAP_TARGETS_DN	299	1.633	0.000	0.115
MARKEY_RB1_ACUTE_LOF_DN	212	1.630	0.000	0.118
ODONNELL_TARGETS_OF_MYC_AND_TFRC_DN	45	1.630	0.006	0.117
CHIANG_LIVER_CANCER_SUBCLASS_INTERFERON_DN	46	1.627	0.006	0.120
PID_FGF_PATHWAY	54	1.627	0.002	0.119
FLECHNER_BIOPSY_KIDNEY_TRANSPLANT_REJECTED_VS_OK_UP	83	1.627	0.002	0.118
TANG_SENESCENCE_TP53_TARGETS_DN	55	1.626	0.006	0.117
CHYLA_CBFA2T3_TARGETS_DN	227	1.626	0.000	0.117
REACTOME_RNA_POL_I_RNA_POL_III_AND_MITOCHONDRIAL_TRANSCRIPTION	111	1.625	0.000	0.117
REACTOME_TRAF6_MEDIATED_IRF7_ACTIVATION	27	1.619	0.014	0.126
SCHURINGA_STAT5A_TARGETS_UP	21	1.618	0.014	0.127
BIOCARTA_LAIR_PATHWAY	17	1.613	0.012	0.134
KOINUMA_COLON_CANCER_MSI_UP	15	1.607	0.014	0.141
XU_HGF_TARGETS_INDUCED_BY_AKT1_48HR_DN	25	1.606	0.008	0.142
WHITEFORD_PEDIATRIC_CANCER_MARKERS	115	1.602	0.000	0.148
MORI_IMMATURE_B_LYMPHOCYTE_DN	90	1.601	0.000	0.149
FIGUEROA_AML_METHYLATION_CLUSTER_5_DN	44	1.601	0.008	0.149
REACTOME_M_G1_TRANSITION	78	1.600	0.002	0.148
REN_BOUND_BY_E2F	60	1.596	0.002	0.154
PID_VEGFR1_PATHWAY	25	1.596	0.010	0.152
BIOCARTA_NO1_PATHWAY	29	1.593	0.014	0.157
MOSERLE_IFNA_RESPONSE	31	1.591	0.012	0.159
MAGRANGEAS_MULTIPLE_MYELOMA_IGG_VS_IGA_DN	24	1.591	0.012	0.158
KEGG_PORPHYRIN_AND_CHLOROPHYLL_METABOLISM	39	1.591	0.011	0.156

SHEPARD_BMYB_TARGETS	71	1.591	0.004	0.155
MARIADASON_RESPONSE_TO_CURCUMIN_SULINDAC_7	18	1.591	0.015	0.154
LUCAS_HNF4A_TARGETS_UP	57	1.589	0.006	0.156
REACTOME_MITOTIC_G1_G1_S_PHASES	129	1.587	0.002	0.158
JOHANSSON_GLIOMAGENESIS_BY_PDGFB_UP	55	1.586	0.004	0.160
OLSSON_E2F3_TARGETS_DN	47	1.584	0.005	0.161
HOFFMANN_LARGE_TO_SMALL_PRE_BII_LYMPHOCYTE_UP	159	1.584	0.000	0.161
SENESE_HDAC2_TARGETS_UP	110	1.583	0.000	0.160
KEGG_ONE_CARBON_POOL_BY_FOLATE	17	1.582	0.010	0.161
MOROSETTI_FACIOSCAPULOHUMERAL_MUSCULAR_DISTROPHY_UP	20	1.582	0.025	0.161
FINETTI_BREAST_CANCER_KINOME_RED	16	1.573	0.019	0.176
PICCALUGA_ANGIOIMMUNOBLASTIC_LYMPHOMA_DN	128	1.573	0.000	0.175
SUZUKI_RESPONSE_TO_TSA_AND_DECITABINE_1A	21	1.571	0.010	0.178
GRAHAM_CML_QUIESCENT_VS_NORMAL_DIVIDING_UP	53	1.571	0.010	0.177
KAMMINGA_EZH2_TARGETS	41	1.569	0.010	0.179
MORI_PRE_BI_LYMPHOCYTE_UP	80	1.568	0.002	0.178
ONDER_CDH1_TARGETS_3_DN	57	1.568	0.006	0.178
PID_AURORA_B_PATHWAY	39	1.567	0.010	0.178
YU_BAP1_TARGETS	28	1.566	0.006	0.178
GYORFFY_DOXORUBICIN_RESISTANCE	46	1.565	0.008	0.179
CAFFAREL_RESPONSE_TO_THC_24HR_5_UP	32	1.564	0.024	0.181
REACTOME_CELL_CYCLE_MITOTIC	302	1.563	0.000	0.181
REACTOME_G1_S_TRANSITION	105	1.562	0.000	0.181
YU_MYC_TARGETS_UP	42	1.562	0.011	0.181
TAKAO_RESPONSE_TO_UVB_RADIATION_UP	83	1.558	0.006	0.187
ALCALAY_AML_BY_NPM1_LOCALIZATION_DN	179	1.558	0.000	0.186
WHITFIELD_CELL_CYCLE_G2_M	205	1.558	0.000	0.185
ST_INTEGRIN_SIGNALING_PATHWAY	82	1.557	0.006	0.185
AMUNDSON_GAMMA_RADIATION_RESPONSE	39	1.557	0.012	0.184
SENESE_HDAC1_AND_HDAC2_TARGETS_UP	224	1.557	0.000	0.183
SASAKI_ADULT_T_CELL_LEUKEMIA	169	1.556	0.000	0.183
JISON_SICKLE_CELL_DISEASE_UP	177	1.554	0.000	0.187
BASAKI_YBX1_TARGETS_UP	272	1.553	0.000	0.188
GENTLES_LEUKEMIC_STEM_CELL_DN	19	1.547	0.027	0.198
ZHU_CMV_ALL_UP	118	1.547	0.008	0.197
BERENJENO_TRANSFORMED_BY_RHOA_FOREVER_UP	19	1.547	0.023	0.196
REACTOME_ASSEMBLY_OF_THE_PRE_REPLICATIVE_COMPLEX	63	1.545	0.011	0.200

Supplemental Table 6. Significantly Enriched Gene Sets from Phase II Analysis of MSigDB C7 Immunologic Gene Sets

		ALL PD-1 Treated		
NAME	SIZE	NES	NOM p-val	FDR q-val
GSE34205_HEALTHY_VS_RSV_INF_INFANT_PBMC_DN	192	2.076	0.000	0.000
GSE15750_DAY6_VS_DAY10_EFF_CD8_TCELL_UP	195	1.991	0.000	0.000
GSE34205_RSV_VS_FLU_INF_INFANT_PBMC_UP	164	1.960	0.000	0.000
GSE15750_DAY6_VS_DAY10_TRAF6KO_EFF_CD8_TCELL_UP	192	1.934	0.000	0.000
GSE36476_CTRL_VS_TSST_ACT_72H_MEMORY_CD4_TCELL_YOUNG_DN	194	1.923	0.000	0.000
GSE27241_WT_VS_RORGT_KO_TH17_POLARIZED_CD4_TCELL_UP	157	1.868	0.000	0.000
GSE13547_CTRL_VS_ANTI_IGM_STIM_BCELL_12H_UP	180	1.852	0.000	0.000
GOLDRATH_NAIVE_VS_EFF_CD8_TCELL_DN	194	1.846	0.000	0.000
GSE14415_INDUCED_VS_NATURAL_TREG_DN	176	1.844	0.000	0.000
GSE39110_DAY3_VS_DAY6_POST_IMMUNIZATION_CD8_TCELL_DN	195	1.813	0.000	0.000
GSE13547_CTRL_VS_ANTI_IGM_STIM_BCELL_2H_UP	176	1.803	0.000	0.000
GSE36476_CTRL_VS_TSST_ACT_72H_MEMORY_CD4_TCELL_OLD_DN	196	1.798	0.000	0.000
GSE36476_CTRL_VS_TSST_ACT_40H_MEMORY_CD4_TCELL_YOUNG_DN	196	1.735	0.000	0.001
GSE36476_CTRL_VS_TSST_ACT_40H_MEMORY_CD4_TCELL_OLD_DN	194	1.727	0.000	0.001
GSE14415_TCONV_VS_FOXP3_KO_INDUCED_TREG_DN	179	1.727	0.000	0.001
GSE17974_IL4_AND_ANTI_IL12_VS_UNTREATED_24H_ACT_CD4_TCELL_UP	152	1.717	0.000	0.001
GSE40274_CTRL_VS_FOXP3_AND_LEF1_TRANSDUCED_ACTIVATED_CD4_TCELL_DN	163	1.716	0.000	0.001
KAECH_DAY8_EFF_VS_MEMORY_CD8_TCELL_UP	191	1.710	0.000	0.002
GSE42021_TREG_PLN_VS_CD24INT_TREG_THYMUS_UP	193	1.696	0.000	0.003
GSE14415_INDUCED_TREG_VS_TCONV_UP	176	1.695	0.000	0.003
GSE37533_PPARG1_FOXP3_VS_FOXP3_TRANSDUCED_CD4_TCELL_DN	191	1.684	0.000	0.004
GSE2405_S_AUREUS_VS_UNTREATED_NEUTROPHIL_DN	194	1.665	0.000	0.007
GSE21774_CD56_BRIGHT_VS_DIM_CD62L_POSITIVE_NK_CELL_UP	192	1.662	0.000	0.007
GSE2770_IL12_ACT_VS_ACT_CD4_TCELL_2H_DN	184	1.659	0.000	0.007
GOLDRATH_EFF_VS_MEMORY_CD8_TCELL_UP	197	1.647	0.000	0.009
GSE33292_WT_VS_TCF1_KO_DN3_THYMOCYTE_DN	199	1.641	0.000	0.010
GSE17186_CD21LOW_VS_CD21HIGH_TRANSITIONAL_BCELL_DN	196	1.638	0.000	0.011
GSE4984_UNTREATED_VS_GALECTIN1_TREATED_DC_UP	171	1.634	0.000	0.011

KAECH_DAY8_EFF_VS_DAY15_EFF_CD8_TCELL_UP	193	1.631	0.000	0.011
KAECH_NAIVE_VS_DAY8_EFF_CD8_TCELL_DN	195	1.627	0.000	0.012
GSE23308_CTRL_VS_CORTICOSTERONE_TREATED_MACROPHAGE_MINERALCORTICOID_REC_KO_DN	190	1.625	0.000	0.012
GSE14415_NATURAL_TREG_VS_TCONV_DN	177	1.613	0.000	0.015
GSE21063_WT_VS_NFATC1_KO_8H_ANTI_IGM_STIM_BCELL_UP	190	1.612	0.000	0.015
GSE2770_UNTREATED_VS_IL4_TREATED_ACT_CD4_TCELL_2H_UP	192	1.610	0.000	0.015
GSE30962_PRIMARY_VS_SECONDARY_ACUTE_LCMV_INF_CD8_TCELL_UP	194	1.606	0.000	0.016
GSE40274_CTRL_VS_EOS_TRANSDUCED_ACTIVATED_CD4_TCELL_UP	171	1.604	0.000	0.016
GSE24634_TEFF_VS_TCONV_DAY7_IN_CULTURE_UP	194	1.603	0.000	0.016
GSE9960_HEALTHY_VS_SEPSIS_PBMC_UP	174	1.601	0.000	0.017
GSE45365_CD8A_DC_VS_CD11B_DC_UP	187	1.600	0.000	0.016
GSE7568_IL4_VS_IL4_AND_DEXAMETHASONE_TREATED_MACROPHAGE_UP	165	1.598	0.000	0.017
GSE45365_HEALTHY_VS_MCMV_INFECTION_CD11B_DC_IFNAR_KO_DN	170	1.587	0.000	0.021
GSE15330_HSC_VS_LYMPHOID_PRIMED_MULTIPOTENT_PROGENITOR_DN	179	1.587	0.000	0.020
GSE22886_NAIVE_BCELL_VS_MONOCYTE_DN	193	1.581	0.000	0.023
GSE3982_CENT_MEMORY_CD4_TCELL_VS_TH1_DN	197	1.578	0.000	0.024
GSE41176_UNSTIM_VS_ANTI_IGM_STIM_BCELL_1H_DN	191	1.576	0.002	0.024
GSE24634_TREG_VS_TCONV_POST_DAY7_IL4_CONVERSION_UP	191	1.575	0.000	0.024
GSE27434_WT_VS_DNMT1_KO_TREG_DN	194	1.573	0.000	0.024
GSE39556_CD8A_DC_VS_NK_CELL_MOUSE_3H_POST_POLYIC_INJ_UP	197	1.571	0.000	0.025
GSE11057_NAIVE_VS_MEMORY_CD4_TCELL_DN	189	1.570	0.000	0.025
GSE21360_PRIMARY_VS_TERTIARY_MEMORY_CD8_TCELL_DN	192	1.570	0.002	0.024
GSE2706_UNSTIM_VS_2H_LPS_AND_R848_DC_DN	173	1.568	0.000	0.025
GSE9509_LPS_VS_LPS_AND_IL10_STIM_IL10_KO_MACROPHAGE_20MIN_DN	192	1.568	0.002	0.024
GSE12845_IGD_POS_BLOOD_VS_PRE_GC_TONSIL_BCELL_DN	198	1.566	0.000	0.025
GSE24634_NAIVE_CD4_TCELL_VS_DAY10_IL4_CONV_TREG_DN	196	1.562	0.000	0.027
GSE9988_ANTI_TREM1_VS_ANTI_TREM1_AND_LPS_MONOCYTE_DN	172	1.560	0.000	0.027
GSE34156_UNTREATED_VS_6H_TLR1_TLR2_LIGAND_TREATED_MONOCYTE_UP	176	1.553	0.000	0.031
GSE13485_DAY1_VS_DAY7_YF17D_VACCINE_PBMC_DN	187	1.553	0.000	0.031
GSE3720_VD1_VS_VD2_GAMMADELTA_TCELL_DN	195	1.548	0.002	0.033

GSE13547_2H_VS_12_H_ANTI_IGM_STIM_BCELL_DN	177	1.546	0.000	0.034
GSE3039_NKT_CELL_VS_B2_BCELL_UP	196	1.546	0.000	0.034
GSE22886_NAIVE_BCELL_VS_NEUTROPHIL_DN	184	1.542	0.002	0.035
GSE16755_CTRL_VS_IFNA_TREATED_MAC_DN	184	1.541	0.002	0.036
GSE4748_CYANOBACTERIUM_LPSLIKE_VS_LPS_AND_CYANOBACTERIUM_LPSLIKE_STIM_DC_3H_DN	170	1.540	0.000	0.036
GSE29618_MONOCYTE_VS_MDC_UP	191	1.539	0.002	0.036
GSE19923_E2A_KO_VS_E2A_AND_HEB_KO_DP_THYMOCYTE_DN	198	1.538	0.002	0.036
GSE6674_ANTI_IGM_VS_PL2_3_STIM_BCELL_UP	188	1.537	0.002	0.036
GSE35685_CD34POS_CD38NEG_VS_CD34POS_CD10POS_BONE_MARROW_DN	194	1.536	0.000	0.037
GSE37301_HEMATOPOIETIC_STEM_CELL_VS_CD4_TCELL_UP	169	1.536	0.000	0.036
GSE3982_EOSINOPHIL_VS_CENT_MEMORY_CD4_TCELL_UP	185	1.535	0.000	0.036
GSE14415_ACT_TCONV_VS_ACT_NATURAL_TREG_DN	173	1.531	0.000	0.039
GSE21546_SAP1A_KO_VS_SAP1A_KO_AND_ELK1_KO_ANTI_CD3_STIM_DP_THYMOCYTES_UP	192	1.529	0.000	0.041
GSE42021_TCONV_PLN_VS_TREG_PRECURSORS_THYMUS_DN	194	1.527	0.000	0.041
GSE18893_TCONV_VS_TREG_24H_TNF_STIM_UP	193	1.525	0.000	0.042
GSE13547_WT_VS_ZFX_KO_BCELL_DN	172	1.525	0.000	0.042
GSE14699_NAIVE_VS_DELETIONAL_TOLERANCE_CD8_TCELL_DN	193	1.520	0.002	0.045
GSE11057_NAIVE_CD4_VS_PBMC_CD4_TCELL_DN	187	1.519	0.000	0.045
GSE7548_NAIVE_VS_DAY7_PCC_IMMUNIZATION_CD4_TCELL_DN	198	1.517	0.000	0.046
GSE16451_IMMATURE_VS_MATURE_NEURON_CELL_LINE_WEST_EQUINE_ENC_VIRUS_UP	191	1.516	0.000	0.047
GSE19888_ADENOSINE_A3R_INH_PRETREAT_AND_ACT_BY_A3R_VS_TCELL_MEMBRANES_ACT_MAST_CELL_UP	191	1.513	0.000	0.049
GSE24142_EARLY_THYMIC_PROGENITOR_VS_DN2_THYMOCYTE_FETAL_DN	193	1.513	0.000	0.049
GSE5679_CTRL_VS_PPARG_LIGAND_ROSIGLITAZONE_TREATED_DC_UP	195	1.512	0.002	0.049
GSE39110_DAY3_VS_DAY6_POST_IMMUNIZATION_CD8_TCELL_WITH_IL2_TREATMENT_UP	192	1.512	0.000	0.048
GSE23568_CTRL_VS_ID3_TRANSDUCED_CD8_TCELL_DN	193	1.509	0.000	0.050
GSE36826_WT_VS_IL1R_KO_SKIN_STAPH_AUREUS_INF_UP	194	1.508	0.000	0.050
GSE36888_UNTREATED_VS_IL2_TREATED_TCELL_6H_UP	177	1.508	0.000	0.050
GSE7460_FOXP3_MUT_VS_WT_ACT_WITH_TGFB_TCONV_UP	189	1.505	0.000	0.052
GSE7219_WT_VS_NIK_NFKB2_KO_LPS_AND_ANTI_CD40_STIM_DC_UP	197	1.502	0.002	0.054
GSE3982_MAST_CELL_VS_NKCELL_UP	198	1.500	0.000	0.055

GSE33425_CD161_INT_VS_NEG_CD8_TCELL_UP	197	1.497	0.002	0.058
GSE12392_CD8A_POS_VS_NEG_SPLEEN_DC_DN	194	1.495	0.000	0.059
GSE21379_TFH_VS_NON_TFH_SAP_KO_CD4_TCELL_UP	194	1.494	0.004	0.060
GSE19401_UNSTIM_VS_PAM2CSK4_STIM_FOLLICULAR_DC_DN	197	1.493	0.003	0.061
GSE13547_2H_VS_12_H_ANTI_IGM_STIM_BCELL_UP	171	1.489	0.002	0.064
GSE29614_CTRL_VS_DAY7_TIV_FLU_VACCINE_PBMC_DN	176	1.489	0.004	0.064
GSE3982_MAC_VS_TH1_DN	190	1.488	0.002	0.064
GSE7852_LN_VS_THYMUS_TCONV_DN	193	1.483	0.002	0.069
GSE40666_WT_VS_STAT1_KO_CD8_TCELL_WITH_IFNA_STIM_90MIN_DN	196	1.480	0.002	0.072
GSE10239_NAIVE_VS_KLRG1HIGH_EFF_CD8_TCELL_DN	188	1.479	0.000	0.073
GSE22886_NAIVE_CD4_TCELL_VS_DC_DN	193	1.479	0.005	0.073
GSE10239_NAIVE_VS_DAY4.5_EFF_CD8_TCELL_DN	191	1.478	0.000	0.073
GSE34006_UNTREATED_VS_A2AR_AGONIST_TREATED_TREG_UP	193	1.477	0.000	0.073
GSE16266_CTRL_VS_HEATSHOCK_AND_LPS_STIM_MEF_UP	194	1.477	0.000	0.072
GSE37533_PPARG2_FOXP3_VS_FOXP3_TRANSDUCED_CD4_TCELL_DN	189	1.476	0.002	0.073
GSE11057_NAIVE_VS_CENT_MEMORY_CD4_TCELL_DN	190	1.475	0.002	0.074
GSE15930_NAIVE_VS_72H_IN_VITRO_STIM_CD8_TCELL_DN	190	1.475	0.004	0.074
GSE37532_WT_VS_PPARG_KO_LN_TREG_DN	111	1.472	0.006	0.076
GSE9006_HEALTHY_VS_TYPE_1_DIABETES_PBMC_4MONTH_POST_DX_UP	195	1.472	0.002	0.076
GSE360_T_GONDII_VS_M_TUBERCULOSIS_DC_DN	186	1.470	0.002	0.077
GSE24634_IL4_VS_CTRL_TREATED_NAIVE_CD4_TCELL_DAY7_UP	190	1.469	0.000	0.078
GSE32034_LY6C_HIGH_VS_LOW_ROSIGLIZATONE_TREATED_MONOCYTE_DN	194	1.469	0.006	0.077
GSE40274_CTRL_VS_FOXP3_AND_GATA1_TRANSDUCED_ACTIVATED_CD4_TCELL_DN	142	1.467	0.008	0.079
GSE37533_PPARG1_FOXP3_VS_PPARG2_FOXP3_TRANSDUCED_CD4_TCELL_PIOGLITAZONE_TREATED_DN	191	1.467	0.005	0.080
GSE33162_UNTREATED_VS_4H_LPS_STIM_HDAC3_KO_MACROPHAGE_DN	197	1.466	0.000	0.080
GSE42021_TREG_VS_TCONV_PLN_UP	189	1.466	0.004	0.080
GSE29618_MONOCYTE_VS_MDC_DAY7_FLU_VACCINE_UP	190	1.465	0.000	0.080
GSE37532_WT_VS_PPARG_KO_VISCERAL_ADIPOSE_TISSUE_TREG_UP	184	1.464	0.004	0.081
GSE40274_CTRL_VS_FOXP3_AND_IRF4_TRANSDUCED_ACTIVATED_CD4_TCELL_DN	157	1.463	0.006	0.082
GSE25085_FETAL_LIVER_VS_FETAL_BM_SP4_THYMIC_IMPLANT_DN	185	1.459	0.002	0.086
GSE2706_UNSTIM_VS_2H_LPS_DC_DN	172	1.459	0.007	0.086

GSE24634_TREG_VS_TCONV_POST_DAY10_IL4_CONVERSION_UP	195	1.457	0.000	0.087
GSE7852_TREG_VS_TCONV_FAT_DN	190	1.457	0.002	0.087
GSE14415_INDUCED_TREG_VS_FAILED_INDUCED_TREG_DN	176	1.457	0.002	0.086
GSE11386_NAIVE_VS_MEMORY_BCELL_UP	180	1.457	0.006	0.086
GSE45365_CD8A_DC_VS_CD11B_DC_IFNAR_KO_MCMV_INFECTION_UP	169	1.456	0.004	0.086
GSE19941_UNSTIM_VS_LPS_STIM_IL10_KO_NFKBP50_KO_MACROPHAGE_UP	190	1.456	0.000	0.086
GSE32164_RESTING_DIFFERENTIATED_VS_ALTERNATIVELY_ACT_M2_MACROPHAGE_UP	196	1.453	0.002	0.089
GSE36888_UNTREATED_VS_IL2_TREATED_STAT5_AB_KNOCKIN_TCELL_17H_UP	175	1.451	0.000	0.091
GSE13485_DAY1_VS_DAY21_YF17D_VACCINE_PBMC_DN	181	1.451	0.004	0.092
GSE32986_GMCSF_VS_GMCSF_AND_CURDLAN_LOWDOSE_STIM_DC_UP	190	1.449	0.002	0.093
GSE3039_ALPHAALPHA_VS_ALPHABETA_CD8_TCELL_DN	190	1.448	0.002	0.094
GSE18281_SUBCAPSULAR_VS_CENTRAL_CORTICAL_REGION_OF_THYMUS_DN	192	1.448	0.002	0.094
GSE19401_NAIVE_VS_IMMUNIZED_MOUSE_PLN_FOLLICULAR_DC_UP	187	1.447	0.006	0.094
GSE36392_TYPE_2_MYELOID_VS_NEUTROPHIL_IL25_TREATED_LUNG_UP	195	1.447	0.006	0.093
GSE19888_ADENOSINE_A3R_INH_VS_ACT_WITH_INHIBITOR_PRETREATMENT_IN_MAST_CELL_UP	188	1.447	0.007	0.093
GSE19941_LPS_VS_LPS_AND_IL10_STIM_IL10_KO_MACROPHAGE_UP	178	1.447	0.004	0.093
GSE3920_IFNA_VS_IFNB_TREATED_ENDOTHELIAL_CELL_DN	162	1.445	0.006	0.095
GSE22935_UNSTIM_VS_24H_MBOVIS_BCG_STIM_MYD88_KO_MACROPHAGE_UP	190	1.445	0.002	0.095
GSE23502_WT_VS_HDC_KO_MYELOID_DERIVED_SUPPRESSOR_CELL_COLON_TUMOR_UP	193	1.444	0.000	0.096
GSE10325_MYELOID_VS_LUPUS_MYELOID_DN	193	1.443	0.002	0.096
GSE40274_CTRL_VS_FOXP3_AND_EOS_TRANSDUCED_ACTIVATED_CD4_TCELL_UP	158	1.442	0.012	0.096
GSE13547_CTRL_VS_ANTI_IGM_STIM_ZFX_KO_BCELL_2H_UP	160	1.442	0.004	0.097
GSE10325_CD4_TCELL_VS_LUPUS_CD4_TCELL_DN	189	1.441	0.000	0.098
GSE34156_NOD2_LIGAND_VS_NOD2_AND_TLR1_TLR2_LIGAND_6H_TREATED_MONOCYTE_DN	195	1.440	0.004	0.099
GSE5589_LPS_VS_LPS_AND_IL6_STIM_IL6_KO_MACROPHAGE_45MIN_UP	196	1.439	0.000	0.099
GSE3720_LPS_VS_PMA_STIM_VD2_GAMMADELTA_TCELL_UP	130	1.439	0.004	0.098
GSE4748_CTRL_VS_LPS_AND_CYANOBACTERIUM_LPSLIKE_STIM_DC_3H_UP	193	1.436	0.000	0.102

GSE6269_FLU_VS_STAPH_AUREUS_INF_PBMC_DN	161	1.436	0.004	0.102
GSE29618_MONOCYTE_VS_PDC_DAY7_FLU_VACCINE_UP	198	1.435	0.004	0.102
GSE19772_CTRL_VS_HCMV_INF_MONOCYTES_UP	192	1.435	0.009	0.102
GSE9509_LPS_VS_LPS_AND_IL10_STIM_IL10_KO_MACROPHAGE_10MIN_UP	190	1.435	0.002	0.101
GSE23502_WT_VS_HDC_KO_MYELOID_DERIVED_SUPPRESSOR_CELL_BM_DN	194	1.434	0.004	0.102
GSE10240_CTRL_VS_IL17_AND_IL22_STIM_PRIMARY_BRONCHIAL_EPITHELIAL_CELLS_UP	188	1.434	0.003	0.102
GSE34392_ST2_KO_VS_WT_DAY8_LCMV_EFFECTOR_CD8_TCELL_DN	195	1.433	0.002	0.102
GSE5589_LPS_VS_LPS_AND_IL10_STIM_IL10_KO_MACROPHAGE_45MIN_DN	187	1.433	0.009	0.102
GSE45365_WT_VS_IFNAR_KO_BCELL_DN	162	1.433	0.011	0.101
GSE17721_ALL_VS_24H_PAM3CSK4_BMDC_DN	191	1.432	0.002	0.101
GSE37532_WT_VS_PPARG_KO_LN_TCONV_DN	171	1.432	0.007	0.101
GSE15330_LYMPHOID_MULTIPOTENT_VS_MEGAKARYOCYTE_ERYTHROID_PROGENITOR_IKAROS_KO_DN	175	1.431	0.006	0.101
GSE29618_MONOCYTE_VS_PDC_UP	190	1.429	0.005	0.105
GSE26488_CTRL_VS_PEPTIDE_INJECTION_HDAC7_DELTAP_TG_OT2_THYMOCYTE_UP	198	1.429	0.002	0.105
GSE34156_NOD2_LIGAND_VS_TLR1_TLR2_LIGAND_6H_TREATED_MONOCYTE_DN	177	1.426	0.004	0.109
GSE3982_MAST_CELL_VS_CENT_MEMORY_CD4_TCELL_UP	191	1.426	0.000	0.109
GSE9037_WT_VS_IRAK4_KO_BMDM_DN	187	1.424	0.004	0.110
GSE21360_NAIVE_VS_QUATERNARY_MEMORY_CD8_TCELL_UP	199	1.423	0.004	0.112
GSE14769_UNSTIM_VS_120MIN_LPS_BMDM_DN	194	1.422	0.006	0.113
GSE369_PRE_VS_POST_IL6_INJECTION_SOCS3_KO_LIVER_UP	193	1.422	0.004	0.113
GSE45365_WT_VS_IFNAR_KO_BCELL_MCMV_INFECTION_DN	164	1.421	0.002	0.113
GSE43863_TFH_VS_LY6C_INT_CXCR5POS_MEMORY_CD4_TCELL_UP	189	1.420	0.002	0.114
GSE10325_BCELL_VS_MYELOID_DN	190	1.419	0.002	0.116
GSE2405_0H_VS_24H_A_PHAGOCYTOPHILUM_STIM_NEUTROPHIL_DN	190	1.419	0.007	0.116
GSE21546_WT_VS_SAP1A_KO_DP_THYMOCYTES_UP	176	1.418	0.011	0.116
GSE45365_HEALTHY_VS_MCMV_INFECTION_CD11B_DC_DN	170	1.418	0.002	0.116
GSE4142_PLASMA_CELL_VS_MEMORY_BCELL_DN	187	1.418	0.002	0.116
GSE17721_0.5H_VS_8H_CPG_BMDC_DN	189	1.417	0.002	0.116
GSE6269_HEALTHY_VS_STAPH_PNEUMO_INF_PBMC_DN	165	1.416	0.002	0.118
GSE19888_CTRL_VS_A3R_INHIBITOR_TREATED_MAST_CELL_DN	175	1.416	0.011	0.117
GSE37416_12H_VS_48H_F_TULARENSIS_LVS_NEUTROPHIL_	188	1.415	0.005	0.117

UP				
GSE27786_NKTCELL_VS_MONO_MAC_DN	194	1.415	0.004	0.117
GSE3982_MAC_VS_NKCELL_UP	195	1.415	0.004	0.116
GSE7460_TCONV_VS_TREG_LN_DN	195	1.415	0.004	0.117
GSE9946_LISTERIA_INF_MATURE_VS_PROSTAGLANDINE2_TREATED_MATURE_DC_DN	108	1.412	0.004	0.121
GSE30971_CTRL_VS_LPS_STIM_MACROPHAGE_WBP7_KO_4H_DN	179	1.411	0.008	0.122
GSE13485_PRE_VS_POST_YF17D_VACCINATION_PBMC_DN	192	1.410	0.007	0.124
GSE13229_IMM_VS_INTMATURE_NKCELL_UP	195	1.410	0.005	0.123
GSE17974_IL4_AND_ANTI_IL12_VS_UNTREATED_12H_ACT_CD4_TCELL_UP	181	1.410	0.014	0.123
GSE7219_UNSTIM_VS_LPS_AND_ANTI_CD40_STIM_NIK_NFKB2_KO_DC_DN	185	1.409	0.002	0.124
GSE34156_UNTREATED_VS_24H_NOD2_LIGAND_TREATED_MONOCYTE_DN	180	1.408	0.007	0.125
GSE14769_UNSTIM_VS_60MIN_LPS_BMDM_DN	190	1.408	0.006	0.124
GSE45739_UNSTIM_VS_ACD3_ACD28_STIM_WT_CD4_TCELL_DN	193	1.407	0.005	0.125
GSE21380_NON_TFH_VS_TFH_CD4_TCELL_DN	192	1.406	0.002	0.126
GSE25088_IL4_VS_IL4_AND_ROSIGLITAZONE_STIM_STAT6_KO_MACROPHAGE_DAY10_UP	185	1.405	0.002	0.127
GSE45365_WT_VS_IFNAR_KO_CD11B_DC_MCMV_INFECTION_DN	157	1.403	0.009	0.130
GSE2770_TGFB_AND_IL4_ACT_VS_ACT_CD4_TCELL_2H_UP	176	1.401	0.008	0.135
GSE25123_CTRL_VS_IL4_STIM_PPARG_KO_MACROPHAGE_UP	192	1.400	0.009	0.136
GSE14769_UNSTIM_VS_80MIN_LPS_BMDM_DN	191	1.400	0.006	0.135
GSE13547_2H_VS_12_H_ANTI_IGM_STIM_ZFX_KO_BCELL_DN	167	1.400	0.011	0.135
GSE7348_UNSTIM_VS_LPS_STIM_MACROPHAGE_DN	165	1.400	0.005	0.134
GSE5589_UNSTIM_VS_180MIN_LPS_AND_IL10_STIM_MACROPHAGE_DN	194	1.400	0.007	0.133
GSE25087_FETAL_VS_ADULT_TREG_UP	190	1.396	0.007	0.140
GSE46606_DAY1_VS_DAY3_CD40L_IL2_IL5_STIMULATED_BCELL_UP	159	1.396	0.009	0.140
GSE18804_SPLEEN_MACROPHAGE_VS_TUMORAL_MACROPHAGE_DN	182	1.396	0.011	0.140
GSE38681_WT_VS_LYL1_KO_LYMPHOID_PRIMED_MULTIPOTENT_PROGENITOR_DN	192	1.391	0.007	0.149
GSE22886_NAIVE_CD4_TCELL_VS_48H_ACT_TH1_DN	196	1.391	0.005	0.149
GSE23505_IL6_IL1_IL23_VS_IL6_IL1_TGFB_TREATED_CD4_TCELL_UP	195	1.390	0.007	0.149
GSE22103_LPS_VS_GMCSF_AND_IFNG_STIM_NEUTROPHIL_DN	193	1.389	0.004	0.152
GSE32164_ALTERNATIVELY_ACT_M2_VS_CMYC_INHIBITE	193	1.389	0.006	0.151

D_MACROPHAGE_DN				
GSE20366_EX_VIVO_VS_HOMEOSTATIC_CONVERSION_TREG_DN	194	1.387	0.009	0.155
GSE5503_MLN_DC_VS_PLN_DC_ACTIVATED_ALLOGENIC_TCELL_UP	190	1.385	0.007	0.159
GSE24634_TEFF_VS_TCONV_DAY5_IN_CULTURE_UP	195	1.384	0.004	0.160
GSE19772_HCMV_INFL_VS_HCMV_INF_MONOCYTES_AND_PI3K_INHIBITION_DN	194	1.384	0.008	0.159
GSE3982_BASOPHIL_VS_EFF_MEMORY_CD4_TCELL_UP	189	1.384	0.009	0.159
GSE9650_EFFECTOR_VS_MEMORY_CD8_TCELL_UP	193	1.383	0.009	0.160
GSE2405_0H_VS_24H_A_PHAGOCYTOPHILUM_STIM_NEUTROPHIL_UP	193	1.382	0.007	0.161
GSE360_HIGH_DOSE_B_MALAYI_VS_M_TUBERCULOSIS_DC_DN	194	1.380	0.010	0.166
GSE5542_UNTREATED_VS_IFNA_TREATED_EPITHELIAL_CELLS_6H_UP	176	1.378	0.008	0.169
GSE2405_0H_VS_12H_A_PHAGOCYTOPHILUM_STIM_NEUTROPHIL_DN	188	1.378	0.014	0.169
GSE3982_NEUTROPHIL_VS_NKCELL_UP	188	1.378	0.007	0.169
GSE17974_IL4_AND_ANTI_IL12_VS_UNTREATED_12H_ACT_CD4_TCELL_DN	172	1.377	0.013	0.171
GSE2405_HEAT_KILLED_LYSATE_VS_LIVE_A_PHAGOCYTOPHILUM_STIM_NEUTROPHIL_9H_UP	190	1.376	0.005	0.171
GSE14769_UNSTIM_VS_240MIN_LPS_BMDM_DN	195	1.376	0.009	0.171
GSE23321_CENTRAL_MEMORY_VS_NAIVE_CD8_TCELL_UP	193	1.376	0.008	0.171
GSE15767_MED_VS_SCS_MAC_LN_UP	189	1.375	0.012	0.172
GSE360_LOW_DOSE_B_MALAYI_VS_M_TUBERCULOSIS_DC_DN	196	1.374	0.011	0.173
GSE1432_CTRL_VS_IFNG_6H_MICROGLIA_DN	193	1.374	0.013	0.172
GSE6674_ANTI_IGM_VS_CPG_STIM_BCELL_UP	190	1.374	0.010	0.172
GSE27786_LSK_VS_ERYTHROBLAST_DN	184	1.374	0.011	0.172
GSE30971_CTRL_VS_LPS_STIM_MACROPHAGE_WBP7_HET_2H_UP	178	1.373	0.011	0.172
GSE24634_IL4_VS_CTRL_TREATED_NAIVE_CD4_TCELL_DAY3_DN	196	1.373	0.009	0.174
GSE41867_DAY8_EFFECTOR_VS_DAY30_EXHAUSTED_CD8_TCELL_LCMV_CLONE13_UP	190	1.371	0.011	0.176
GSE17974_IL4_AND_ANTI_IL12_VS_UNTREATED_72H_ACT_CD4_TCELL_UP	161	1.371	0.009	0.176
GSE15930_NAIVE_VS_72H_IN_VITRO_STIM_TRICHOSTATIN A_CD8_TCELL_DN	192	1.370	0.015	0.178
GSE34515_CD16_POS_MONOCYTE_VS_DC_DN	189	1.370	0.021	0.178
GSE2405_0H_VS_3H_A_PHAGOCYTOPHILUM_STIM_NEUTROPHIL_UP	195	1.369	0.007	0.178
GSE37532_TREG_VS_TCONV_PPARG_KO_CD4_TCELL_FROM_LN_UP	193	1.369	0.009	0.178

GSE10325_LUPUS_BCELL_VS_LUPUS_MYELOID_DN	192	1.368	0.005	0.181
GSE24671_BAKIMULC_VS_SENDAI_VIRUS_INFECTED_MOUSE_SPLENOCYTES_UP	195	1.366	0.007	0.183
GSE17721_CPG_VS_GARDIQUIMOD_6H_BMDC_UP	195	1.366	0.009	0.183
GSE3039_B2_VS_B1_BCELL_UP	194	1.365	0.002	0.185
GSE3982_MAST_CELL_VS_BCELL_UP	194	1.365	0.011	0.185
GSE30971_CTRL_VS_LPS_STIM_MACROPHAGE_WBP7_KO_2H_UP	179	1.364	0.010	0.186
GSE26023_PHD3_KO_VS_WT_NEUTROPHIL_HYPOXIA_DN	188	1.364	0.016	0.185
GSE3982_EFF_MEMORY_CD4_TCELL_VS_TH1_DN	191	1.364	0.007	0.186
GSE22229_UNTREATED_VS_IMMUNOSUPP_THERAPY_RENAL_TRANSPLANT_PATIENT_PBMC_DN	192	1.363	0.015	0.187
GSE21546_WT_VS_ELK1_KO_DP_THYMOCYTES_UP	194	1.363	0.017	0.187
GSE30962_ACUTE_VS_CHRONIC_LCMV_SECONDARY_INF_CD8_TCELL_DN	195	1.363	0.006	0.186
GSE37301_COMMON_LYMPHOID_PROGENITOR_VS_PRO_BCELL_DN	194	1.363	0.009	0.185
GSE5679_CTRL_VS_PPARG_LIGAND_ROSIGLITAZONE_AND_RARA_AGONIST_AM580_TREATED_DC_DN	195	1.362	0.007	0.185
GSE29949_MICROGLIA_BRAIN_VS_CD8_POS_DC_SPLEEN_DN	191	1.362	0.018	0.186
GSE6674_CPG_VS_CPG_AND_ANTI_IGM_STIM_BCELL_UP	190	1.360	0.016	0.189
GSE411_100MIN_VS_400MIN_IL6_STIM_SOCS3_KO_MACROPHAGE_UP	198	1.360	0.005	0.189
GSE2405_HEAT_KILLED_VS_LIVE_A_PHAGOCYTOPHILUM_STIM_NEUTROPHIL_9H_DN	192	1.360	0.006	0.189
GSE22886_NAIVE_CD4_TCELL_VS_MONOCYTE_DN	193	1.359	0.004	0.192
GSE19941_IL10_KO_VS_IL10_KO_AND_NFKBP50_KO_LPS_AND_IL10_STIM_MACROPHAGE_DN	160	1.356	0.010	0.197
GSE22886_NAIVE_VS_MEMORY_TCELL_DN	195	1.356	0.012	0.197
GSE360_L_DONOVANI_VS_B_MALAYI_LOW_DOSE_DC_UP	194	1.356	0.007	0.197
GSE17721_0.5H_VS_4H_CPG_BMDC_DN	190	1.355	0.006	0.198
GSE9650_NAIVE_VS_EFF_CD8_TCELL_DN	193	1.354	0.012	0.199

Supplemental Table 7. Significantly Enriched Gene Sets from Phase II Analysis of MSigDB Hallmark Gene Sets

		ALL PD-1 Treated		
NAME	SIZE	NES	NOM p-val	FDR q-val
HALLMARK_HEME_METABOLISM	198	2.353	0.000	0.000
HALLMARK_E2F_TARGETS	199	1.765	0.000	0.005
HALLMARK_INTERFERON_ALPHA_RESPONSE	93	1.740	0.000	0.005
HALLMARK_G2M_CHECKPOINT	199	1.726	0.000	0.004
HALLMARK_INTERFERON_GAMMA_RESPONSE	189	1.564	0.000	0.020
HALLMARK_IL2_STAT5_SIGNALING	197	1.462	0.004	0.053
HALLMARK_OXIDATIVE_PHOSPHORYLATION	196	1.416	0.004	0.078
HALLMARK_ESTROGEN_RESPONSE_LATE	198	1.391	0.008	0.087
HALLMARK_HYPOXIA	200	1.358	0.007	0.110
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY	47	1.357	0.062	0.100

Supplemental Table 8. Gene Sets Positively Enriched in Phase I and Phase II from Analysis of MSigDB Hallmark and C2 Curated Gene Sets

	Phase I PD-1 Ab Treated		Phase II PD-1 Ab Treated	
NAME	NES	FDR q-val	NES	FDR q-val
HALLMARK_HEME_METABOLISM	1.858	0.001	2.353	0.000
HALLMARK_E2F_TARGETS	1.570	0.022	1.765	0.005
GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_UP	2.027	0.001	2.031	0.000
GRAHAM_NORMAL_QUIESCENT_VS_NORMAL_DIVIDING_DN	1.961	0.003	1.945	0.001
ROSTY_CERVICAL_CANCER_PROLIFERATION_CLUSTER	1.879	0.030	2.066	0.000
WINNEPENNINCKX_MELANOMA_METASTASIS_UP	1.843	0.054	1.723	0.061
LEE_EARLY_T_LYMPHOCYTE_UP	1.832	0.052	1.737	0.049
ISHIDA_E2F_TARGETS	1.793	0.094	1.898	0.006
KONG_E2F3_TARGETS	1.782	0.098	1.825	0.017
STEINER_ERYTHROCYTE_MEMBRANE_GENES	1.782	0.086	1.819	0.018
SOTIRIOU_BREAST_CANCER_GRADE_1_VS_3_UP	1.777	0.085	2.061	0.000
ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_6HR	1.770	0.087	1.676	0.091
KANG_DOXORUBICIN_RESISTANCE_UP	1.766	0.086	1.753	0.044
ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_24HR	1.743	0.120	1.683	0.084
ZHAN_MULTIPLE_MYELOMA_PR_UP	1.731	0.137	1.857	0.009
MOLENAAR_TARGETS_OF_CCND1_AND_CDK4_DN	1.719	0.157	1.645	0.106
WHITEFORD_PEDIATRIC_CANCER_MARKERS	1.715	0.146	1.602	0.148
HORIUCHI_WTAP_TARGETS_DN	1.710	0.141	1.633	0.115
ONDER_CDH1_TARGETS_3_DN	1.688	0.173	1.568	0.178
GRAHAM_CML_QUIESCENT_VS_NORMAL_QUIESCENT_UP	1.682	0.179	1.969	0.001
ODONNELL_TFRC_TARGETS_DN	1.682	0.171	1.671	0.097
CHANG_CYCLING_GENES	1.668	0.198	1.868	0.010
FERREIRA_EWINGS_SARCOMA_UNSTABLE_VS_STABLE_UP	1.666	0.195	1.640	0.111

CHAPTER IV

DISCUSSION & FUTURE DIRECTIONS

SUMMARY OF WORK

The work presented in this dissertation characterized the development of a novel MVA viral vector for use in immunizations against HIV and therapeutic approaches to establish a functional cure for chronic HIV infection. MVA has been tested as a vaccine vector in the context of a variety of infectious diseases such as HIV as well as solid tumor cancers due to its safety and immunogenicity profile. Here, we characterized the development of an MVA vector expressing the anti-apoptotic gene B13R which demonstrated significantly reduced apoptosis of infected cells, in particular antigen presenting cells, augmented vaccine-induced HIV-specific humoral immunity *in vivo* and delayed anti-viral responses during the early course of infection (Figure 1). The development of this modified MVA vector additionally contributes to the field a novel vector for use in improving vaccine-induced humoral responses to other pathologies.

Our work utilizing combination anti-retroviral therapy and PD-1 blockade established a foundation regarding safety and therapeutic potential of the novel regimen. The use of PD-1 blockade in HIV infected patients is limited primarily to patients that also present with cancer co-morbidities due to the dearth of safety and efficacy data regarding administering PD-1 blockade to patients on ART which is the clinical state most patients would be if they were to receive this treatment. We show for the first time in the non-human primate model that administration of PD-1 blockade prior to ART initiation as well as during the suppressive ART phase was well-tolerated in our chronically infected rhesus macaques, improved anti-viral cellular responses, disrupted the latent SIV reservoir, and limited viral reemergence after ART treatment interruption (Figure 2). This study has important implications in furthering HIV functional cure research regarding development of novel regimens to co-administer with PD-1 blockade to improve both anti-viral responses as well as reactivation of the latent reservoir to accomplish durable remission of HIV.

DEVELOPMENT OF MVA EXPRESSING ANTI-APOPTOTIC B13R GENE

MVA AND ANTIGEN PRESENTING CELLS

We have shown that MVA infectivity of immune cell subsets is directed more highly towards antigen presenting cells (APCs) than B cells and subsequently more than T cell subsets in rhesus macaques. Additionally, it is in these plasmacytoid DCs (pDCs) and conventional DCs (cDCs) that we see the effect of improved viability after MVA-B13R infection. The susceptibility to cell death after infection may be related to the differential expression of anti-viral sensors in the different cell subsets. Plasmacytoid dendritic cells (pDCs) are known to express the receptors toll-like receptor 7 (TLR7) and TLR9 in endosomal locations to recognize single-stranded RNA and unmethylated CpG DNA, respectively. cDCs express a variety of innate viral sensors including cGAS and TLR9. Results from Dai *et al.* indicate that cDCs are the main source of the type I IFN response after MVA infection and that cGAS is the main cytosolic DNA sensor that recognizes MVA infection in cDCs (512). Despite identification of cGAS as the main MVA sensor, there is still a role that TLR9 and the signaling adaptor molecule MyD88 play in sensing and activating of cDCs after MVA infection (512). In their study using bone marrow derived dendritic cells (BMDCs) from TLR9 or MyD88 knockout mice, infection with MVA resulted in reduced though not abolished type I IFN production *in vitro* compared to MVA infection of wild-type BMDCs, indicating a minor but significant role for the TLR9/MyD88 signaling pathway in MVA sensing in DCs. The differential expression levels of cGAS and TLR9 between pDCs and cDCs may contribute to the relative differences observed in apoptosis induced after MVA infection compared to other immune cell subsets. In our experiment, after 20 hours of rhesus macaque cells, MVA infection of pDCs induced 40-50% caspase 3 activation of infected cells whereas approximately 90% of infected cDCs activated caspase 3 at the same time.

Discovery of the role and functionality of cGAS sensing of MVA was performed in mice and used mouse *in vitro* derived DC populations so the effects of MVA infection on these cells may not completely translate when compared to our data of infection of rhesus macaque cell subsets. Despite this however,

the data indicate the possibility that different DC subsets maintain different levels of anti-viral sensors which contribute to the degree of anti-viral response induced as determined by type I IFN responses. We postulate the improved viability observed after infection with MVA-B13R in the APCs is due to the reduced anti-viral sensing occurring at the early stages of infection. This is supported by our RNA-Seq data in which anti-viral genes and antigen presentation and processing related genes were expressed at a relatively lower level in MVA-B13R immunized animals compared to MVA immunized mice at one day after immunization.

DISCERNING MECHANISM OF RESPONSE

Further analysis of the innate responses generated by MVA versus MVA-B13R would better elucidate the mechanisms of how MVA-B13R is capable of enhancing humoral responses during vaccinations. As a method to detect infected cells *in vivo*, future studies could harvest draining lymph node cells after mice immunization and determine MVA infectivity by flow cytometry. The monoclonal antibody against the MVA E3 protein may be detectable in various immune cells if infection has occurred. This would allow for real-time detection of infection and allow us to determine potential differences in cell infectivity and viability or changes in DC activation levels after immunization with MVA or MVA-B13R. An *ex vivo* assay would also allow us to begin to answer this question. Infection of immune cells *ex vivo* to test for changes in activation status and cytokine production into the supernatant would be informative as we probe these early response time points.

Additionally, to better understand the intracellular dynamics of MVA or MVA-B13R infected cells versus bystander cells, sorting of specific cell subsets for RNA-Seq analysis would be informative. As we had used bulk draining lymph node cells in our RNA-Seq analysis, there remains the potential that significant genes and gene signature profiles may be diluted and rendered non-significant by uninfected cell subsets. While there are many studies that indicate the beneficial nature of type I IFNs during an acute response in regard to improving humoral responses (515-518), we show that these responses may be further improved

in a delayed setting. MVA-B13R vaccinations had reduced levels of IFN signaling relative to MVA immunizations at day 1 but become comparable by day 2 and 6. This slower ramping up of the immune response during MVA-B13R vaccination could be how MVA-B13R influences the humoral response. *In vivo* studies to test this hypothesis may include immunization with an MVA construct in an environment with suboptimal type I IFN signaling. This may be achieved through low level blockade of the type I IFN receptor with a monoclonal antibody. Titrations and kinetics of this blockade would be important to optimize to allow for only partial and transiently low levels of type I IFN signaling.

FURTHER IMPROVEMENT OF IMMUNE RESPONSES

Immunogenicity studies in the BALB/c mouse model demonstrated improved antigen-specific humoral responses from our MVA-B13R vaccine vector though T cell responses did not improve. Use of homologous vaccinations with MVA in the clinic have shown that antibody responses are well induced but there remains room for improvement regarding vaccine-induced cellular responses (456). Combining the MVA vector in a heterologous prime/boost vaccination regimen such as with DNA has been shown to enhance both CD4+ and CD8+ T cell responses (565). Combination DNA/MVA-B13R vaccinations is a modality worthy of consideration for the future development of this vector. Preliminary studies of this regimen indicate an improvement in Envelope-specific humoral responses after two DNA immunization followed by one MVA-B13R immunization. This may also be beneficial in avoiding MVA vector-specific responses that may detract from further immunogen-specific responses. The influence on DNA priming regarding vaccine-specific cellular response remain an area to be explored.

Further enhancement of humoral responses is also an area of interest. The last decade has seen intense development of novel immunogens for generation of HIV Envelope-specific responses that are better at neutralizing HIV or targeting Envelope epitopes associated with vaccine-mediated protection. Development of novel protein immunogens that better mimic the native state of the Envelope trimer such as the BG505 SOSIP gp140 construct have been showing promise as a soluble protein capable of eliciting

bNAbs (566). Use of next-generation designed trimers that can improve humoral responses to epitopes of interest for the induction of bNAbs in combination with the MVA-B13R viral vector has great potential. Additionally, incorporation of immunogens into MVA-B13R that express epitopes hypothesized to mediate protection from infection such as the V2-hotspot of the HIV Envelope as detected in the RV144 Thai trial could greatly improve the qualitative nature of the humoral response (567).

Another potential avenue of improving vaccine responses include further modification of the MVA genome. Infection with MVA-B13R during *in vitro* studies demonstrated a reduction of apoptosis and a concomitant increase in necrosis. Potentially impeding both the apoptotic pathway during MVA infection with B13R expression and impeding the necroptosis pathway with a murine cytomegalovirus (MCMV)-derived immune modulatory gene such as M45 (viral inhibitor of RIP activation which inhibits RIP1/RIP3-mediated necroptosis) could provide a synergistic effect on extending viability of the infected cells (568). Alternatively, exogenous expression of the pro-survival factor Bcl2 or its other family members into the MVA-B13R genome may also provide overall survival benefit to infected cells. Altogether, there remains many possibilities to capitalize on the enhanced immunogenicity observed in our novel MVA-B13R vector construct.

COMBINATION PD-1 BLOCKADE AND ANTIRETROVIRAL THERAPY

AUTOIMMUNITY AND SAFETY CONCERNS

There is considerable risk with use of any checkpoint inhibitor blockade therapy as this approach aims to activate the immune system in an antigen non-specific manner. Nearly 90% of patients in a phase III study utilizing CTLA-4 blockade for metastatic melanoma experienced adverse events of various severities and ranging from gastrointestinal disorders and pneumonitis to immune-related events such as colitis (569). Use of PD-1 or PD-L1 blockade also resulted in a range of 70-90% antibody-related adverse events of various grades (570, 571). Treatment interruption or discontinuation along with treatments to

manage the adverse events typically led to improvement or resolution of events in all patients. This in addition to the mostly low-grade events that occur make these therapies relatively well-tolerated though concern generally remains. Use of blockade therapies for chronic infections such as HIV and hepatitis B virus however will need to be optimized for reducing dosages to the minimal effective doses and the shortest duration to prevent the potential of off-target effects.

The efficacy of PD-1 blockade for HIV infected patients will also be of incredible interest while under ART therapy. As seen in the differences in responses between phase I and phase II of our PD-1 blockade and ART combination study, the immunological and virologic environment under ART is incredibly different compared to the absence of ART. We observed fewer overall changes in cellular responses regarding both proliferation and cytokine producing potential during phase II which was marked by a lack of viremia or antigen while blockade was being administered. The overall reduction in systemic hyperimmune activation as well during phase II compared to phase I likely contributed to the lowered responses observed. The reduced inflammation and lack of antigen of a patient under ART is a dramatically different immunological environment compared to a cancer patient combating a tumor. The likelihood of epitope spread therefore is likely to be less in a HIV+ patient under ART.

POTENTIAL BIOMARKERS OF BLOCKADE RESPONSE

Our PD-1 blockade therapy administered in combination with ART has set the framework for understanding the safety and dynamics of immune responses that occur during PD-1 blockade in the setting of chronic infection. Along with the immunological and virologic outcomes described in Chapter III, unanswered questions and room for improvement remain. The early control of viremia by single PD-1 blockade treated animals that was subsequently loss and the long-term control of viremia by only a subset of double PD-1 blockade treated animals invites the question as to why some treated animals maintained the benefit of blockade while others did not. This question would be of interest to answer in a clinical

setting as well to avoid unnecessary blockade therapy exposure to patients that can be classified as non-responders prior to treatment.

Literature on PD-1 blockade efficacy for cancer patients indicates potential biomarkers of interest. In cancer, some studies saw a correlation with the level of PD-L1 expression on tumor cells and treatment efficacy (572). In the chronic HIV/SIV infection setting, it would be of interest to assess the expression levels of PD-1 ligands on APCs and B cells prior to treatment in an effort to determine blockade efficacy. Higher expression of PD-1 ligands on relevant immune cell subsets would indicate that the cellular exhaustion experienced by an individual is mediated by the PD-1 signaling dynamics and that its disruption in this setting would be advantageous. The degree of neoantigens displayed by tumor cells also has been shown to be associated with improved treatment efficacy to PD-1 blockade. This may speak to the overall immunogenicity a patient may have for viral antigens or the breadth of virus-specific responses a host may be able to mount. These responses by SIV-infected rhesus macaques can be determined early during acute infection and a relationship to post-treatment interruption viral control could be determined in future studies. Other immunological factors that have shown to be associated with blockade efficacy in cancer patients are high eosinophil and lymphocyte blood counts, increased serum TGF-β levels, and increased Th1 and CTLA-4 (but not FoxP3) gene expression levels prior to treatment (572).

Recent studies have also described CD28 as the main target of SHP2 dephosphorylation stemming from PD-1 signaling (573, 574). Results indicate that cells responding to blockade in cancer patients were predominantly CD28-expressing cells making CD28 a potential biomarker of interest (573). Higher expression of CD28 as a marker of PD-1 blockade responsiveness is in line with the mechanisms of inhibition of PD-1 which prevents downstream signaling of CD28. Removing the inhibition on CD28 signaling is important to regaining cellular activation but having an adequate expression level of CD28 to provide the co-stimulatory signaling would also be important to generating a response. The biomarkers

described here indicate that patients who already possess these and other effective pre-treatment immune responses are likely to benefit from most from blockade therapy in cancer and would be of considerable interest to determine if similar trends hold in chronic infection scenarios.

Of interest is a recent study which showed the impact of host microbiota on the efficacy of PD-1 blockade during cancer treatment. Routy *et al*. described an abnormal gut microbiome composition, perturbed due to antibiotics in this instance, inhibited the benefits of PD-1 blockade therapy in patients with advanced cancer (557). In mice, the authors showed that use of antibiotics compromised blockade efficacy in a cancer mouse model as well as in cancer patients and that this was related to microbiota composition. In particular, the presence of the bacterial species *Akkermansia muciniphila* resulted in improvement in control of tumors in mice. This study has the potential for significant impact regarding concurrent treatments given to cancer patients and patients with chronic infections. The usage or not of a course of antibiotics during PD-1 immune checkpoint blockade treatment could be more easily managed relative to some of the other potential biomarkers discussed. As we further optimize the PD-1 blockade and ART combination regimen, these and related factors should be considered for monitoring as a method to characterize responders and non-responders prior to treatment.

FUTURE COMBINATION PD-1 BLOCKADE AND ANTIRETROVIRAL THERAPY STUDIES

Our results highlight the potential impact that PD-1 blockade has on disruption of the viral reservoir while under suppressive ART therapy. While detection of plasma viremia during this phase did not show significant differences between the PD-1 blockade treated and control groups, the results indicate the potential influence blockade may have had on reactivation of the latent reservoir. Lim *et al*. has recently shown that administration of TLR7 agonists induced transient viremia in SIV infected animals under suppressive ART and that the majority of these viral blips occurred within 24 to 72 hours of treatment administration (281). Due to limitations regarding sampling frequency and volume for the animals in our study, we were unable to test for viremia in our animals as frequently as the TLR7 study and thus sampled

for plasma viremia on a weekly basis. It is possible that PD-1 blockade may have led to viral reactivation to a degree that was detectable in the plasma in our animals, but we were unable to capture the relevant time points. Additionally, the kinetics of transient viral reactivation are likely to be short due to the fact that during ART treatment, cells cannot become newly infected. Thus, the relatively small pool of reservoir cells contributing to induced viremia is likely able to be controlled by anti-viral immune responses more rapidly than in a primary or acute infection period. It would be beneficial to be able to analyze these early times points during which viremia may occur as a method to detect reactivation of virus and potential reservoir disruption.

The impact of PD-1 blockade has a differential impact on the tissues we assessed. This is highlighted by the changes observed for cytotoxic CD8+ T cells and CXCR5 expressing CD4+ T cells detected in the lymph node of treated animals which were not observed in the peripheral blood compartment. There are many components that contribute to the efficacy of blockade, but the location of where we interrogate these responses are also of importance. The lymphoid tissues, which include the gut-associated lymphoid tissue, maintain a different architecture, immune dynamic, and population of cell subsets (including PD-1 high expressing Tfh cells) that has the potential to benefit from PD-1 blockade. While there are limitations on the number of lymph node biopsies rhesus macaque can undergo, the recently developed fine-needle aspirate method of sampling the lymph node environment would be highly informative regarding lymphoid tissue dynamics during PD-1 blockade.

COMBINATION INHIBITORY RECEPTOR BLOCKADE

The rationale behind the regimen utilized in our study was in line with other studies utilizing the “shock and kill” approach towards an HIV functional cure (271, 272). The shock attempts to reactivate the cells that comprise the latent viral reservoir and lead to expression of viral antigens thereby making the cell visible to the immune system for immune clearance. The kill component refers to various strategies to elicit improved T cell responses capable of detecting and eliminating virally infected cells. Our study

provides the groundwork that this approach using PD-1 blockade with ART is feasible and safe for use and provides a roadmap to achieving the results of a functional cure.

There remains room for improvement in both of this aims however. Reactivation of virus during the suppressive ART phase (phase II of our study) was modest, though we only sampled for plasma viremia on a weekly basis, and control of reemerged viremia only occurred for some treated animals. It has been well documented that during HIV infection, there is upregulation of a variety of immune checkpoint markers beyond PD-1 including CTLA-4, TIGIT, TIM-3, and LAG-3 on T cells (205, 311, 312). In a study determining the efficacy of CTLA-4 blockade in SIV infection of macaques, treated animals had increased levels of HIV replication and T cell activation though did not result in improved virus-specific cellular responses (575). When CTLA-4 blockade was administered with ART, animals showed a decrease in the level of SIV RNA detected in their lymph nodes as well as some improvement in their anti-viral cellular responses (576). Together these studies indicate the potential for use of CTLA-4 with ART as a latency reversing agent in addition to a therapy that improves virus-specific responses. The use of CTLA-4 blockade via a monoclonal antibody (ipilimumab) is currently licensed for use in cancers. In the context of melanoma, combination PD-1 (nivolumab) and CTLA-4 blockade showed efficacy greater than either blockade alone though the use of the combination therapy also resulted in increased toxicity in patients (577). The use of PD-1 and CTLA-4 combination blockade during suppressive ART is of interest as these markers would allow for targeting and activation of different cell subsets that contribute to the reservoir (544). Providing data regarding safety due to concerns of enhanced autoimmunity after treatment will be of importance in further development of this approach.

A new generation of coinhibitory receptor blockade therapies are undergoing development in the clinic. Beyond PD-1 and CTLA-4, these therapies target LAG-3, TIM-3, and TIGIT to reverse immune exhaustion during chronic pathologies such as cancers and potentially viral infections (578). Given the importance of CTLA-4 and PD-1 on maintaining self-tolerance as evidenced when the receptors are

genetically deleted or blocked *in vivo*, blockade of these receptors are predicted to be associated with a greater likelihood of autoimmune toxicity events (578). Blockade of coinhibitory receptors that are less involved with immune tolerance mechanisms such as LAG-3, TIM-3, and TIGIT, are predicted then to have a better clinical safety profile than PD-1 or CTLA-4 blockade. LAG-3 expression on T cells in blood and lymph nodes during HIV infection is associated with plasma viremia levels and inversely related to CD4+ T cell counts (579). HIV-specific high TIGIT expressing cells observed in HIV infected individuals co-expressed PD-1, were inversely correlated with polyfunctionality, and had diminished expression of the co-stimulatory receptor CD226 (580). *Ex vivo* blockade of TIGIT and PD-1 resulted in improvement of HIV-specific cellular functionality (312). While still in early development in the clinic, the potential use of these markers as targets of blockade therapy in HIV infected individuals on ART would be of interest as the concern for safety and reduction of severe adverse effects likely resulting from autoimmunity will be paramount.

ENHANCED TARGETING OF VIRALLY INFECTED CELLS

Strategies to improve the immune mediated clearance of HIV infected cells include enhancement of anti-viral cellular responses to target and eliminate these cells by use of vaccinations. Traditionally, vaccines have been administered as a preventative measure against pathogens by inducing antigen-specific cellular and humoral responses. The same concepts are applied to the HIV functional cure strategy of "shock and kill" by aiming to approve the killing portion of the approach. Early studies that sought to enhance anti-viral responses in patients through structured ART treatment interruptions. Patients would be interrupted from ART, allowed to have their virus reemerge and induce an HIV-specific response which researchers hypothesized would lead to better control of viremia and reduction in the reservoir in the absence of ART though this strategy proved unsuccessful (581, 582). More focused strategies to induce anti-viral responses through vaccinations have since been under development.

The use of a variety of vaccine modalities and adjuvants have been studied (272, 583, 584). Studies in the non-human primate SIV infection model include vaccinations with DNA, viral vectors such as MVA, and DC-based vaccine strategies in the presence or absence of ART (583, 585-587). These studies demonstrated improved cytokine production and cytotoxic functionality of T cells though only a modest effect on control of viremia after ART interruption. A recent clinical study performed to test safety and efficacy of administration of an adjuvanted DNA prime and vesicular stomatitis virus (VSV) boost in patients who started ART early in infection was observed to be safe and well tolerated though improvements in immune enhancement were modest and there were no changes observed regarding control of viremia after ART interruption (588). It would be of interest to test the combination of these therapeutic vaccinations with PD-1 blockade and ART therapy to aid in reversal of exhaustion of virus-specific T cells already present and expand virus-specific cells.

Innovations in immune therapy over the last decade has the potential to improve targeting of the latent viral reservoir for reactivation and elimination (584). Chimeric antigen receptor (CAR) T cells are T cells engineered to express an antigen receptor such as that of an antibody which is fused to a T cell activation signaling domain on its intracellular domain. The advantage of CAR T cells being that targeted epitopes are not MHC-restricted. Early CAR T cells utilized for HIV involved a CAR T cell with the CD4 extracellular domain to allow for binding to HIV Envelope gp120 on infected cell surfaces and this was fused to the CD3 signaling domain. This however did not reduce the level of plasma viremia in infected individuals nor impact the viral reservoir (589, 590). Improvements in signaling capacities of CAR T cells have led to second generation CD4 CAR T cells with greater functionality observed *in vitro* and in a humanized mouse model compared to first generation constructs (591). Additionally, the discovery of more potent and broadly neutralizing antibodies to HIV as potential CAR candidates are underway (592). Further engineering of SIV-specific CAR T cells that co-express the CXCR5 receptor that have the potential to home to the sites where the viral reservoir is known to reside would be of immense interest to

use in combination with PD-1 blockade (593). Targeted elimination of the cells by improving their homing to relevant viral sites could have a major impact on reduction of the reservoir.

Bispecific T cell engagers (BiTEs) have been studied in the context of cancer therapy. BiTEs have a tumor-specific variable fragment and a CD3-specific fragment joined by a linker allowing for redirection of effector T cells to eliminate cells expressing the tumor-associated antigen (584). Improvement of BiTEs led to dual-affinity re-targeting proteins (DARTs) which have improved storage and serum stability and enhanced potency as well (594, 595). HIV-specific BiTEs and DARTs contain a single-chain variable fragment (scFV) from an anti-CD3 antibody in combination with scFVs from the bNAbs B12, VRC01, 17b, or CD4 extracellular domains (596). Results from studies with HIV-specific BiTEs and DARTs substantiate their ability and potential clinical use in targeting of latently infected cells for elimination. Utilizing the innovations in therapeutic vaccines and enhanced targeting of latently infected cells in combination with PD-1 blockade which has been demonstrated to improve anti-viral responses has the potential for achieving the goals of a functional cure.

COMBINATION PD-1 BLOCKADE AND MVA-B13R VACCINATION

Of interest would be the combination of both of the strategies outlined in this dissertation: use of PD-1 blockade therapy under ART in combination with a therapeutic vaccine that utilizes the MVA-B13R vector to elicit greater magnitude and durability of virus-specific responses. Using a heterologous prime/boost regimen for therapeutic vaccination with DNA as the priming agent and MVA-B13R as the boost has the potential for greater induction of HIV-specific responses. DNA priming to an MVA boost has been shown to be capable of inducing higher antigen-specific cellular responses. The timing of PD-1 blockade administration during this phase would also be important. Blockade administered during effector phases of an immune response has the potential to greatly augment vaccine-induced responses even further. Determining the synergistic effect of the combination of MVA-B13R vaccination with PD-1 blockade could lead to significant improvement in generating responses against the latent reservoir.

In summary, this dissertation has added to the field of vaccine development and HIV functional cure. We have developed an MVA vector expressing the anti-apoptotic gene B13R that improved vaccine-induced immune responses and contributes to the ongoing work of enhancing MVA immunogenicity for use in vaccinations for a wide range of conditions. Furthermore, we established a foundation for further development of the PD-1 blockade and ART treatment combination as a functional cure strategy against HIV by describing the safety and therapeutic potential of this novel regimen. We believe our findings will contribute to further optimization of both MVA vaccine vector development and functional cure approaches.

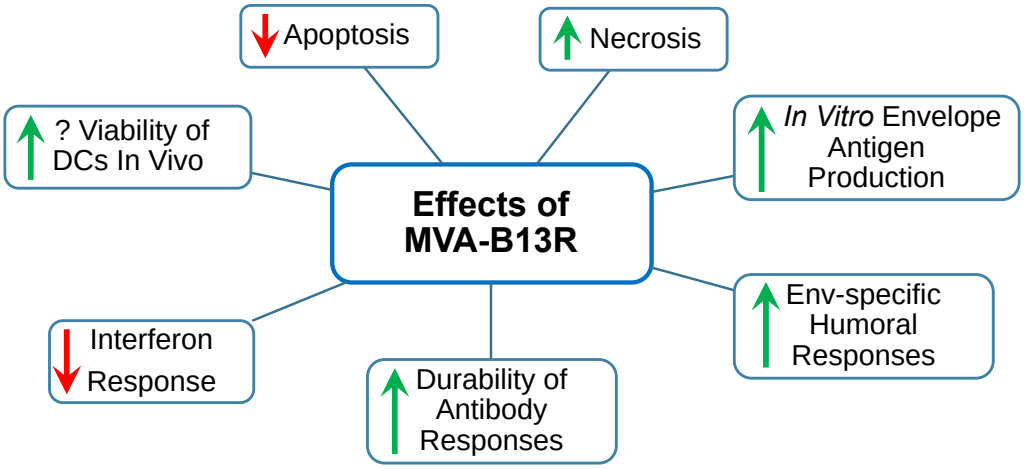


Figure 1. Summary of Conclusions for Development of MVA Expressing Anti-Apoptotic B13R Gene.

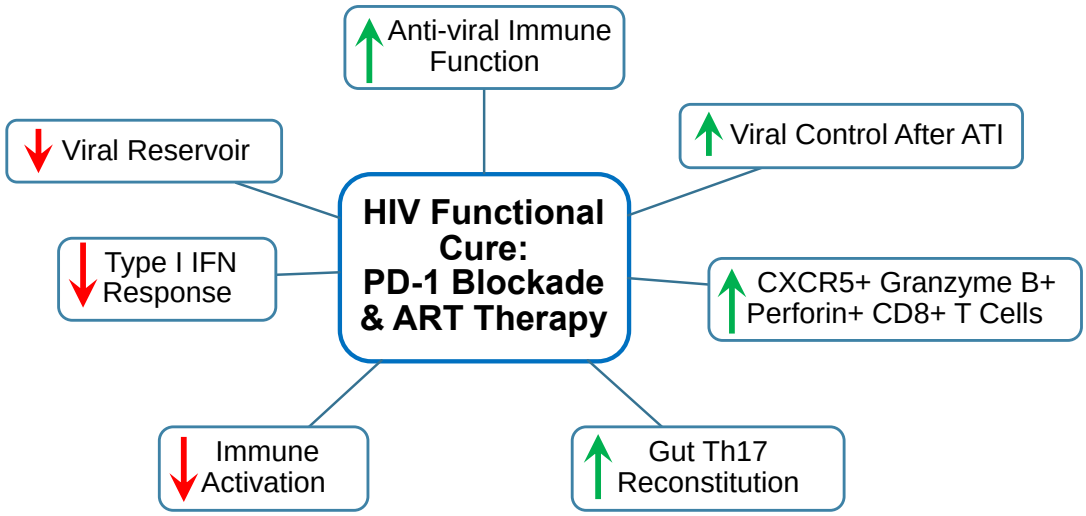


Figure 2. Summary of Conclusions for Combination PD-1 Blockade And Anti-retroviral Therapy.

CHAPTER V

BIBLIOGRAPHY

1. WHO. HIV/AIDS Key Facts 2018 [cited 2018 October 14]. Available from: http://www.who.int/en/news-room/fact-sheets/detail/hiv-aids.

2. UNAIDS. Global HIV & AIDS statistics — 2018 fact sheet 2018 [cited 2018 October 14]. Available from: http://www.unaids.org/en/resources/fact-sheet.

3. Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. The New England journal of medicine. 1981;305(24):1425-31. Epub 1981/12/10. doi: 10.1056/NEJM198112103052401. PubMed PMID: 6272109.

4. Barre-Sinoussi F, Ross AL, Delfraissy JF. Past, present and future: 30 years of HIV research. Nature reviews Microbiology. 2013;11(12):877-83. Epub 2013/10/29. doi: 10.1038/nrmicro3132. PubMed PMID: 24162027.

5. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vezinet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L. 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. PubMed PMID: 6189183.

6. Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science. 1984;224(4648):497-500. Epub 1984/05/04. PubMed PMID: 6200935.

7. Levy JA, Hoffman AD, Kramer SM, Landis JA, Shimabukuro JM, Oshiro LS. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science. 1984;225(4664):840-2. Epub 1984/08/24. PubMed PMID: 6206563.

8. CDC. HIV in the United States by Geography [cited 2018 October 14]. Available from: https://www.cdc.gov/hiv/statistics/overview/geographicdistribution.html.

9. CDC. HIV in the United States: At A Glance [cited 2018 October 14]. Available from: https://www.cdc.gov/hiv/statistics/overview/ataglance.html.

10. 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; PMCID: PMC3234451.

11. Peeters M, Courgnaud V, Abela B, Auzel P, Pourrut X, Bibollet-Ruche F, Loul S, Liegeois F, Butel C, Koulagna D, Mpoudi-Ngole E, Shaw GM, Hahn BH, Delaporte E. Risk to human health from a plethora of simian immunodeficiency viruses in primate bushmeat. Emerg Infect Dis. 2002;8(5):451-7. Epub 2002/05/09. doi: 10.3201/eid0805.010522. PubMed PMID: 11996677; PMCID: PMC2732488.

12. Deeks SG, Overbaugh J, Phillips A, Buchbinder S. HIV infection. Nat Rev Dis Primers. 2015;1:15035. Epub 2015/01/01. doi: 10.1038/nrdp.2015.35. PubMed PMID: 27188527.

13. Hemelaar J. The origin and diversity of the HIV-1 pandemic. Trends in molecular medicine. 2012;18(3):182-92. Epub 2012/01/14. doi: 10.1016/j.molmed.2011.12.001. PubMed PMID: 22240486.

14. Chinen J, Shearer WT. Molecular virology and immunology of HIV infection. The Journal of allergy and clinical immunology. 2002;110(2):189-98. Epub 2002/08/10. PubMed PMID: 12170257.

15. Frankel AD, Young JA. HIV-1: fifteen proteins and an RNA. Annu Rev Biochem. 1998;67:1-25. Epub 1998/10/06. doi: 10.1146/annurev.biochem.67.1.1. PubMed PMID: 9759480.

16. Luciw P. Human immunodeficiency virus and its replication. In: BM F, editor. Fields Virology. 3rd ed. Philadelphia: Lippincott-Raven Publishers; 1996.

17. Freed EO. HIV-1 assembly, release and maturation. Nature reviews Microbiology. 2015;13(8):484-96. Epub 2015/06/30. doi: 10.1038/nrmicro3490. PubMed PMID: 26119571.

18. Maddon PJ, Dalgleish AG, McDougal JS, Clapham PR, Weiss RA, Axel R. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell. 1986;47(3):333-48. Epub 1986/11/07. PubMed PMID: 3094962.

19. Klatzmann D, Champagne E, Chamaret S, Gruest J, Guetard D, Hercend T, Gluckman JC, Montagnier L. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature. 1984;312(5996):767-8. Epub 1984/12/20. PubMed PMID: 6083454.

20. Dalgleish AG, Beverley PC, Clapham PR, Crawford DH, Greaves MF, Weiss RA. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature. 1984;312(5996):763-7. Epub 1984/12/20. PubMed PMID: 6096719.

21. Weiss RA. Thirty years on: HIV receptor gymnastics and the prevention of infection. BMC Biol. 2013;11:57. Epub 2013/05/23. doi: 10.1186/1741-7007-11-57. PubMed PMID: 23692808; PMCID: PMC3660199.

22. Feng Y, Broder CC, Kennedy PE, Berger EA. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science. 1996;272(5263):872-7. Epub 1996/05/10. PubMed PMID: 8629022.

23. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, MacDonald ME, Stuhlmann H, Koup RA, Landau NR. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell. 1996;86(3):367-77. Epub 1996/08/09. PubMed PMID: 8756719.

24. Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, Lapoumeroulie C, Cognaux J, Forceille C, Muyldermans G, Verhofstede C, Burtonboy G, Georges M, Imai T, Rana S, Yi Y, Smyth RJ, Collman RG, Doms RW, Vassart G, Parmentier M. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature. 1996;382(6593):722-5. Epub 1996/08/22. doi: 10.1038/382722a0. PubMed PMID: 8751444.

25. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gomperts E, Donfield S, Vlahov D, Kaslow R, Saah A, Rinaldo C, Detels R, O'Brien SJ. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. Science. 1996;273(5283):1856-62. Epub 1996/09/27. PubMed PMID: 8791590.

26. Wu L, KewalRamani VN. Dendritic-cell interactions with HIV: infection and viral dissemination. Nature reviews Immunology. 2006;6(11):859-68. Epub 2006/10/26. doi: 10.1038/nri1960. PubMed PMID: 17063186; PMCID: PMC1796806.

27. Karn J, Stoltzfus CM. Transcriptional and posttranscriptional regulation of HIV-1 gene expression. Cold Spring Harb Perspect Med. 2012;2(2):a006916. Epub 2012/02/23. doi: 10.1101/cshperspect.a006916. PubMed PMID: 22355797; PMCID: PMC3281586.

28. Rittner K, Churcher MJ, Gait MJ, Karn J. The human immunodeficiency virus long terminal repeat includes a specialised initiator element which is required for Tat-responsive transcription. J Mol Biol. 1995;248(3):562-80. Epub 1995/05/05. doi: 10.1006/jmbi.1995.0243. PubMed PMID: 7752225.

29. Sodroski J, Patarca R, Rosen C, Wong-Staal F, Haseltine W. Location of the trans-activating region on the genome of human T-cell lymphotropic virus type III. Science. 1985;229(4708):74-7. Epub 1985/07/05. PubMed PMID: 2990041.

30. Sodroski J, Rosen C, Wong-Staal F, Salahuddin SZ, Popovic M, Arya S, Gallo RC, Haseltine WA. Trans-acting transcriptional regulation of human T-cell leukemia virus type III long terminal repeat. Science. 1985;227(4683):171-3. Epub 1985/01/11. PubMed PMID: 2981427.

31. Kao SY, Calman AF, Luciw PA, Peterlin BM. Anti-termination of transcription within the long terminal repeat of HIV-1 by tat gene product. Nature. 1987;330(6147):489-93. Epub 1987/12/03. doi: 10.1038/330489a0. PubMed PMID: 2825027.

32. Sodroski J, Goh WC, Rosen C, Dayton A, Terwilliger E, Haseltine W. A second post-transcriptional trans-activator gene required for HTLV-III replication. Nature. 1986;321(6068):412-7. Epub 1986/05/22. doi: 10.1038/321412a0. PubMed PMID: 3012355.

33. Malim MH, Hauber J, Le SY, Maizel JV, Cullen BR. The HIV-1 rev trans-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. Nature. 1989;338(6212):254-7. Epub 1989/03/16. doi: 10.1038/338254a0. PubMed PMID: 2784194.

34. Sheehy AM, Gaddis NC, Choi JD, Malim MH. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature. 2002;418(6898):646-50. Epub 2002/08/09. doi: 10.1038/nature00939. PubMed PMID: 12167863.

35. Goila-Gaur R, Strebel K. HIV-1 Vif, APOBEC, and intrinsic immunity. Retrovirology. 2008;5:51. Epub 2008/06/26. doi: 10.1186/1742-4690-5-51. PubMed PMID: 18577210; PMCID: PMC2443170.

36. Kitamura S, Ode H, Iwatani Y. Structural Features of Antiviral APOBEC3 Proteins are Linked to Their Functional Activities. Front Microbiol. 2011;2:258. Epub 2011/12/29. doi: 10.3389/fmicb.2011.00258. PubMed PMID: 22203821; PMCID: PMC3243911.

37. Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature. 2008;451(7177):425-30. Epub 2008/01/18. doi: 10.1038/nature06553. PubMed PMID: 18200009.

38. Van Damme N, Goff D, Katsura C, Jorgenson RL, Mitchell R, Johnson MC, Stephens EB, Guatelli J. The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. Cell host & microbe. 2008;3(4):245-52. Epub 2008/03/18. doi: 10.1016/j.chom.2008.03.001. PubMed PMID: 18342597; PMCID: PMC2474773.

39. Bachand F, Yao XJ, Hrimech M, Rougeau N, Cohen EA. Incorporation of Vpr into human immunodeficiency virus type 1 requires a direct interaction with the p6 domain of the p55 gag precursor. The Journal of biological chemistry. 1999;274(13):9083-91. Epub 1999/03/20. PubMed PMID: 10085158.

40. Paillart JC, Gottlinger HG. Opposing effects of human immunodeficiency virus type 1 matrix mutations support a myristyl switch model of gag membrane targeting. Journal of virology. 1999;73(4):2604-12. Epub 1999/03/12. PubMed PMID: 10074105; PMCID: PMC104015.

41. Selig L, Pages JC, Tanchou V, Preveral S, Berlioz-Torrent C, Liu LX, Erdtmann L, Darlix J, Benarous R, Benichou S. Interaction with the p6 domain of the gag precursor mediates incorporation into

virions of Vpr and Vpx proteins from primate lentiviruses. Journal of virology. 1999;73(1):592-600. Epub 1998/12/16. PubMed PMID: 9847364; PMCID: PMC103865.

42. Guenzel CA, Herate C, Benichou S. HIV-1 Vpr-a still "enigmatic multitasker". Front Microbiol. 2014;5:127. Epub 2014/04/20. doi: 10.3389/fmicb.2014.00127. PubMed PMID: 24744753; PMCID: PMC3978352.

43. He J, Choe S, Walker R, Di Marzio P, Morgan DO, Landau NR. Human immunodeficiency virus type 1 viral protein R (Vpr) arrests cells in the G2 phase of the cell cycle by inhibiting p34cdc2 activity. Journal of virology. 1995;69(11):6705-11. Epub 1995/11/01. PubMed PMID: 7474080; PMCID: PMC189580.

44. Jowett JB, Planelles V, Poon B, Shah NP, Chen ML, Chen IS. The human immunodeficiency virus type 1 vpr gene arrests infected T cells in the G2 + M phase of the cell cycle. Journal of virology. 1995;69(10):6304-13. Epub 1995/10/01. PubMed PMID: 7666531; PMCID: PMC189529.

45. Re F, Braaten D, Franke EK, Luban J. Human immunodeficiency virus type 1 Vpr arrests the cell cycle in G2 by inhibiting the activation of p34cdc2-cyclin B. Journal of virology. 1995;69(11):6859-64. Epub 1995/11/01. PubMed PMID: 7474100; PMCID: PMC189600.

46. Heinzinger NK, Bukrinsky MI, Haggerty SA, Ragland AM, Kewalramani V, Lee MA, Gendelman HE, Ratner L, Stevenson M, Emerman M. The Vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. Proceedings of the National Academy of Sciences of the United States of America. 1994;91(15):7311-5. Epub 1994/07/19. PubMed PMID: 8041786; PMCID: PMC44389.

47. Popov S, Rexach M, Ratner L, Blobel G, Bukrinsky M. Viral protein R regulates docking of the HIV-1 preintegration complex to the nuclear pore complex. The Journal of biological chemistry. 1998;273(21):13347-52. Epub 1998/05/28. PubMed PMID: 9582382.

48. Fujita M, Nomaguchi M, Adachi A, Otsuka M. SAMHD1-Dependent and -Independent Functions of HIV-2/SIV Vpx Protein. Front Microbiol. 2012;3:297. Epub 2012/08/22. doi: 10.3389/fmicb.2012.00297. PubMed PMID: 22908011; PMCID: PMC3415948.

49. Khamsri B, Murao F, Yoshida A, Sakurai A, Uchiyama T, Shirai H, Matsuo Y, Fujita M, Adachi A. Comparative study on the structure and cytopathogenic activity of HIV Vpr/Vpx proteins. Microbes Infect. 2006;8(1):10-5. Epub 2005/09/13. doi: 10.1016/j.micinf.2005.05.020. PubMed PMID: 16153874.

50. Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, Segeral E, Yatim A, Emiliani S, Schwartz O, Benkirane M. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature. 2011;474(7353):654-7. Epub 2011/05/27. doi: 10.1038/nature10117. PubMed PMID: 21613998; PMCID: PMC3595993.

51. Foster JL, Garcia JV. HIV-1 Nef: at the crossroads. Retrovirology. 2008;5:84. Epub 2008/09/24. doi: 10.1186/1742-4690-5-84. PubMed PMID: 18808677; PMCID: PMC2563024.

52. Aiken C, Krause L, Chen YL, Trono D. Mutational analysis of HIV-1 Nef: identification of two mutants that are temperature-sensitive for CD4 downregulation. Virology. 1996;217(1):293-300. Epub 1996/03/01. doi: 10.1006/viro.1996.0116. PubMed PMID: 8599214.

53. Anderson SJ, Lenburg M, Landau NR, Garcia JV. The cytoplasmic domain of CD4 is sufficient for its down-regulation from the cell surface by human immunodeficiency virus type 1 Nef. Journal of virology. 1994;68(5):3092-101. Epub 1994/05/01. PubMed PMID: 8151774; PMCID: PMC236799.

54. Garcia JV, Miller AD. Serine phosphorylation-independent downregulation of cell-surface CD4 by nef. Nature. 1991;350(6318):508-11. Epub 1991/04/11. doi: 10.1038/350508a0. PubMed PMID: 2014052.

55. Lundquist CA, Tobiume M, Zhou J, Unutmaz D, Aiken C. Nef-mediated downregulation of CD4 enhances human immunodeficiency virus type 1 replication in primary T lymphocytes. Journal of virology. 2002;76(9):4625-33. Epub 2002/04/05. PubMed PMID: 11932428; PMCID: PMC155097.

56. Mangasarian A, Foti M, Aiken C, Chin D, Carpentier JL, Trono D. The HIV-1 Nef protein acts as a connector with sorting pathways in the Golgi and at the plasma membrane. Immunity. 1997;6(1):67-77. Epub 1997/01/01. PubMed PMID: 9052838.

57. Atkins KM, Thomas L, Youker RT, Harriff MJ, Pissani F, You H, Thomas G. HIV-1 Nef binds PACS-2 to assemble a multikinase cascade that triggers major histocompatibility complex class I (MHC-

I) down-regulation: analysis using short interfering RNA and knock-out mice. The Journal of biological chemistry. 2008;283(17):11772-84. Epub 2008/02/26. doi: 10.1074/jbc.M707572200. PubMed PMID: 18296443; PMCID: PMC2431057.

58. Greenberg ME, Iafrate AJ, Skowronski J. The SH3 domain-binding surface and an acidic motif in HIV-1 Nef regulate trafficking of class I MHC complexes. The EMBO journal. 1998;17(10):2777-89. Epub 1998/06/10. doi: 10.1093/emboj/17.10.2777. PubMed PMID: 9582271; PMCID: PMC1170618.

59. Noviello CM, Benichou S, Guatelli JC. Cooperative binding of the class I major histocompatibility complex cytoplasmic domain and human immunodeficiency virus type 1 Nef to the endosomal AP-1 complex via its mu subunit. Journal of virology. 2008;82(3):1249-58. Epub 2007/12/07. doi: 10.1128/JVI.00660-07. PubMed PMID: 18057255; PMCID: PMC2224416.

60. Wonderlich ER, Williams M, Collins KL. The tyrosine binding pocket in the adaptor protein 1 (AP-1) mu1 subunit is necessary for Nef to recruit AP-1 to the major histocompatibility complex class I cytoplasmic tail. The Journal of biological chemistry. 2008;283(6):3011-22. Epub 2007/12/13. doi: 10.1074/jbc.M707760200. PubMed PMID: 18073204.

61. Schwartz O, Marechal V, Le Gall S, Lemonnier F, Heard JM. Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein. Nature medicine. 1996;2(3):338-42. Epub 1996/03/01. PubMed PMID: 8612235.

62. Arora VK, Molina RP, Foster JL, Blakemore JL, Chernoff J, Fredericksen BL, Garcia JV. Lentivirus Nef specifically activates Pak2. Journal of virology. 2000;74(23):11081-7. Epub 2000/11/09. PubMed PMID: 11070003; PMCID: PMC113188.

63. Renkema GH, Manninen A, Mann DA, Harris M, Saksela K. Identification of the Nef-associated kinase as p21-activated kinase 2. Curr Biol. 1999;9(23):1407-10. Epub 1999/12/23. PubMed PMID: 10607567.

64. Simmons A, Aluvihare V, McMichael A. Nef triggers a transcriptional program in T cells imitating single-signal T cell activation and inducing HIV virulence mediators. Immunity. 2001;14(6):763-77. Epub 2001/06/23. PubMed PMID: 11420046.

65. Wei BL, Arora VK, Raney A, Kuo LS, Xiao GH, O'Neill E, Testa JR, Foster JL, Garcia JV. Activation of p21-activated kinase 2 by human immunodeficiency virus type 1 Nef induces merlin phosphorylation. Journal of virology. 2005;79(23):14976-80. Epub 2005/11/12. doi: 10.1128/JVI.79.23.14976-14980.2005. PubMed PMID: 16282498; PMCID: PMC1287594.

66. Campbell EM, Nunez R, Hope TJ. Disruption of the actin cytoskeleton can complement the ability of Nef to enhance human immunodeficiency virus type 1 infectivity. Journal of virology. 2004;78(11):5745-55. Epub 2004/05/14. doi: 10.1128/JVI.78.11.5745-5755.2004. PubMed PMID: 15140972; PMCID: PMC415815.

67. Aiken C. Pseudotyping human immunodeficiency virus type 1 (HIV-1) by the glycoprotein of vesicular stomatitis virus targets HIV-1 entry to an endocytic pathway and suppresses both the requirement for Nef and the sensitivity to cyclosporin A. Journal of virology. 1997;71(8):5871-7. Epub 1997/08/01. PubMed PMID: 9223476; PMCID: PMC191842.

68. Chowers MY, Spina CA, Kwoh TJ, Fitch NJ, Richman DD, Guatelli JC. Optimal infectivity in vitro of human immunodeficiency virus type 1 requires an intact nef gene. Journal of virology. 1994;68(5):2906-14. Epub 1994/05/01. PubMed PMID: 8151761; PMCID: PMC236779.

69. Luo T, Livingston RA, Garcia JV. Infectivity enhancement by human immunodeficiency virus type 1 Nef is independent of its association with a cellular serine/threonine kinase. Journal of virology. 1997;71(12):9524-30. Epub 1997/11/26. PubMed PMID: 9371615; PMCID: PMC230259.

70. Miller MD, Warmerdam MT, Gaston I, Greene WC, Feinberg MB. The human immunodeficiency virus-1 nef gene product: a positive factor for viral infection and replication in primary lymphocytes and macrophages. The Journal of experimental medicine. 1994;179(1):101-13. Epub 1994/01/01. PubMed PMID: 8270859; PMCID: PMC2191317.

71. Pizzato M, Helander A, Popova E, Calistri A, Zamborlini A, Palu G, Gottlinger HG. Dynamin 2 is required for the enhancement of HIV-1 infectivity by Nef. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(16):6812-7. Epub 2007/04/07. doi: 10.1073/pnas.0607622104. PubMed PMID: 17412836; PMCID: PMC1871867.

72. Usami Y, Wu Y, Gottlinger HG. SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. Nature. 2015;526(7572):218-23. Epub 2015/09/30. doi: 10.1038/nature15400. PubMed PMID: 26416733; PMCID: PMC4600458.

73. Rosa A, Chande A, Ziglio S, De Sanctis V, Bertorelli R, Goh SL, McCauley SM, Nowosielska A, Antonarakis SE, Luban J, Santoni FA, Pizzato M. HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation. Nature. 2015;526(7572):212-7. Epub 2015/09/30. doi: 10.1038/nature15399. PubMed PMID: 26416734; PMCID: PMC4861059.

74. Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, Sun C, Grayson T, Wang S, Li H, Wei X, Jiang C, Kirchherr JL, Gao F, Anderson JA, Ping LH, Swanstrom R, Tomaras GD, Blattner WA, Goepfert PA, Kilby JM, Saag MS, Delwart EL, Busch MP, Cohen MS, Montefiori DC, Haynes BF, Gaschen B, Athreya GS, Lee HY, Wood N, Seoighe C, Perelson AS, Bhattacharya T, Korber BT, Hahn BH, Shaw GM. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(21):7552-7. Epub 2008/05/21. doi: 10.1073/pnas.0802203105. PubMed PMID: 18490657; PMCID: PMC2387184.

75. Moir S, Chun TW, Fauci AS. Pathogenic mechanisms of HIV disease. Annual review of pathology. 2011;6:223-48. Epub 2010/11/03. doi: 10.1146/annurev-pathol-011110-130254. PubMed PMID: 21034222.

76. Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment, and prevention. Lancet. 2014;384(9939):258-71. Epub 2014/06/09. doi: 10.1016/S0140-6736(14)60164-1. PubMed PMID: 24907868.

77. Zhang Z, Schuler T, Zupancic M, Wietgrefe S, Staskus KA, Reimann KA, Reinhart TA, Rogan M, Cavert W, Miller CJ, Veazey RS, Notermans D, Little S, Danner SA, Richman DD, Havlir D, Wong J, Jordan HL, Schacker TW, Racz P, Tenner-Racz K, Letvin NL, Wolinsky S, Haase AT. Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. Science. 1999;286(5443):1353-7. Epub 1999/11/13. PubMed PMID: 10558989.

78. Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, Boden D, Racz P, Markowitz M. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. The Journal of experimental medicine. 2004;200(6):761-70. Epub 2004/09/15. doi: 10.1084/jem.20041196. PubMed PMID: 15365095; PMCID: PMC2211967.

79. Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature. 2005;434(7037):1093-7. Epub 2005/03/29. doi: 10.1038/nature03501. PubMed PMID: 15793563.

80. Li Q, Duan L, Estes JD, Ma ZM, Rourke T, Wang Y, Reilly C, Carlis J, Miller CJ, Haase AT. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature. 2005;434(7037):1148-52. Epub 2005/03/29. doi: 10.1038/nature03513. PubMed PMID: 15793562.

81. Guadalupe M, Reay E, Sankaran S, Prindiville T, Flamm J, McNeil A, Dandekar S. 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. Journal of virology. 2003;77(21):11708-17. Epub 2003/10/15. PubMed PMID: 14557656; PMCID: PMC229357.

82. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, Nguyen PL, Khoruts A, Larson M, Haase AT, Douek DC. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. The Journal of experimental medicine. 2004;200(6):749-59. Epub 2004/09/15. doi: 10.1084/jem.20040874. PubMed PMID: 15365096; PMCID: PMC2211962.

83. Gurunathan S, Habib RE, Baglyos L, Meric C, Plotkin S, Dodet B, Corey L, Tartaglia J. Use of predictive markers of HIV disease progression in vaccine trials. Vaccine. 2009;27(14):1997-2015. Epub 2009/02/10. doi: 10.1016/j.vaccine.2009.01.039. PubMed PMID: 19200450.

84. Piatak M, Jr., Saag MS, Yang LC, Clark SJ, Kappes JC, Luk KC, Hahn BH, Shaw GM, Lifson JD. 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. PubMed PMID: 8096089.

85. Little SJ, McLean AR, Spina CA, Richman DD, Havlir DV. Viral dynamics of acute HIV-1 infection. The Journal of experimental medicine. 1999;190(6):841-50. Epub 1999/09/28. PubMed PMID: 10499922; PMCID: PMC2195636.

86. Mellors JW, Rinaldo CR, Jr., Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science. 1996;272(5265):1167-70. Epub 1996/05/24. PubMed PMID: 8638160.

87. Douek DC, Roederer M, Koup RA. Emerging concepts in the immunopathogenesis of AIDS. Annual review of medicine. 2009;60:471-84. Epub 2008/10/25. doi: 10.1146/annurev.med.60.041807.123549. PubMed PMID: 18947296; PMCID: PMC2716400.

88. Doitsh G, Galloway NL, Geng X, Yang Z, Monroe KM, Zepeda O, Hunt PW, Hatano H, Sowinski S, Munoz-Arias I, Greene WC. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature. 2014;505(7484):509-14. Epub 2013/12/21. doi: 10.1038/nature12940. PubMed PMID: 24356306; PMCID: PMC4047036.

89. Pitcher CJ, Quittner C, Peterson DM, Connors M, Koup RA, Maino VC, Picker LJ. HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nature medicine. 1999;5(5):518-25. Epub 1999/05/06. doi: 10.1038/8400. PubMed PMID: 10229228.

90. Soghoian DZ, Jessen H, Flanders M, Sierra-Davidson K, Cutler S, Pertel T, Ranasinghe S, Lindqvist M, Davis I, Lane K, Rychert J, Rosenberg ES, Piechocka-Trocha A, Brass AL, Brenchley JM, Walker BD, Streeck H. HIV-specific cytolytic CD4 T cell responses during acute HIV infection predict disease outcome. Science translational medicine. 2012;4(123):123ra25. Epub 2012/03/02. doi: 10.1126/scitranslmed.3003165. PubMed PMID: 22378925; PMCID: PMC3918726.

91. Schieffer M, Jessen HK, Oster AF, Pissani F, Soghoian DZ, Lu R, Jessen AB, Zedlack C, Schultz BT, Davis I, Ranasinghe S, Rosenberg ES, Alter G, Schumann RR, Streeck H. Induction of Gag-specific CD4 T cell responses during acute HIV infection is associated with improved viral control. Journal of

virology. 2014;88(13):7357-66. Epub 2014/04/18. doi: 10.1128/JVI.00728-14. PubMed PMID: 24741089; PMCID: PMC4054446.

92. Kaufmann DE, Bailey PM, Sidney J, Wagner B, Norris PJ, Johnston MN, Cosimi LA, Addo MM, Lichterfeld M, Altfeld M, Frahm N, Brander C, Sette A, Walker BD, Rosenberg ES. Comprehensive analysis of human immunodeficiency virus type 1-specific CD4 responses reveals marked immunodominance of gag and nef and the presence of broadly recognized peptides. Journal of virology. 2004;78(9):4463-77. Epub 2004/04/14. PubMed PMID: 15078927; PMCID: PMC387674.

93. Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, Walker BD. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science. 1997;278(5342):1447-50. Epub 1997/12/31. PubMed PMID: 9367954.

94. Ranasinghe S, Flanders M, Cutler S, Soghoian DZ, Ghebremichael M, Davis I, Lindqvist M, Pereyra F, Walker BD, Heckerman D, Streeck H. HIV-specific CD4 T cell responses to different viral proteins have discordant associations with viral load and clinical outcome. Journal of virology. 2012;86(1):277-83. Epub 2011/10/28. doi: 10.1128/JVI.05577-11. PubMed PMID: 22031937; PMCID: PMC3255877.

95. Ranasinghe S, Cutler S, Davis I, Lu R, Soghoian DZ, Qi Y, Sidney J, Kranias G, Flanders MD, Lindqvist M, Kuhl B, Alter G, Deeks SG, Walker BD, Gao X, Sette A, Carrington M, Streeck H. Association of HLA-DRB1-restricted CD4(+) T cell responses with HIV immune control. Nature medicine. 2013;19(7):930-3. Epub 2013/06/25. doi: 10.1038/nm.3229. PubMed PMID: 23793098; PMCID: PMC3974408.

96. Julg B, Moodley ES, Qi Y, Ramduth D, Reddy S, Mncube Z, Gao X, Goulder PJ, Detels R, Ndung'u T, Walker BD, Carrington M. Possession of HLA class II DRB1*1303 associates with reduced viral loads in chronic HIV-1 clade C and B infection. The Journal of infectious diseases. 2011;203(6):803-9. Epub 2011/01/25. doi: 10.1093/infdis/jiq122. PubMed PMID: 21257739; PMCID: PMC3071131.

97. Rosenberg ES, Altfeld M, Poon SH, Phillips MN, Wilkes BM, Eldridge RL, Robbins GK, D'Aquila RT, Goulder PJ, Walker BD. Immune control of HIV-1 after early treatment of acute infection. Nature. 2000;407(6803):523-6. Epub 2000/10/12. doi: 10.1038/35035103. PubMed PMID: 11029005.

98. Chevalier MF, Didier C, Girard PM, Manea ME, Campa P, Barre-Sinoussi F, Scott-Algara D, Weiss L. CD4 T-Cell Responses in Primary HIV Infection: Interrelationship with Immune Activation and Virus Burden. Frontiers in immunology. 2016;7:395. Epub 2016/10/18. doi: 10.3389/fimmu.2016.00395. PubMed PMID: 27746782; PMCID: PMC5040706.

99. Walker B, McMichael A. The T-cell response to HIV. Cold Spring Harb Perspect Med. 2012;2(11). Epub 2012/09/25. doi: 10.1101/cshperspect.a007054. PubMed PMID: 23002014; PMCID: PMC3543107.

100. Demers KR, Reuter MA, Betts MR. CD8(+) T-cell effector function and transcriptional regulation during HIV pathogenesis. Immunological reviews. 2013;254(1):190-206. Epub 2013/06/19. doi: 10.1111/imr.12069. PubMed PMID: 23772621; PMCID: PMC3693771.

101. 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. PubMed PMID: 2431484.

102. Dalod M, Dupuis M, Deschemin JC, Goujard C, Deveau C, Meyer L, Ngo N, Rouzioux C, Guillet JG, Delfraissy JF, Sinet M, Venet A. Weak anti-HIV CD8(+) T-cell effector activity in HIV primary infection. The Journal of clinical investigation. 1999;104(10):1431-9. Epub 1999/11/24. doi: 10.1172/JCI7162. PubMed PMID: 10562305; PMCID: PMC409838.

103. Maecker HT, Dunn HS, Suni MA, Khatamzas E, Pitcher CJ, Bunde T, Persaud N, Trigona W, Fu TM, Sinclair E, Bredt BM, McCune JM, Maino VC, Kern F, Picker LJ. Use of overlapping peptide mixtures as antigens for cytokine flow cytometry. J Immunol Methods. 2001;255(1-2):27-40. Epub 2001/07/27. PubMed PMID: 11470284.

104. Altman JD, Moss PA, Goulder PJ, Barouch DH, McHeyzer-Williams MG, Bell JI, McMichael AJ, Davis MM. Phenotypic analysis of antigen-specific T lymphocytes. Science. 1996;274(5284):94-6. Epub 1996/10/04. PubMed PMID: 8810254.

105. 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. Journal of virology. 1994;68(9):6103-10. Epub 1994/09/01. PubMed PMID: 8057491; PMCID: PMC237022.

106. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, Farthing C, Ho DD. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. Journal of virology. 1994;68(7):4650-5. Epub 1994/07/01. PubMed PMID: 8207839; PMCID: 236393.

107. Phillips RE, Rowland-Jones S, Nixon DF, Gotch FM, Edwards JP, Ogunlesi AO, Elvin JG, Rothbard JA, Bangham CR, Rizza CR, et al. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. Nature. 1991;354(6353):453-9. Epub 1991/12/12. doi: 10.1038/354453a0. PubMed PMID: 1721107.

108. Salazar-Gonzalez JF, Salazar MG, Keele BF, Learn GH, Giorgi EE, Li H, Decker JM, Wang S, Baalwa J, Kraus MH, Parrish NF, Shaw KS, Guffey MB, Bar KJ, Davis KL, Ochsenbauer-Jambor C, Kappes JC, Saag MS, Cohen MS, Mulenga J, Derdeyn CA, Allen S, Hunter E, Markowitz M, Hraber P, Perelson AS, Bhattacharya T, Haynes BF, Korber BT, Hahn BH, Shaw GM. Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. The Journal of experimental medicine. 2009;206(6):1273-89. Epub 2009/06/03. doi: 10.1084/jem.20090378. PubMed PMID: 19487424; PMCID: PMC2715054.

109. Shaw GM, Hunter E. HIV transmission. Cold Spring Harb Perspect Med. 2012;2(11). Epub 2012/10/09. doi: 10.1101/cshperspect.a006965. PubMed PMID: 23043157; PMCID: PMC3543106.

110. Goonetilleke N, Liu MK, Salazar-Gonzalez JF, Ferrari G, Giorgi E, Ganusov VV, Keele BF, Learn GH, Turnbull EL, Salazar MG, Weinhold KJ, Moore S, B CCC, Letvin N, Haynes BF, Cohen MS,

Hraber P, Bhattacharya T, Borrow P, Perelson AS, Hahn BH, Shaw GM, Korber BT, McMichael AJ. The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. The Journal of experimental medicine. 2009;206(6):1253-72. Epub 2009/06/03. doi: 10.1084/jem.20090365. PubMed PMID: 19487423; PMCID: PMC2715063.

111. Lyles RH, Munoz A, Yamashita TE, Bazmi H, Detels R, Rinaldo CR, Margolick JB, Phair JP, Mellors JW. Natural history of human immunodeficiency virus type 1 viremia after seroconversion and proximal to AIDS in a large cohort of homosexual men. Multicenter AIDS Cohort Study. The Journal of infectious diseases. 2000;181(3):872-80. Epub 2000/03/18. doi: 10.1086/315339. PubMed PMID: 10720507.

112. Lichterfeld M, Yu XG, Cohen D, Addo MM, Malenfant J, Perkins B, Pae E, Johnston MN, Strick D, Allen TM, Rosenberg ES, Korber B, Walker BD, Altfeld M. HIV-1 Nef is preferentially recognized by CD8 T cells in primary HIV-1 infection despite a relatively high degree of genetic diversity. Aids. 2004;18(10):1383-92. Epub 2004/06/17. PubMed PMID: 15199314.

113. Turnbull EL, Wong M, Wang S, Wei X, Jones NA, Conrod KE, Aldam D, Turner J, Pellegrino P, Keele BF, Williams I, Shaw GM, Borrow P. Kinetics of expansion of epitope-specific T cell responses during primary HIV-1 infection. Journal of immunology. 2009;182(11):7131-45. Epub 2009/05/21. doi: 10.4049/jimmunol.0803658. PubMed PMID: 19454710.

114. Altfeld M, Rosenberg ES, Shankarappa R, Mukherjee JS, Hecht FM, Eldridge RL, Addo MM, Poon SH, Phillips MN, Robbins GK, Sax PE, Boswell S, Kahn JO, Brander C, Goulder PJ, Levy JA, Mullins JI, Walker BD. Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. The Journal of experimental medicine. 2001;193(2):169-80. Epub 2001/01/10. PubMed PMID: 11148221; PMCID: PMC2193337.

115. Altfeld M, Kalife ET, Qi Y, Streeck H, Lichterfeld M, Johnston MN, Burgett N, Swartz ME, Yang A, Alter G, Yu XG, Meier A, Rockstroh JK, Allen TM, Jessen H, Rosenberg ES, Carrington M, Walker BD. HLA Alleles Associated with Delayed Progression to AIDS Contribute Strongly to the Initial

CD8(+) T Cell Response against HIV-1. PLoS medicine. 2006;3(10):e403. Epub 2006/11/02. doi: 10.1371/journal.pmed.0030403. PubMed PMID: 17076553; PMCID: PMC1626551.

116. Streeck H, Jolin JS, Qi Y, Yassine-Diab B, Johnson RC, Kwon DS, Addo MM, Brumme C, Routy JP, Little S, Jessen HK, Kelleher AD, Hecht FM, Sekaly RP, Rosenberg ES, Walker BD, Carrington M, Altfeld M. Human immunodeficiency virus type 1-specific CD8+ T-cell responses during primary infection are major determinants of the viral set point and loss of CD4+ T cells. Journal of virology. 2009;83(15):7641-8. Epub 2009/05/22. doi: 10.1128/JVI.00182-09. PubMed PMID: 19458000; PMCID: PMC2708622.

117. Lichterfeld M, Yu XG, Mui SK, Williams KL, Trocha A, Brockman MA, Allgaier RL, Waring MT, Koibuchi T, Johnston MN, Cohen D, Allen TM, Rosenberg ES, Walker BD, Altfeld M. Selective depletion of high-avidity human immunodeficiency virus type 1 (HIV-1)-specific CD8+ T cells after early HIV-1 infection. Journal of virology. 2007;81(8):4199-214. Epub 2007/02/09. doi: 10.1128/JVI.01388-06. PubMed PMID: 17287271; PMCID: PMC1866095.

118. Gao X, Nelson GW, Karacki P, Martin MP, Phair J, Kaslow R, Goedert JJ, Buchbinder S, Hoots K, Vlahov D, O'Brien SJ, Carrington M. Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. The New England journal of medicine. 2001;344(22):1668-75. Epub 2001/06/02. doi: 10.1056/NEJM200105313442203. PubMed PMID: 11386265.

119. Kaslow RA, Carrington M, Apple R, Park L, Munoz A, Saah AJ, Goedert JJ, Winkler C, O'Brien SJ, Rinaldo C, Detels R, Blattner W, Phair J, Erlich H, Mann DL. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. Nature medicine. 1996;2(4):405-11. Epub 1996/04/01. PubMed PMID: 8597949.

120. Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L, Hallahan CW, Selig SM, Schwartz D, Sullivan J, Connors M. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(6):2709-14. Epub 2000/03/01. doi: 10.1073/pnas.050567397. PubMed PMID: 10694578; PMCID: PMC15994.

121. Hendel H, Caillat-Zucman S, Lebuanec H, Carrington M, O'Brien S, Andrieu JM, Schachter F, Zagury D, Rappaport J, Winkler C, Nelson GW, Zagury JF. New class I and II HLA alleles strongly associated with opposite patterns of progression to AIDS. Journal of immunology. 1999;162(11):6942-6. Epub 1999/06/03. PubMed PMID: 10352317.

122. Migueles SA, Connors M. Frequency and function of HIV-specific CD8(+) T cells. Immunology letters. 2001;79(1-2):141-50. Epub 2001/10/12. PubMed PMID: 11595301.

123. Migueles SA, Connors M. The Role of CD4(+) and CD8(+) T Cells in Controlling HIV Infection. Current infectious disease reports. 2002;4(5):461-7. Epub 2002/09/14. PubMed PMID: 12228034.

124. O'Brien SJ, Gao X, Carrington M. HLA and AIDS: a cautionary tale. Trends in molecular medicine. 2001;7(9):379-81. Epub 2001/09/01. PubMed PMID: 11530315.

125. Costello C, Tang J, Rivers C, Karita E, Meizen-Derr J, Allen S, Kaslow RA. HLA-B*5703 independently associated with slower HIV-1 disease progression in Rwandan women. Aids. 1999;13(14):1990-1. Epub 1999/10/08. PubMed PMID: 10513667.

126. Klein MR, van der Burg SH, Hovenkamp E, Holwerda AM, Drijfhout JW, Melief CJ, Miedema F. Characterization of HLA-B57-restricted human immunodeficiency virus type 1 Gag- and RT-specific cytotoxic T lymphocyte responses. The Journal of general virology. 1998;79 (Pt 9):2191-201. Epub 1998/09/25. doi: 10.1099/0022-1317-79-9-2191. PubMed PMID: 9747728.

127. Goulder PJ, Bunce M, Krausa P, McIntyre K, Crowley S, Morgan B, Edwards A, Giangrande P, Phillips RE, McMichael AJ. Novel, cross-restricted, conserved, and immunodominant cytotoxic T lymphocyte epitopes in slow progressors in HIV type 1 infection. AIDS research and human retroviruses. 1996;12(18):1691-8. Epub 1996/12/10. doi: 10.1089/aid.1996.12.1691. PubMed PMID: 8959245.

128. Gillespie GM, Kaul R, Dong T, Yang HB, Rostron T, Bwayo JJ, Kiama P, Peto T, Plummer FA, McMichael AJ, Rowland-Jones SL. Cross-reactive cytotoxic T lymphocytes against a HIV-1 p24 epitope in slow progressors with B*57. Aids. 2002;16(7):961-72. Epub 2002/04/16. PubMed PMID: 11953462.

129. Flores-Villanueva PO, Yunis EJ, Delgado JC, Vittinghoff E, Buchbinder S, Leung JY, Uglialoro AM, Clavijo OP, Rosenberg ES, Kalams SA, Braun JD, Boswell SL, Walker BD, Goldfeld AE. Control

of HIV-1 viremia and protection from AIDS are associated with HLA-Bw4 homozygosity. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(9):5140-5. Epub 2001/04/20. doi: 10.1073/pnas.071548198. PubMed PMID: 11309482; PMCID: PMC33177.

130. Keet IP, Tang J, Klein MR, LeBlanc S, Enger C, Rivers C, Apple RJ, Mann D, Goedert JJ, Miedema F, Kaslow RA. Consistent associations of HLA class I and II and transporter gene products with progression of human immunodeficiency virus type 1 infection in homosexual men. The Journal of infectious diseases. 1999;180(2):299-309. Epub 1999/07/09. doi: 10.1086/314862. PubMed PMID: 10395843.

131. Altfeld M, Addo MM, Rosenberg ES, Hecht FM, Lee PK, Vogel M, Yu XG, Draenert R, Johnston MN, Strick D, Allen TM, Feeney ME, Kahn JO, Sekaly RP, Levy JA, Rockstroh JK, Goulder PJ, Walker BD. Influence of HLA-B57 on clinical presentation and viral control during acute HIV-1 infection. Aids. 2003;17(18):2581-91. Epub 2003/12/20. doi: 10.1097/01.aids.0000096870.36052.b6. PubMed PMID: 14685052.

132. Jin X, Bauer DE, Tuttleton SE, Lewin S, Gettie A, Blanchard J, Irwin CE, Safrit JT, Mittler J, Weinberger L, Kostrikis LG, Zhang L, Perelson AS, Ho DD. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. The Journal of experimental medicine. 1999;189(6):991-8. Epub 1999/03/17. PubMed PMID: 10075982; PMCID: PMC2193038.

133. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, Racz P, Tenner-Racz K, Dalesandro M, Scallon BJ, Ghrayeb J, Forman MA, Montefiori DC, Rieber EP, Letvin NL, Reimann KA. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science. 1999;283(5403):857-60. Epub 1999/02/05. PubMed PMID: 9933172.

134. Cartwright EK, Spicer L, Smith SA, Lee D, Fast R, Paganini S, Lawson BO, Nega M, Easley K, Schmitz JE, Bosinger SE, Paiardini M, Chahroudi A, Vanderford TH, Estes JD, Lifson JD, Derdeyn CA, Silvestri G. CD8(+) Lymphocytes Are Required for Maintaining Viral Suppression in SIV-Infected

Macaques Treated with Short-Term Antiretroviral Therapy. Immunity. 2016;45(3):656-68. Epub 2016/09/23. doi: 10.1016/j.immuni.2016.08.018. PubMed PMID: 27653601; PMCID: PMC5087330.

135. Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, Abraham J, Lederman MM, Benito JM, Goepfert PA, Connors M, Roederer M, Koup RA. 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; PMCID: PMC1895811.

136. Streeck H, Brumme ZL, Anastario M, Cohen KW, Jolin JS, Meier A, Brumme CJ, Rosenberg ES, Alter G, Allen TM, Walker BD, Altfeld M. Antigen load and viral sequence diversification determine the functional profile of HIV-1-specific CD8+ T cells. PLoS medicine. 2008;5(5):e100. Epub 2008/05/09. doi: 10.1371/journal.pmed.0050100. PubMed PMID: 18462013; PMCID: PMC2365971.

137. 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. PubMed PMID: 8525373.

138. Copeland KF. The role of CD8+ T cell soluble factors in human immunodeficiency virus infection. Curr Med Chem. 2002;9(20):1781-90. Epub 2002/10/09. PubMed PMID: 12369877.

139. Hersperger AR, Pereyra F, Nason M, Demers K, Sheth P, Shin LY, Kovacs CM, Rodriguez B, Sieg SF, Teixeira-Johnson L, Gudonis D, Goepfert PA, Lederman MM, Frank I, Makedonas G, Kaul R, Walker BD, Betts MR. Perforin expression directly ex vivo by HIV-specific CD8 T-cells is a correlate of HIV elite control. PLoS pathogens. 2010;6(5):e1000917. Epub 2010/06/05. doi: 10.1371/journal.ppat.1000917. PubMed PMID: 20523897; PMCID: PMC2877741.

140. Betts MR, Harari A. Phenotype and function of protective T cell immune responses in HIV. Current opinion in HIV and AIDS. 2008;3(3):349-55. Epub 2009/04/18. doi: 10.1097/COH.0b013e3282fbaa81. PubMed PMID: 19372989.

141. Shacklett BL, Anton PA. HIV Infection and Gut Mucosal Immune Function: Updates on Pathogenesis with Implications for Management and Intervention. Current infectious disease reports.

2010;12(1):19-27. Epub 2010/02/23. doi: 10.1007/s11908-009-0072-9. PubMed PMID: 20174448; PMCID: PMC2821616.

142. Ling B, Mohan M, Lackner AA, Green LC, Marx PA, Doyle LA, Veazey RS. The large intestine as a major reservoir for simian immunodeficiency virus in macaques with long-term, nonprogressing infection. The Journal of infectious diseases. 2010;202(12):1846-54. Epub 2010/11/06. doi: 10.1086/657413. PubMed PMID: 21050120; PMCID: PMC3058301.

143. Smit-McBride Z, Mattapallil JJ, McChesney M, Ferrick D, Dandekar S. Gastrointestinal T lymphocytes retain high potential for cytokine responses but have severe CD4(+) T-cell depletion at all stages of simian immunodeficiency virus infection compared to peripheral lymphocytes. Journal of virology. 1998;72(8):6646-56. Epub 1998/07/11. PubMed PMID: 9658111; PMCID: PMC109855.

144. Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, Rosenzweig M, Johnson RP, Desrosiers RC, Lackner AA. 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. PubMed PMID: 9545219.

145. Veazey R, Lackner A. The mucosal immune system and HIV-1 infection. AIDS Rev. 2003;5(4):245-52. Epub 2004/03/12. PubMed PMID: 15012003.

146. Veazey RS, Mansfield KG, Tham IC, Carville AC, Shvetz DE, Forand AE, Lackner AA. Dynamics of CCR5 expression by CD4(+) T cells in lymphoid tissues during simian immunodeficiency virus infection. Journal of virology. 2000;74(23):11001-7. Epub 2000/11/09. PubMed PMID: 11069995; PMCID: PMC113180.

147. Ivanov, II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009;139(3):485-98. Epub 2009/10/20. doi: 10.1016/j.cell.2009.09.033. PubMed PMID: 19836068; PMCID: PMC2796826.

148. Brenchley JM, Paiardini M, Knox KS, Asher AI, Cervasi B, Asher TE, Scheinberg P, Price DA, Hage CA, Kholi LM, Khoruts A, Frank I, Else J, Schacker T, Silvestri G, Douek DC. 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; PMCID: PMC2556618.

149. Cecchinato V, Franchini G. Th17 cells in pathogenic simian immunodeficiency virus infection of macaques. Current opinion in HIV and AIDS. 2010;5(2):141-5. Epub 2010/06/15. doi: 10.1097/COH.0b013e32833653ec. PubMed PMID: 20543591; PMCID: PMC2898129.

150. Cecchinato V, Trindade CJ, Laurence A, Heraud JM, Brenchley JM, Ferrari MG, Zaffiri L, Tryniszewska E, Tsai WP, Vaccari M, Parks RW, Venzon D, Douek DC, O'Shea JJ, Franchini G. Altered balance between Th17 and Th1 cells at mucosal sites predicts AIDS progression in simian immunodeficiency virus-infected macaques. Mucosal immunology. 2008;1(4):279-88. doi: 10.1038/mi.2008.14. PubMed PMID: 19079189; PMCID: 2997489.

151. Favre D, Lederer S, Kanwar B, Ma ZM, Proll S, Kasakow Z, Mold J, Swainson L, Barbour JD, Baskin CR, Palermo R, Pandrea I, Miller CJ, Katze MG, McCune JM. Critical loss of the balance between Th17 and T regulatory cell populations in pathogenic SIV infection. PLoS pathogens. 2009;5(2):e1000295. Epub 2009/02/14. doi: 10.1371/journal.ppat.1000295. PubMed PMID: 19214220; PMCID: PMC2635016.

152. Klatt NR, Brenchley JM. Th17 cell dynamics in HIV infection. Current opinion in HIV and AIDS. 2010;5(2):135-40. Epub 2010/06/15. doi: 10.1097/COH.0b013e3283364846. PubMed PMID: 20543590; PMCID: PMC2886291.

153. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR, Rodriguez B, Teixeira-Johnson L, Landay A, Martin JN, Hecht FM, Picker LJ, Lederman MM, Deeks SG, Douek DC. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nature medicine. 2006;12(12):1365-71. Epub 2006/11/23. doi: 10.1038/nm1511. PubMed PMID: 17115046.

154. Schacker TW, Brenchley JM, Beilman GJ, Reilly C, Pambuccian SE, Taylor J, Skarda D, Larson M, Douek DC, Haase AT. Lymphatic tissue fibrosis is associated with reduced numbers of naive CD4+ T

cells in human immunodeficiency virus type 1 infection. Clinical and vaccine immunology : CVI. 2006;13(5):556-60. Epub 2006/05/10. doi: 10.1128/CVI.13.5.556-560.2006. PubMed PMID: 16682476; PMCID: PMC1459657.

155. Campbell DJ, Koch MA. Phenotypical and functional specialization of FOXP3+ regulatory T cells. Nature reviews Immunology. 2011;11(2):119-30. Epub 2011/01/27. doi: 10.1038/nri2916. PubMed PMID: 21267013; PMCID: PMC3289970.

156. Boasso A, Vaccari M, Hryniewicz A, Fuchs D, Nacsa J, Cecchinato V, Andersson J, Franchini G, Shearer GM, Chougnet C. Regulatory T-cell markers, indoleamine 2,3-dioxygenase, and virus levels in spleen and gut during progressive simian immunodeficiency virus infection. Journal of virology. 2007;81(21):11593-603. Epub 2007/08/24. doi: 10.1128/JVI.00760-07. PubMed PMID: 17715231; PMCID: PMC2168803.

157. Veazey RS, Gauduin MC, Mansfield KG, Tham IC, Altman JD, Lifson JD, Lackner AA, Johnson RP. Emergence and kinetics of simian immunodeficiency virus-specific CD8(+) T cells in the intestines of macaques during primary infection. Journal of virology. 2001;75(21):10515-9. Epub 2001/10/03. doi: 10.1128/JVI.75.21.10515-10519.2001. PubMed PMID: 11581423; PMCID: PMC114629.

158. Veazey RS, Lifson JD, Schmitz JE, Kuroda MJ, Piatak M, Jr., Pandrea I, Purcell J, Bohm R, Blanchard J, Williams KC, Lackner AA. Dynamics of Simian immunodeficiency virus-specific cytotoxic T-cell responses in tissues. Journal of medical primatology. 2003;32(4-5):194-200. Epub 2003/09/23. PubMed PMID: 14498979.

159. Critchfield JW, Young DH, Hayes TL, Braun JV, Garcia JC, Pollard RB, Shacklett BL. Magnitude and complexity of rectal mucosa HIV-1-specific CD8+ T-cell responses during chronic infection reflect clinical status. PloS one. 2008;3(10):e3577. Epub 2008/11/01. doi: 10.1371/journal.pone.0003577. PubMed PMID: 18974782; PMCID: PMC2570490.

160. Ferre AL, Hunt PW, Critchfield JW, Young DH, Morris MM, Garcia JC, Pollard RB, Yee HF, Jr., Martin JN, Deeks SG, Shacklett BL. Mucosal immune responses to HIV-1 in elite controllers: a

potential correlate of immune control. Blood. 2009;113(17):3978-89. Epub 2008/12/26. doi: 10.1182/blood-2008-10-182709. PubMed PMID: 19109229; PMCID: PMC2673124.

161. Crotty S. A brief history of T cell help to B cells. Nature reviews Immunology. 2015;15(3):185-9. Epub 2015/02/14. doi: 10.1038/nri3803. PubMed PMID: 25677493; PMCID: PMC4414089.

162. Dimopoulos Y, Moysi E, Petrovas C. The Lymph Node in HIV Pathogenesis. Curr HIV/AIDS Rep. 2017;14(4):133-40. Epub 2017/07/08. doi: 10.1007/s11904-017-0359-7. PubMed PMID: 28685417.

163. Lindqvist M, van Lunzen J, Soghoian DZ, Kuhl BD, Ranasinghe S, Kranias G, Flanders MD, Cutler S, Yudanin N, Muller MI, Davis I, Farber D, Hartjen P, Haag F, Alter G, Schulze zur Wiesch J, Streeck H. Expansion of HIV-specific T follicular helper cells in chronic HIV infection. The Journal of clinical investigation. 2012;122(9):3271-80. doi: 10.1172/JCI64314. PubMed PMID: 22922259; PMCID: 3428098.

164. Perreau M, Savoye AL, De Crignis E, Corpataux JM, Cubas R, Haddad EK, De Leval L, Graziosi C, Pantaleo G. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. The Journal of experimental medicine. 2013;210(1):143-56. doi: 10.1084/jem.20121932. PubMed PMID: 23254284; PMCID: 3549706.

165. Xu Y, Weatherall C, Bailey M, Alcantara S, De Rose R, Estaquier J, Wilson K, Suzuki K, Corbeil J, Cooper DA, Kent SJ, Kelleher AD, Zaunders J. Simian immunodeficiency virus infects follicular helper CD4 T cells in lymphoid tissues during pathogenic infection of pigtail macaques. Journal of virology. 2013;87(7):3760-73. doi: 10.1128/JVI.02497-12. PubMed PMID: 23325697; PMCID: 3624224.

166. Petrovas C, Yamamoto T, Gerner MY, Boswell KL, Wloka K, Smith EC, Ambrozak DR, Sandler NG, Timmer KJ, Sun X, Pan L, Poholek A, Rao SS, Brenchley JM, Alam SM, Tomaras GD, Roederer M, Douek DC, Seder RA, Germain RN, Haddad EK, Koup RA. CD4 T follicular helper cell dynamics during SIV infection. The Journal of clinical investigation. 2012;122(9):3281-94. doi: 10.1172/JCI63039. PubMed PMID: 22922258; PMCID: PMC3428091.

167. Xu H, Wang X, Malam N, Lackner AA, Veazey RS. Persistent Simian Immunodeficiency Virus Infection Causes Ultimate Depletion of Follicular Th Cells in AIDS. Journal of immunology. 2015;195(9):4351-7. Epub 2015/09/27. doi: 10.4049/jimmunol.1501273. PubMed PMID: 26408660; PMCID: PMC4610871.

168. Xu H, Wang X, Malam N, Aye PP, Alvarez X, Lackner AA, Veazey RS. Persistent Simian Immunodeficiency Virus Infection Drives Differentiation, Aberrant Accumulation, and Latent Infection of Germinal Center Follicular T Helper Cells. Journal of virology. 2016;90(3):1578-87. Epub 2015/11/27. doi: 10.1128/JVI.02471-15. PubMed PMID: 26608323; PMCID: PMC4719631.

169. Altfeld M, van Lunzen J, Frahm N, Yu XG, Schneider C, Eldridge RL, Feeney ME, Meyer-Olson D, Stellbrink HJ, Walker BD. Expansion of pre-existing, lymph node-localized CD8+ T cells during supervised treatment interruptions in chronic HIV-1 infection. The Journal of clinical investigation. 2002;109(6):837-43. Epub 2002/03/20. doi: 10.1172/JCI14789. PubMed PMID: 11901192; PMCID: PMC150914.

170. Connick E, Mattila T, Folkvord JM, Schlichtemeier R, Meditz AL, Ray MG, McCarter MD, Mawhinney S, Hage A, White C, Skinner PJ. CTL fail to accumulate at sites of HIV-1 replication in lymphoid tissue. Journal of immunology. 2007;178(11):6975-83. Epub 2007/05/22. PubMed PMID: 17513747.

171. Fukazawa Y, Lum R, Okoye AA, Park H, Matsuda K, Bae JY, Hagen SI, Shoemaker R, Deleage C, Lucero C, Morcock D, Swanson T, Legasse AW, Axthelm MK, Hesselgesser J, Geleziunas R, Hirsch VM, Edlefsen PT, Piatak M, Jr., Estes JD, Lifson JD, Picker LJ. B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers. Nature medicine. 2015;21(2):132-9. doi: 10.1038/nm.3781. PubMed PMID: 25599132; PMCID: 4320022.

172. Crotty S. Follicular helper CD4 T cells (TFH). Annual review of immunology. 2011;29:621-63. doi: 10.1146/annurev-immunol-031210-101400. PubMed PMID: 21314428.

173. Vinuesa CG, Cyster JG. How T cells earn the follicular rite of passage. Immunity. 2011;35(5):671-80. Epub 2011/11/29. doi: 10.1016/j.immuni.2011.11.001. PubMed PMID: 22118524.

174. Sasikala-Appukuttan AK, Kim HO, Kinzel NJ, Hong JJ, Smith AJ, Wagstaff R, Reilly C, Piatak M, Jr., Lifson JD, Reeves RK, Johnson RP, Haase AT, Skinner PJ. Location and dynamics of the immunodominant CD8 T cell response to SIVDeltanef immunization and SIVmac251 vaginal challenge. PloS one. 2013;8(12):e81623. Epub 2013/12/19. doi: 10.1371/journal.pone.0081623. PubMed PMID: 24349100; PMCID: PMC3857218.

175. Tjernlund A, Zhu J, Laing K, Diem K, McDonald D, Vazquez J, Cao J, Ohlen C, McElrath MJ, Picker LJ, Corey L. In situ detection of Gag-specific CD8+ cells in the GI tract of SIV infected Rhesus macaques. Retrovirology. 2010;7:12. Epub 2010/02/18. doi: 10.1186/1742-4690-7-12. PubMed PMID: 20158906; PMCID: PMC2834607.

176. Connick E, Folkvord JM, Lind KT, Rakasz EG, Miles B, Wilson NA, Santiago ML, Schmitt K, Stephens EB, Kim HO, Wagstaff R, Li S, Abdelaal HM, Kemp N, Watkins DI, MaWhinney S, Skinner PJ. Compartmentalization of simian immunodeficiency virus replication within secondary lymphoid tissues of rhesus macaques is linked to disease stage and inversely related to localization of virus-specific CTL. Journal of immunology. 2014;193(11):5613-25. Epub 2014/11/02. doi: 10.4049/jimmunol.1401161. PubMed PMID: 25362178; PMCID: PMC4239212.

177. Leong YA, Chen Y, Ong HS, Wu D, Man K, Deleage C, Minnich M, Meckiff BJ, Wei Y, Hou Z, Zotos D, Fenix KA, Atnerkar A, Preston S, Chipman JG, Beilman GJ, Allison CC, Sun L, Wang P, Xu J, Toe JG, Lu HK, Tao Y, Palendira U, Dent AL, Landay AL, Pellegrini M, Comerford I, McColl SR, Schacker TW, Long HM, Estes JD, Busslinger M, Belz GT, Lewin SR, Kallies A, Yu D. CXCR5(+) follicular cytotoxic T cells control viral infection in B cell follicles. Nature immunology. 2016;17(10):1187-96. Epub 2016/08/04. doi: 10.1038/ni.3543. PubMed PMID: 27487330.

178. Bronnimann MP, Skinner PJ, Connick E. The B-Cell Follicle in HIV Infection: Barrier to a Cure. Frontiers in immunology. 2018;9:20. Epub 2018/02/10. doi: 10.3389/fimmu.2018.00020. PubMed PMID: 29422894; PMCID: PMC5788973.

179. Li S, Folkvord JM, Rakasz EG, Abdelaal HM, Wagstaff RK, Kovacs KJ, Kim HO, Sawahata R, MaWhinney S, Masopust D, Connick E, Skinner PJ. Simian Immunodeficiency Virus-Producing Cells in

Follicles Are Partially Suppressed by CD8+ Cells In Vivo. Journal of virology. 2016;90(24):11168-80. Epub 2016/11/01. doi: 10.1128/JVI.01332-16. PubMed PMID: 27707919; PMCID: PMC5126374.

180. He R, Hou S, Liu C, Zhang A, Bai Q, Han M, Yang Y, Wei G, Shen T, Yang X, Xu L, Chen X, Hao Y, Wang P, Zhu C, Ou J, Liang H, Ni T, Zhang X, Zhou X, Deng K, Chen Y, Luo Y, Xu J, Qi H, Wu Y, Ye L. Follicular CXCR5- expressing CD8(+) T cells curtail chronic viral infection. Nature. 2016;537(7620):412-28. Epub 2016/08/09. doi: 10.1038/nature19317. PubMed PMID: 27501245.

181. Petrovas C, Ferrando-Martinez S, Gerner MY, Casazza JP, Pegu A, Deleage C, Cooper A, Hataye J, Andrews S, Ambrozak D, Del Rio Estrada PM, Boritz E, Paris R, Moysi E, Boswell KL, Ruiz-Mateos E, Vagios I, Leal M, Ablanedo-Terrazas Y, Rivero A, Gonzalez-Hernandez LA, McDermott AB, Moir S, Reyes-Teran G, Docobo F, Pantaleo G, Douek DC, Betts MR, Estes JD, Germain RN, Mascola JR, Koup RA. Follicular CD8 T cells accumulate in HIV infection and can kill infected cells in vitro via bispecific antibodies. Science translational medicine. 2017;9(373). Epub 2017/01/20. doi: 10.1126/scitranslmed.aag2285. PubMed PMID: 28100833; PMCID: PMC5497679.

182. Mylvaganam GH, Rios D, Abdelaal HM, Iyer S, Tharp G, Mavigner M, Hicks S, Chahroudi A, Ahmed R, Bosinger SE, Williams IR, Skinner PJ, Velu V, Amara RR. Dynamics of SIV-specific CXCR5+ CD8 T cells during chronic SIV infection. Proceedings of the National Academy of Sciences of the United States of America. 2017;114(8):1976-81. Epub 2017/02/06. doi: 10.1073/pnas.1621418114. PubMed PMID: 28159893; PMCID: PMC5338410.

183. Miles B, Miller SM, Folkvord JM, Levy DN, Rakasz EG, Skinner PJ, Connick E. Follicular Regulatory CD8 T Cells Impair the Germinal Center Response in SIV and Ex Vivo HIV Infection. PLoS pathogens. 2016;12(10):e1005924. Epub 2016/10/08. doi: 10.1371/journal.ppat.1005924. PubMed PMID: 27716848; PMCID: PMC5055335.

184. Kim HJ, Verbinnen B, Tang X, Lu L, Cantor H. Inhibition of follicular T-helper cells by CD8(+) regulatory T cells is essential for self tolerance. Nature. 2010;467(7313):328-32. Epub 2010/09/17. doi: 10.1038/nature09370. PubMed PMID: 20844537; PMCID: PMC3395240.

185. Kim HJ, Wang X, Radfar S, Sproule TJ, Roopenian DC, Cantor H. CD8+ T regulatory cells express the Ly49 Class I MHC receptor and are defective in autoimmune prone B6-Yaa mice. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(5):2010-5. Epub 2011/01/15. doi: 10.1073/pnas.1018974108. PubMed PMID: 21233417; PMCID: PMC3033298.

186. Madec Y, Boufassa F, Porter K, Meyer L, Collaboration C. Spontaneous control of viral load and CD4 cell count progression among HIV-1 seroconverters. Aids. 2005;19(17):2001-7. Epub 2005/11/02. PubMed PMID: 16260907.

187. Okulicz JF, Marconi VC, Landrum ML, Wegner S, Weintrob A, Ganesan A, Hale B, Crum-Cianflone N, Delmar J, Barthel V, Quinnan G, Agan BK, Dolan MJ, Infectious Disease Clinical Research Program HIVWG. Clinical outcomes of elite controllers, viremic controllers, and long-term nonprogressors in the US Department of Defense HIV natural history study. The Journal of infectious diseases. 2009;200(11):1714-23. Epub 2009/10/27. doi: 10.1086/646609. PubMed PMID: 19852669.

188. Boufassa F, Saez-Cirion A, Lechenadec J, Zucman D, Avettand-Fenoel V, Venet A, Rouzioux C, Delfraissy JF, Lambotte O, Meyer L, Group AEHCS. CD4 dynamics over a 15 year-period among HIV controllers enrolled in the ANRS French observatory. PloS one. 2011;6(4):e18726. Epub 2011/05/03. doi: 10.1371/journal.pone.0018726. PubMed PMID: 21533035; PMCID: PMC3080877.

189. Grabar S, Selinger-Leneman H, Abgrall S, Pialoux G, Weiss L, Costagliola D. Prevalence and comparative characteristics of long-term nonprogressors and HIV controller patients in the French Hospital Database on HIV. Aids. 2009;23(9):1163-9. Epub 2009/05/16. doi: 10.1097/QAD.0b013e32832b44c8. PubMed PMID: 19444075.

190. Pereyra F, Addo MM, Kaufmann DE, Liu Y, Miura T, Rathod A, Baker B, Trocha A, Rosenberg R, Mackey E, Ueda P, Lu Z, Cohen D, Wrin T, Petropoulos CJ, Rosenberg ES, Walker BD. Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. The Journal of infectious diseases. 2008;197(4):563-71. Epub 2008/02/16. doi: 10.1086/526786. PubMed PMID: 18275276.

191. Bailey JR, Williams TM, Siliciano RF, Blankson JN. Maintenance of viral suppression in HIV-1-infected HLA-B*57+ elite suppressors despite CTL escape mutations. The Journal of experimental medicine. 2006;203(5):1357-69. Epub 2006/05/10. doi: 10.1084/jem.20052319. PubMed PMID: 16682496; PMCID: PMC2121215.

192. Hatano H, Delwart EL, Norris PJ, Lee TH, Dunn-Williams J, Hunt PW, Hoh R, Stramer SL, Linnen JM, McCune JM, Martin JN, Busch MP, Deeks SG. Evidence for persistent low-level viremia in individuals who control human immunodeficiency virus in the absence of antiretroviral therapy. Journal of virology. 2009;83(1):329-35. Epub 2008/10/24. doi: 10.1128/JVI.01763-08. PubMed PMID: 18945778; PMCID: PMC2612329.

193. Pereyra F, Palmer S, Miura T, Block BL, Wiegand A, Rothchild AC, Baker B, Rosenberg R, Cutrell E, Seaman MS, Coffin JM, Walker BD. Persistent low-level viremia in HIV-1 elite controllers and relationship to immunologic parameters. The Journal of infectious diseases. 2009;200(6):984-90. Epub 2009/08/07. doi: 10.1086/605446. PubMed PMID: 19656066; PMCID: PMC3725728.

194. Dinoso JB, Kim SY, Siliciano RF, Blankson JN. A comparison of viral loads between HIV-1-infected elite suppressors and individuals who receive suppressive highly active antiretroviral therapy. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;47(1):102-4. Epub 2008/05/23. doi: 10.1086/588791. PubMed PMID: 18494606; PMCID: PMC2564994.

195. Lambotte O, Boufassa F, Madec Y, Nguyen A, Goujard C, Meyer L, Rouzioux C, Venet A, Delfraissy JF, Group S-HS. HIV controllers: a homogeneous group of HIV-1-infected patients with spontaneous control of viral replication. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2005;41(7):1053-6. Epub 2005/09/06. doi: 10.1086/433188. PubMed PMID: 16142675.

196. Goujard C, Chaix ML, Lambotte O, Deveau C, Sinet M, Guergnon J, Courgnaud V, Rouzioux C, Delfraissy JF, Venet A, Meyer L, Agence Nationale de Recherche sur le Sida PSG. Spontaneous control of viral replication during primary HIV infection: when is "HIV controller" status established? Clinical

infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;49(6):982-6. Epub 2009/08/18. doi: 10.1086/605504. PubMed PMID: 19681706.

197. Blankson JN, Bailey JR, Thayil S, Yang HC, Lassen K, Lai J, Gandhi SK, Siliciano JD, Williams TM, Siliciano RF. Isolation and characterization of replication-competent human immunodeficiency virus type 1 from a subset of elite suppressors. Journal of virology. 2007;81(5):2508-18. Epub 2006/12/08. doi: 10.1128/JVI.02165-06. PubMed PMID: 17151109; PMCID: PMC1865922.

198. Lewin SR, Rouzioux C. HIV cure and eradication: how will we get from the laboratory to effective clinical trials? Aids. 2011;25(7):885-97. Epub 2011/03/23. doi: 10.1097/QAD.0b013e3283467041. PubMed PMID: 21422987.

199. 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.

200. Horton H, Frank I, Baydo R, Jalbert E, Penn J, Wilson S, McNevin JP, McSweyn MD, Lee D, Huang Y, De Rosa SC, McElrath MJ. Preservation of T cell proliferation restricted by protective HLA alleles is critical for immune control of HIV-1 infection. Journal of immunology. 2006;177(10):7406-15. Epub 2006/11/04. PubMed PMID: 17082660.

201. Saez-Cirion A, Lacabaratz C, Lambotte O, Versmisse P, Urrutia A, Boufassa F, Barre-Sinoussi F, Delfraissy JF, Sinet M, Pancino G, Venet A, Agence Nationale de Recherches sur le Sida EPHIVCSG. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(16):6776-81. Epub 2007/04/13. doi: 10.1073/pnas.0611244104. PubMed PMID: 17428922; PMCID: PMC1851664.

202. Migueles SA, Laborico AC, Shupert WL, Sabbaghian MS, Rabin R, Hallahan CW, Van Baarle D, Kostense S, Miedema F, McLaughlin M, Ehler L, Metcalf J, Liu S, Connors M. HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. Nature immunology. 2002;3(11):1061-8. Epub 2002/10/09. doi: 10.1038/ni845. PubMed PMID: 12368910.

203. Migueles SA, Osborne CM, Royce C, Compton AA, Joshi RP, Weeks KA, Rood JE, Berkley AM, Sacha JB, Cogliano-Shutta NA, Lloyd M, Roby G, Kwan R, McLaughlin M, Stallings S, Rehm C, O'Shea MA, Mican J, Packard BZ, Komoriya A, Palmer S, Wiegand AP, Maldarelli F, Coffin JM, Mellors JW, Hallahan CW, Follman DA, Connors M. Lytic granule loading of CD8+ T cells is required for HIV-infected cell elimination associated with immune control. Immunity. 2008;29(6):1009-21. Epub 2008/12/09. doi: 10.1016/j.immuni.2008.10.010. PubMed PMID: 19062316; PMCID: PMC2622434.

204. Ferre AL, Lemongello D, Hunt PW, Morris MM, Garcia JC, Pollard RB, Yee HF, Jr., Martin JN, Deeks SG, Shacklett BL. 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. Journal of virology. 2010;84(19):10354-65. Epub 2010/07/30. doi: 10.1128/JVI.00803-10. PubMed PMID: 20668079; PMCID: PMC2937770.

205. Kaufmann DE, Kavanagh DG, Pereyra F, Zaunders JJ, Mackey EW, Miura T, Palmer S, Brockman M, Rathod A, Piechocka-Trocha A, Baker B, Zhu B, Le Gall S, Waring MT, Ahern R, Moss K, Kelleher AD, Coffin JM, Freeman GJ, Rosenberg ES, Walker BD. Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction. Nature immunology. 2007;8(11):1246-54. Epub 2007/10/02. doi: 10.1038/ni1515. PubMed PMID: 17906628.

206. Emu B, Sinclair E, Favre D, Moretto WJ, Hsue P, Hoh R, Martin JN, Nixon DF, McCune JM, Deeks SG. Phenotypic, functional, and kinetic parameters associated with apparent T-cell control of human immunodeficiency virus replication in individuals with and without antiretroviral treatment. Journal of virology. 2005;79(22):14169-78. Epub 2005/10/29. doi: 10.1128/JVI.79.22.14169-14178.2005. PubMed PMID: 16254352; PMCID: PMC1280210.

207. Potter SJ, Lacabaratz C, Lambotte O, Perez-Patrigeon S, Vingert B, Sinet M, Colle JH, Urrutia A, Scott-Algara D, Boufassa F, Delfraissy JF, Theze J, Venet A, Chakrabarti LA. Preserved central memory and activated effector memory CD4+ T-cell subsets in human immunodeficiency virus controllers: an

ANRS EP36 study. Journal of virology. 2007;81(24):13904-15. Epub 2007/10/12. doi: 10.1128/JVI.01401-07. PubMed PMID: 17928341; PMCID: PMC2168869.

208. Weiss RA, Clapham PR, Weber JN, Dalgleish AG, Lasky LA, Berman PW. Variable and conserved neutralization antigens of human immunodeficiency virus. Nature. 1986;324(6097):572-5. Epub 1986/12/11. doi: 10.1038/324572a0. PubMed PMID: 2431324.

209. Shibata R, Igarashi T, Haigwood N, Buckler-White A, Ogert R, Ross W, Willey R, Cho MW, Martin MA. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. Nature medicine. 1999;5(2):204-10. doi: 10.1038/5568. PubMed PMID: 9930869.

210. Tomaras GD, Yates NL, Liu P, Qin L, Fouda GG, Chavez LL, Decamp AC, Parks RJ, Ashley VC, Lucas JT, Cohen M, Eron J, Hicks CB, Liao HX, Self SG, Landucci G, Forthal DN, Weinhold KJ, Keele BF, Hahn BH, Greenberg ML, Morris L, Karim SS, Blattner WA, Montefiori DC, Shaw GM, Perelson AS, Haynes BF. Initial B-cell responses to transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia. Journal of virology. 2008;82(24):12449-63. Epub 2008/10/10. doi: 10.1128/JVI.01708-08. PubMed PMID: 18842730; PMCID: PMC2593361.

211. Bonsignori M, Moody MA, Parks RJ, Holl TM, Kelsoe G, Hicks CB, Vandergrift N, Tomaras GD, Haynes BF. HIV-1 envelope induces memory B cell responses that correlate with plasma antibody levels after envelope gp120 protein vaccination or HIV-1 infection. Journal of immunology. 2009;183(4):2708-17. Epub 2009/07/25. doi: 10.4049/jimmunol.0901068. PubMed PMID: 19625640; PMCID: PMC3089979.

212. Morris L, Binley JM, Clas BA, Bonhoeffer S, Astill TP, Kost R, Hurley A, Cao Y, Markowitz M, Ho DD, Moore JP. HIV-1 antigen-specific and -nonspecific B cell responses are sensitive to combination antiretroviral therapy. The Journal of experimental medicine. 1998;188(2):233-45. Epub 1998/07/22. PubMed PMID: 9670036; PMCID: PMC2212446.

213. Mascola JR, Haynes BF. HIV-1 neutralizing antibodies: understanding nature's pathways. Immunological reviews. 2013;254(1):225-44. Epub 2013/06/19. doi: 10.1111/imr.12075. PubMed PMID: 23772623; PMCID: PMC3738265.

214. Richman DD, Wrin T, Little SJ, Petropoulos CJ. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(7):4144-9. Epub 2003/03/20. doi: 10.1073/pnas.0630530100. PubMed PMID: 12644702; PMCID: PMC153062.

215. Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, Salazar-Gonzalez JF, Salazar MG, Kilby JM, Saag MS, Komarova NL, Nowak MA, Hahn BH, Kwong PD, Shaw GM. Antibody neutralization and escape by HIV-1. Nature. 2003;422(6929):307-12. doi: 10.1038/nature01470. PubMed PMID: 12646921.

216. Burton DR, Stanfield RL, Wilson IA. Antibody vs. HIV in a clash of evolutionary titans. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(42):14943-8. Epub 2005/10/13. doi: 10.1073/pnas.0505126102. PubMed PMID: 16219699; PMCID: PMC1257708.

217. Kong L, Sattentau QJ. Antigenicity and Immunogenicity in HIV-1 Antibody-Based Vaccine Design. J AIDS Clin Res. 2012;S8:3. Epub 2012/12/12. doi: 10.4172/2155-6113. PubMed PMID: 23227445; PMCID: PMC3515071.

218. Kwong PD, Doyle ML, Casper DJ, Cicala C, Leavitt SA, Majeed S, Steenbeke TD, Venturi M, Chaiken I, Fung M, Katinger H, Parren PW, Robinson J, Van Ryk D, Wang L, Burton DR, Freire E, Wyatt R, Sodroski J, Hendrickson WA, Arthos J. HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. Nature. 2002;420(6916):678-82. Epub 2002/12/13. doi: 10.1038/nature01188. PubMed PMID: 12478295.

219. Overbaugh J, Morris L. The Antibody Response against HIV-1. Cold Spring Harb Perspect Med. 2012;2(1):a007039. Epub 2012/02/09. doi: 10.1101/cshperspect.a007039. PubMed PMID: 22315717; PMCID: PMC3253031.

220. Pantophlet R, Burton DR. GP120: target for neutralizing HIV-1 antibodies. Annual review of immunology. 2006;24:739-69. Epub 2006/03/23. doi: 10.1146/annurev.immunol.24.021605.090557. PubMed PMID: 16551265.

221. Moore PL, Gray ES, Morris L. Specificity of the autologous neutralizing antibody response. Current opinion in HIV and AIDS. 2009;4(5):358-63. Epub 2010/01/06. doi: 10.1097/COH.0b013e32832ea7e8. PubMed PMID: 20048698; PMCID: PMC3004050.

222. Haynes BF, Moody MA, Liao HX, Verkoczy L, Tomaras GD. B cell responses to HIV-1 infection and vaccination: pathways to preventing infection. Trends in molecular medicine. 2011;17(2):108-16. Epub 2010/11/30. doi: 10.1016/j.molmed.2010.10.008. PubMed PMID: 21112250; PMCID: PMC3053087.

223. Doria-Rose NA, Klein RM, Daniels MG, O'Dell S, Nason M, Lapedes A, Bhattacharya T, Migueles SA, Wyatt RT, Korber BT, Mascola JR, Connors M. Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. Journal of virology. 2010;84(3):1631-6. Epub 2009/11/20. doi: 10.1128/JVI.01482-09. PubMed PMID: 19923174; PMCID: PMC2812355.

224. Gray ES, Madiga MC, Moore PL, Mlisana K, Abdool Karim SS, Binley JM, Shaw GM, Mascola JR, Morris L. Broad neutralization of human immunodeficiency virus type 1 mediated by plasma antibodies against the gp41 membrane proximal external region. Journal of virology. 2009;83(21):11265-74. Epub 2009/08/21. doi: 10.1128/JVI.01359-09. PubMed PMID: 19692477; PMCID: PMC2772769.

225. Stamatatos L, Morris L, Burton DR, Mascola JR. Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? Nature medicine. 2009;15(8):866-70. Epub 2009/06/16. doi: 10.1038/nm.1949. PubMed PMID: 19525964.

226. Haynes BF, Fleming J, St Clair EW, Katinger H, Stiegler G, Kunert R, Robinson J, Scearce RM, Plonk K, Staats HF, Ortel TL, Liao HX, Alam SM. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science. 2005;308(5730):1906-8. Epub 2005/04/30. doi: 10.1126/science.1111781. PubMed PMID: 15860590.

227. McElrath MJ, Haynes BF. Induction of immunity to human immunodeficiency virus type-1 by vaccination. Immunity. 2010;33(4):542-54. Epub 2010/10/30. doi: 10.1016/j.immuni.2010.09.011. PubMed PMID: 21029964; PMCID: 3031162.

228. Possas C, Antunes AMS, Lins Mendes FM, Veloso V, Martins RM, Homma A. HIV cure: global overview of bNAbs' patents and related scientific publications. Expert Opin Ther Pat. 2018;28(7):551-60. Epub 2018/07/03. doi: 10.1080/13543776.2018.1495708. PubMed PMID: 29962249.

229. Huber M, Trkola A. Humoral immunity to HIV-1: neutralization and beyond. J Intern Med. 2007;262(1):5-25. Epub 2007/06/30. doi: 10.1111/j.1365-2796.2007.01819.x. PubMed PMID: 17598812.

230. Robinson HL. Non-neutralizing antibodies in prevention of HIV infection. Expert opinion on biological therapy. 2013;13(2):197-207. doi: 10.1517/14712598.2012.743527. PubMed PMID: 23130709.

231. Florese RH, Demberg T, Xiao P, Kuller L, Larsen K, Summers LE, Venzon D, Cafaro A, Ensoli B, Robert-Guroff M. Contribution of nonneutralizing vaccine-elicited antibody activities to improved protective efficacy in rhesus macaques immunized with Tat/Env compared with multigenic vaccines. Journal of immunology. 2009;182(6):3718-27. Epub 2009/03/07. doi: 10.4049/jimmunol.0803115. PubMed PMID: 19265150; PMCID: PMC2744397.

232. Alpert MD, Harvey JD, Lauer WA, Reeves RK, Piatak M, Jr., Carville A, Mansfield KG, Lifson JD, Li W, Desrosiers RC, Johnson RP, Evans DT. ADCC develops over time during persistent infection with live-attenuated SIV and is associated with complete protection against SIV(mac)251 challenge. PLoS pathogens. 2012;8(8):e1002890. Epub 2012/08/29. doi: 10.1371/journal.ppat.1002890. PubMed PMID: 22927823; PMCID: PMC3426556.

233. Paredes R, Lalama CM, Ribaudo HJ, Schackman BR, Shikuma C, Giguel F, Meyer WA, 3rd, Johnson VA, Fiscus SA, D'Aquila RT, Gulick RM, Kuritzkes DR, Team ACTGAS. Pre-existing minority drug-resistant HIV-1 variants, adherence, and risk of antiretroviral treatment failure. The Journal of infectious diseases. 2010;201(5):662-71. Epub 2010/01/28. doi: 10.1086/650543. PubMed PMID: 20102271; PMCID: PMC2825289.

234. Hileman CO, Funderburg NT. Inflammation, Immune Activation, and Antiretroviral Therapy in HIV. Curr HIV/AIDS Rep. 2017;14(3):93-100. Epub 2017/04/24. doi: 10.1007/s11904-017-0356-x. PubMed PMID: 28434169; PMCID: PMC5514315.

235. Clotet B, Feinberg J, van Lunzen J, Khuong-Josses MA, Antinori A, Dumitru I, Pokrovskiy V, Fehr J, Ortiz R, Saag M, Harris J, Brennan C, Fujiwara T, Min S, Team INGS. Once-daily dolutegravir versus darunavir plus ritonavir in antiretroviral-naive adults with HIV-1 infection (FLAMINGO): 48 week results from the randomised open-label phase 3b study. Lancet. 2014;383(9936):2222-31. Epub 2014/04/05. doi: 10.1016/S0140-6736(14)60084-2. PubMed PMID: 24698485.

236. Walmsley SL, Antela A, Clumeck N, Duiculescu D, Eberhard A, Gutierrez F, Hocqueloux L, Maggiolo F, Sandkovsky U, Granier C, Pappa K, Wynne B, Min S, Nichols G, Investigators S. Dolutegravir plus abacavir-lamivudine for the treatment of HIV-1 infection. The New England journal of medicine. 2013;369(19):1807-18. Epub 2013/11/08. doi: 10.1056/NEJMoa1215541. PubMed PMID: 24195548.

237. Raffi F, Jaeger H, Quiros-Roldan E, Albrecht H, Belonosova E, Gatell JM, Baril JG, Domingo P, Brennan C, Almond S, Min S, extended S-SG. Once-daily dolutegravir versus twice-daily raltegravir in antiretroviral-naive adults with HIV-1 infection (SPRING-2 study): 96 week results from a randomised, double-blind, non-inferiority trial. The Lancet Infectious diseases. 2013;13(11):927-35. Epub 2013/10/01. doi: 10.1016/S1473-3099(13)70257-3. PubMed PMID: 24074642.

238. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, Kovacs C, Gange SJ, Siliciano RF. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nature medicine. 2003;9(6):727-8. Epub 2003/05/20. doi: 10.1038/nm880. PubMed PMID: 12754504.

239. Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC, Koup RA, Mellors JW, Connick E, Conway B, Kilby M, Wang L, Whitcomb JM, Hellmann NS, Richman DD. Antiretroviral-drug resistance among patients recently infected with HIV. The New England journal of medicine. 2002;347(6):385-94. Epub 2002/08/09. doi: 10.1056/NEJMoa013552. PubMed PMID: 12167680.

240. Vercauteren J, Wensing AM, van de Vijver DA, Albert J, Balotta C, Hamouda O, Kucherer C, Struck D, Schmit JC, Asjo B, Bruckova M, Camacho RJ, Clotet B, Coughlan S, Grossman Z, Horban A, Korn K, Kostrikis L, Nielsen C, Paraskevis D, Poljak M, Puchhammer-Stockl E, Riva C, Ruiz L, Salminen M, Schuurman R, Sonnerborg A, Stanekova D, Stanojevic M, Vandamme AM, Boucher CA. Transmission of drug-resistant HIV-1 is stabilizing in Europe. The Journal of infectious diseases. 2009;200(10):1503-8. Epub 2009/10/20. doi: 10.1086/644505. PubMed PMID: 19835478.

241. Rhee SY, Blanco JL, Jordan MR, Taylor J, Lemey P, Varghese V, Hamers RL, Bertagnolio S, Rinke de Wit TF, Aghokeng AF, Albert J, Avi R, Avila-Rios S, Bessong PO, Brooks JI, Boucher CA, Brumme ZL, Busch MP, Bussmann H, Chaix ML, Chin BS, D'Aquin TT, De Gascun CF, Derache A, Descamps D, Deshpande AK, Djoko CF, Eshleman SH, Fleury H, Frange P, Fujisaki S, Harrigan PR, Hattori J, Holguin A, Hunt GM, Ichimura H, Kaleebu P, Katzenstein D, Kiertiburanakul S, Kim JH, Kim SS, Li Y, Lutsar I, Morris L, Ndembi N, Ng KP, Paranjape RS, Peeters M, Poljak M, Price MA, Ragonnet-Cronin ML, Reyes-Teran G, Rolland M, Sirivichayakul S, Smith DM, Soares MA, Soriano VV, Ssemwanga D, Stanojevic M, Stefani MA, Sugiura W, Sungkanuparph S, Tanuri A, Tee KK, Truong HM, van de Vijver DA, Vidal N, Yang C, Yang R, Yebra G, Ioannidis JP, Vandamme AM, Shafer RW. Geographic and temporal trends in the molecular epidemiology and genetic mechanisms of transmitted HIV-1 drug resistance: an individual-patient- and sequence-level meta-analysis. PLoS medicine. 2015;12(4):e1001810. Epub 2015/04/08. doi: 10.1371/journal.pmed.1001810. PubMed PMID: 25849352; PMCID: PMC4388826.

242. Pham QD, Wilson DP, Law MG, Kelleher AD, Zhang L. Global burden of transmitted HIV drug resistance and HIV-exposure categories: a systematic review and meta-analysis. Aids. 2014;28(18):2751-62. Epub 2014/12/11. doi: 10.1097/QAD.0000000000000494. PubMed PMID: 25493601.

243. Chun TW, Finzi D, Margolick J, Chadwick K, Schwartz D, Siliciano RF. In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. Nature medicine. 1995;1(12):1284-90. Epub 1995/12/01. PubMed PMID: 7489410.

244. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, Lloyd AL, Nowak MA, Fauci AS. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(24):13193-7. Epub 1997/12/16. PubMed PMID: 9371822; PMCID: PMC24285.

245. Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, Smith K, Lisziewicz J, Lori F, Flexner C, Quinn TC, Chaisson RE, Rosenberg E, Walker B, Gange S, Gallant J, Siliciano RF. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nature medicine. 1999;5(5):512-7. Epub 1999/05/06. doi: 10.1038/8394. PubMed PMID: 10229227.

246. Pedersen C, Lindhardt BO, Jensen BL, Lauritzen E, Gerstoft J, Dickmeiss E, Gaub J, Scheibel E, Karlsmark T. Clinical course of primary HIV infection: consequences for subsequent course of infection. BMJ. 1989;299(6692):154-7. Epub 1989/07/15. PubMed PMID: 2569901; PMCID: PMC1837064.

247. Pierson T, McArthur J, Siliciano RF. Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. Annual review of immunology. 2000;18:665-708. Epub 2000/06/03. doi: 10.1146/annurev.immunol.18.1.665. PubMed PMID: 10837072.

248. Margolis DM, Archin NM. Proviral Latency, Persistent Human Immunodeficiency Virus Infection, and the Development of Latency Reversing Agents. The Journal of infectious diseases. 2017;215(suppl_3):S111-S8. Epub 2017/05/19. doi: 10.1093/infdis/jiw618. PubMed PMID: 28520964; PMCID: PMC5410986.

249. Margolis DM, Garcia JV, Hazuda DJ, Haynes BF. Latency reversal and viral clearance to cure HIV-1. Science. 2016;353(6297):aaf6517. Epub 2016/07/28. doi: 10.1126/science.aaf6517. PubMed PMID: 27463679; PMCID: PMC5021637.

250. Crooks AM, Bateson R, Cope AB, Dahl NP, Griggs MK, Kuruc JD, Gay CL, Eron JJ, Margolis DM, Bosch RJ, Archin NM. Precise Quantitation of the Latent HIV-1 Reservoir: Implications for Eradication Strategies. The Journal of infectious diseases. 2015;212(9):1361-5. Epub 2015/04/17. doi: 10.1093/infdis/jiv218. PubMed PMID: 25877550; PMCID: PMC4601910.

251. Whitney JB, Hill AL, Sanisetty S, Penaloza-MacMaster P, Liu J, Shetty M, Parenteau L, Cabral C, Shields J, Blackmore S, Smith JY, Brinkman AL, Peter LE, Mathew SI, Smith KM, Borducchi EN, Rosenbloom DI, Lewis MG, Hattersley J, Li B, Hesselgesser J, Geleziunas R, Robb ML, Kim JH, Michael NL, Barouch DH. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. Nature. 2014;512(7512):74-7. doi: 10.1038/nature13594. PubMed PMID: 25042999; PMCID: 4126858.

252. Strain MC, Little SJ, Daar ES, Havlir DV, Gunthard HF, Lam RY, Daly OA, Nguyen J, Ignacio CC, Spina CA, Richman DD, Wong JK. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. The Journal of infectious diseases. 2005;191(9):1410-8. Epub 2005/04/06. doi: 10.1086/428777. PubMed PMID: 15809898.

253. Ananworanich J, Chomont N, Fletcher JL, Pinyakorn S, Schuetz A, Sereti I, Rerknimitr R, Dewar R, Kroon E, Vandergeeten C, Trichavaroj R, Chomchey N, Chalermchai T, Michael NL, Kim JH, Phanuphak P, Phanuphak N. Markers of HIV reservoir size and immune activation after treatment in acute HIV infection with and without raltegravir and maraviroc intensification. Journal of virus eradication. 2015;1(2):116-22. Epub 2016/02/03. PubMed PMID: 26835516; PMCID: PMC4729387.

254. Archin NM, Vaidya NK, Kuruc JD, Liberty AL, Wiegand A, Kearney MF, Cohen MS, Coffin JM, Bosch RJ, Gay CL, Eron JJ, Margolis DM, Perelson AS. Immediate antiviral therapy appears to restrict resting CD4+ cell HIV-1 infection without accelerating the decay of latent infection. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(24):9523-8. Epub 2012/05/31. doi: 10.1073/pnas.1120248109. PubMed PMID: 22645358; PMCID: PMC3386138.

255. Okoye AA, Hansen SG, Vaidya M, Fukazawa Y, Park H, Duell DM, Lum R, Hughes CM, Ventura AB, Ainslie E, Ford JC, Morrow D, Gilbride RM, Legasse AW, Hesselgesser J, Geleziunas R, Li Y, Oswald K, Shoemaker R, Fast R, Bosche WJ, Borate BR, Edlefsen PT, Axthelm MK, Picker LJ, Lifson JD. Early antiretroviral therapy limits SIV reservoir establishment to delay or prevent post-treatment viral rebound. Nature medicine. 2018;24(9):1430-40. Epub 2018/08/08. doi: 10.1038/s41591-018-0130-7. PubMed PMID: 30082858.

256. Eriksson S, Graf EH, Dahl V, Strain MC, Yukl SA, Lysenko ES, Bosch RJ, Lai J, Chioma S, Emad F, Abdel-Mohsen M, Hoh R, Hecht F, Hunt P, Somsouk M, Wong J, Johnston R, Siliciano RF, Richman DD, O'Doherty U, Palmer S, Deeks SG, Siliciano JD. Comparative analysis of measures of viral reservoirs in HIV-1 eradication studies. PLoS pathogens. 2013;9(2):e1003174. Epub 2013/03/06. doi: 10.1371/journal.ppat.1003174. PubMed PMID: 23459007; PMCID: PMC3573107.

257. Henrich TJ, Deeks SG, Pillai SK. Measuring the Size of the Latent Human Immunodeficiency Virus Reservoir: The Present and Future of Evaluating Eradication Strategies. The Journal of infectious diseases. 2017;215(suppl_3):S134-S41. Epub 2017/05/19. doi: 10.1093/infdis/jiw648. PubMed PMID: 28520968; PMCID: PMC5410981.

258. Soriano-Sarabia N, Bateson RE, Dahl NP, Crooks AM, Kuruc JD, Margolis DM, Archin NM. Quantitation of replication-competent HIV-1 in populations of resting CD4+ T cells. Journal of virology. 2014;88(24):14070-7. Epub 2014/09/26. doi: 10.1128/JVI.01900-14. PubMed PMID: 25253353; PMCID: PMC4249150.

259. Pallikkuth S, Sharkey M, Babic DZ, Gupta S, Stone GW, Fischl MA, Stevenson M, Pahwa S. Peripheral T Follicular Helper Cells Are the Major HIV Reservoir within Central Memory CD4 T Cells in Peripheral Blood from Chronically HIV-Infected Individuals on Combination Antiretroviral Therapy. Journal of virology. 2015;90(6):2718-28. Epub 2015/12/18. doi: 10.1128/JVI.02883-15. PubMed PMID: 26676775; PMCID: PMC4810658.

260. Banga R, Procopio FA, Noto A, Pollakis G, Cavassini M, Ohmiti K, Corpataux JM, de Leval L, Pantaleo G, Perreau M. PD-1(+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. Nature medicine. 2016;22(7):754-61. doi: 10.1038/nm.4113. PubMed PMID: 27239760.

261. Fromentin R, Bakeman W, Lawani MB, Khoury G, Hartogensis W, DaFonseca S, Killian M, Epling L, Hoh R, Sinclair E, Hecht FM, Bacchetti P, Deeks SG, Lewin SR, Sekaly RP, Chomont N. CD4+ T Cells Expressing PD-1, TIGIT and LAG-3 Contribute to HIV Persistence during ART. PLoS

pathogens. 2016;12(7):e1005761. doi: 10.1371/journal.ppat.1005761. PubMed PMID: 27415008; PMCID: PMC4944956.

262. Williams SA, Greene WC. Host factors regulating post-integration latency of HIV. Trends Microbiol. 2005;13(4):137-9. Epub 2005/04/09. doi: 10.1016/j.tim.2005.02.006. PubMed PMID: 15817380.

263. Wagner TA, McLaughlin S, Garg K, Cheung CY, Larsen BB, Styrchak S, Huang HC, Edlefsen PT, Mullins JI, Frenkel LM. HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. Science. 2014;345(6196):570-3. Epub 2014/07/12. doi: 10.1126/science.1256304. PubMed PMID: 25011556; PMCID: PMC4230336.

264. Maldarelli F, Wu X, Su L, Simonetti FR, Shao W, Hill S, Spindler J, Ferris AL, Mellors JW, Kearney MF, Coffin JM, Hughes SH. HIV latency. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science. 2014;345(6193):179-83. Epub 2014/06/28. doi: 10.1126/science.1254194. PubMed PMID: 24968937; PMCID: PMC4262401.

265. Simonetti FR, Sobolewski MD, Fyne E, Shao W, Spindler J, Hattori J, Anderson EM, Watters SA, Hill S, Wu X, Wells D, Su L, Luke BT, Halvas EK, Besson G, Penrose KJ, Yang Z, Kwan RW, Van Waes C, Uldrick T, Citrin DE, Kovacs J, Polis MA, Rehm CA, Gorelick R, Piatak M, Keele BF, Kearney MF, Coffin JM, Hughes SH, Mellors JW, Maldarelli F. Clonally expanded CD4+ T cells can produce infectious HIV-1 in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2016;113(7):1883-8. Epub 2016/02/10. doi: 10.1073/pnas.1522675113. PubMed PMID: 26858442; PMCID: PMC4763755.

266. Cohn LB, Silva IT, Oliveira TY, Rosales RA, Parrish EH, Learn GH, Hahn BH, Czartoski JL, McElrath MJ, Lehmann C, Klein F, Caskey M, Walker BD, Siliciano JD, Siliciano RF, Jankovic M, Nussenzweig MC. HIV-1 integration landscape during latent and active infection. Cell. 2015;160(3):420-32. Epub 2015/01/31. doi: 10.1016/j.cell.2015.01.020. PubMed PMID: 25635456; PMCID: PMC4371550.

267. Archin NM, Liberty AL, Kashuba AD, Choudhary SK, Kuruc JD, Crooks AM, Parker DC, Anderson EM, Kearney MF, Strain MC, Richman DD, Hudgens MG, Bosch RJ, Coffin JM, Eron JJ, Hazuda DJ, Margolis DM. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. Nature. 2012;487(7408):482-5. Epub 2012/07/28. doi: 10.1038/nature11286. PubMed PMID: 22837004; PMCID: PMC3704185.

268. Elliott JH, Wightman F, Solomon A, Ghneim K, Ahlers J, Cameron MJ, Smith MZ, Spelman T, McMahon J, Velayudham P, Brown G, Roney J, Watson J, Prince MH, Hoy JF, Chomont N, Fromentin R, Procopio FA, Zeidan J, Palmer S, Odevall L, Johnstone RW, Martin BP, Sinclair E, Deeks SG, Hazuda DJ, Cameron PU, Sekaly RP, Lewin SR. Activation of HIV transcription with short-course vorinostat in HIV-infected patients on suppressive antiretroviral therapy. PLoS pathogens. 2014;10(10):e1004473. Epub 2014/11/14. doi: 10.1371/journal.ppat.1004473. PubMed PMID: 25393648; PMCID: PMC4231123.

269. Rasmussen TA, Tolstrup M, Brinkmann CR, Olesen R, Erikstrup C, Solomon A, Winckelmann A, Palmer S, Dinarello C, Buzon M, Lichterfeld M, Lewin SR, Ostergaard L, Sogaard OS. Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. The lancet HIV. 2014;1(1):e13-21. Epub 2015/10/02. doi: 10.1016/S2352-3018(14)70014-1. PubMed PMID: 26423811.

270. Sogaard OS, Graversen ME, Leth S, Olesen R, Brinkmann CR, Nissen SK, Kjaer AS, Schleimann MH, Denton PW, Hey-Cunningham WJ, Koelsch KK, Pantaleo G, Krogsgaard K, Sommerfelt M, Fromentin R, Chomont N, Rasmussen TA, Ostergaard L, Tolstrup M. The Depsipeptide Romidepsin Reverses HIV-1 Latency In Vivo. PLoS pathogens. 2015;11(9):e1005142. Epub 2015/09/18. doi: 10.1371/journal.ppat.1005142. PubMed PMID: 26379282; PMCID: PMC4575032.

271. Kim Y, Anderson JL, Lewin SR. Getting the "Kill" into "Shock and Kill": Strategies to Eliminate Latent HIV. Cell host & microbe. 2018;23(1):14-26. Epub 2018/01/13. doi: 10.1016/j.chom.2017.12.004. PubMed PMID: 29324227.

272. Mylvaganam GH, Silvestri G, Amara RR. HIV therapeutic vaccines: moving towards a functional cure. Current opinion in immunology. 2015;35:1-8. Epub 2015/05/23. doi: 10.1016/j.coi.2015.05.001. PubMed PMID: 25996629; PMCID: PMC4553139.

273. Drummond DC, Noble CO, Kirpotin DB, Guo Z, Scott GK, Benz CC. Clinical development of histone deacetylase inhibitors as anticancer agents. Annu Rev Pharmacol Toxicol. 2005;45:495-528. Epub 2005/04/12. doi: 10.1146/annurev.pharmtox.45.120403.095825. PubMed PMID: 15822187.

274. Manson McManamy ME, Hakre S, Verdin EM, Margolis DM. Therapy for latent HIV-1 infection: the role of histone deacetylase inhibitors. Antivir Chem Chemother. 2014;23(4):145-9. Epub 2013/12/10. doi: 10.3851/IMP2551. PubMed PMID: 24318952; PMCID: PMC3947511.

275. Archin NM, Espeseth A, Parker D, Cheema M, Hazuda D, Margolis DM. Expression of latent HIV induced by the potent HDAC inhibitor suberoylanilide hydroxamic acid. AIDS research and human retroviruses. 2009;25(2):207-12. Epub 2009/02/26. doi: 10.1089/aid.2008.0191. PubMed PMID: 19239360; PMCID: PMC2853863.

276. Contreras X, Schweneker M, Chen CS, McCune JM, Deeks SG, Martin J, Peterlin BM. Suberoylanilide hydroxamic acid reactivates HIV from latently infected cells. The Journal of biological chemistry. 2009;284(11):6782-9. Epub 2009/01/13. doi: 10.1074/jbc.M807898200. PubMed PMID: 19136668; PMCID: PMC2652322.

277. Nabel G, Baltimore D. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature. 1987;326(6114):711-3. Epub 1987/04/16. doi: 10.1038/326711a0. PubMed PMID: 3031512.

278. Kinoshita S, Su L, Amano M, Timmerman LA, Kaneshima H, Nolan GP. The T cell activation factor NF-ATc positively regulates HIV-1 replication and gene expression in T cells. Immunity. 1997;6(3):235-44. Epub 1997/03/01. PubMed PMID: 9075924.

279. Pache L, Dutra MS, Spivak AM, Marlett JM, Murry JP, Hwang Y, Maestre AM, Manganaro L, Vamos M, Teriete P, Martins LJ, Konig R, Simon V, Bosque A, Fernandez-Sesma A, Cosford ND, Bushman FD, Young JA, Planelles V, Chanda SK. BIRC2/cIAP1 Is a Negative Regulator of HIV-1

Transcription and Can Be Targeted by Smac Mimetics to Promote Reversal of Viral Latency. Cell host & microbe. 2015;18(3):345-53. Epub 2015/09/12. doi: 10.1016/j.chom.2015.08.009. PubMed PMID: 26355217; PMCID: PMC4617541.

280. Novis CL, Archin NM, Buzon MJ, Verdin E, Round JL, Lichterfeld M, Margolis DM, Planelles V, Bosque A. Reactivation of latent HIV-1 in central memory CD4(+) T cells through TLR-1/2 stimulation. Retrovirology. 2013;10:119. Epub 2013/10/26. doi: 10.1186/1742-4690-10-119. PubMed PMID: 24156240; PMCID: PMC3826617.

281. Lim SY, Osuna CE, Hraber PT, Hesselgesser J, Gerold JM, Barnes TL, Sanisetty S, Seaman MS, Lewis MG, Geleziunas R, Miller MD, Cihlar T, Lee WA, Hill AL, Whitney JB. TLR7 agonists induce transient viremia and reduce the viral reservoir in SIV-infected rhesus macaques on antiretroviral therapy. Science translational medicine. 2018;10(439). doi: 10.1126/scitranslmed.aao4521. PubMed PMID: 29720451; PMCID: PMC5973480.

282. Borducchi EN, Liu J, Nkolola JP, Cadena AM, Yu WH, Fischinger S, Broge T, Abbink P, Mercado NB, Chandrashekar A, Jetton D, Peter L, McMahan K, Moseley ET, Bekerman E, Hesselgesser J, Li W, Lewis MG, Alter G, Geleziunas R, Barouch DH. Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. Nature. 2018;563(7731):360-4. Epub 2018/10/05. doi: 10.1038/s41586-018-0600-6. PubMed PMID: 30283138; PMCID: PMC6237629.

283. Tsai A, Irrinki A, Kaur J, Cihlar T, Kukolj G, Sloan DD, Murry JP. Toll-Like Receptor 7 Agonist GS-9620 Induces HIV Expression and HIV-Specific Immunity in Cells from HIV-Infected Individuals on Suppressive Antiretroviral Therapy. Journal of virology. 2017;91(8). Epub 2017/02/10. doi: 10.1128/JVI.02166-16. PubMed PMID: 28179531; PMCID: PMC5375698.

284. Offersen R, Nissen SK, Rasmussen TA, Ostergaard L, Denton PW, Sogaard OS, Tolstrup M. A Novel Toll-Like Receptor 9 Agonist, MGN1703, Enhances HIV-1 Transcription and NK Cell-Mediated Inhibition of HIV-1-Infected Autologous CD4+ T Cells. Journal of virology. 2016;90(9):4441-53. Epub 2016/02/19. doi: 10.1128/JVI.00222-16. PubMed PMID: 26889036; PMCID: PMC4836316.

285. Vibholm L, Schleimann MH, Hojen JF, Benfield T, Offersen R, Rasmussen K, Olesen R, Dige A, Agnholt J, Grau J, Buzon M, Wittig B, Lichterfeld M, Petersen AM, Deng X, Abdel-Mohsen M, Pillai SK, Rutsaert S, Trypsteen W, De Spiegelaere W, Vandekerchove L, Ostergaard L, Rasmussen TA, Denton PW, Tolstrup M, Sogaard OS. Short-Course Toll-Like Receptor 9 Agonist Treatment Impacts Innate Immunity and Plasma Viremia in Individuals With Human Immunodeficiency Virus Infection. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017;64(12):1686-95. Epub 2017/03/23. doi: 10.1093/cid/cix201. PubMed PMID: 28329286; PMCID: PMC5849129.

286. Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. Nature reviews Immunology. 2018;18(3):153-67. Epub 2017/10/11. doi: 10.1038/nri.2017.108. PubMed PMID: 28990585.

287. Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, Yagita H, Honjo T. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. International immunology. 1996;8(5):765-72. Epub 1996/05/01. PubMed PMID: 8671665.

288. Duraiswamy J, Ibegbu CC, Masopust D, Miller JD, Araki K, Doho GH, Tata P, Gupta S, Zilliox MJ, Nakaya HI, Pulendran B, Haining WN, Freeman GJ, Ahmed R. Phenotype, function, and gene expression profiles of programmed death-1(hi) CD8 T cells in healthy human adults. Journal of immunology. 2011;186(7):4200-12. Epub 2011/03/09. doi: 10.4049/jimmunol.1001783. PubMed PMID: 21383243; PMCID: PMC3723805.

289. Lucas JA, Menke J, Rabacal WA, Schoen FJ, Sharpe AH, Kelley VR. Programmed death ligand 1 regulates a critical checkpoint for autoimmune myocarditis and pneumonitis in MRL mice. Journal of immunology. 2008;181(4):2513-21. Epub 2008/08/08. PubMed PMID: 18684942; PMCID: PMC2587295.

290. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity. 1999;11(2):141-51. Epub 1999/09/15. PubMed PMID: 10485649.

291. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, Sasayama S, Mizoguchi A, Hiai H, Minato N, Honjo T. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. Science. 2001;291(5502):319-22. Epub 2001/02/24. doi: 10.1126/science.291.5502.319. PubMed PMID: 11209085.

292. Wang J, Yoshida T, Nakaki F, Hiai H, Okazaki T, Honjo T. Establishment of NOD-Pdcd1-/- mice as an efficient animal model of type I diabetes. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(33):11823-8. Epub 2005/08/10. doi: 10.1073/pnas.0505497102. PubMed PMID: 16087865; PMCID: PMC1188011.

293. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. 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.

294. Crawford A, Angelosanto JM, Kao C, Doering TA, Odorizzi PM, Barnett BE, Wherry EJ. Molecular and transcriptional basis of CD4(+) T cell dysfunction during chronic infection. Immunity. 2014;40(2):289-302. Epub 2014/02/18. doi: 10.1016/j.immuni.2014.01.005. PubMed PMID: 24530057; PMCID: PMC3990591.

295. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nature medicine. 1999;5(12):1365-9. Epub 1999/12/02. doi: 10.1038/70932. PubMed PMID: 10581077.

296. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR, Honjo T. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. The Journal of experimental medicine. 2000;192(7):1027-34. Epub 2000/10/04. PubMed PMID: 11015443; PMCID: PMC2193311.

297. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annual review of immunology. 2005;23:515-48. Epub 2005/03/18. doi: 10.1146/annurev.immunol.23.021704.115611. PubMed PMID: 15771580.

298. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, Iwai Y, Long AJ, Brown JA, Nunes R, Greenfield EA, Bourque K, Boussiotis VA, Carter LL, Carreno BM, Malenkovich N, Nishimura H, Okazaki T, Honjo T, Sharpe AH, Freeman GJ. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nature immunology. 2001;2(3):261-8. Epub 2001/02/27. doi: 10.1038/85330. PubMed PMID: 11224527.

299. Tseng SY, Otsuji M, Gorski K, Huang X, Slansky JE, Pai SI, Shalabi A, Shin T, Pardoll DM, Tsuchiya H. B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. The Journal of experimental medicine. 2001;193(7):839-46. Epub 2001/04/03. PubMed PMID: 11283156; PMCID: PMC2193370.

300. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annual review of immunology. 2008;26:677-704. Epub 2008/01/05. doi: 10.1146/annurev.immunol.26.021607.090331. PubMed PMID: 18173375.

301. Riley JL. PD-1 signaling in primary T cells. Immunological reviews. 2009;229(1):114-25. Epub 2009/05/12. doi: 10.1111/j.1600-065X.2009.00767.x. PubMed PMID: 19426218; PMCID: PMC3424066.

302. Schildberg FA, Klein SR, Freeman GJ, Sharpe AH. Coinhibitory Pathways in the B7-CD28 Ligand-Receptor Family. Immunity. 2016;44(5):955-72. Epub 2016/05/19. doi: 10.1016/j.immuni.2016.05.002. PubMed PMID: 27192563; PMCID: PMC4905708.

303. Wykes MN, Lewin SR. Immune checkpoint blockade in infectious diseases. Nature reviews Immunology. 2018;18(2):91-104. Epub 2017/10/11. doi: 10.1038/nri.2017.112. PubMed PMID: 28990586; PMCID: PMC5991909.

304. Wherry EJ. T cell exhaustion. Nature immunology. 2011;12(6):492-9. Epub 2011/07/09. PubMed PMID: 21739672.

305. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. Trends Immunol. 2015;36(4):265-76. Epub 2015/03/24. doi: 10.1016/j.it.2015.02.008. PubMed PMID: 25797516; PMCID: PMC4393798.

306. Odorizzi PM, Pauken KE, Paley MA, Sharpe A, Wherry EJ. Genetic absence of PD-1 promotes accumulation of terminally differentiated exhausted CD8+ T cells. The Journal of experimental medicine. 2015;212(7):1125-37. Epub 2015/06/03. doi: 10.1084/jem.20142237. PubMed PMID: 26034050; PMCID: PMC4493417.

307. Fuller MJ, Callendret B, Zhu B, Freeman GJ, Hasselschwert DL, Satterfield W, Sharpe AH, Dustin LB, Rice CM, Grakoui A, Ahmed R, Walker CM. Immunotherapy of chronic hepatitis C virus infection with antibodies against programmed cell death-1 (PD-1). Proceedings of the National Academy of Sciences of the United States of America. 2013;110(37):15001-6. Epub 2013/08/28. doi: 10.1073/pnas.1312772110. PubMed PMID: 23980172; PMCID: PMC3773803.

308. Velu V, Titanji K, Zhu B, Husain S, Pladevega A, Lai L, Vanderford TH, Chennareddi L, Silvestri G, Freeman GJ, Ahmed R, Amara RR. Enhancing SIV-specific immunity in vivo by PD-1 blockade. Nature. 2009;458(7235):206-10. doi: 10.1038/nature07662. PubMed PMID: 19078956; PMCID: 2753387.

309. Gardiner D, Lalezari J, Lawitz E, DiMicco M, Ghalib R, Reddy KR, Chang KM, Sulkowski M, Marro SO, Anderson J, He B, Kansra V, McPhee F, Wind-Rotolo M, Grasela D, Selby M, Korman AJ, Lowy I. A randomized, double-blind, placebo-controlled assessment of BMS-936558, a fully human monoclonal antibody to programmed death-1 (PD-1), in patients with chronic hepatitis C virus infection. PloS one. 2013;8(5):e63818. Epub 2013/05/30. doi: 10.1371/journal.pone.0063818. PubMed PMID: 23717490; PMCID: PMC3661719.

310. Zhang JY, Zhang Z, Wang X, Fu JL, Yao J, Jiao Y, Chen L, Zhang H, Wei J, Jin L, Shi M, Gao GF, Wu H, Wang FS. 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.

311. Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, Boulassel MR, Delwart E, Sepulveda H, Balderas RS, Routy JP, Haddad EK, Sekaly RP. Upregulation of PD-1 expression on

HIV-specific CD8+ T cells leads to reversible immune dysfunction. Nature medicine. 2006;12(10):1198-202. Epub 2006/08/19. doi: 10.1038/nm1482. PubMed PMID: 16917489.

312. Chew GM, Fujita T, Webb GM, Burwitz BJ, Wu HL, Reed JS, Hammond KB, Clayton KL, Ishii N, Abdel-Mohsen M, Liegler T, Mitchell BI, Hecht FM, Ostrowski M, Shikuma CM, Hansen SG, Maurer M, Korman AJ, Deeks SG, Sacha JB, Ndhlovu LC. TIGIT Marks Exhausted T Cells, Correlates with Disease Progression, and Serves as a Target for Immune Restoration in HIV and SIV Infection. PLoS pathogens. 2016;12(1):e1005349. Epub 2016/01/08. doi: 10.1371/journal.ppat.1005349. PubMed PMID: 26741490; PMCID: PMC4704737.

313. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, Mackey EW, Miller JD, Leslie AJ, DePierres C, Mncube Z, Duraiswamy J, Zhu B, Eichbaum Q, Altfeld M, Wherry EJ, Coovadia HM, Goulder PJ, Klenerman P, Ahmed R, Freeman GJ, Walker BD. 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.

314. Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, Precopio ML, Schacker T, Roederer M, Douek DC, Koup RA. PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. The Journal of experimental medicine. 2006;203(10):2281-92. Epub 2006/09/07. doi: 10.1084/jem.20061496. PubMed PMID: 16954372; PMCID: PMC2118095.

315. Hoffmann M, Pantazis N, Martin GE, Hickling S, Hurst J, Meyerowitz J, Willberg CB, Robinson N, Brown H, Fisher M, Kinloch S, Babiker A, Weber J, Nwokolo N, Fox J, Fidler S, Phillips R, Frater J, Spartac, Investigators C. Exhaustion of Activated CD8 T Cells Predicts Disease Progression in Primary HIV-1 Infection. PLoS pathogens. 2016;12(7):e1005661. Epub 2016/07/16. doi: 10.1371/journal.ppat.1005661. PubMed PMID: 27415828; PMCID: PMC4945085.

316. Shive CL, Clagett B, McCausland MR, Mudd JC, Funderburg NT, Freeman ML, Younes SA, Ferrari BM, Rodriguez B, McComsey GA, Calabrese LH, Sieg SF, Lederman MM. Inflammation Perturbs the IL-7 Axis, Promoting Senescence and Exhaustion that Broadly Characterize Immune Failure

in Treated HIV Infection. Journal of acquired immune deficiency syndromes. 2016;71(5):483-92. Epub 2015/12/03. doi: 10.1097/QAI.0000000000000913. PubMed PMID: 26627102; PMCID: PMC4788576.

317. Hurst J, Hoffmann M, Pace M, Williams JP, Thornhill J, Hamlyn E, Meyerowitz J, Willberg C, Koelsch KK, Robinson N, Brown H, Fisher M, Kinloch S, Cooper DA, Schechter M, Tambussi G, Fidler S, Babiker A, Weber J, Kelleher AD, Phillips RE, Frater J. Immunological biomarkers predict HIV-1 viral rebound after treatment interruption. Nature communications. 2015;6:8495. Epub 2015/10/10. doi: 10.1038/ncomms9495. PubMed PMID: 26449164; PMCID: PMC4633715.

318. Akhmetzyanova I, Drabczyk M, Neff CP, Gibbert K, Dietze KK, Werner T, Liu J, Chen L, Lang KS, Palmer BE, Dittmer U, Zelinskyy G. PD-L1 Expression on Retrovirus-Infected Cells Mediates Immune Escape from CD8+ T Cell Killing. PLoS pathogens. 2015;11(10):e1005224. Epub 2015/10/21. doi: 10.1371/journal.ppat.1005224. PubMed PMID: 26484769; PMCID: PMC4617866.

319. Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, Boucher G, Boulassel MR, Ghattas G, Brenchley JM, Schacker TW, Hill BJ, Douek DC, Routy JP, Haddad EK, Sekaly RP. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nature medicine. 2009;15(8):893-900. doi: 10.1038/nm.1972. PubMed PMID: 19543283; PMCID: 2859814.

320. Cockerham LR, Jain V, Sinclair E, Glidden DV, Hartogenesis W, Hatano H, Hunt PW, Martin JN, Pilcher CD, Sekaly R, McCune JM, Hecht FM, Deeks SG. Programmed death-1 expression on CD4(+) and CD8(+) T cells in treated and untreated HIV disease. Aids. 2014;28(12):1749-58. doi: 10.1097/QAD.0000000000000314. PubMed PMID: 24871455; PMCID: PMC4206412.

321. Hatano H, Jain V, Hunt PW, Lee TH, Sinclair E, Do TD, Hoh R, Martin JN, McCune JM, Hecht F, Busch MP, Deeks SG. Cell-based measures of viral persistence are associated with immune activation and programmed cell death protein 1 (PD-1)-expressing CD4+ T cells. The Journal of infectious diseases. 2013;208(1):50-6. Epub 2012/10/24. doi: 10.1093/infdis/jis630. PubMed PMID: 23089590; PMCID: PMC3666131.

322. Khoury G, Fromentin R, Solomon A, Hartogensis W, Killian M, Hoh R, Somsouk M, Hunt PW, Girling V, Sinclair E, Bacchetti P, Anderson JL, Hecht FM, Deeks SG, Cameron PU, Chomont N, Lewin SR. Human Immunodeficiency Virus Persistence and T-Cell Activation in Blood, Rectal, and Lymph Node Tissue in Human Immunodeficiency Virus-Infected Individuals Receiving Suppressive Antiretroviral Therapy. The Journal of infectious diseases. 2017;215(6):911-9. Epub 2017/04/30. doi: 10.1093/infdis/jix039. PubMed PMID: 28453847; PMCID: PMC5407052.

323. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proceedings of the National Academy of Sciences of the United States of America. 2002;99(19):12293-7. Epub 2002/09/10. doi: 10.1073/pnas.192461099. PubMed PMID: 12218188; PMCID: PMC129438.

324. Iwai Y, Terawaki S, Honjo T. PD-1 blockade inhibits hematogenous spread of poorly immunogenic tumor cells by enhanced recruitment of effector T cells. International immunology. 2005;17(2):133-44. Epub 2004/12/22. doi: 10.1093/intimm/dxh194. PubMed PMID: 15611321.

325. Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, Krzysiek R, Knutson KL, Daniel B, Zimmermann MC, David O, Burow M, Gordon A, Dhurandhar N, Myers L, Berggren R, Hemminki A, Alvarez RD, Emilie D, Curiel DT, Chen L, Zou W. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. Nature medicine. 2003;9(5):562-7. Epub 2003/04/22. doi: 10.1038/nm863. PubMed PMID: 12704383.

326. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, Rietz C, Flies DB, Lau JS, Zhu G, Tamada K, Chen L. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer research. 2005;65(3):1089-96. Epub 2005/02/12. PubMed PMID: 15705911.

327. Strome SE, Dong H, Tamura H, Voss SG, Flies DB, Tamada K, Salomao D, Cheville J, Hirano F, Lin W, Kasperbauer JL, Ballman KV, Chen L. B7-H1 blockade augments adoptive T-cell immunotherapy for squamous cell carcinoma. Cancer research. 2003;63(19):6501-5. Epub 2003/10/16. PubMed PMID: 14559843.

328. Dyavar Shetty R, Velu V, Titanji K, Bosinger SE, Freeman GJ, Silvestri G, Amara RR. PD-1 blockade during chronic SIV infection reduces hyperimmune activation and microbial translocation in rhesus macaques. The Journal of clinical investigation. 2012;122(5):1712-6. Epub 2012/04/24. doi: 10.1172/JCI60612. PubMed PMID: 22523065; PMCID: PMC3336983.

329. Gill AL, Green SA, Abdullah S, Le Saout C, Pittaluga S, Chen H, Turnier R, Lifson J, Godin S, Qin J, Sneller MC, Cuillerot JM, Sabzevari H, Lane HC, Catalfamo M. Programed death-1/programed death-ligand 1 expression in lymph nodes of HIV infected patients: results of a pilot safety study in rhesus macaques using anti-programed death-ligand 1 (Avelumab). Aids. 2016;30(16):2487-93. Epub 2016/10/19. doi: 10.1097/QAD.0000000000001217. PubMed PMID: 27490642; PMCID: PMC5051527.

330. Gay CL, Bosch RJ, Ritz J, Hataye JM, Aga E, Tressler RL, Mason SW, Hwang CK, Grasela DM, Ray N, Cyktor JC, Coffin JM, Acosta EP, Koup RA, Mellors JW, Eron JJ, Team ACTS. Clinical Trial of the Anti-PD-L1 Antibody BMS-936559 in HIV-1 Infected Participants on Suppressive Antiretroviral Therapy. The Journal of infectious diseases. 2017;215(11):1725-33. Epub 2017/04/22. doi: 10.1093/infdis/jix191. PubMed PMID: 28431010; PMCID: PMC5790148.

331. Shankarappa R, Margolick JB, Gange SJ, Rodrigo AG, Upchurch D, Farzadegan H, Gupta P, Rinaldo CR, Learn GH, He X, Huang XL, Mullins JI. Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus type 1 infection. Journal of virology. 1999;73(12):10489-502. PubMed PMID: 10559367; PMCID: 113104.

332. Korber B, Gaschen B, Yusim K, Thakallapally R, Kesmir C, Detours V. Evolutionary and immunological implications of contemporary HIV-1 variation. British medical bulletin. 2001;58:19-42. PubMed PMID: 11714622.

333. Excler JL, Robb ML, Kim JH. HIV-1 vaccines: challenges and new perspectives. Human vaccines & immunotherapeutics. 2014;10(6):1734-46. doi: 10.4161/hv.28462. PubMed PMID: 24637946.

334. Phanuphak N, Lo YR, Shao Y, Solomon SS, O'Connell RJ, Tovanabutra S, Chang D, Kim JH, Excler JL. HIV Epidemic in Asia: Implications for HIV Vaccine and Other Prevention Trials. AIDS

research and human retroviruses. 2015;31(11):1060-76. doi: 10.1089/aid.2015.0049. PubMed PMID: 26107771; PMCID: 4651036.

335. Excler JL, Ake J, Robb ML, Kim JH, Plotkin SA. Nonneutralizing functional antibodies: a new "old" paradigm for HIV vaccines. Clinical and vaccine immunology : CVI. 2014;21(8):1023-36. doi: 10.1128/CVI.00230-14. PubMed PMID: 24920599; PMCID: PMC4135913.

336. Su B, Moog C. Which Antibody Functions are Important for an HIV Vaccine? Frontiers in immunology. 2014;5:289. doi: 10.3389/fimmu.2014.00289. PubMed PMID: 24995008; PMCID: PMC4062070.

337. Burton DR, Ahmed R, Barouch DH, Butera ST, Crotty S, Godzik A, Kaufmann DE, McElrath MJ, Nussenzweig MC, Pulendran B, Scanlan CN, Schief WR, Silvestri G, Streeck H, Walker BD, Walker LM, Ward AB, Wilson IA, Wyatt R. A Blueprint for HIV Vaccine Discovery. Cell host & microbe. 2012;12(4):396-407. doi: 10.1016/j.chom.2012.09.008. PubMed PMID: 23084910; PMCID: 3513329.

338. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF, rgp HIVVSG. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. The Journal of infectious diseases. 2005;191(5):654-65. doi: 10.1086/428404. PubMed PMID: 15688278.

339. Gilbert PB, Peterson ML, Follmann D, Hudgens MG, Francis DP, Gurwith M, Heyward WL, Jobes DV, Popovic V, Self SG, Sinangil F, Burke D, Berman PW. Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. The Journal of infectious diseases. 2005;191(5):666-77. doi: 10.1086/428405. PubMed PMID: 15688279.

340. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F, Hu D, Tappero JW, Choopanya K, Bangkok Vaccine Evaluation G. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. The Journal of infectious diseases. 2006;194(12):1661-71. doi: 10.1086/508748. PubMed PMID: 17109337.

341. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, Gilbert PB, Lama JR, Marmor M, Del Rio C, McElrath MJ, Casimiro DR, Gottesdiener KM, Chodakewitz JA, Corey L, Robertson MN, Step Study Protocol T. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet. 2008;372(9653):1881-93. doi: 10.1016/S0140-6736(08)61591-3. PubMed PMID: 19012954; PMCID: 2721012.

342. McElrath MJ, De Rosa SC, Moodie Z, Dubey S, Kierstead L, Janes H, Defawe OD, Carter DK, Hural J, Akondy R, Buchbinder SP, Robertson MN, Mehrotra DV, Self SG, Corey L, Shiver JW, Casimiro DR, Step Study Protocol T. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. Lancet. 2008;372(9653):1894-905. doi: 10.1016/S0140-6736(08)61592-5. PubMed PMID: 19012957; PMCID: 2774110.

343. Gray GE, Allen M, Moodie Z, Churchyard G, Bekker LG, Nchabeleng M, Mlisana K, Metch B, de Bruyn G, Latka MH, Roux S, Mathebula M, Naicker N, Ducar C, Carter DK, Puren A, Eaton N, McElrath MJ, Robertson M, Corey L, Kublin JG, team HPs. Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. The Lancet Infectious diseases. 2011;11(7):507-15. Epub 2011/05/17. doi: 10.1016/S1473-3099(11)70098-6. PubMed PMID: 21570355; PMCID: PMC3417349.

344. Gray GE, Moodie Z, Metch B, Gilbert PB, Bekker LG, Churchyard G, Nchabeleng M, Mlisana K, Laher F, Roux S, Mngadi K, Innes C, Mathebula M, Allen M, McElrath MJ, Robertson M, Kublin J, Corey L, team HPs. Recombinant adenovirus type 5 HIV gag/pol/nef vaccine in South Africa: unblinded, long-term follow-up of the phase 2b HVTN 503/Phambili study. The Lancet Infectious diseases. 2014;14(5):388-96. Epub 2014/02/25. doi: 10.1016/S1473-3099(14)70020-9. PubMed PMID: 24560541; PMCID: PMC4174314.

345. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, Grove D, Koblin BA, Buchbinder SP, Keefer MC, Tomaras GD, Frahm N, Hural J, Anude C, Graham BS, Enama ME, Adams

E, DeJesus E, Novak RM, Frank I, Bentley C, Ramirez S, Fu R, Koup RA, Mascola JR, Nabel GJ, Montefiori DC, Kublin J, McElrath MJ, Corey L, Gilbert PB, Team HS. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. The New England journal of medicine. 2013;369(22):2083-92. doi: 10.1056/NEJMoa1310566. PubMed PMID: 24099601; PMCID: PMC4030634.

346. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, Benenson M, Gurunathan S, Tartaglia J, McNeil JG, Francis DP, Stablein D, Birx DL, Chunsuttiwat S, Khamboonruang C, Thongcharoen P, Robb ML, Michael NL, Kunasol P, Kim JH, Investigators M-T. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. The New England journal of medicine. 2009;361(23):2209-20. Epub 2009/10/22. doi: 10.1056/NEJMoa0908492. PubMed PMID: 19843557.

347. Vaccari M, Poonam P, Franchini G. Phase III HIV vaccine trial in Thailand: a step toward a protective vaccine for HIV. Expert review of vaccines. 2010;9(9):997-1005. Epub 2010/09/09. doi: 10.1586/erv.10.104. PubMed PMID: 20822342.

348. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, Liao HX, DeVico AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang Y, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Bailer RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen X, Koup RA, Pitisuttithum P, Kaewkungwal J, Nitayaphan S, Rerks-Ngarm S, Michael NL, Kim JH. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. The New England journal of medicine. 2012;366(14):1275-86. doi: 10.1056/NEJMoa1113425. PubMed PMID: 22475592; PMCID: 3371689.

349. Kim JH, Excler JL, Michael NL. Lessons from the RV144 Thai phase III HIV-1 vaccine trial and the search for correlates of protection. Annual review of medicine. 2015;66:423-37. Epub 2014/10/24. doi: 10.1146/annurev-med-052912-123749. PubMed PMID: 25341006.

350. Gottardo R, Bailer RT, Korber BT, Gnanakaran S, Phillips J, Shen X, Tomaras GD, Turk E, Imholte G, Eckler L, Wenschuh H, Zerweck J, Greene K, Gao H, Berman PW, Francis D, Sinangil F, Lee

C, Nitayaphan S, Rerks-Ngarm S, Kaewkungwal J, Pitisuttithum P, Tartaglia J, Robb ML, Michael NL, Kim JH, Zolla-Pazner S, Haynes BF, Mascola JR, Self S, Gilbert P, Montefiori DC. Plasma IgG to linear epitopes in the V2 and V3 regions of HIV-1 gp120 correlate with a reduced risk of infection in the RV144 vaccine efficacy trial. PloS one. 2013;8(9):e75665. Epub 2013/10/03. doi: 10.1371/journal.pone.0075665. PubMed PMID: 24086607; PMCID: PMC3784573.

351. Montefiori DC, Karnasuta C, Huang Y, Ahmed H, Gilbert P, de Souza MS, McLinden R, Tovanabutra S, Laurence-Chenine A, Sanders-Buell E, Moody MA, Bonsignori M, Ochsenbauer C, Kappes J, Tang H, Greene K, Gao H, LaBranche CC, Andrews C, Polonis VR, Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Self SG, Berman PW, Francis D, Sinangil F, Lee C, Tartaglia J, Robb ML, Haynes BF, Michael NL, Kim JH. Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. The Journal of infectious diseases. 2012;206(3):431-41. Epub 2012/05/29. doi: 10.1093/infdis/jis367. PubMed PMID: 22634875; PMCID: PMC3392187.

352. Yates NL, Liao HX, Fong Y, deCamp A, Vandergrift NA, Williams WT, Alam SM, Ferrari G, Yang ZY, Seaton KE, Berman PW, Alpert MD, Evans DT, O'Connell RJ, Francis D, Sinangil F, Lee C, Nitayaphan S, Rerks-Ngarm S, Kaewkungwal J, Pitisuttithum P, Tartaglia J, Pinter A, Zolla-Pazner S, Gilbert PB, Nabel GJ, Michael NL, Kim JH, Montefiori DC, Haynes BF, Tomaras GD. Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. Science translational medicine. 2014;6(228):228ra39. Epub 2014/03/22. doi: 10.1126/scitranslmed.3007730. PubMed PMID: 24648342; PMCID: PMC4116665.

353. Roussilhon C, Oeuvray C, Muller-Graf C, Tall A, Rogier C, Trape JF, Theisen M, Balde A, Perignon JL, Druilhe P. Long-term clinical protection from falciparum malaria is strongly associated with IgG3 antibodies to merozoite surface protein 3. PLoS medicine. 2007;4(11):e320. Epub 2007/11/16. doi: 10.1371/journal.pmed.0040320. PubMed PMID: 18001147; PMCID: PMC2071934.

354. Kam YW, Simarmata D, Chow A, Her Z, Teng TS, Ong EK, Renia L, Leo YS, Ng LF. Early appearance of neutralizing immunoglobulin G3 antibodies is associated with chikungunya virus clearance

and long-term clinical protection. The Journal of infectious diseases. 2012;205(7):1147-54. Epub 2012/03/06. doi: 10.1093/infdis/jis033. PubMed PMID: 22389226; PMCID: PMC3295607.

355. Moss B. Poxviridae: The viruses and their replication. In: Knipe DM HP, editor. Fields virology. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 2905–46.

356. Afonso CL, Tulman ER, Lu Z, Zsak L, Kutish GF, Rock DL. The genome of fowlpox virus. Journal of virology. 2000;74(8):3815-31. Epub 2000/03/23. PubMed PMID: 10729156; PMCID: PMC111890.

357. Seet BT, Johnston JB, Brunetti CR, Barrett JW, Everett H, Cameron C, Sypula J, Nazarian SH, Lucas A, McFadden G. Poxviruses and immune evasion. Annual review of immunology. 2003;21:377-423. doi: 10.1146/annurev.immunol.21.120601.141049. PubMed PMID: 12543935.

358. Moss B. Poxvirus cell entry: how many proteins does it take? Viruses. 2012;4(5):688-707. Epub 2012/07/04. doi: 10.3390/v4050688. PubMed PMID: 22754644; PMCID: PMC3386626.

359. Condit RC, Moussatche N, Traktman P. In a nutshell: structure and assembly of the vaccinia virion. Advances in virus research. 2006;66:31-124. Epub 2006/08/01. doi: 10.1016/S0065-3527(06)66002-8. PubMed PMID: 16877059.

360. Hollinshead M, Vanderplasschen A, Smith GL, Vaux DJ. Vaccinia virus intracellular mature virions contain only one lipid membrane. Journal of virology. 1999;73(2):1503-17. Epub 1999/01/09. PubMed PMID: 9882356; PMCID: PMC103975.

361. Cyrklaff M, Risco C, Fernandez JJ, Jimenez MV, Esteban M, Baumeister W, Carrascosa JL. Cryo-electron tomography of vaccinia virus. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(8):2772-7. Epub 2005/02/09. doi: 10.1073/pnas.0409825102. PubMed PMID: 15699328; PMCID: PMC549483.

362. Hiller G, Weber K. Golgi-derived membranes that contain an acylated viral polypeptide are used for vaccinia virus envelopment. Journal of virology. 1985;55(3):651-9. Epub 1985/09/01. PubMed PMID: 4020961; PMCID: PMC255032.

363. Schmelz M, Sodeik B, Ericsson M, Wolffe EJ, Shida H, Hiller G, Griffiths G. Assembly of vaccinia virus: the second wrapping cisterna is derived from the trans Golgi network. Journal of virology. 1994;68(1):130-47. Epub 1994/01/01. PubMed PMID: 8254722; PMCID: PMC236272.

364. Tooze J, Hollinshead M, Reis B, Radsak K, Kern H. Progeny vaccinia and human cytomegalovirus particles utilize early endosomal cisternae for their envelopes. Eur J Cell Biol. 1993;60(1):163-78. Epub 1993/02/01. PubMed PMID: 8385018.

365. Ward BM, Moss B. Vaccinia virus intracellular movement is associated with microtubules and independent of actin tails. Journal of virology. 2001;75(23):11651-63. Epub 2001/11/02. doi: 10.1128/JVI.75.23.11651-11663.2001. PubMed PMID: 11689647; PMCID: PMC114752.

366. Smith GL, Vanderplasschen A, Law M. The formation and function of extracellular enveloped vaccinia virus. The Journal of general virology. 2002;83(Pt 12):2915-31. Epub 2002/12/06. doi: 10.1099/0022-1317-83-12-2915. PubMed PMID: 12466468.

367. Hsiao JC, Chung CS, Chang W. Vaccinia virus envelope D8L protein binds to cell surface chondroitin sulfate and mediates the adsorption of intracellular mature virions to cells. Journal of virology. 1999;73(10):8750-61. Epub 1999/09/11. PubMed PMID: 10482629; PMCID: PMC112896.

368. Chung CS, Hsiao JC, Chang YS, Chang W. A27L protein mediates vaccinia virus interaction with cell surface heparan sulfate. Journal of virology. 1998;72(2):1577-85. Epub 1998/01/28. PubMed PMID: 9445060; PMCID: PMC124638.

369. Hsiao JC, Chung CS, Chang W. Cell surface proteoglycans are necessary for A27L protein-mediated cell fusion: identification of the N-terminal region of A27L protein as the glycosaminoglycan-binding domain. Journal of virology. 1998;72(10):8374-9. Epub 1998/09/12. PubMed PMID: 9733888; PMCID: PMC110218.

370. Lin CL, Chung CS, Heine HG, Chang W. Vaccinia virus envelope H3L protein binds to cell surface heparan sulfate and is important for intracellular mature virion morphogenesis and virus infection in vitro and in vivo. Journal of virology. 2000;74(7):3353-65. Epub 2000/03/09. PubMed PMID: 10708453; PMCID: PMC111837.

371. Vazquez MI, Esteban M. Identification of functional domains in the 14-kilodalton envelope protein (A27L) of vaccinia virus. Journal of virology. 1999;73(11):9098-109. Epub 1999/10/09. PubMed PMID: 10516016; PMCID: PMC112942.

372. Chiu WL, Lin CL, Yang MH, Tzou DL, Chang W. Vaccinia virus 4c (A26L) protein on intracellular mature virus binds to the extracellular cellular matrix laminin. Journal of virology. 2007;81(5):2149-57. Epub 2006/12/15. doi: 10.1128/JVI.02302-06. PubMed PMID: 17166913; PMCID: PMC1865921.

373. Moss B. Membrane fusion during poxvirus entry. Semin Cell Dev Biol. 2016;60:89-96. Epub 2016/07/18. doi: 10.1016/j.semcdb.2016.07.015. PubMed PMID: 27423915; PMCID: PMC5161597.

374. Hsiao JC, Chu LW, Lo YT, Lee SP, Chen TJ, Huang CY, Ping YH, Chang W. Intracellular Transport of Vaccinia Virus in HeLa Cells Requires WASH-VPEF/FAM21-Retromer Complexes and Recycling Molecules Rab11 and Rab22. Journal of virology. 2015;89(16):8365-82. Epub 2015/06/05. doi: 10.1128/JVI.00209-15. PubMed PMID: 26041286; PMCID: PMC4524218.

375. Noris M, Remuzzi G. Overview of complement activation and regulation. Semin Nephrol. 2013;33(6):479-92. Epub 2013/10/29. doi: 10.1016/j.semnephrol.2013.08.001. PubMed PMID: 24161035; PMCID: PMC3820029.

376. Kotwal GJ, Isaacs SN, McKenzie R, Frank MM, Moss B. Inhibition of the complement cascade by the major secretory protein of vaccinia virus. Science. 1990;250(4982):827-30. Epub 1990/11/09. PubMed PMID: 2237434.

377. Isaacs SN, Kotwal GJ, Moss B. Vaccinia virus complement-control protein prevents antibody-dependent complement-enhanced neutralization of infectivity and contributes to virulence. Proceedings of the National Academy of Sciences of the United States of America. 1992;89(2):628-32. Epub 1992/01/15. PubMed PMID: 1731333; PMCID: PMC48292.

378. Rosengard AM, Liu Y, Nie Z, Jimenez R. Variola virus immune evasion design: expression of a highly efficient inhibitor of human complement. Proceedings of the National Academy of Sciences of the

United States of America. 2002;99(13):8808-13. Epub 2002/05/30. doi: 10.1073/pnas.112220499. PubMed PMID: 12034872; PMCID: PMC124380.

379. Miller CG, Shchelkunov SN, Kotwal GJ. The cowpox virus-encoded homolog of the vaccinia virus complement control protein is an inflammation modulatory protein. Virology. 1997;229(1):126-33. Epub 1997/03/03. doi: 10.1006/viro.1996.8396. PubMed PMID: 9123854.

380. Kotwal GJ, Miller CG, Justus DE. The inflammation modulatory protein (IMP) of cowpox virus drastically diminishes the tissue damage by down-regulating cellular infiltration resulting from complement activation. Mol Cell Biochem. 1998;185(1-2):39-46. Epub 1998/09/24. PubMed PMID: 9746210.

381. Miller CG, Justus DE, Jayaraman S, Kotwal GJ. Severe and prolonged inflammatory response to localized cowpox virus infection in footpads of C5-deficient mice: investigation of the role of host complement in poxvirus pathogenesis. Cellular immunology. 1995;162(2):326-32. Epub 1995/05/01. doi: 10.1006/cimm.1995.1086. PubMed PMID: 7743560.

382. Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. Annual review of immunology. 2014;32:513-45. Epub 2014/02/22. doi: 10.1146/annurev-immunol-032713-120231. PubMed PMID: 24555472; PMCID: PMC4313732.

383. Symons JA, Alcami A, Smith GL. Vaccinia virus encodes a soluble type I interferon receptor of novel structure and broad species specificity. Cell. 1995;81(4):551-60. Epub 1995/05/19. PubMed PMID: 7758109.

384. Liptakova H, Kontsekova E, Alcami A, Smith GL, Kontsek P. Analysis of an interaction between the soluble vaccinia virus-coded type I interferon (IFN)-receptor and human IFN-alpha1 and IFN-alpha2. Virology. 1997;232(1):86-90. Epub 1997/05/26. PubMed PMID: 9185591.

385. Vancova I, La Bonnardiere C, Kontsek P. Vaccinia virus protein B18R inhibits the activity and cellular binding of the novel type interferon-delta. The Journal of general virology. 1998;79 (Pt 7):1647-9. Epub 1998/07/29. doi: 10.1099/0022-1317-79-7-1647. PubMed PMID: 9680126.

386. Muller U, Steinhoff U, Reis LF, Hemmi S, Pavlovic J, Zinkernagel RM, Aguet M. Functional role of type I and type II interferons in antiviral defense. Science. 1994;264(5167):1918-21. Epub 1994/06/24. PubMed PMID: 8009221.

387. Huang S, Hendriks W, Althage A, Hemmi S, Bluethmann H, Kamijo R, Vilcek J, Zinkernagel RM, Aguet M. Immune response in mice that lack the interferon-gamma receptor. Science. 1993;259(5102):1742-5. Epub 1993/03/19. PubMed PMID: 8456301.

388. van den Broek MF, Muller U, Huang S, Aguet M, Zinkernagel RM. Antiviral defense in mice lacking both alpha/beta and gamma interferon receptors. Journal of virology. 1995;69(8):4792-6. Epub 1995/08/01. PubMed PMID: 7609046; PMCID: PMC189290.

389. Upton C, Mossman K, McFadden G. Encoding of a homolog of the IFN-gamma receptor by myxoma virus. Science. 1992;258(5086):1369-72. Epub 1992/11/20. PubMed PMID: 1455233.

390. Mossman K, Upton C, Buller RM, McFadden G. Species specificity of ectromelia virus and vaccinia virus interferon-gamma binding proteins. Virology. 1995;208(2):762-9. Epub 1995/04/20. doi: 10.1006/viro.1995.1208. PubMed PMID: 7747448.

391. Alcami A, Smith GL. Vaccinia, cowpox, and camelpox viruses encode soluble gamma interferon receptors with novel broad species specificity. Journal of virology. 1995;69(8):4633-9. Epub 1995/08/01. PubMed PMID: 7609027; PMCID: PMC189264.

392. Sroller V, Ludvikova V, Maresova L, Hainz P, Nemeckova S. Effect of IFN-gamma receptor gene deletion on vaccinia virus virulence. Arch Virol. 2001;146(2):239-49. Epub 2001/04/24. PubMed PMID: 11315635.

393. Najarro P, Traktman P, Lewis JA. Vaccinia virus blocks gamma interferon signal transduction: viral VH1 phosphatase reverses Stat1 activation. Journal of virology. 2001;75(7):3185-96. Epub 2001/03/10. doi: 10.1128/JVI.75.7.3185-3196.2001. PubMed PMID: 11238845; PMCID: PMC114112.

394. Katze MG, He Y, Gale M, Jr. Viruses and interferon: a fight for supremacy. Nature reviews Immunology. 2002;2(9):675-87. Epub 2002/09/05. doi: 10.1038/nri888. PubMed PMID: 12209136.

395. Davies MV, Chang HW, Jacobs BL, Kaufman RJ. The E3L and K3L vaccinia virus gene products stimulate translation through inhibition of the double-stranded RNA-dependent protein kinase by different mechanisms. Journal of virology. 1993;67(3):1688-92. Epub 1993/03/01. PubMed PMID: 8094759; PMCID: PMC237544.

396. Chang HW, Watson JC, Jacobs BL. The E3L gene of vaccinia virus encodes an inhibitor of the interferon-induced, double-stranded RNA-dependent protein kinase. Proceedings of the National Academy of Sciences of the United States of America. 1992;89(11):4825-9. Epub 1992/06/01. PubMed PMID: 1350676; PMCID: PMC49180.

397. Sharp TV, Moonan F, Romashko A, Joshi B, Barber GN, Jagus R. The vaccinia virus E3L gene product interacts with both the regulatory and the substrate binding regions of PKR: implications for PKR autoregulation. Virology. 1998;250(2):302-15. Epub 1998/10/30. doi: 10.1006/viro.1998.9365. PubMed PMID: 9792841.

398. Xiang Y, Condit RC, Vijaysri S, Jacobs B, Williams BR, Silverman RH. Blockade of interferon induction and action by the E3L double-stranded RNA binding proteins of vaccinia virus. Journal of virology. 2002;76(10):5251-9. Epub 2002/04/23. PubMed PMID: 11967338; PMCID: PMC136119.

399. Smith EJ, Marie I, Prakash A, Garcia-Sastre A, Levy DE. IRF3 and IRF7 phosphorylation in virus-infected cells does not require double-stranded RNA-dependent protein kinase R or Ikappa B kinase but is blocked by Vaccinia virus E3L protein. The Journal of biological chemistry. 2001;276(12):8951-7. Epub 2001/01/02. doi: 10.1074/jbc.M008717200. PubMed PMID: 11124948.

400. Beattie E, Tartaglia J, Paoletti E. Vaccinia virus-encoded eIF-2 alpha homolog abrogates the antiviral effect of interferon. Virology. 1991;183(1):419-22. Epub 1991/07/01. PubMed PMID: 1711259.

401. Carroll K, Elroy-Stein O, Moss B, Jagus R. Recombinant vaccinia virus K3L gene product prevents activation of double-stranded RNA-dependent, initiation factor 2 alpha-specific protein kinase. The Journal of biological chemistry. 1993;268(17):12837-42. Epub 1993/06/15. PubMed PMID: 8099586.

402. Beattie E, Denzler KL, Tartaglia J, Perkus ME, Paoletti E, Jacobs BL. Reversal of the interferon-sensitive phenotype of a vaccinia virus lacking E3L by expression of the reovirus S4 gene. Journal of virology. 1995;69(1):499-505. Epub 1995/01/01. PubMed PMID: 7527085; PMCID: PMC188598.

403. Beattie E, Kauffman EB, Martinez H, Perkus ME, Jacobs BL, Paoletti E, Tartaglia J. Host-range restriction of vaccinia virus E3L-specific deletion mutants. Virus Genes. 1996;12(1):89-94. Epub 1996/01/01. PubMed PMID: 8879125.

404. Brandt TA, Jacobs BL. Both carboxy- and amino-terminal domains of the vaccinia virus interferon resistance gene, E3L, are required for pathogenesis in a mouse model. Journal of virology. 2001;75(2):850-6. Epub 2001/01/03. doi: 10.1128/JVI.75.2.850-856.2001. PubMed PMID: 11134298; PMCID: PMC113981.

405. Fang ZY, Limbach K, Tartaglia J, Hammonds J, Chen X, Spearman P. Expression of vaccinia E3L and K3L genes by a novel recombinant canarypox HIV vaccine vector enhances HIV-1 pseudovirion production and inhibits apoptosis in human cells. Virology. 2001;291(2):272-84. Epub 2002/03/07. doi: 10.1006/viro.2001.1209. PubMed PMID: 11878896.

406. Afonina IS, Muller C, Martin SJ, Beyaert R. Proteolytic Processing of Interleukin-1 Family Cytokines: Variations on a Common Theme. Immunity. 2015;42(6):991-1004. Epub 2015/06/18. doi: 10.1016/j.immuni.2015.06.003. PubMed PMID: 26084020.

407. Ray CA, Black RA, Kronheim SR, Greenstreet TA, Sleath PR, Salvesen GS, Pickup DJ. Viral inhibition of inflammation: cowpox virus encodes an inhibitor of the interleukin-1 beta converting enzyme. Cell. 1992;69(4):597-604. Epub 1992/05/15. PubMed PMID: 1339309.

408. Alcami A, Smith GL. A soluble receptor for interleukin-1 beta encoded by vaccinia virus: a novel mechanism of virus modulation of the host response to infection. Cell. 1992;71(1):153-67. Epub 1992/10/02. PubMed PMID: 1394428.

409. Spriggs MK, Hruby DE, Maliszewski CR, Pickup DJ, Sims JE, Buller RM, VanSlyke J. Vaccinia and cowpox viruses encode a novel secreted interleukin-1-binding protein. Cell. 1992;71(1):145-52. Epub 1992/10/02. PubMed PMID: 1339315.

410. Alcami A, Smith GL. A mechanism for the inhibition of fever by a virus. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(20):11029-34. Epub 1996/10/01. PubMed PMID: 8855303; PMCID: PMC38278.

411. Born TL, Morrison LA, Esteban DJ, VandenBos T, Thebeau LG, Chen N, Spriggs MK, Sims JE, Buller RM. A poxvirus protein that binds to and inactivates IL-18, and inhibits NK cell response. Journal of immunology. 2000;164(6):3246-54. Epub 2000/03/08. PubMed PMID: 10706717.

412. Bowie A, Kiss-Toth E, Symons JA, Smith GL, Dower SK, O'Neill LA. A46R and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(18):10162-7. Epub 2000/08/02. doi: 10.1073/pnas.160027697. PubMed PMID: 10920188; PMCID: PMC27775.

413. Xu X, Nash P, McFadden G. Myxoma virus expresses a TNF receptor homolog with two distinct functions. Virus Genes. 2000;21(1-2):97-109. Epub 2000/10/07. PubMed PMID: 11022793.

414. Cunnion KM. Tumor necrosis factor receptors encoded by poxviruses. Mol Genet Metab. 1999;67(4):278-82. Epub 1999/08/13. doi: 10.1006/mgme.1999.2878. PubMed PMID: 10444338.

415. Hu FQ, Smith CA, Pickup DJ. Cowpox virus contains two copies of an early gene encoding a soluble secreted form of the type II TNF receptor. Virology. 1994;204(1):343-56. Epub 1994/10/01. doi: 10.1006/viro.1994.1539. PubMed PMID: 8091665.

416. Smith CA, Hu FQ, Smith TD, Richards CL, Smolak P, Goodwin RG, Pickup DJ. Cowpox virus genome encodes a second soluble homologue of cellular TNF receptors, distinct from CrmB, that binds TNF but not LT alpha. Virology. 1996;223(1):132-47. Epub 1996/09/01. doi: 10.1006/viro.1996.0462. PubMed PMID: 8806547.

417. Loparev VN, Parsons JM, Knight JC, Panus JF, Ray CA, Buller RM, Pickup DJ, Esposito JJ. A third distinct tumor necrosis factor receptor of orthopoxviruses. Proceedings of the National Academy of Sciences of the United States of America. 1998;95(7):3786-91. Epub 1998/05/09. PubMed PMID: 9520445; PMCID: PMC19915.

418. Saraiva M, Alcami A. CrmE, a novel soluble tumor necrosis factor receptor encoded by poxviruses. Journal of virology. 2001;75(1):226-33. Epub 2000/12/19. doi: 10.1128/JVI.75.1.226-233.2001. PubMed PMID: 11119592; PMCID: PMC113916.

419. Nagata S. Apoptosis and Clearance of Apoptotic Cells. Annual review of immunology. 2018;36:489-517. Epub 2018/02/06. doi: 10.1146/annurev-immunol-042617-053010. PubMed PMID: 29400998.

420. Bertin J, Armstrong RC, Ottilie S, Martin DA, Wang Y, Banks S, Wang GH, Senkevich TG, Alnemri ES, Moss B, Lenardo MJ, Tomaselli KJ, Cohen JI. Death effector domain-containing herpesvirus and poxvirus proteins inhibit both Fas- and TNFR1-induced apoptosis. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(4):1172-6. Epub 1997/02/18. PubMed PMID: 9037025; PMCID: PMC19763.

421. Hu S, Vincenz C, Buller M, Dixit VM. A novel family of viral death effector domain-containing molecules that inhibit both CD-95- and tumor necrosis factor receptor-1-induced apoptosis. The Journal of biological chemistry. 1997;272(15):9621-4. Epub 1997/04/11. PubMed PMID: 9092488.

422. Thome M, Schneider P, Hofmann K, Fickenscher H, Meinl E, Neipel F, Mattmann C, Burns K, Bodmer JL, Schroter M, Scaffidi C, Krammer PH, Peter ME, Tschopp J. Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. Nature. 1997;386(6624):517-21. Epub 1997/04/03. doi: 10.1038/386517a0. PubMed PMID: 9087414.

423. Thompson JP, Turner PC, Ali AN, Crenshaw BC, Moyer RW. The effects of serpin gene mutations on the distinctive pathobiology of cowpox and rabbitpox virus following intranasal inoculation of Balb/c mice. Virology. 1993;197(1):328-38. Epub 1993/11/01. doi: 10.1006/viro.1993.1594. PubMed PMID: 8212568.

424. Quan LT, Caputo A, Bleackley RC, Pickup DJ, Salvesen GS. Granzyme B is inhibited by the cowpox virus serpin cytokine response modifier A. The Journal of biological chemistry. 1995;270(18):10377-9. Epub 1995/05/05. PubMed PMID: 7737968.

425. Tewari M, Telford WG, Miller RA, Dixit VM. CrmA, a poxvirus-encoded serpin, inhibits cytotoxic T-lymphocyte-mediated apoptosis. The Journal of biological chemistry. 1995;270(39):22705-8. Epub 1995/09/29. PubMed PMID: 7559394.

426. Screpanti V, Wallin RP, Ljunggren HG, Grandien A. A central role for death receptor-mediated apoptosis in the rejection of tumors by NK cells. Journal of immunology. 2001;167(4):2068-73. Epub 2001/08/08. PubMed PMID: 11489989.

427. Dobbelstein M, Shenk T. Protection against apoptosis by the vaccinia virus SPI-2 (B13R) gene product. Journal of virology. 1996;70(9):6479-85. PubMed PMID: 8709286; PMCID: 190684.

428. Kettle S, Alcami A, Khanna A, Ehret R, Jassoy C, Smith GL. Vaccinia virus serpin B13R (SPI-2) inhibits interleukin-1beta-converting enzyme and protects virus-infected cells from TNF- and Fas-mediated apoptosis, but does not prevent IL-1beta-induced fever. The Journal of general virology. 1997;78 (Pt 3):677-85. PubMed PMID: 9049422.

429. Macen J, Takahashi A, Moon KB, Nathaniel R, Turner PC, Moyer RW. Activation of caspases in pig kidney cells infected with wild-type and CrmA/SPI-2 mutants of cowpox and rabbitpox viruses. Journal of virology. 1998;72(5):3524-33. Epub 1998/04/29. PubMed PMID: 9557631; PMCID: PMC109571.

430. Everett H, Barry M, Lee SF, Sun X, Graham K, Stone J, Bleackley RC, McFadden G. M11L: a novel mitochondria-localized protein of myxoma virus that blocks apoptosis of infected leukocytes. The Journal of experimental medicine. 2000;191(9):1487-98. Epub 2000/05/03. PubMed PMID: 10790424; PMCID: PMC2213443.

431. Panicali D, Davis SW, Weinberg RL, Paoletti E. Construction of live vaccines by using genetically engineered poxviruses: biological activity of recombinant vaccinia virus expressing influenza virus hemagglutinin. Proceedings of the National Academy of Sciences of the United States of America. 1983;80(17):5364-8. Epub 1983/09/01. PubMed PMID: 6310573; PMCID: 384256.

432. Smith GL, Mackett M, Moss B. Infectious vaccinia virus recombinants that express hepatitis B virus surface antigen. Nature. 1983;302(5908):490-5. Epub 1983/04/07. PubMed PMID: 6835382.

433. Smith GL, Murphy BR, Moss B. Construction and characterization of an infectious vaccinia virus recombinant that expresses the influenza hemagglutinin gene and induces resistance to influenza virus infection in hamsters. Proceedings of the National Academy of Sciences of the United States of America. 1983;80(23):7155-9. Epub 1983/12/01. PubMed PMID: 6580632; PMCID: 390012.

434. Moss B. Vaccinia virus: a tool for research and vaccine development. Science. 1991;252(5013):1662-7. Epub 1991/06/21. PubMed PMID: 2047875.

435. Smith GL, Moss B. Infectious poxvirus vectors have capacity for at least 25 000 base pairs of foreign DNA. Gene. 1983;25(1):21-8. Epub 1983/11/01. PubMed PMID: 6229451.

436. Kennedy RB, Ovsyannikova I, Poland GA. Smallpox vaccines for biodefense. Vaccine. 2009;27 Suppl 4:D73-9. Epub 2009/10/20. doi: 10.1016/j.vaccine.2009.07.103. PubMed PMID: 19837292; PMCID: 2764553.

437. Volz A, Sutter G. Modified Vaccinia Virus Ankara: History, Value in Basic Research, and Current Perspectives for Vaccine Development. Advances in virus research. 2017;97:187-243. Epub 2017/01/07. doi: 10.1016/bs.aivir.2016.07.001. PubMed PMID: 28057259.

438. Herrlich A, Mayr A. [Comparative experimental works on cow pox virus vaccines]. Arch Hyg Bakteriol. 1954;138(7):479-504. Epub 1954/11/01. PubMed PMID: 13218616.

439. Stickl H, Hochstein-Mintzel V. [Intracutaneous smallpox vaccination with a weak pathogenic vaccinia virus ("MVA virus")]. Munch Med Wochenschr. 1971;113(35):1149-53. Epub 1971/08/27. PubMed PMID: 5109577.

440. Antoine G, Scheiflinger F, Dorner F, Falkner FG. The Complete Genomic Sequence of the Modified Vaccinia Ankara Strain: Comparison with Other Orthopoxviruses. Virology. 1998;244(2):365-96. doi: http://dx.doi.org/10.1006/viro.1998.9123.

441. Meisinger-Henschel C, Schmidt M, Lukassen S, Linke B, Krause L, Konietzny S, Goesmann A, Howley P, Chaplin P, Suter M, Hausmann J. Genomic sequence of chorioallantois vaccinia virus Ankara, the ancestor of modified vaccinia virus Ankara. The Journal of general virology. 2007;88(Pt 12):3249-59. Epub 2007/11/21. doi: 10.1099/vir.0.83156-0. PubMed PMID: 18024893.

442. Mahnel H, Mayr A. [Experiences with immunization against orthopox viruses of humans and animals using vaccine strain MVA]. Berl Munch Tierarztl Wochenschr. 1994;107(8):253-6. Epub 1994/08/01. PubMed PMID: 7945180.

443. Blanchard TJ, Alcami A, Andrea P, Smith GL. Modified vaccinia virus Ankara undergoes limited replication in human cells and lacks several immunomodulatory proteins: implications for use as a human vaccine. The Journal of general virology. 1998;79 (Pt 5):1159-67. Epub 1998/05/29. doi: 10.1099/0022-1317-79-5-1159. PubMed PMID: 9603331.

444. Sutter G, Moss B. Nonreplicating vaccinia vector efficiently expresses recombinant genes. Proceedings of the National Academy of Sciences of the United States of America. 1992;89(22):10847-51. Epub 1992/11/15. PubMed PMID: 1438287; PMCID: PMC50439.

445. Hanke T, Blanchard TJ, Schneider J, Ogg GS, Tan R, Becker M, Gilbert SC, Hill AV, Smith GL, McMichael A. Immunogenicities of intravenous and intramuscular administrations of modified vaccinia virus Ankara-based multi-CTL epitope vaccine for human immunodeficiency virus type 1 in mice. The Journal of general virology. 1998;79 (Pt 1):83-90. Epub 1998/02/14. doi: 10.1099/0022-1317-79-1-83. PubMed PMID: 9460927.

446. Schneider J, Gilbert SC, Blanchard TJ, Hanke T, Robson KJ, Hannan CM, Becker M, Sinden R, Smith GL, Hill AV. Enhanced immunogenicity for CD8+ T cell induction and complete protective efficacy of malaria DNA vaccination by boosting with modified vaccinia virus Ankara. Nature medicine. 1998;4(4):397-402. Epub 1998/04/18. PubMed PMID: 9546783.

447. Wyatt LS, Belyakov IM, Earl PL, Berzofsky JA, Moss B. Enhanced cell surface expression, immunogenicity and genetic stability resulting from a spontaneous truncation of HIV Env expressed by a recombinant MVA. Virology. 2008;372(2):260-72. doi: 10.1016/j.virol.2007.10.033. PubMed PMID: 18048074; PMCID: 2289778.

448. Burgers WA, Shephard E, Monroe JE, Greenhalgh T, Binder A, Hurter E, Van Harmelen JH, Williamson C, Williamson AL. Construction, characterization, and immunogenicity of a multigene

modified vaccinia Ankara (MVA) vaccine based on HIV type 1 subtype C. AIDS research and human retroviruses. 2008;24(2):195-206. doi: 10.1089/aid.2007.0205. PubMed PMID: 18240957.

449. Cottingham MG, Carroll MW. Recombinant MVA vaccines: dispelling the myths. Vaccine. 2013;31(39):4247-51. Epub 2013/03/26. doi: 10.1016/j.vaccine.2013.03.021. PubMed PMID: 23523407.

450. Wyatt LS, Earl PL, Xiao W, Americo JL, Cotter CA, Vogt J, Moss B. Elucidating and minimizing the loss by recombinant vaccinia virus of human immunodeficiency virus gene expression resulting from spontaneous mutations and positive selection. Journal of virology. 2009;83(14):7176-84. doi: 10.1128/JVI.00687-09. PubMed PMID: 19420086; PMCID: 2704791.

451. Waibler Z, Anzaghe M, Ludwig H, Akira S, Weiss S, Sutter G, Kalinke U. Modified vaccinia virus Ankara induces Toll-like receptor-independent type I interferon responses. Journal of virology. 2007;81(22):12102-10. Epub 2007/09/15. doi: 10.1128/JVI.01190-07. PubMed PMID: 17855554; PMCID: PMC2168990.

452. Lynch HE, Ray CA, Oie KL, Pollara JJ, Petty IT, Sadler AJ, Williams BR, Pickup DJ. Modified vaccinia virus Ankara can activate NF-kappaB transcription factors through a double-stranded RNA-activated protein kinase (PKR)-dependent pathway during the early phase of virus replication. Virology. 2009;391(2):177-86. Epub 2009/07/15. doi: 10.1016/j.virol.2009.06.012. PubMed PMID: 19596385; PMCID: PMC2765328.

453. Baden LR, Walsh SR, Seaman MS, Cohen YZ, Johnson JA, Licona JH, Filter RD, Kleinjan JA, Gothing JA, Jennings J, Peter L, Nkolola J, Abbink P, Borducchi EN, Kirilova M, Stephenson KE, Pegu P, Eller MA, Trinh HV, Rao M, Ake JA, Sarnecki M, Nijs S, Callewaert K, Schuitemaker H, Hendriks J, Pau MG, Tomaka F, Korber BT, Alter G, Dolin R, Earl PL, Moss B, Michael NL, Robb ML, Barouch DH, Group IRH-VAS. First-in-Human Randomized Controlled Trial of Mosaic HIV-1 Immunogens Delivered via a Modified Vaccinia Ankara Vector. The Journal of infectious diseases. 2018. Epub 2018/04/19. doi: 10.1093/infdis/jiy212. PubMed PMID: 29669026.

454. Njuguna IN, Ambler G, Reilly M, Ondondo B, Kanyugo M, Lohman-Payne B, Gichuhi C, Borthwick N, Black A, Mehedi SR, Sun J, Maleche-Obimbo E, Chohan B, John-Stewart GC, Jaoko W,

Hanke T. PedVacc 002: a phase I/II randomized clinical trial of MVA.HIVA vaccine administered to infants born to human immunodeficiency virus type 1-positive mothers in Nairobi. Vaccine. 2014;32(44):5801-8. Epub 2014/09/01. doi: 10.1016/j.vaccine.2014.08.034. PubMed PMID: 25173484; PMCID: PMC4414927.

455. Currier JR, Ngauy V, de Souza MS, Ratto-Kim S, Cox JH, Polonis VR, Earl P, Moss B, Peel S, Slike B, Sriplienchan S, Thongcharoen P, Paris RM, Robb ML, Kim J, Michael NL, Marovich MA. Phase I safety and immunogenicity evaluation of MVA-CMDR, a multigenic, recombinant modified vaccinia Ankara-HIV-1 vaccine candidate. PloS one. 2010;5(11):e13983. Epub 2010/11/19. doi: 10.1371/journal.pone.0013983. PubMed PMID: 21085591; PMCID: PMC2981570.

456. Goepfert PA, Elizaga ML, Seaton K, Tomaras GD, Montefiori DC, Sato A, Hural J, DeRosa SC, Kalams SA, McElrath MJ, Keefer MC, Baden LR, Lama JR, Sanchez J, Mulligan MJ, Buchbinder SP, Hammer SM, Koblin BA, Pensiero M, Butler C, Moss B, Robinson HL, Group HS, National Institutes of A, Infectious Diseases HIVVTN. Specificity and 6-month durability of immune responses induced by DNA and recombinant modified vaccinia Ankara vaccines expressing HIV-1 virus-like particles. The Journal of infectious diseases. 2014;210(1):99-110. doi: 10.1093/infdis/jiu003. PubMed PMID: 24403557; PMCID: 4072895.

457. Lai L, Kwa SF, Kozlowski PA, Montefiori DC, Nolen TL, Hudgens MG, Johnson WE, Ferrari G, Hirsch VM, Felber BK, Pavlakis GN, Earl PL, Moss B, Amara RR, Robinson HL. SIVmac239 MVA vaccine with and without a DNA prime, similar prevention of infection by a repeated dose SIVsmE660 challenge despite different immune responses. Vaccine. 2012;30(9):1737-45. doi: 10.1016/j.vaccine.2011.12.026. PubMed PMID: 22178526; PMCID: 3278564.

458. La Rosa C, Longmate J, Martinez J, Zhou Q, Kaltcheva TI, Tsai W, Drake J, Carroll M, Wussow F, Chiuppesi F, Hardwick N, Dadwal S, Aldoss I, Nakamura R, Zaia JA, Diamond DJ. MVA vaccine encoding CMV antigens safely induces durable expansion of CMV-specific T cells in healthy adults. Blood. 2017;129(1):114-25. Epub 2016/10/21. doi: 10.1182/blood-2016-07-729756. PubMed PMID: 27760761; PMCID: PMC5216266.

459. Domi A, Feldmann F, Basu R, McCurley N, Shifflett K, Emanuel J, Hellerstein MS, Guirakhoo F, Orlandi C, Flinko R, Lewis GK, Hanley PW, Feldmann H, Robinson HL, Marzi A. A Single Dose of Modified Vaccinia Ankara expressing Ebola Virus Like Particles Protects Nonhuman Primates from Lethal Ebola Virus Challenge. Scientific reports. 2018;8(1):864. Epub 2018/01/18. doi: 10.1038/s41598-017-19041-y. PubMed PMID: 29339750; PMCID: PMC5770434.

460. Lazaro-Frias A, Gomez-Medina S, Sanchez-Sampedro L, Ljungberg K, Ustav M, Liljestrom P, Munoz-Fontela C, Esteban M, Garcia-Arriaza J. Distinct Immunogenicity and Efficacy of Poxvirus-Based Vaccine Candidates against Ebola Virus Expressing GP and VP40 Proteins. Journal of virology. 2018;92(11). Epub 2018/03/09. doi: 10.1128/JVI.00363-18. PubMed PMID: 29514907; PMCID: PMC5952144.

461. Heery CR, Palena C, McMahon S, Donahue RN, Lepone LM, Grenga I, Dirmeier U, Cordes L, Marte J, Dahut W, Singh H, Madan RA, Fernando RI, Hamilton DH, Schlom J, Gulley JL. Phase I Study of a Poxviral TRICOM-Based Vaccine Directed Against the Transcription Factor Brachyury. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017;23(22):6833-45. Epub 2017/09/01. doi: 10.1158/1078-0432.CCR-17-1087. PubMed PMID: 28855356; PMCID: PMC5690815.

462. Taylor GS, Jia H, Harrington K, Lee LW, Turner J, Ladell K, Price DA, Tanday M, Matthews J, Roberts C, Edwards C, McGuigan L, Hartley A, Wilson S, Hui EP, Chan AT, Rickinson AB, Steven NM. A recombinant modified vaccinia ankara vaccine encoding Epstein-Barr Virus (EBV) target antigens: a phase I trial in UK patients with EBV-positive cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(19):5009-22. Epub 2014/08/16. doi: 10.1158/1078-0432.CCR-14-1122-T. PubMed PMID: 25124688; PMCID: PMC4340506.

463. Kreijtz JH, Goeijenbier M, Moesker FM, van den Dries L, Goeijenbier S, De Gruyter HL, Lehmann MH, Mutsert G, van de Vijver DA, Volz A, Fouchier RA, van Gorp EC, Rimmelzwaan GF, Sutter G, Osterhaus AD. Safety and immunogenicity of a modified-vaccinia-virus-Ankara-based influenza A H5N1 vaccine: a randomised, double-blind phase 1/2a clinical trial. The Lancet Infectious

diseases. 2014;14(12):1196-207. Epub 2014/12/03. doi: 10.1016/S1473-3099(14)70963-6. PubMed PMID: 25455987.

464. Hardwick NR, Carroll M, Kaltcheva T, Qian D, Lim D, Leong L, Chu P, Kim J, Chao J, Fakih M, Yen Y, Espenschied J, Ellenhorn JD, Diamond DJ, Chung V. p53MVA therapy in patients with refractory gastrointestinal malignancies elevates p53-specific CD8+ T-cell responses. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(17):4459-70. Epub 2014/07/06. doi: 10.1158/1078-0432.CCR-13-3361. PubMed PMID: 24987057; PMCID: PMC4155000.

465. Hui EP, Taylor GS, Jia H, Ma BB, Chan SL, Ho R, Wong WL, Wilson S, Johnson BF, Edwards C, Stocken DD, Rickinson AB, Steven NM, Chan AT. Phase I trial of recombinant modified vaccinia ankara encoding Epstein-Barr viral tumor antigens in nasopharyngeal carcinoma patients. Cancer research. 2013;73(6):1676-88. Epub 2013/01/26. doi: 10.1158/0008-5472.CAN-12-2448. PubMed PMID: 23348421.

466. Pathan AA, Minassian AM, Sander CR, Rowland R, Porter DW, Poulton ID, Hill AV, Fletcher HA, McShane H. Effect of vaccine dose on the safety and immunogenicity of a candidate TB vaccine, MVA85A, in BCG vaccinated UK adults. Vaccine. 2012;30(38):5616-24. Epub 2012/07/14. doi: 10.1016/j.vaccine.2012.06.084. PubMed PMID: 22789508; PMCID: PMC3424417.

467. von Krempelhuber A, Vollmar J, Pokorny R, Rapp P, Wulff N, Petzold B, Handley A, Mateo L, Siersbol H, Kollaritsch H, Chaplin P. A randomized, double-blind, dose-finding Phase II study to evaluate immunogenicity and safety of the third generation smallpox vaccine candidate IMVAMUNE. Vaccine. 2010;28(5):1209-16. Epub 2009/12/01. doi: 10.1016/j.vaccine.2009.11.030. PubMed PMID: 19944151; PMCID: 2814951.

468. Perdiguero B, Gomez CE, Najera JL, Sorzano CO, Delaloye J, Gonzalez-Sanz R, Jimenez V, Roger T, Calandra T, Pantaleo G, Esteban M. Deletion of the viral anti-apoptotic gene F1L in the HIV/AIDS vaccine candidate MVA-C enhances immune responses against HIV-1 antigens. PloS one. 2012;7(10):e48524. Epub 2012/11/03. doi: 10.1371/journal.pone.0048524. PubMed PMID: 23119046; PMCID: 3485360.

469. Garcia-Arriaza J, Najera JL, Gomez CE, Tewabe N, Sorzano CO, Calandra T, Roger T, Esteban M. A candidate HIV/AIDS vaccine (MVA-B) lacking vaccinia virus gene C6L enhances memory HIV-1-specific T-cell responses. PloS one. 2011;6(8):e24244. Epub 2011/09/13. doi: 10.1371/journal.pone.0024244. PubMed PMID: 21909386; PMCID: 3164197.

470. Falivene J, Del Medico Zajac MP, Pascutti MF, Rodriguez AM, Maeto C, Perdiguero B, Gomez CE, Esteban M, Calamante G, Gherardi MM. Improving the MVA vaccine potential by deleting the viral gene coding for the IL-18 binding protein. PloS one. 2012;7(2):e32220. Epub 2012/03/03. doi: 10.1371/journal.pone.0032220. PubMed PMID: 22384183; PMCID: 3285208.

471. Garcia-Arriaza J, Gomez CE, Sorzano CO, Esteban M. Deletion of the vaccinia virus N2L gene encoding an inhibitor of IRF3 improves the immunogenicity of modified vaccinia virus Ankara expressing HIV-1 antigens. Journal of virology. 2014;88(6):3392-410. Epub 2014/01/07. doi: 10.1128/JVI.02723-13. PubMed PMID: 24390336; PMCID: 3957918.

472. Rehm KE, Roper RL. Deletion of the A35 gene from Modified Vaccinia Virus Ankara increases immunogenicity and isotype switching. Vaccine. 2011;29(17):3276-83. Epub 2011/03/01. doi: 10.1016/j.vaccine.2011.02.023. PubMed PMID: 21352940; PMCID: 3078999.

473. Garcia-Arriaza J, Najera JL, Gomez CE, Sorzano CO, Esteban M. Immunogenic profiling in mice of a HIV/AIDS vaccine candidate (MVA-B) expressing four HIV-1 antigens and potentiation by specific gene deletions. PloS one. 2010;5(8):e12395. Epub 2010/09/03. doi: 10.1371/journal.pone.0012395. PubMed PMID: 20811493; PMCID: 2927552.

474. Garber DA, O'Mara LA, Gangadhara S, McQuoid M, Zhang X, Zheng R, Gill K, Verma M, Yu T, Johnson B, Li B, Derdeyn CA, Ibegbu C, Altman JD, Hunter E, Feinberg MB. Deletion of specific immune-modulatory genes from modified vaccinia virus Ankara-based HIV vaccines engenders improved immunogenicity in rhesus macaques. Journal of virology. 2012;86(23):12605-15. Epub 2012/09/14. doi: 10.1128/JVI.00246-12. PubMed PMID: 22973033; PMCID: 3497691.

475. Baden LR, Walsh SR, Seaman MS, Cohen YZ, Johnson JA, Licona JH, Filter RD, Kleinjan JA, Gothing JA, Jennings J, Peter L, Nkolola J, Abbink P, Borducchi EN, Kirilova M, Stephenson KE, Pegu

P, Eller MA, Trinh HV, Rao M, Ake JA, Sarnecki M, Nijs S, Callewaert K, Schuitemaker H, Hendriks J, Pau MG, Tomaka F, Korber BT, Alter G, Dolin R, Earl PL, Moss B, Michael NL, Robb ML, Barouch DH, Group IRH-VAS. First-in-Human Randomized, Controlled Trial of Mosaic HIV-1 Immunogens Delivered via a Modified Vaccinia Ankara Vector. The Journal of infectious diseases. 2018;218(4):633-44. Epub 2018/04/19. doi: 10.1093/infdis/jiy212. PubMed PMID: 29669026; PMCID: PMC6047429.

476. Mehendale S, Thakar M, Sahay S, Kumar M, Shete A, Sathyamurthi P, Verma A, Kurle S, Shrotri A, Gilmour J, Goyal R, Dally L, Sayeed E, Zachariah D, Ackland J, Kochhar S, Cox JH, Excler JL, Kumaraswami V, Paranjape R, Ramanathan VD. Safety and immunogenicity of DNA and MVA HIV-1 subtype C vaccine prime-boost regimens: a phase I randomised Trial in HIV-uninfected Indian volunteers. PloS one. 2013;8(2):e55831. doi: 10.1371/journal.pone.0055831. PubMed PMID: 23418465; PMCID: 3572184.

477. Nilsson C, Godoy-Ramirez K, Hejdeman B, Brave A, Gudmundsdotter L, Hallengard D, Currier JR, Wieczorek L, Hasselrot K, Earl PL, Polonis VR, Marovich MA, Robb ML, Sandstrom E, Wahren B, Biberfeld G. Broad and potent cellular and humoral immune responses after a second late HIV-modified vaccinia virus ankara vaccination in HIV-DNA-primed and HIV-modified vaccinia virus Ankara-boosted Swedish vaccinees. AIDS research and human retroviruses. 2014;30(3):299-311. doi: 10.1089/AID.2013.0149. PubMed PMID: 24090081; PMCID: 3938943.

478. Vasan S, Schlesinger SJ, Chen Z, Hurley A, Lombardo A, Than S, Adesanya P, Bunce C, Boaz M, Boyle R, Sayeed E, Clark L, Dugin D, Boente-Carrera M, Schmidt C, Fang Q, LeiBa, Huang Y, Zaharatos GJ, Gardiner DF, Caskey M, Seamons L, Ho M, Dally L, Smith C, Cox J, Gill D, Gilmour J, Keefer MC, Fast P, Ho DD. Phase 1 safety and immunogenicity evaluation of ADMVA, a multigenic, modified vaccinia Ankara-HIV-1 B'/C candidate vaccine. PloS one. 2010;5(1):e8816. Epub 2010/01/30. doi: 10.1371/journal.pone.0008816. PubMed PMID: 20111599; PMCID: PMC2810329.

479. Keefer MC, Frey SE, Elizaga M, Metch B, De Rosa SC, Barroso PF, Tomaras G, Cardinali M, Goepfert P, Kalichman A, Philippon V, McElrath MJ, Jin X, Ferrari G, Defawe OD, Mazzara GP, Montefiori D, Pensiero M, Panicali DL, Corey L, Network NHVT. A phase I trial of preventive HIV

vaccination with heterologous poxviral-vectors containing matching HIV-1 inserts in healthy HIV-uninfected subjects. Vaccine. 2011;29(10):1948-58. Epub 2011/01/11. doi: 10.1016/j.vaccine.2010.12.104. PubMed PMID: 21216311; PMCID: PMC3043112.

480. Gorse GJ, Newman MJ, deCamp A, Hay CM, De Rosa SC, Noonan E, Livingston BD, Fuchs JD, Kalams SA, Cassis-Ghavami FL, Network NHVT. DNA and modified vaccinia virus Ankara vaccines encoding multiple cytotoxic and helper T-lymphocyte epitopes of human immunodeficiency virus type 1 (HIV-1) are safe but weakly immunogenic in HIV-1-uninfected, vaccinia virus-naive adults. Clinical and vaccine immunology : CVI. 2012;19(5):649-58. doi: 10.1128/CVI.00038-12. PubMed PMID: 22398243; PMCID: 3346329.

481. Antrobus RD, Berthoud TK, Mullarkey CE, Hoschler K, Coughlan L, Zambon M, Hill AV, Gilbert SC. Coadministration of seasonal influenza vaccine and MVA-NP+M1 simultaneously achieves potent humoral and cell-mediated responses. Molecular therapy : the journal of the American Society of Gene Therapy. 2014;22(1):233-8. Epub 2013/07/09. doi: 10.1038/mt.2013.162. PubMed PMID: 23831594; PMCID: PMC3978791.

482. Di Bisceglie AM, Janczweska-Kazek E, Habersetzer F, Mazur W, Stanciu C, Carreno V, Tanasescu C, Flisiak R, Romero-Gomez M, Fich A, Bataille V, Toh ML, Hennequi M, Zerr P, Honnet G, Inchauspe G, Agathon D, Limacher JM, Wedemeyer H. Efficacy of immunotherapy with TG4040, peg-interferon, and ribavirin in a Phase 2 study of patients with chronic HCV infection. Gastroenterology. 2014;147(1):119-31 e3. Epub 2014/03/25. doi: 10.1053/j.gastro.2014.03.007. PubMed PMID: 24657484.

483. Hodgson SH, Ewer KJ, Bliss CM, Edwards NJ, Rampling T, Anagnostou NA, de Barra E, Havelock T, Bowyer G, Poulton ID, de Cassan S, Longley R, Illingworth JJ, Douglas AD, Mange PB, Collins KA, Roberts R, Gerry S, Berrie E, Moyle S, Colloca S, Cortese R, Sinden RE, Gilbert SC, Bejon P, Lawrie AM, Nicosia A, Faust SN, Hill AV. Evaluation of the efficacy of ChAd63-MVA vectored vaccines expressing circumsporozoite protein and ME-TRAP against controlled human malaria infection in malaria-naive individuals. The Journal of infectious diseases. 2015;211(7):1076-86. Epub 2014/10/23. doi: 10.1093/infdis/jiu579. PubMed PMID: 25336730; PMCID: PMC4354983.

484. Hodgson SH, Choudhary P, Elias SC, Milne KH, Rampling TW, Biswas S, Poulton ID, Miura K, Douglas AD, Alanine DG, Illingworth JJ, de Cassan SC, Zhu D, Nicosia A, Long CA, Moyle S, Berrie E, Lawrie AM, Wu Y, Ellis RD, Hill AV, Draper SJ. Combining viral vectored and protein-in-adjuvant vaccines against the blood-stage malaria antigen AMA1: report on a phase 1a clinical trial. Molecular therapy : the journal of the American Society of Gene Therapy. 2014;22(12):2142-54. Epub 2014/08/27. doi: 10.1038/mt.2014.157. PubMed PMID: 25156127; PMCID: PMC4250079.

485. Sheehy SH, Duncan CJ, Elias SC, Collins KA, Ewer KJ, Spencer AJ, Williams AR, Halstead FD, Moretz SE, Miura K, Epp C, Dicks MD, Poulton ID, Lawrie AM, Berrie E, Moyle S, Long CA, Colloca S, Cortese R, Gilbert SC, Nicosia A, Hill AV, Draper SJ. Phase Ia clinical evaluation of the Plasmodium falciparum blood-stage antigen MSP1 in ChAd63 and MVA vaccine vectors. Molecular therapy : the journal of the American Society of Gene Therapy. 2011;19(12):2269-76. Epub 2011/08/25. doi: 10.1038/mt.2011.176. PubMed PMID: 21862998; PMCID: PMC3242658.

486. Dunachie SJ, Berthoud T, Keating SM, Hill AV, Fletcher HA. MIG and the regulatory cytokines IL-10 and TGF-beta1 correlate with malaria vaccine immunogenicity and efficacy. PloS one. 2010;5(9):e12557. Epub 2010/09/15. doi: 10.1371/journal.pone.0012557. PubMed PMID: 20838432; PMCID: PMC2933226.

487. Garber DA, O'Mara LA, Zhao J, Gangadhara S, An I, Feinberg MB. Expanding the repertoire of Modified Vaccinia Ankara-based vaccine vectors via genetic complementation strategies. PloS one. 2009;4(5):e5445. doi: 10.1371/journal.pone.0005445. PubMed PMID: 19421328; PMCID: 2674217.

488. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. British journal of cancer. 1972;26(4):239-57. PubMed PMID: 4561027; PMCID: 2008650.

489. Fadok VA, Bratton DL, Frasch SC, Warner ML, Henson PM. The role of phosphatidylserine in recognition of apoptotic cells by phagocytes. Cell death and differentiation. 1998;5(7):551-62. doi: 10.1038/sj.cdd.4400404. PubMed PMID: 10200509.

490. Spel L, Boelens JJ, Nierkens S, Boes M. Antitumor immune responses mediated by dendritic cells: How signals derived from dying cancer cells drive antigen cross-presentation. Oncoimmunology. 2013;2(11):e26403. doi: 10.4161/onci.26403. PubMed PMID: 24482744; PMCID: 3894247.

491. Sasaki S, Amara RR, Oran AE, Smith JM, Robinson HL. Apoptosis-mediated enhancement of DNA-raised immune responses by mutant caspases. Nature biotechnology. 2001;19(6):543-7. doi: 10.1038/89289. PubMed PMID: 11385458.

492. Kettle S, Blake NW, Law KM, Smith GL. Vaccinia virus serpins B13R (SPI-2) and B22R (SPI-1) encode M(r) 38.5 and 40K, intracellular polypeptides that do not affect virus virulence in a murine intranasal model. Virology. 1995;206(1):136-47. PubMed PMID: 7831769.

493. Zhou Q, Snipas S, Orth K, Muzio M, Dixit VM, Salvesen GS. Target protease specificity of the viral serpin CrmA. Analysis of five caspases. The Journal of biological chemistry. 1997;272(12):7797-800. PubMed PMID: 9065443.

494. Palucka K, Banchereau J, Mellman I. Designing vaccines based on biology of human dendritic cell subsets. Immunity. 2010;33(4):464-78. Epub 2010/10/30. doi: 10.1016/j.immuni.2010.10.007. PubMed PMID: 21029958; PMCID: PMC2975953.

495. Karrich JJ, Jachimowski LC, Uittenbogaart CH, Blom B. The plasmacytoid dendritic cell as the Swiss army knife of the immune system: molecular regulation of its multifaceted functions. Journal of immunology. 2014;193(12):5772-8. Epub 2014/12/07. doi: 10.4049/jimmunol.1401541. PubMed PMID: 25480956; PMCID: PMC4258904.

496. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, Chen CJ, Dunbar PR, Wadley RB, Jeet V, Vulink AJ, Hart DN, Radford KJ. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. The Journal of experimental medicine. 2010;207(6):1247-60. Epub 2010/05/19. doi: 10.1084/jem.20092140. PubMed PMID: 20479116; PMCID: PMC2882828.

497. Meixlsperger S, Leung CS, Ramer PC, Pack M, Vanoaica LD, Breton G, Pascolo S, Salazar AM, Dzionek A, Schmitz J, Steinman RM, Munz C. CD141+ dendritic cells produce prominent amounts of

IFN-alpha after dsRNA recognition and can be targeted via DEC-205 in humanized mice. Blood. 2013;121(25):5034-44. Epub 2013/03/14. doi: 10.1182/blood-2012-12-473413. PubMed PMID: 23482932; PMCID: PMC3689250.

498. Segura E, Valladeau-Guilemond J, Donnadieu MH, Sastre-Garau X, Soumelis V, Amigorena S. Characterization of resident and migratory dendritic cells in human lymph nodes. The Journal of experimental medicine. 2012;209(4):653-60. Epub 2012/03/21. doi: 10.1084/jem.20111457. PubMed PMID: 22430490; PMCID: PMC3328358.

499. Bachem A, Guttler S, Hartung E, Ebstein F, Schaefer M, Tannert A, Salama A, Movassaghi K, Opitz C, Mages HW, Henn V, Kloetzel PM, Gurka S, Kroczek RA. Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. The Journal of experimental medicine. 2010;207(6):1273-81. Epub 2010/05/19. doi: 10.1084/jem.20100348. PubMed PMID: 20479115; PMCID: PMC2882837.

500. Supek F, Bosnjak M, Skunca N, Smuc T. REVIGO summarizes and visualizes long lists of gene ontology terms. PloS one. 2011;6(7):e21800. Epub 2011/07/27. doi: 10.1371/journal.pone.0021800. PubMed PMID: 21789182; PMCID: PMC3138752.

501. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(43):15545-50. Epub 2005/10/04. doi: 10.1073/pnas.0506580102. PubMed PMID: 16199517; PMCID: PMC1239896.

502. Pantaleo G, Esteban M, Jacobs B, Tartaglia J. Poxvirus vector-based HIV vaccines. Current opinion in HIV and AIDS. 2010;5(5):391-6. Epub 2010/10/28. doi: 10.1097/COH.0b013e32833d1e87. PubMed PMID: 20978379.

503. Sanchez-Sampedro L, Perdiguero B, Mejias-Perez E, Garcia-Arriaza J, Di Pilato M, Esteban M. The evolution of poxvirus vaccines. Viruses. 2015;7(4):1726-803. Epub 2015/04/09. doi: 10.3390/v7041726. PubMed PMID: 25853483; PMCID: PMC4411676.

504. Gomez CE, Najera JL, Krupa M, Perdiguero B, Esteban M. MVA and NYVAC as vaccines against emergent infectious diseases and cancer. Curr Gene Ther. 2011;11(3):189-217. PubMed PMID: 21453284.

505. Pasparakis M, Vandenabeele P. Necroptosis and its role in inflammation. Nature. 2015;517(7534):311-20. Epub 2015/01/17. doi: 10.1038/nature14191. PubMed PMID: 25592536.

506. Silke J, Rickard JA, Gerlic M. The diverse role of RIP kinases in necroptosis and inflammation. Nature immunology. 2015;16(7):689-97. doi: 10.1038/ni.3206. PubMed PMID: 26086143.

507. Rock KL, Kono H. The inflammatory response to cell death. Annual review of pathology. 2008;3:99-126. doi: 10.1146/annurev.pathmechdis.3.121806.151456. PubMed PMID: 18039143; PMCID: 3094097.

508. Tam HH, Melo MB, Kang M, Pelet JM, Ruda VM, Foley MH, Hu JK, Kumari S, Crampton J, Baldeon AD, Sanders RW, Moore JP, Crotty S, Langer R, Anderson DG, Chakraborty AK, Irvine DJ. Sustained antigen availability during germinal center initiation enhances antibody responses to vaccination. Proceedings of the National Academy of Sciences of the United States of America. 2016;113(43):E6639-E48. Epub 2016/10/30. doi: 10.1073/pnas.1606050113. PubMed PMID: 27702895; PMCID: PMC5086995.

509. Postigo A, Cross JR, Downward J, Way M. Interaction of F1L with the BH3 domain of Bak is responsible for inhibiting vaccinia-induced apoptosis. Cell death and differentiation. 2006;13(10):1651-62. Epub 2006/01/28. doi: 10.1038/sj.cdd.4401853. PubMed PMID: 16439990.

510. Wasilenko ST, Banadyga L, Bond D, Barry M. The vaccinia virus F1L protein interacts with the proapoptotic protein Bak and inhibits Bak activation. Journal of virology. 2005;79(22):14031-43. Epub 2005/10/29. doi: 10.1128/JVI.79.22.14031-14043.2005. PubMed PMID: 16254338; PMCID: PMC1280199.

511. Zhai D, Yu E, Jin C, Welsh K, Shiau CW, Chen L, Salvesen GS, Liddington R, Reed JC. Vaccinia virus protein F1L is a caspase-9 inhibitor. The Journal of biological chemistry.

2010;285(8):5569-80. Epub 2009/12/22. doi: 10.1074/jbc.M109.078113. PubMed PMID: 20022954; PMCID: PMC2820784.

512. Dai P, Wang W, Cao H, Avogadri F, Dai L, Drexler I, Joyce JA, Li XD, Chen Z, Merghoub T, Shuman S, Deng L. Modified vaccinia virus Ankara triggers type I IFN production in murine conventional dendritic cells via a cGAS/STING-mediated cytosolic DNA-sensing pathway. PLoS pathogens. 2014;10(4):e1003989. Epub 2014/04/20. doi: 10.1371/journal.ppat.1003989. PubMed PMID: 24743339; PMCID: PMC3990710.

513. Zhang Z, Yuan B, Bao M, Lu N, Kim T, Liu YJ. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. Nature immunology. 2011;12(10):959-65. Epub 2011/09/06. doi: 10.1038/ni.2091. PubMed PMID: 21892174; PMCID: PMC3671854.

514. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35(4):495-516. Epub 2007/06/15. doi: 10.1080/01926230701320337. PubMed PMID: 17562483; PMCID: PMC2117903.

515. Swanson CL, Wilson TJ, Strauch P, Colonna M, Pelanda R, Torres RM. Type I IFN enhances follicular B cell contribution to the T cell-independent antibody response. The Journal of experimental medicine. 2010;207(7):1485-500. Epub 2010/06/23. doi: 10.1084/jem.20092695. PubMed PMID: 20566717; PMCID: PMC2901065.

516. Coro ES, Chang WL, Baumgarth N. Type I IFN receptor signals directly stimulate local B cells early following influenza virus infection. Journal of immunology. 2006;176(7):4343-51. Epub 2006/03/21. PubMed PMID: 16547272.

517. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF. Type i interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity. 2001;14(4):461-70. Epub 2001/05/05. PubMed PMID: 11336691.

518. Jego G, Palucka AK, Blanck JP, Chalouni C, Pascual V, Banchereau J. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. Immunity. 2003;19(2):225-34. Epub 2003/08/23. PubMed PMID: 12932356.

519. McLellan JS, Pancera M, Carrico C, Gorman J, Julien JP, Khayat R, Louder R, Pejchal R, Sastry M, Dai K, O'Dell S, Patel N, Shahzad-ul-Hussan S, Yang Y, Zhang B, Zhou T, Zhu J, Boyington JC, Chuang GY, Diwanji D, Georgiev I, Kwon YD, Lee D, Louder MK, Moquin S, Schmidt SD, Yang ZY, Bonsignori M, Crump JA, Kapiga SH, Sam NE, Haynes BF, Burton DR, Koff WC, Walker LM, Phogat S, Wyatt R, Orwenyo J, Wang LX, Arthos J, Bewley CA, Mascola JR, Nabel GJ, Schief WR, Ward AB, Wilson IA, Kwong PD. Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. Nature. 2011;480(7377):336-43. Epub 2011/11/25. doi: 10.1038/nature10696. PubMed PMID: 22113616; PMCID: PMC3406929.

520. Bosinger SE, Li Q, Gordon SN, Klatt NR, Duan L, Xu L, Francella N, Sidahmed A, Smith AJ, Cramer EM, Zeng M, Masopust D, Carlis JV, Ran L, Vanderford TH, Paiardini M, Isett RB, Baldwin DA, Else JG, Staprans SI, Silvestri G, Haase AT, Kelvin DJ. Global genomic analysis reveals rapid control of a robust innate response in SIV-infected sooty mangabeys. The Journal of clinical investigation. 2009;119(12):3556-72. Epub 2009/12/05. doi: 10.1172/JCI40115. PubMed PMID: 19959874; PMCID: PMC2786806.

521. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-504. Epub 2003/11/05. doi: 10.1101/gr.1239303. PubMed PMID: 14597658; PMCID: PMC403769.

522. Velu V, Kannanganat S, Ibegbu C, Chennareddi L, Villinger F, Freeman GJ, Ahmed R, Amara RR. Elevated expression levels of inhibitory receptor programmed death 1 on simian immunodeficiency virus-specific CD8 T cells during chronic infection but not after vaccination. Journal of virology. 2007;81(11):5819-28. doi: 10.1128/JVI.00024-07. PubMed PMID: 17376899; PMCID: 1900286.

523. Petrovas C, Price DA, Mattapallil J, Ambrozak DR, Geldmacher C, Cecchinato V, Vaccari M, Tryniszewska E, Gostick E, Roederer M, Douek DC, Morgan SH, Davis SJ, Franchini G, Koup RA. SIV-specific CD8+ T cells express high levels of PD1 and cytokines but have impaired proliferative capacity

in acute and chronic SIVmac251 infection. Blood. 2007;110(3):928-36. doi: 10.1182/blood-2007-01-069112. PubMed PMID: 17440051; PMCID: 1924769.

524. Porichis F, Hart MG, Massa A, Everett HL, Morou A, Richard J, Brassard N, Veillette M, Hassan M, Ly NL, Routy JP, Freeman GJ, Dube M, Finzi A, Kaufmann DE. Immune Checkpoint Blockade Restores HIV-Specific CD4 T Cell Help for NK Cells. Journal of immunology. 2018. Epub 2018/06/24. doi: 10.4049/jimmunol.1701551. PubMed PMID: 29934472.

525. Ndhlovu ZM, Kamya P, Mewalal N, Kloverpris HN, Nkosi T, Pretorius K, Laher F, Ogunshola F, Chopera D, Shekhar K, Ghebremichael M, Ismail N, Moodley A, Malik A, Leslie A, Goulder PJ, Buus S, Chakraborty A, Dong K, Ndung'u T, Walker BD. Magnitude and Kinetics of CD8+ T Cell Activation during Hyperacute HIV Infection Impact Viral Set Point. Immunity. 2015;43(3):591-604. doi: 10.1016/j.immuni.2015.08.012. PubMed PMID: 26362266; PMCID: PMC4575777.

526. Radebe M, Gounder K, Mokgoro M, Ndhlovu ZM, Mncube Z, Mkhize L, van der Stok M, Jaggernath M, Walker BD, Ndung'u T. Broad and persistent Gag-specific CD8+ T-cell responses are associated with viral control but rarely drive viral escape during primary HIV-1 infection. Aids. 2015;29(1):23-33. doi: 10.1097/QAD.0000000000000508. PubMed PMID: 25387316.

527. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, Savage KJ, Hernberg MM, Lebbe C, Charles J, Mihalcioiu C, Chiarion-Sileni V, Mauch C, Cognetti F, Arance A, Schmidt H, Schadendorf D, Gogas H, Lundgren-Eriksson L, Horak C, Sharkey B, Waxman IM, Atkinson V, Ascierto PA. Nivolumab in previously untreated melanoma without BRAF mutation. The New England journal of medicine. 2015;372(4):320-30. doi: 10.1056/NEJMoa1412082. PubMed PMID: 25399552.

528. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, Hoeller C, Khushalani NI, Miller WH, Jr., Lao CD, Linette GP, Thomas L, Lorigan P, Grossmann KF, Hassel JC, Maio M, Sznol M, Ascierto PA, Mohr P, Chmielowski B, Bryce A, Svane IM, Grob JJ, Krackhardt AM, Horak C, Lambert A, Yang AS, Larkin J. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3

trial. Lancet Oncol. 2015;16(4):375-84. doi: 10.1016/S1470-2045(15)70076-8. PubMed PMID: 25795410.

529. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, Hodi FS, Schachter J, Pavlick AC, Lewis KD, Cranmer LD, Blank CU, O'Day SJ, Ascierto PA, Salama AK, Margolin KA, Loquai C, Eigentler TK, Gangadhar TC, Carlino MS, Agarwala SS, Moschos SJ, Sosman JA, Goldinger SM, Shapira-Frommer R, Gonzalez R, Kirkwood JM, Wolchok JD, Eggermont A, Li XN, Zhou W, Zernhelt AM, Lis J, Ebbinghaus S, Kang SP, Daud A. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol. 2015;16(8):908-18. doi: 10.1016/S1470-2045(15)00083-2. PubMed PMID: 26115796.

530. Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, Hamid O, Robert C, Ascierto PA, Richards JM, Lebbe C, Ferraresi V, Smylie M, Weber JS, Maio M, Bastholt L, Mortier L, Thomas L, Tahir S, Hauschild A, Hassel JC, Hodi FS, Taitt C, de Pril V, de Schaetzen G, Suciu S, Testori A. Prolonged Survival in Stage III Melanoma with Ipilimumab Adjuvant Therapy. The New England journal of medicine. 2016;375(19):1845-55. doi: 10.1056/NEJMoa1611299. PubMed PMID: 27717298; PMCID: PMC5648545.

531. Wightman F, Solomon A, Kumar SS, Urriola N, Gallagher K, Hiener B, Palmer S, McNeil C, Garsia R, Lewin SR. Effect of ipilimumab on the HIV reservoir in an HIV-infected individual with metastatic melanoma. Aids. 2015;29(4):504-6. doi: 10.1097/QAD.0000000000000562. PubMed PMID: 25628259.

532. Simeone E, Gentilcore G, Giannarelli D, Grimaldi AM, Caraco C, Curvietto M, Esposito A, Paone M, Palla M, Cavalcanti E, Sandomenico F, Petrillo A, Botti G, Fulciniti F, Palmieri G, Queirolo P, Marchetti P, Ferraresi V, Rinaldi G, Pistillo MP, Ciliberto G, Mozzillo N, Ascierto PA. Immunological and biological changes during ipilimumab treatment and their potential correlation with clinical response and survival in patients with advanced melanoma. Cancer immunology, immunotherapy : CII. 2014;63(7):675-83. doi: 10.1007/s00262-014-1545-8. PubMed PMID: 24695951.

533. Mylvaganam GH, Velu V, Hong JJ, Sadagopal S, Kwa S, Basu R, Lawson B, Villinger F, Amara RR. Diminished Viral Control during Simian Immunodeficiency Virus Infection Is Associated with Aberrant PD-1hi CD4 T Cell Enrichment in the Lymphoid Follicles of the Rectal Mucosa. Journal of immunology. 2014;193(9):4527-36. doi: 10.4049/jimmunol.1401222. PubMed PMID: 25246494; PMCID: 4201952.

534. Jiang G, Mendes EA, Kaiser P, Wong DP, Tang Y, Cai I, Fenton A, Melcher GP, Hildreth JE, Thompson GR, Wong JK, Dandekar S. Synergistic Reactivation of Latent HIV Expression by Ingenol-3-Angelate, PEP005, Targeted NF-kB Signaling in Combination with JQ1 Induced p-TEFb Activation. PLoS pathogens. 2015;11(7):e1005066. doi: 10.1371/journal.ppat.1005066. PubMed PMID: 26225771; PMCID: PMC4520526.

535. Chan JK, Greene WC. NF-kappaB/Rel: agonist and antagonist roles in HIV-1 latency. Current opinion in HIV and AIDS. 2011;6(1):12-8. doi: 10.1097/COH.0b013e32834124fd. PubMed PMID: 21242888; PMCID: PMC3493179.

536. DaFonseca S, Chomont, N., El Far, M., Boulassel, R., Routy, J. and Sekaly, R., editor. Purging the HIV-1 reservoir through the disruption of the PD-1 pathway. Journal of the International AIDS Society; 2010.

537. Pauken KE, Sammons MA, Odorizzi PM, Manne S, Godec J, Khan O, Drake AM, Chen Z, Sen DR, Kurachi M, Barnitz RA, Bartman C, Bengsch B, Huang AC, Schenkel JM, Vahedi G, Haining WN, Berger SL, Wherry EJ. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. Science. 2016;354(6316):1160-5. Epub 2016/10/30. doi: 10.1126/science.aaf2807. PubMed PMID: 27789795; PMCID: PMC5484795.

538. Im SJ, Hashimoto M, Gerner MY, Lee J, Kissick HT, Burger MC, Shan Q, Hale JS, Lee J, Nasti TH, Sharpe AH, Freeman GJ, Germain RN, Nakaya HI, Xue HH, Ahmed R. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. Nature. 2016. doi: 10.1038/nature19330. PubMed PMID: 27501248.

539. Sandler NG, Bosinger SE, Estes JD, Zhu RT, Tharp GK, Boritz E, Levin D, Wijeyesinghe S, Makamdop KN, del Prete GQ, Hill BJ, Timmer JK, Reiss E, Yarden G, Darko S, Contijoch E, Todd JP, Silvestri G, Nason M, Norgren RB, Jr., Keele BF, Rao S, Langer JA, Lifson JD, Schreiber G, Douek DC. Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. Nature. 2014;511(7511):601-5. doi: 10.1038/nature13554. PubMed PMID: 25043006.

540. Jacquelin B, Mayau V, Targat B, Liovat AS, Kunkel D, Petitjean G, Dillies MA, Roques P, Butor C, Silvestri G, Giavedoni LD, Lebon P, Barre-Sinoussi F, Benecke A, Muller-Trutwin MC. Nonpathogenic SIV infection of African green monkeys induces a strong but rapidly controlled type I IFN response. The Journal of clinical investigation. 2009;119(12):3544-55. doi: 10.1172/JCI40093. PubMed PMID: 19959873; PMCID: PMC2786805.

541. Stacey AR, Norris PJ, Qin L, Haygreen EA, Taylor E, Heitman J, Lebedeva M, DeCamp A, Li D, Grove D, Self SG, Borrow P. Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. Journal of virology. 2009;83(8):3719-33. doi: 10.1128/JVI.01844-08. PubMed PMID: 19176632; PMCID: PMC2663284.

542. Cheng L, Ma J, Li J, Li D, Li G, Li F, Zhang Q, Yu H, Yasui F, Ye C, Tsao LC, Hu Z, Su L, Zhang L. Blocking type I interferon signaling enhances T cell recovery and reduces HIV-1 reservoirs. The Journal of clinical investigation. 2017;127(1):269-79. doi: 10.1172/JCI90745. PubMed PMID: 27941247; PMCID: PMC5199717.

543. Harris LD, Tabb B, Sodora DL, Paiardini M, Klatt NR, Douek DC, Silvestri G, Muller-Trutwin M, Vasile-Pandrea I, Apetrei C, Hirsch V, Lifson J, Brenchley JM, Estes JD. Downregulation of robust acute type I interferon responses distinguishes nonpathogenic simian immunodeficiency virus (SIV) infection of natural hosts from pathogenic SIV infection of rhesus macaques. Journal of virology. 2010;84(15):7886-91. doi: 10.1128/JVI.02612-09. PubMed PMID: 20484518; PMCID: PMC2897601.

544. McGary CS, Deleage C, Harper J, Micci L, Ribeiro SP, Paganini S, Kuri-Cervantes L, Benne C, Ryan ES, Balderas R, Jean S, Easley K, Marconi V, Silvestri G, Estes JD, Sekaly RP, Paiardini M.

CTLA-4(+)PD-1(-) Memory CD4(+) T Cells Critically Contribute to Viral Persistence in Antiretroviral Therapy-Suppressed, SIV-Infected Rhesus Macaques. Immunity. 2017;47(4):776-88 e5. Epub 2017/10/19. doi: 10.1016/j.immuni.2017.09.018. PubMed PMID: 29045906; PMCID: PMC5679306.

545. d'Ettorre G, Baroncelli S, Micci L, Ceccarelli G, Andreotti M, Sharma P, Fanello G, Fiocca F, Cavallari EN, Giustini N, Mallano A, Galluzzo CM, Vella S, Mastroianni CM, Silvestri G, Paiardini M, Vullo V. Reconstitution of intestinal CD4 and Th17 T cells in antiretroviral therapy suppressed HIV-infected subjects: implication for residual immune activation from the results of a clinical trial. PloS one. 2014;9(10):e109791. doi: 10.1371/journal.pone.0109791. PubMed PMID: 25340778; PMCID: 4207675.

546. Ortiz AM, Klase ZA, DiNapoli SR, Vujkovic-Cvijin I, Carmack K, Perkins MR, Calantone N, Vinton CL, Riddick NE, Gallagher J, Klatt NR, McCune JM, Estes JD, Paiardini M, Brenchley JM. IL-21 and probiotic therapy improve Th17 frequencies, microbial translocation, and microbiome in ARV-treated, SIV-infected macaques. Mucosal immunology. 2015. doi: 10.1038/mi.2015.75. PubMed PMID: 26286233.

547. Pallikkuth S, Micci L, Ende ZS, Iriele RI, Cervasi B, Lawson B, McGary CS, Rogers KA, Else JG, Silvestri G, Easley K, Estes JD, Villinger F, Pahwa S, Paiardini M. Maintenance of intestinal Th17 cells and reduced microbial translocation in SIV-infected rhesus macaques treated with interleukin (IL)-21. PLoS pathogens. 2013;9(7):e1003471. doi: 10.1371/journal.ppat.1003471. PubMed PMID: 23853592; PMCID: 3701718.

548. Micci L, Ryan ES, Fromentin R, Bosinger SE, Harper JL, He T, Paganini S, Easley KA, Chahroudi A, Benne C, Gumber S, McGary CS, Rogers KA, Deleage C, Lucero C, Byrareddy SN, Apetrei C, Estes JD, Lifson JD, Piatak M, Jr., Chomont N, Villinger F, Silvestri G, Brenchley JM, Paiardini M. Interleukin-21 combined with ART reduces inflammation and viral reservoir in SIV-infected macaques. The Journal of clinical investigation. 2015;125(12):4497-513. doi: 10.1172/JCI81400. PubMed PMID: 26551680; PMCID: 4665780.

549. Shen A, Zink MC, Mankowski JL, Chadwick K, Margolick JB, Carruth LM, Li M, Clements JE, Siliciano RF. Resting CD4+ T lymphocytes but not thymocytes provide a latent viral reservoir in a simian

immunodeficiency virus-Macaca nemestrina model of human immunodeficiency virus type 1-infected patients on highly active antiretroviral therapy. Journal of virology. 2003;77(8):4938-49. PubMed PMID: 12663799; PMCID: 152116.

550. Jones RB, Mueller S, O'Connor R, Rimpel K, Sloan DD, Karel D, Wong HC, Jeng EK, Thomas AS, Whitney JB, Lim SY, Kovacs C, Benko E, Karandish S, Huang SH, Buzon MJ, Lichterfeld M, Irrinki A, Murry JP, Tsai A, Yu H, Geleziunas R, Trocha A, Ostrowski MA, Irvine DJ, Walker BD. A Subset of Latency-Reversing Agents Expose HIV-Infected Resting CD4+ T-Cells to Recognition by Cytotoxic T-Lymphocytes. PLoS pathogens. 2016;12(4):e1005545. doi: 10.1371/journal.ppat.1005545. PubMed PMID: 27082643; PMCID: PMC4833318.

551. Nasr N, Alshehri AA, Wright TK, Shahid M, Heiner BM, Harman AN, Botting RA, Helbig KJ, Beard MR, Suzuki K, Kelleher AD, Hertzog P, Cunningham AL. Mechanism of Interferon-Stimulated Gene Induction in HIV-1-Infected Macrophages. Journal of virology. 2017;91(20). Epub 2017/08/05. doi: 10.1128/JVI.00744-17. PubMed PMID: 28768867; PMCID: PMC5625512.

552. Rempel H, Sun B, Calosing C, Pillai SK, Pulliam L. Interferon-alpha drives monocyte gene expression in chronic unsuppressed HIV-1 infection. Aids. 2010;24(10):1415-23. Epub 2010/05/25. doi: 10.1097/QAD.0b013e32833ac623. PubMed PMID: 20495440; PMCID: PMC2991092.

553. Said EA, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M, Hill BJ, Noto A, Ancuta P, Peretz Y, Fonseca SG, Van Grevenynghe J, Boulassel MR, Bruneau J, Shoukry NH, Routy JP, Douek DC, Haddad EK, Sekaly RP. Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. Nature medicine. 2010;16(4):452-9. Epub 2010/03/09. doi: 10.1038/nm.2106. PubMed PMID: 20208540; PMCID: PMC4229134.

554. Evans VA, van der Sluis RM, Solomon A, Dantanarayana A, McNeil C, Garsia R, Palmer S, Fromentin R, Chomont N, Sekaly RP, Cameron PU, Lewin SR. Programmed cell death-1 contributes to the establishment and maintenance of HIV-1 latency. Aids. 2018;32(11):1491-7. doi: 10.1097/QAD.0000000000001849. PubMed PMID: 29746296; PMCID: PMC6026054.

555. Alsaab HO, Sau S, Alzhrani R, Tatiparti K, Bhise K, Kashaw SK, Iyer AK. PD-1 and PD-L1 Checkpoint Signaling Inhibition for Cancer Immunotherapy: Mechanism, Combinations, and Clinical Outcome. Front Pharmacol. 2017;8:561. Epub 2017/09/08. doi: 10.3389/fphar.2017.00561. PubMed PMID: 28878676; PMCID: PMC5572324.

556. Jenkins RW, Barbie DA, Flaherty KT. Mechanisms of resistance to immune checkpoint inhibitors. British journal of cancer. 2018;118(1):9-16. Epub 2018/01/11. doi: 10.1038/bjc.2017.434. PubMed PMID: 29319049; PMCID: PMC5765236.

557. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, Fidelle M, Flament C, Poirier-Colame V, Opolon P, Klein C, Iribarren K, Mondragon L, Jacquelot N, Qu B, Ferrere G, Clemenson C, Mezquita L, Masip JR, Naltet C, Brosseau S, Kaderbhai C, Richard C, Rizvi H, Levenez F, Galleron N, Quinquis B, Pons N, Ryffel B, Minard-Colin V, Gonin P, Soria JC, Deutsch E, Loriot Y, Ghiringhelli F, Zalcman G, Goldwasser F, Escudier B, Hellmann MD, Eggermont A, Raoult D, Albiges L, Kroemer G, Zitvogel L. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science. 2018;359(6371):91-7. Epub 2017/11/04. doi: 10.1126/science.aan3706. PubMed PMID: 29097494.

558. Laforge M, Silvestre R, Rodrigues V, Garibal J, Campillo-Gimenez L, Mouhamad S, Monceaux V, Cumont MC, Rabezanahary H, Pruvost A, Cordeiro-da-Silva A, Hurtrel B, Silvestri G, Senik A, Estaquier J. The anti-caspase inhibitor Q-VD-OPH prevents AIDS disease progression in SIV-infected rhesus macaques. The Journal of clinical investigation. 2018;128(4):1627-40. Epub 2018/03/20. doi: 10.1172/JCI95127. PubMed PMID: 29553486; PMCID: PMC5873886.

559. Dorfman DM, Brown JA, Shahsafaei A, Freeman GJ. Programmed death-1 (PD-1) is a marker of germinal center-associated T cells and angioimmunoblastic T-cell lymphoma. Am J Surg Pathol. 2006;30(7):802-10. Epub 2006/07/05. doi: 10.1097/01.pas.0000209855.28282.ce. PubMed PMID: 16819321; PMCID: PMC3137919.

560. Naito M, Hainz U, Burkhardt UE, Fu B, Ahove D, Stevenson KE, Rajasagi M, Zhu B, Alonso A, Witten E, Matsuoka K, Neuberg D, Duke-Cohan JS, Wu CJ, Freeman GJ. CD40L-Tri, a novel

formulation of recombinant human CD40L that effectively activates B cells. Cancer immunology, immunotherapy : CII. 2013;62(2):347-57. Epub 2012/08/29. doi: 10.1007/s00262-012-1331-4. PubMed PMID: 22926059; PMCID: PMC3569584.

561. Iyer SS, Gangadhara S, Victor B, Gomez R, Basu R, Hong JJ, Labranche C, Montefiori DC, Villinger F, Moss B, Amara RR. Codelivery of Envelope Protein in Alum with MVA Vaccine Induces CXCR3-Biased CXCR5+ and CXCR5- CD4 T Cell Responses in Rhesus Macaques. Journal of immunology. 2015;195(3):994-1005. doi: 10.4049/jimmunol.1500083. PubMed PMID: 26116502; PMCID: 4506863.

562. Amara RR, Villinger F, Altman JD, Lydy SL, O'Neil SP, Staprans SI, Montefiori DC, Xu Y, Herndon JG, Wyatt LS, Candido MA, Kozyr NL, Earl PL, Smith JM, Ma HL, Grimm BD, Hulsey ML, Miller J, McClure HM, McNicholl JM, Moss B, Robinson HL. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. Science. 2001;292(5514):69-74. PubMed PMID: 11393868.

563. Sereti I, Estes JD, Thompson WL, Morcock DR, Fischl MA, Croughs T, Beq S, Lafaye de Micheaux S, Yao MD, Ober A, Wilson EM, Natarajan V, Imamichi H, Boulassel MR, Lederman MM, Routy JP. Decreases in colonic and systemic inflammation in chronic HIV infection after IL-7 administration. PLoS pathogens. 2014;10(1):e1003890. doi: 10.1371/journal.ppat.1003890. PubMed PMID: 24497828; PMCID: PMC3907377.

564. Roederer M, Nozzi JL, Nason MC. SPICE: exploration and analysis of post-cytometric complex multivariate datasets. Cytometry Part A : the journal of the International Society for Analytical Cytology. 2011;79(2):167-74. doi: 10.1002/cyto.a.21015. PubMed PMID: 21265010; PMCID: 3072288.

565. Chea LS, Amara RR. Immunogenicity and efficacy of DNA/MVA HIV vaccines in rhesus macaque models. Expert review of vaccines. 2017;16(10):973-85. Epub 2017/08/26. doi: 10.1080/14760584.2017.1371594. PubMed PMID: 28838267; PMCID: PMC6120759.

566. Sanders RW, Derking R, Cupo A, Julien JP, Yasmeen A, de Val N, Kim HJ, Blattner C, de la Pena AT, Korzun J, Golabek M, de Los Reyes K, Ketas TJ, van Gils MJ, King CR, Wilson IA, Ward AB,

Klasse PJ, Moore JP. A next-generation cleaved, soluble HIV-1 Env trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. PLoS pathogens. 2013;9(9):e1003618. doi: 10.1371/journal.ppat.1003618. PubMed PMID: 24068931; PMCID: PMC3777863.

567. Zolla-Pazner S, deCamp AC, Cardozo T, Karasavvas N, Gottardo R, Williams C, Morris DE, Tomaras G, Rao M, Billings E, Berman P, Shen X, Andrews C, O'Connell RJ, Ngauy V, Nitayaphan S, de Souza M, Korber B, Koup R, Bailer RT, Mascola JR, Pinter A, Montefiori D, Haynes BF, Robb ML, Rerks-Ngarm S, Michael NL, Gilbert PB, Kim JH. Analysis of V2 antibody responses induced in vaccinees in the ALVAC/AIDSVAX HIV-1 vaccine efficacy trial. PloS one. 2013;8(1):e53629. doi: 10.1371/journal.pone.0053629. PubMed PMID: 23349725; PMCID: 3547933.

568. Upton JW, Kaiser WJ, Mocarski ES. Virus inhibition of RIP3-dependent necrosis. Cell host & microbe. 2010;7(4):302-13. Epub 2010/04/24. doi: 10.1016/j.chom.2010.03.006. PubMed PMID: 20413098; PMCID: PMC4279434.

569. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbe C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. The New England journal of medicine. 2010;363(8):711-23. Epub 2010/06/08. doi: 10.1056/NEJMoa1003466. PubMed PMID: 20525992; PMCID: PMC3549297.

570. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. The New England journal of medicine. 2012;366(26):2443-54. Epub 2012/06/05. doi: 10.1056/NEJMoa1200690. PubMed PMID: 22658127; PMCID: PMC3544539.

571. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. The New England journal of medicine. 2012;366(26):2455-65. Epub 2012/06/05. doi: 10.1056/NEJMoa1200694. PubMed PMID: 22658128; PMCID: PMC3563263.

572. Seidel JA, Otsuka A, Kabashima K. Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, Efficacy, and Limitations. Front Oncol. 2018;8:86. Epub 2018/04/13. doi: 10.3389/fonc.2018.00086. PubMed PMID: 29644214; PMCID: PMC5883082.

573. Kamphorst AO, Wieland A, Nasti T, Yang S, Zhang R, Barber DL, Konieczny BT, Daugherty CZ, Koenig L, Yu K, Sica GL, Sharpe AH, Freeman GJ, Blazar BR, Turka LA, Owonikoko TK, Pillai RN, Ramalingam SS, Araki K, Ahmed R. Rescue of exhausted CD8 T cells by PD-1-targeted therapies is CD28-dependent. Science. 2017;355(6332):1423-7. Epub 2017/03/11. doi: 10.1126/science.aaf0683. PubMed PMID: 28280249; PMCID: PMC5595217.

574. Hui E, Cheung J, Zhu J, Su X, Taylor MJ, Wallweber HA, Sasmal DK, Huang J, Kim JM, Mellman I, Vale RD. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. Science. 2017;355(6332):1428-33. Epub 2017/03/11. doi: 10.1126/science.aaf1292. PubMed PMID: 28280247; PMCID: PMC6286077.

575. Cecchinato V, Tryniszewska E, Ma ZM, Vaccari M, Boasso A, Tsai WP, Petrovas C, Fuchs D, Heraud JM, Venzon D, Shearer GM, Koup RA, Lowy I, Miller CJ, Franchini G. Immune activation driven by CTLA-4 blockade augments viral replication at mucosal sites in simian immunodeficiency virus infection. Journal of immunology. 2008;180(8):5439-47. Epub 2008/04/09. PubMed PMID: 18390726; PMCID: PMC2768121.

576. Hryniewicz A, Boasso A, Edghill-Smith Y, Vaccari M, Fuchs D, Venzon D, Nacsa J, Betts MR, Tsai WP, Heraud JM, Beer B, Blanset D, Chougnet C, Lowy I, Shearer GM, Franchini G. 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; PMCID: PMC1895471.

577. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haanen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, Long GV, Callahan MK, Postow MA, Grossmann K, Sznol M, Dreno B, Bastholt L, Yang A, Rollin LM, Horak C, Hodi FS, Wolchok JD. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. The New England journal of medicine. 2015;373(1):23-34. Epub 2015/06/02. doi: 10.1056/NEJMoa1504030. PubMed PMID: 26027431; PMCID: PMC5698905.

578. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: Co-inhibitory Receptors with Specialized Functions in Immune Regulation. Immunity. 2016;44(5):989-1004. Epub 2016/05/19. doi: 10.1016/j.immuni.2016.05.001. PubMed PMID: 27192565; PMCID: PMC4942846.

579. Tian X, Zhang A, Qiu C, Wang W, Yang Y, Qiu C, Liu A, Zhu L, Yuan S, Hu H, Wang W, Wei Q, Zhang X, Xu J. The upregulation of LAG-3 on T cells defines a subpopulation with functional exhaustion and correlates with disease progression in HIV-infected subjects. Journal of immunology. 2015;194(8):3873-82. Epub 2015/03/18. doi: 10.4049/jimmunol.1402176. PubMed PMID: 25780040.

580. Tauriainen J, Scharf L, Frederiksen J, Naji A, Ljunggren HG, Sonnerborg A, Lund O, Reyes-Teran G, Hecht FM, Deeks SG, Betts MR, Buggert M, Karlsson AC. Perturbed CD8(+) T cell TIGIT/CD226/PVR axis despite early initiation of antiretroviral treatment in HIV infected individuals. Scientific reports. 2017;7:40354. Epub 2017/01/14. doi: 10.1038/srep40354. PubMed PMID: 28084312; PMCID: PMC5233961.

581. Oxenius A, Price DA, Gunthard HF, Dawson SJ, Fagard C, Perrin L, Fischer M, Weber R, Plana M, Garcia F, Hirschel B, McLean A, Phillips RE. Stimulation of HIV-specific cellular immunity by structured treatment interruption fails to enhance viral control in chronic HIV infection. Proceedings of the National Academy of Sciences of the United States of America. 2002;99(21):13747-52. Epub 2002/10/09. doi: 10.1073/pnas.202372199. PubMed PMID: 12370434; PMCID: PMC129766.

582. Ortiz GM, Wellons M, Brancato J, Vo HT, Zinn RL, Clarkson DE, Van Loon K, Bonhoeffer S, Miralles GD, Montefiori D, Bartlett JA, Nixon DF. Structured antiretroviral treatment interruptions in chronically HIV-1-infected subjects. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(23):13288-93. Epub 2001/11/01. doi: 10.1073/pnas.221452198. PubMed PMID: 11687611; PMCID: PMC60863.

583. Ramirez LA, Arango T, Boyer J. Therapeutic and prophylactic DNA vaccines for HIV-1. Expert opinion on biological therapy. 2013;13(4):563-73. Epub 2013/03/13. doi: 10.1517/14712598.2013.758709. PubMed PMID: 23477730.

584. Yang H, Wallace Z, Dorrell L. Therapeutic Targeting of HIV Reservoirs: How to Give T Cells a New Direction. Frontiers in immunology. 2018;9:2861. Epub 2018/12/20. doi: 10.3389/fimmu.2018.02861. PubMed PMID: 30564246; PMCID: PMC6288286.

585. Fuller DH, Rajakumar PA, Wu MS, McMahon CW, Shipley T, Fuller JT, Bazmi A, Trichel AM, Allen TM, Mothe B, Haynes JR, Watkins DI, Murphey-Corb M. 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.

586. Hel Z, Nacsa J, Tryniszewska E, Tsai WP, Parks RW, Montefiori DC, Felber BK, Tartaglia J, Pavlakis GN, Franchini G. Containment of simian immunodeficiency virus infection in vaccinated macaques: correlation with the magnitude of virus-specific pre- and postchallenge CD4+ and CD8+ T cell responses. Journal of immunology. 2002;169(9):4778-87. Epub 2002/10/23. PubMed PMID: 12391187.

587. De Rose R, Fernandez CS, Smith MZ, Batten CJ, Alcantara S, Peut V, Rollman E, Loh L, Mason RD, Wilson K, Law MG, Handley AJ, Kent SJ. Control of viremia and prevention of AIDS following immunotherapy of SIV-infected macaques with peptide-pulsed blood. PLoS pathogens. 2008;4(5):e1000055. Epub 2008/05/03. doi: 10.1371/journal.ppat.1000055. PubMed PMID: 18451982; PMCID: PMC2323103.

588. Sneller MC, Justement JS, Gittens KR, Petrone ME, Clarridge KE, Proschan MA, Kwan R, Shi V, Blazkova J, Refsland EW, Morris DE, Cohen KW, McElrath MJ, Xu R, Egan MA, Eldridge JH, Benko E, Kovacs C, Moir S, Chun TW, Fauci AS. A randomized controlled safety/efficacy trial of therapeutic vaccination in HIV-infected individuals who initiated antiretroviral therapy early in infection. Science translational medicine. 2017;9(419). Epub 2017/12/08. doi: 10.1126/scitranslmed.aan8848. PubMed PMID: 29212716.

589. Mitsuyasu RT, Anton PA, Deeks SG, Scadden DT, Connick E, Downs MT, Bakker A, Roberts MR, June CH, Jalali S, Lin AA, Pennathur-Das R, Hege KM. Prolonged survival and tissue trafficking following adoptive transfer of CD4zeta gene-modified autologous CD4(+) and CD8(+) T cells in human immunodeficiency virus-infected subjects. Blood. 2000;96(3):785-93. Epub 2000/07/27. PubMed PMID: 10910888.

590. Deeks SG, Wagner B, Anton PA, Mitsuyasu RT, Scadden DT, Huang C, Macken C, Richman DD, Christopherson C, June CH, Lazar R, Broad DF, Jalali S, Hege KM. A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy. Molecular therapy : the journal of the American Society of Gene Therapy. 2002;5(6):788-97. Epub 2002/05/25. doi: 10.1006/mthe.2002.0611. PubMed PMID: 12027564.

591. Leibman RS, Richardson MW, Ellebrecht CT, Maldini CR, Glover JA, Secreto AJ, Kulikovskaya I, Lacey SF, Akkina SR, Yi Y, Shaheen F, Wang J, Dufendach KA, Holmes MC, Collman RG, Payne AS, Riley JL. Supraphysiologic control over HIV-1 replication mediated by CD8 T cells expressing a re-engineered CD4-based chimeric antigen receptor. PLoS pathogens. 2017;13(10):e1006613. Epub 2017/10/13. doi: 10.1371/journal.ppat.1006613. PubMed PMID: 29023549; PMCID: PMC5638568.

592. Ali A, Kitchen SG, Chen ISY, Ng HL, Zack JA, Yang OO. HIV-1-Specific Chimeric Antigen Receptors Based on Broadly Neutralizing Antibodies. Journal of virology. 2016;90(15):6999-7006. Epub 2016/05/27. doi: 10.1128/JVI.00805-16. PubMed PMID: 27226366; PMCID: PMC4944295.

593. Haran KP, Hajduczki A, Pampusch MS, Mwakalundwa G, Vargas-Inchaustegui DA, Rakasz EG, Connick E, Berger EA, Skinner PJ. Simian Immunodeficiency Virus (SIV)-Specific Chimeric Antigen

Receptor-T Cells Engineered to Target B Cell Follicles and Suppress SIV Replication. Frontiers in immunology. 2018;9:492. Epub 2018/04/05. doi: 10.3389/fimmu.2018.00492. PubMed PMID: 29616024; PMCID: PMC5869724.

594. Johnson S, Burke S, Huang L, Gorlatov S, Li H, Wang W, Zhang W, Tuaillon N, Rainey J, Barat B, Yang Y, Jin L, Ciccarone V, Moore PA, Koenig S, Bonvini E. Effector cell recruitment with novel Fv-based dual-affinity re-targeting protein leads to potent tumor cytolysis and in vivo B-cell depletion. J Mol Biol. 2010;399(3):436-49. Epub 2010/04/13. doi: 10.1016/j.jmb.2010.04.001. PubMed PMID: 20382161.

595. Moore PA, Zhang W, Rainey GJ, Burke S, Li H, Huang L, Gorlatov S, Veri MC, Aggarwal S, Yang Y, Shah K, Jin L, Zhang S, He L, Zhang T, Ciccarone V, Koenig S, Bonvini E, Johnson S. Application of dual affinity retargeting molecules to achieve optimal redirected T-cell killing of B-cell lymphoma. Blood. 2011;117(17):4542-51. Epub 2011/02/09. doi: 10.1182/blood-2010-09-306449. PubMed PMID: 21300981.

596. Brozy J, Schlaepfer E, Mueller CKS, Rochat MA, Rampini SK, Myburgh R, Raum T, Kufer P, Baeuerle PA, Muenz M, Speck RF. Antiviral Activity of HIV gp120-Targeting Bispecific T Cell Engager Antibody Constructs. Journal of virology. 2018;92(14). Epub 2018/05/04. doi: 10.1128/JVI.00491-18. PubMed PMID: 29720517; PMCID: PMC6026749.