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# Evaluation of the Humoral/Fc-mediated Immune Responses to an Adenovirus-26 Viral Vector/gp140 Subunit Combined Vaccine Regimen as a Prophylactic HIV-1

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# Evaluation of the Humoral/Fc-mediated Immune Responses to an Adenovirus-26 Viral Vector/gp140 Subunit Combined Vaccine Regimen as a Prophylactic HIV-1


## Abstract

Background and Introduction: HIV is one of the most problematic pandemics to date, currently infecting upwards of 38 million people worldwide ("The Global HIV/AIDS Epidemic," 2020). Although infection and mortality rates have generally decreased, current prophylactic (preventive) measures against HIV-1 acquisition have shown major weaknesses that could be remedied with a vaccine (Pitisuttithum & Marovich, 2020). Manufacturing an effective, prophylactic HIV-1 vaccine, however, is not without challenges - namely design/selection of vaccine-delivered immunogens (antigens) and elicitation of proper immune responses to HIV-1 antigens (Ng'uni et al., 2020). Fortunately, despite past, unsuccessful research, studies within the past 10-15 years have begun to elucidate immune correlates of protection against HIV-1 acquisition, specifically IgG1/IgG3 antibody (Ab) production, as well as non-neutralizing, Fc-Mediated effector functions (Rerks-Ngarm et al., 2009). Excitingly, studies released in the last 3 years, which use Adenovirus serotype-26 (Ad26) vector (Custers et al., 2020) show promising signs that an effective HIV-1 vaccine could become a reality (Baden et al., 2020; Barouch et al., 2018). Thus, for this presentation, I hope to show that Johnson & Johnson's two new Ad26-based vaccines (combined with booster vaccines) sufficiently elicit key immune correlates of protection to warrant an Ad26 vector-based regimen's testing past Phase 1/2a clinical trials. Results and Conclusions: Recent studies that demonstrate the abilities of an Ad26-based vaccine regimen to elicit key immune correlates of protection (while remaining generally safe) are the APPROACH and TRAVERSE studies. From the APPROACH study, it's demonstrated that a regimen consisting of two Ad26 priming injections, followed by two Ad26 boosting injections and two subunit boosting injections (Ad26/Ad26 + HD gp140), elicits more IgG1/IgG3 Abs and ADCP (antibody-dependent cellular phagocytosis) when compared to six alternative candidate regimens. Additionally, when this Ad26/Ad26 + HD gp140 regimen was administered to rhesus macaques, a 94% reduction in disease acquisition was observed (Barouch et al., 2018). Building off of the APPROACH study, the TRAVERSE study demonstrates the effects on immunogenicity when the Ad26 vector's valency is increased. Increase in valency resulted in increased IgG1/IgG3 Ab production, as well as increased phagocytosis.. Promisingly, the breadth of HIV-1 antigens recognized by IgG1/IgG3 Abs also increased when vaccine valency increased as well (Baden et al., 2020). From the results of these two studies then, and the researchers' conclusions from them, it seems appropriate to advance these two new Ad26-based regimens into further clinical trials (Phase 2 and 3).



## Keywords

HIV, viral vectors, vaccine

# Evaluation of the Humoral/Fc-mediated Immune Responses to an Adenovirus-26 Viral Vector/gp140 Subunit Combined Vaccine Regimen as a Prophylactic HIV-1

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## The Global Need for an HIV-1 Vaccine

In order to understand the relevance of a prospective, prophylactic HIV-1 (Human Immunodeficiency Virus type-1) vaccine, as well as the context in which it is being developed and assessed, it is crucial to first have a general understanding of the current, world-wide HIV-1 pandemic. According to data on HIV.gov, it is currently estimated that around 38 million people were infected with both HIV-1 and HIV-2 as of 2019 (though most of these infections are due to HIV-1). Around 1.8 million of these infected individuals (also as of 2019) were children. Additionally, 1.7 million of these overall 38 million were calculated to have become infected during 2019 alone. Fortunately, however, this yearly rate of new infections has decreased a total of 23% since 2010. Regardless, it is also estimated that around 690,000 individuals died, in 2019, due to complications/opportunistic pathogen infections related to HIV/AIDS - down by about 60% since 2004, but still illustrating the urgent need for a preventative vaccine against HIV infection. ("The Global HIV/AIDS Epidemic", 2020).

Most of the noticeable decrease in HIV-related infections has been largely attributed to the effectiveness of PrEP (Pre-exposure Prophylaxis) treatments, such as the well-known medication called Truvada©, which has been measured to reduce the risk of acquiring HIV-1 by an impressive >90%. Oral preventative medications, like Truvada©, appear then - at least at first glance - to negate the need for further research into developing a prophylactic HIV1 vaccine. Afterall, scientists have been attempting to develop a preventative HIV-1 vaccine for well over 35 years now. Why bother attempting to discover an elusive vaccine strategy against HIV-1, especially when an existing medication seems to be fulfilling that niche already? The reason for the continued interest in a prophylactic vaccine, despite the

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successes of medications like Truvada©, is that PrEP treatments have a couple crucial flaws that could be remedied with a vaccine. First, and most importantly, a consistent lack of patient adherence to the strict dosage schedule of PrEP treatments remains a pertinent issue (whether this be due to simple forgetfulness of patients, poorly informed patients, or more cultural factors) leading to significantly decreased protection against infection (Pitisuttithum & Marovich, 2020). In fact, according to an NIH article, during a PrEP study called "iPrEX", researchers discovered that Truvada©, when administered to 2,500 men who have sex with other men (MSM), only provided around a 44% reduction in infection risk when it was not taken as prescribed (daily), as opposed to around a 92% reduction in infection risk when taken daily (National Institutes of Health, 2020). Another critical problem with PrEP, is that it isn't universally available to everyone, only being available to citizens of countries in which the PrEP medication is licensed. For example, Truvada© is only licensed in a handful of countries. Finally, PrEP, oddly enough, has demonstrated varying degrees of effectiveness in women - though it isn't known if this is due to functional differences in PrEP stemming from physiological differences in men or women, or if this is due to differences in adherence between men and women (Pitisuttithum & Marovich, 2020). Therefore, it is evident that a preventative vaccine against HIV-1 is still a crucially needed goal in the fight against the HIV-1 pandemic, especially because an HIV-1 vaccine regimen would be far less burdensome upon the individual - likely only needing a priming shot and a few booster shots to provide protection rather than taking a medication daily (Barouch et al., 2018).

# HIV-1 Pathogenesis: From MALT Infection to AIDS Development

Now having a general understanding of the need for a vaccine, it also important to acquire a broad understanding of what HIV-1 (Human immunodeficiency virus type 1) is, how it's transmitted, the mechanism of its pathogenesis, the disease(s) it causes, and the immune system's response to HIV-1. Understanding these topics will provide background on the disease a prophylactic HIV-1 vaccine would prevent, as well as providing helpful context regarding the environment and challenges in which a vaccine would have to successfully operate in. To begin with, HIV-1 is a retrovirus from the viral family, *Retroviridae*. Being a retrovirus, HIV-1's genome is composed exclusively of RNA rather than DNA. Though there is no unanimous consensus regarding the exact origins of this virus in humans, it is known that HIV began as a virus that infected African primates and later mutated to begin infecting humans within the last 100 years. One favored explanation as to the zoonotic transmission of this virus to humans, suggests that HIV was passed to humans via the consumption of infected chimpanzee meat. It wasn't until the 1980's however, that the virus began spreading globally (Engelman & Cherepanov, 2012). Today, due to the genetic diversity of

HIV-1 strains, HIV-1 is categorized into groups M, N, O, and P (though only group M is of major concern due to it being responsible for the worldwide pandemic). There are also several subtypes ("clades") of HIV-1 including, most-notably, Clade C - which is responsible for over half of all HIV-1 infections world-wide and is especially concentrated in sub-Saharan Africa (Shaw & Hunter, 2012).

Now that the virus is capable of pathogenicity in humans, it is transmitted between humans via a multitude of ways - namely, through infectious bodily fluids. Examples of these fluids include blood, semen, rectal fluids, vaginal fluids, and breast milk (Centers for Disease Control and Prevention). Transmission of HIV, however, is not a unilateral process - not only must an uninfected individual encounter infectious fluid, but those infectious fluids must be able to cross mechanical barriers of a new host's immune system. The most commonly breached mechanical barriers are mucous membranes in various regions of the body. Specifically, one of the most vulnerable mucosal membranes to HIV-1 infection is the anal/rectal mucosa - though vaginal epithelium, urethral epithelium, and the penile cutaneous membrane are also vulnerable to HIV-1 but to a lesser degree. Anal/rectal mucosa is particularly susceptible, because the epithelial layer within this mucosa is composed of only a single layer of simple columnar epithelium (as opposed to the stratified squamous epithelium of the vaginal mucosa and the keratinized stratified squamous epithelium of the penile skin.) This means that micro lacerations, which expose underlying, vulnerable MALT (mucosa-associated lymphoid tissues) underneath the mucosal layers, have a greater propensity to form within the thin anal/rectal mucosa's epithelium as opposed to other, thicker mucosa's epithelial layers (Fox & Fiddler, 2010; Gonzalez et al., 2019).

Since the anal/rectal mucosa is so susceptible, it serves as a good example for the first mechanistic phases of HIV-1 pathogenesis. As mentioned previously, one way in which HIV-1 can cross the anal/rectal mucosa is via micro lacerations (physical tears) in the mucosa itself, allowing HIV-1 viral particles to gain access to the rectal MALT, subsequently leading to the beginning of Acute Infection in resting memory CD4+ T-cells (bystander Helper T-cells) within the MALT. There are, however, alternative means by which HIV-1 viral particles can gain access to the underlying MALT. First, viral particles can simply be transported across the simple columnar epithelium via transcytosis, subsequently coming out of the basal aspect of the epithelium and into the vulnerable MALT. Alternatively, intraepithelial lymphocytes (specifically CD4+ T-cell subtypes) can be directly infected by viral particles approaching the epithelium from the rectal lumen. Furthermore, viral particles can take advantage of microfold cells (Mcells), which normally transcytosis potential antigens to the underlying MALT, via the M-cells' tendency to simply transcytosis the viral particles through the epithelium and into the MALT. Finally, and most interestingly, HIV-1 can utilize the normal immunological function of local, mucosa-resident Dendritic Cells (DCs) in order to pass through the epithelium to gain access to local MALT, as well achieving longer-distance dissemination within the body (Fox & Fiddler, 2010). HIV-1 is capable of binding to a specific Type II Fc$\gamma\gamma$R (Fc-receptor) on DCs called

DC-SIGN (Dendritic Cell-specific Intercellular Adhesion Molecule-3-Grabbing Nonintegrin). Upon binding of gp120 to this receptor, HIV-1 viral particles are endocytosed into the DC and undergo MHC-I and MHC-II antigen processing (Moris et al., 2004) in order that the now activated DC may present (via cross-presentation) viral peptide fragments to both naive CD4+ Tcells and naive CD8+ T-cells in nearby lymph nodes (LNs). Although this process will allow for the activation of the Adaptive Immune system, HIV-1 viral particles can also become embedded and stuck within the numerous glycoproteins on the outside of the activated DCs. As the DCs presenting HIV-1 peptide fragments migrate to nearby LNs, HIV-1 particles can ride along on the outside of the DC, effectively hitching a ride towards vulnerable CD4+ T-cells located within the nearby LNs. Thus, once the activated DC begins presenting to naive Helper Tcells during the clonal selection process, HIV-1 viral particles come in contact with vulnerable Helper T-cells and can subsequently infect those Helper T-cells (the overall process is termed the "trans-infection pathway.") Alternatively, DCs, - due to their own expression of CD4 - are able to be infected by HIV-1 particles (though to a lesser extent than Helper T-cells and typically only in inactivated DCs). This subsequently results in what is known as the "de novo pathway" of infection, whereby new HIV-1 particles bud out from infected DCs and infect nearby Helper T-cells in the local MALT of LNs (Cavrois et al., 2007). Therefore, to this point in the infection process, HIV-1 viral particles have made it past the mucosal barriers and have gained access to vulnerable Helper T-cells in either the MALT or the LNs. It is from here that damage to the immune system can begin and the body's natural immune response to HIV-1 can also start.

Upon exposure of Helper T-cells to HIV-1, envelope glycoprotein spikes (composed of trimers of gp120-gp41 heterodimers) bind CD4 and the coreceptor, CCR5 (or CXCR4). Which coreceptor is utilized depends on the tropism of the HIV-1 strain. Some strains are M-Tropic (utilizing CCR5), some are T-Tropic (utilize CXCR4), and others are Dual-Tropic (utilize CCR5 or CXCR4). More specifically, gp120 subunits bind first to CD4 receptors, thereby inducing aggregation of CD4 and CCR5 (or CXCR4), subsequently leading to the interaction of gp120 subunits and the coreceptor. Gp41 then aids in pulling upon the cell's plasma membrane in order to facilitate the injection of the virus' core into the Helper T-cell (Li & Clercq, 2016; Picchio et al., 1998). Then, once viral Reverse Transcriptase begins converting HIV-1's RNA genome into cDNA, three different intracellular responses are possible within the Helper T-cell (or other CD4+ cell). If the Helper T-cell happens to have already been activated, whether in response to HIV-1 or another antigen, then the cDNA product of Reverse Transcription can act as a PAMP (pathogen-associated molecular pattern) to an intracellular PRR (pattern recognition receptor) known as IFI16 (interferon-inducible protein 16), which then activates a signaling cascade leading to the activation of NF$\kappa\kappa$B - leading to the production of Type-1 IFNs that can activate Natural Killer (NK) cells, as well as warning nearby cells and interfering with viral replication (part of the innate immune response to HIV-1) (Altfeld & Gale, 2015). What's fascinating, however, is that if the infected Helper T-cells isn't previously activated, such as a resting Helper T-cells in the MALT (known as "bystander" Helper T-cells), then the binding of HIV-1 cDNA to IFI16 can

cause the activation of the inflammasome protein complex. The inflammasome complex then activates caspase-1 enzymes to mediate pyroptosis of the resting Helper T-cell. Infact, it has been suggested that the mass-suicide of many bystanders Helper Tcells is the primary driving force of disease progression to AIDS (Doitsh & Greene, 2016). This suicide, however, doesn't occur in all resting Helper T-cells that are infected. Recognition of viral cDNA by IFI16 appears to occur only when there is incomplete reverse transcription or when there are mutations in the viral genome that result in disruptions in HIV-1 capsid (Altfeld & Gale, 2015; Doitsh & Greene, 2016). In cases where viral cDNA goes undetected then, the cDNA can be incorporated into the host cell's genome, thereby leading to the establishment of "viral reservoirs" that can produce new viral particles once the host cell is eventually activated in the future (host cell activation, in fact, is what allows the HIV-1 to maintain and increase in population within a host (Siliciano & Greene, 2011). After partially discussing the complex innate immune response to HIV-1 infection (a topic that will be revisited shortly), as well as the primary means by which Helper T-cells population diminish during HIV-1 infection, it makes sense to go on to briefly discuss the adaptive immune response to HIV-1 infection.

Once DCs have internalized HIV-1 particles and processed them via the MHC-I or MHC-II pathway (as discussed previously), the DCs present HIV-1 peptide fragments to naive T-cells. Once clonal selection and clonal activation of HIV-1-specific Helper and Cytotoxic T-cells occurs, effector T-cells begin their futile attempts to begin combating the spread of the HIV-1 infection. Effector cytotoxic T-cells (CTLs) function similarly to HIV-1 as they do in response to other viral infections. They seek out infected cells (CD4+ leukocytes) that display HIV-1 viral peptides (ones that the particular CTL clone recognizes) being presented on MHC-1 complexes and induce apoptosis of the infected cells. This process is also partially responsible for the overall depletion of Helper T-cell populations (Mohan et al., 2014). Effector Helper T-cells, on the other hand, function primarily in order to help activate HIV-1-recognizing B-cells - which have themselves encountered, endocytosed, processed, and begun presenting HIV-1 peptide fragments on MHCII themselves - that, in turn, produce antibodies against HIV-1 (most notably IgGs and IgAs). Although these Abs can operate to neutralize the HIV-1 particles, as will be discussed in greater detail later, it appears that it is actually non-neutralizing (Fc-mediating), polyfunctional Abs that have the greatest effect in combating HIV-1 infection (Rappocciolo et al., 2006; Su et al., 2019). In past studies on the progression of the humoral response to HIV-1, it appears that non-neutralizing polyfunctional Abs specific to epitopes on gp120 and gp41 are produced first (IgG1s and IgG3s facilitating Fc-mediated effector functions), followed by neutralizing antibodies (NAbs) that are effective in neutralizing the specific strains of HIV-1 that they recognize; though they inevitably struggle to keep up with the constant antigenic drift that continually occurs due to rapidly accumulating mutations, resulting in frequent appearances of "escape variants" that can go undetected by the host's immune response until another primary response capable of recognizing the escape variants can be mounted. Finally, after anywhere from 2-5 years post infection, broadly neutralizing antibodies

(bNAbs) can be produced in some individuals, which can neutralize a broad spectrum of HIV-1 strains (Su et al., 2019; Corti et al., 2010). Now after this brief, yet hopefully sufficient, overview of HIV-1 pathogenesis and immune response mechanisms, it would also be helpful to briefly summarize the disease progression that inevitably results from HIV-1 infection.

Once HIV-1 has entered the body and begun establishing viral reservoirs, Acute HIV-1 Infection occurs. This stage can be accompanied with or without any noticeable signs/symptoms. If symptoms are present, they are usually flu-like in nature as the body begins its immune response against HIV-1. This stage also demonstrates a relatively large "viral load" (measurable amount of virus in the blood). The patient at this stage is considered contagious. The next stage of disease progression is Chronic HIV-1 Infection. Although by this stage the patient is still potentially contagious, the replication rate of HIV-1 decreases significantly, but by this point the CD4+ immune cell populations (most noticeably the Helper T-cell populations) are decreasing - even though most patients are asymptomatic at this point. The third and final stage of disease progression is acquired immunodeficiency syndrome (AIDS). Here, the CD4+ cell count is less than 200 cells/mm^3 of blood, and the individual is increasingly susceptible to death brought on by a broad array of pathogens. For example, *Mycobacterium tuberculosis* is the leading cause of AIDS-related death, owing to the disruption of TB-containing granulomas within tissues like the lungs. Patients with AIDS are also less capable of combating fungal infections caused by pathogens such as *Candida albicans* and *Cryptococcus neoformans* (Vaillant & Naik, 2020). Having in mind, then, the fact that HIV-1 pathogenesis has been the focus of many research projects over the past 35+ years of the HIV-1 global pandemic, it must be asked: "Why has no prophylactic HIV-1 been developed in the decades that global society's been aware of the virus?"

# Over 35 Years of Failure: Why hasn't a Prophylactic HIV-1 Vaccine been Developed Yet?

There are two key answers to this previous question: 1.) the incredible genetic diversity of HIV-1 and 2.) uncertainty regarding which immune responses are responsible for combating HIV-1 infection. The influenza virus is an excellent example of a virus that scientists are struggling to create a universal vaccine against, due primarily to regular antigenic drift that is brought about by the accumulation of relatively small mutations, subsequently leading to a 12% change in genetic diversity between strains each year (Fischer et al., 2007). HIV-1 on the other hand, can demonstrate about 10% genetic variation between strains in a single infected individual and around 35% genetic variation among different HIV-1 clades. Genetic variation in the same clade alone can reach as high as 20% (Corti et al., 2010). This substantial genetic variation introduces a massive problem for vaccine developers who are attempting to immunize patients with antigens (or antigen-

carrying vectors) that would allow the patient's body to build immunological memory against that antigen (and by extension the pathogen to which that antigen is a part). Since variations in genetic sequences, brought about by constant mutations by Reverse Transcriptase, necessarily entail variations in amino acid sequence, which, in turn, entail potential variations in viral antigens' peptide sequences and epitopes, there are simply an overwhelming number of different HIV-1 variants that the body's immune system's B & T-Cells must be able to identify in order to combat the HIV-1 infection. Thus, scientists have been attempting to uncover ways to provide the body with antigens that would "teach" the body how to recognize the incredibly diverse strains of HIV-1. However, finding natural, or synthesizing synthetic, antigens that will stimulate the immune system to react (immunogens) and start building immunologic memory against the numerous HIV-1 strains has been incredibly difficult - especially if a more universal prophylactic HIV-1 vaccine is desired (Ng'uni et al., 2020). Furthermore, even if antigens that prime the immune system for broad coverage of HIV-1 strains are developed, the continuous mutation of HIV-1 is still a major issue. As mentioned before, as antigenic drift occurs in HIV-1, the body's Abs and memory cells are less and less likely to recognize HIV-1 particles due to changing epitopes and peptide sequences, necessitating the immune system to conduct another primary response as "escape mutants" of HIV-1 (variants that the immune system cannot recognize) remain one step ahead of the immune system's ability to recognize them - though some vulnerable strains can still be eliminated by the body (Su et al., 2019).

One way that scientists have been looking to overcome the obstacle of needing to immunize people with antigens that provide broad immunological recognition of circulating HIV-1 strains, as well as the problem of escape mutants, is the "mosaic antigen" method. In this process, several short epitope-encoding DNA sequences (usually around 27 nucleotides long) are selected by a computer program, which chooses these sequences based on which combinations would provide epitopes with the least amount of variability among HIV-1 strains - thereby leading to broader coverage. These selected DNA sequences are then spliced together to constitute a larger DNA sequence that now encodes a sort of "frankenstein antigen" containing epitopes representing multiple HIV-1 clades. These synthetic antigens therefore not only help teach the immune system to recognize more conserved epitopes belonging to multiple HIV-1 clades that may be encountered, but hopefully also help build memory against potential, future escape mutants before those escape mutants even appear - thereby allowing the immune system to be one step ahead of the virus (Fischer et al., 2007). With this potential answer to the problem of HIV-1's pre-existent and ever-developing genetic diversity, it's important to now briefly discuss the aforementioned second challenge to HIV-1 vaccine development.

The second challenge to developing an effective HIV-1 vaccine, is the uncertainty regarding which immune responses should be elicited in order to fight HIV-1 infection. This confusion directly impacts vaccine development, because the particular antigens that are included within a vaccine can impact the type of immune responses that are elicited. For example,

inclusion of only Env (HIV-1 envelope) antigens within a vaccine may only elicit a neutralizing antibody (NAb) response, whereas inclusion of antigens that are encoded by the Gag and Pol HIV-1 genes could also illicit more of a non-neutralizing Ab humoral response (Su et al., 2019). What's remarkable, however, is the fact that debate over which immune responses should be targeted for elicitation by a vaccine has been continuing since the beginning of HIV-1 vaccine development. In fact, towards the beginning of HIV-1 vaccine development, there was an emphasis on attempting to elicit a predominantly humoral response to the vaccine in order to provide protective immunity against HIV-1 acquisition and construct populations of memory B-cells specific to HIV-1. However, as the years of research progressed, a new interest in attempting to elicit more of a cellular immune response (specifically be CTLs) came to the forefront of research; but this too began to fall out of favor after several failed research attempts to elicit CTL responses capable of effectively controlling HIV-1 infection. Though the subject of HIV-1 vaccine research began to seem rather bleak, in 2009, a groundbreaking study performed in Thailand, called the RV144 trial, was published and demonstrated that a viral vector (recombinant canarypox)/gp120 subunit combined vaccine regimen demonstrated moderate efficacy (estimated 60% at 12 months and 31.2% at 42 months) against HIV-1 acquisition in humans. To date, this RV144 vaccine regimen is the only vaccine/vaccine regimen that has demonstrated efficacy against HIV-1, and it naturally sparked a renewed hope in being able to develop a more effective HIV-1 vaccine focused on eliciting the immune responses that were correlated with reduced risk in the RV144 trial (Ng'uni et al., 2020; Zolla-Pazner & Gilbert, 2019; Haynes et al., 2012). The importance of this RV144 trial, and the influence it has wielded upon successive HIV-1 vaccine studies, cannot be overstated. Just a cursory read-through of only a few research articles having to do with HIV-1 vaccine development (ones published since 2009) will demonstrate repeated references to this study, and it is this RV144 study that has proven instrumental to the two vaccine studies that will follow later in this paper. Before moving forwards, however, it would be helpful to briefly describe the findings of the RV144 trial that have influenced much of the more recent research into development of a vaccine.

# Lessons from the RV144 HIV Vaccine Trial

The first critical finding from RV144, was the fact that it appeared IgG Abs, specific to the V1V2 region of the gp120 subunits, were inversely correlated with risk of HIV-1 infection, while IgA Abs specific to other gp120/gp41 epitopes were positively correlated with infection risk - leading to the hypothesis that the IgA Abs were possibly competing with the IgG Abs when binding to HIV-1 particles. The RV144 study also showed that Fc-mediated effector functions, specifically ADCC (Antibody-dependent cellular cytotoxicity), was also inversely correlated with HIV-1 infection risk. Since, therefore, IgG Abs (in particular, IgG1 and IgG3) were known to mediate effector functions like ADCC, it was suggested, based on

the funding's of this study, that non-neutralizing Ab functions (Fc-mediated effector functions), mediated by IgG1 and IgG3, were primarily responsible for the reduction in infection risk as a result of the RV144, rather than neutralizing Ab functions (which normally play an instrumental role in combating other kinds of viral infections.) In other words, RV144 demonstrated that the goal of protective immunity can be achieved without the work of neutralizing Abs (Haynes et al., 2012; Kim et al., 2014). The RV144 trial, however, isn't alone in suggesting the primacy of non-neutralizing, Fc-mediated effector functions in protection against (and control of) HIV-1 infection. Research prior to the RV144 trial already demonstrated that the speed at which HIV-1

disease progression occurs appears to be correlated with the concentrations of ADCC-mediated Abs observed, with those showing higher concentrations of ADCC-mediated Abs progressing much slower toward AIDS than individuals who possessed lower concentrations of ADCC mediating Abs (Baum et al., 1996). Additionally, studies in rhesus macaques, with a very similar virus to HIV-1 (called "SHIV" for Simian-human immunodeficiency virus), have demonstrated that another Fc-mediated function, known as ADCP (Antibody-Dependent Cellular

Phagocytosis), not only increases in activity during successive challenges with virus but is also correlated positively with protection (Barouch et al., 2013). Furthermore, another crucial discovery that has been made concerns the nature of HIV-1-specific immune responses in people known as "elite controllers." Elite controllers are individuals who can effectively control HIV-1 disease progression without the assistance of any antiretroviral therapy (ART). In fact, viremia in these patients is usually very low, if not completely undetectable. Both ADCC activity and Fc-mediating Ab titers have been shown to be more frequent (ADCC) and larger in concentration (Fc-mediating Abs) in Elite controllers as compared to chronically infected nonelite controllers. In fact, in one study ADCC activity was observed to be present in all (100%) of tested elite controllers, but only present in around 40% of non-elite controllers. Additionally, in the same study, ADCC-mediating Ab titers were observed to be at least 10 times higher in the elite controller participants than in the non-elite controller participants (Lambotte et al., 2009). It should be noted, however, that there remains uncertainty as to whether or not the magnitude of ADCC activity and ADCC-mediating Ab titers strictly determines how well an elite controller can suppress viremia. Intriguingly, some studies have demonstrated that elite controllers do not appear to necessarily have increased ADCC activity or higher ADCC mediating Ab titers compared to non-elite controllers. Rather, it seems as though the ability of an elite controller to suppress viremia is based more so on how well the immune system can coordinate the different Fc-mediated effector functions (like ADCC and ADCP) in a synchronized attack against HIV-1 (Ackerman et al., 2016). Since these Fc-mediated effector functions appear so critical to the immune response against HIV-1 (in particular ADCC and ADCP), it would be beneficial to briefly discuss how each of these mechanisms operate.

ADCC (Antibody-dependent cellular cytotoxicity) is a process that is mediated by NK cells and IgG1/IgG3 antibodies. It is focused on the elimination of HIV-1-infected host cells (especially the Helper T-cells). Being an Fc-mediated process, ADCC makes use of two key domains on the IgG1/IgG3 antibodies. First, ADCC utilizes the Fragment Antigen-Binding (Fab) domain on the IgG Ab, which is responsible for binding an antigen's epitope that it recognizes. Epitopes commonly recognized by ADCC-mediating Abs tend to be located on gp120 (such as the V1V2 region, V3 binding loop, or CD4 binding site) or on gp41. The other domain on the IgG Ab that's critical is the Fragment Crystallizable (Fc) domain, which is located in the constant region of the IgG Ab and binds to the Fc-Receptor (Fc$\gamma\gamma$R) on the NK cell. Either when an HIV-1 particle is still attached to the surface of a Helper T-cell when attempting to enter the cell, or when HIV-1 epitopes' peptide fragments are presented on MHC-I by an infected Helper T-cell, the Fab domain on an IgG1 or IgG3 Ab will bind to the viral epitope. Subsequently, as a NK cell passes by the infected cell, the IgG1's (or IgG3's) Fc domain will meet and bind to the Fc$\gamma\gamma$R on the surface of the NK cell. This induces the NK to degranulate, thereby releasing perforin and granzyme enzymes towards the infected cell. The perforin naturally forms holes in the plasma membrane of the infected cell, allowing granzyme enzymes into the cytoplasm of the infected cell, where they subsequently induce apoptosis of the infected cell (Su et al., 2019 & Spicer et al., 2017). ADCC, however, doesn't work alone to combat HIV-1 infection - it's assisted by ADCP.

ADCP (antibody-dependent cellular phagocytosis) is another key Fc-mediated function. ADCP also utilizes the Fab and Fc domains on IgG1 and IgG3 Abs, though the cells that mediate this function are different. Rather than NK cells accomplishing ADCP, macrophages, neutrophils, and DCs are the cells that carry out ADCP. During this process, one of two events can occur. In the first event that can occur, ADCC-mediating Abs can bind free-floating HIV-1 viral particles via the Ab's Fab domain (similar epitopes to the ADCC process are recognized here). If multiple similar Abs bind to one viral particle, an immune complex will form that can be, in turn, phagocytosed by a passing macrophage, PMN, or DC. Alternatively, in the second possible event, the ADCC-mediating Abs can begin coating the surface of a virally infected cell (such as when there are viral particles attached to the cell's surface attempting entry) by binding to viral particles, or MHC-expressed viral epitope fragments, with their Fab domains. Then the Abs' Fc domains can bind to Fc$\gamma\gamma$Rs on passing phagocytes, inducing those phagocytes to consume, and subsequently destroy, the virally infected cells (Su et al., 2019 & Zirui Tay et al, 2019). After explaining both ADCC and ADCP, it must be reiterated that these processes are currently presumed to be crucial to preventing and suppressing HIV-1 infection based upon past studies into the correlates of immune protection against HIV-1. Therefore, a vaccine designed to elicit these immune responses is understood to be desirable, but what type of vaccine would need to be used in order to deliver the proper antigens/immunogens to the body to elicit such immune responses and build immunological memory against HIV-1?

# Adenovirus 26 as a HIV-1 Vaccine Vector

Since live-attenuated and inactivated vaccine strategies have been deemed too risky to pursue as a basis for a prophylactic HIV-1 vaccine, another strategy must be implemented instead. The vaccine strategy that has proven to be safe and most effective in attempting to elicit an immune response to HIV-1 is the viral vector-based vaccine strategy. In fact, this was the strategy that was employed in the RV144 trial, which utilized a recombinant canarypox based vaccine (Ng'uni et al., 2020). Despite the moderate success of the canarypox vector, the viral vector that has been studied, and utilized, most recently is Adenovirus serotype-26 virus

(Ad26). Ad26 is a DNA virus from the *Adenoviridae* family and was first identified back in 1956. What is particularly helpful about this serotype of adenovirus, is the fact that it is far less common than other serotypes of Adenoviruses, such as the far more common Ad5. This fact is important, since it means that people are less likely to already have immunological memory against Ad26, thereby preventing the body from destroying the Ad26 viral vector before it can deliver its immunogenic, transgenic cargo (Custers et al., 2020; Vrba et al., 2020). What's even more fascinating, is the fact that studies, such one conducted by Baden et al. in 2013, demonstrated two important findings about the Ad26 vector they were testing. First, they determined that the Ad26 vector was immunogenic without the need of an accompanying adjuvant. Secondly, and most importantly, Baden et al. discovered that although participants' bodies not only made Abs specific to the HIV-1 antigens the Ad26 vectors were expressing, but also increasingly against the Ad26 vectors, results did not appear to demonstrate that the increase in Ad26-specific Abs interfered noticeably with the titers of HIV-1-specific Abs that were also being produced. Therefore, it was shown that when the body is exposed to an Ad26 vector, it will produce an immune response to both the vector and the expressed antigens, however, the immune response's focus on the vector doesn't seem to prevent the vector from completing its goal of building immunological memory against the HIV-1 antigens (Baden et al., 2013). Despite the apparent effectiveness of the Ad26 viral vector, however, it should also be mentioned that the vector vaccines are also usually accompanied by subunit-based vaccines that are given as part of the booster portion of the regimen as well. Although the subunits included in these vaccines tended to be gp120 monomers in the past, an increasingly common subunit in recent trials is gp140. Gp140 is simply a synthetic mimic of the envelope glycoprotein spike on the surface of HIV-1's envelope. Thus, gp140 contains both gp120 and gp41 subunits, however, the portion of the gp41 subunits that normally anchor the spike into the virus' envelope, gp140's transmembrane domain, is removed to the make the complex free-floating and soluble (disulfide bonds may even be added to help support the complex). Besides the subunits themselves, gp140 subunit vaccines also include an adjuvant, typically Alum, in addition to other excipient substances to suspend the adjuvant and subunits (Kovacs et al., 2014.)

Focusing back on the Ad26 vector's mechanism of action, although Ad26 is known for only causing very minor cold-like symptoms at worst, scientists still want to ensure the Ad26

vectors cannot replicate within the host. Therefore, a region of Ad26's genome that is critical for its replication, the E1 region, is removed - thereby making the Ad26 vectors "replication incompetent." Interestingly, the location where the E1 region used to be is where the "transgene cassette" (the DNA coding for the HIV-1 antigens) is later placed. Then, since the virus is unable to replicate, when the Ad26 vectors need to be replicated in order to construct the vaccine, "complement cell lines" (such as HEK293 and PER.C6 - which themselves contain the needed E1 region) are used to replicate the Ad26 vectors in. From there, the Ad26 vectors will inevitably be injected into a patient. Once inside the body, the vectors will typically target a number of different cell types, especially epithelial cells. The vectors utilize the Coxsackie and Adenovirus Receptors on the surface of target cells in order to achieve entrance into the target cells. From there, the vector's DNA genome is released into the cytosol of the target cell and is later shuttled into the cell's nucleus (though not integrated like HIV-1's genome.) From there, the target cells begin synthesizing HIV-1 antigens from the Ad26 vector DNA, which are then secreted by the target cells into the extracellular space (Custers et al., 2020; Rauch et al., 2018). Subsequently, DCs can come across the secreted antigens (as well as the gp140 subunits from the accompanying subunit vaccine), endocytose them, process them via the MHC-I and MHC-II pathways, and then begin activating an immune response - B7 expression would have been most-likely already activated via the Ad26 vector itself or by the subunit vaccine's adjuvant.

Thus, with all of this crucial background information in mind, it is important to now look at two key studies, published within the last few years, that detail how well an Ad26 viral vector vaccine, paired with a gp140 subunit vaccine, can elicit the previously mentioned immune correlates of protection against HIV-1 (namely, IgG production, ADCC and/or ADCP), as well as provide insight into how researchers have come to determine the most optimal Ad26 vector/gp140 subunit vaccine regimen. The intention of reviewing the following two critical articles, is to help answer whether or not a combination Ad26 Viral Vector/gp140 subunit prophylactic vaccine regimen elicits sufficient immune correlates of protection against HIV-1 infection to warrant further testing past Phase 1/2a Clinical Trials.

# Analysis

The first study that will be discussed is Barouch et al.'s study, published in 2018, that details the findings of both a human phase 1/2a clinical trial (named APPROACH), as well as contemporary study carried out by the same researchers on rhesus monkeys (called NHP 1319). The explicit goal of these two concurrent studies (combined into one overarching published study), were to help determine which Ad26 viral vector-based vaccine regimens, out of a collection of 7 different combinations, was most optimal for use in future clinical trials. It is important to note that this was the first phase 1/2a clinical trial of a non-prototype Ad26vectored HIV-1 vaccine. Naturally, as a phase 1/2a trial, the researchers were hoping to discover which vaccine combination was the most

immunogenic (specifically, which produced the greatest amounts of the currently understood immune correlates of protection against HIV1 infection), while also remaining safe and well-tolerated (Barouch et al., 2018). First, it's important to briefly explain Barouch et al.'s methods for conducting these two concurrent studies.

In order to conduct the APPROACH human trial, Barouch et al. adopted a multicenter, randomized, double-blind, placebo-controlled model. To achieve this model, a relatively wide diversity of participants from different countries/continents was obtained. The researchers recruited a total of 393 participants (ages 18-50) from 12 different, participating clinical locations in Thailand, South Africa, eastern Africa, and the United States. Specifically, 58 participants were from Thailand, 56 from South Africa, 129 from eastern Africa, and 150 from the U.S. This inclusion of participants from different locations around the world, was important due to the fact that the long-term goal of Barouch et al. - as well as many other researchers - is to develop a universal, prophylactic HIV-1 vaccine. Thus, Barouch et al. surely understood that they needed to determine whether or not any differences in safety or immunogenicity between different ethnicities/nationalities existed. Regardless of where the participants came from, however, only healthy/HIV-1-uninfected individuals were recruited for this study. This step worked to further limited study variables - particularly ensuring that any immunogenicity that was measured post-vaccination, was not a result of prior infection with HIV-1. Next, Barouch et al. randomly assigned all the participants into eight different groups (7 test groups and one placebo-control group). To ensure that one ethnicity/nationality wasn't entirely in one of these aforementioned groups, the researchers stratified their sample of 393 participants by region, thereby assigning a relatively equal proportion of randomized individuals into one of each of the trial's eight groups. The seven test (non-placebo) groups were all similar in the fact that they all began with two replication-incompetent Ad26 viral vector priming vaccinations - trivalent Ad26.Mos.HIV vaccine with three different vectors: one expressing 1 Env mosaic antigen and 2 expressing different Gag-Pol mosaic antigens - (given at a dosage of 5x10^10 vp/0.5mL) at week 0 (beginning) and week 12. The groups differed, however, based upon what booster vaccines were given at weeks 24 and 48. Three of the test groups were boosted with the same vaccine used to prime each group (the Ad26 vector vaccine at the same priming dosage), as well as with either a high dose (250μg), low dose (50μg), or absence of Clade C Env gp140 subunit vaccine (with Alum adjuvant). On the other hand, another three groups were boosted at weeks 24 and 48 by an MVA (modified vaccinia ankara) vaccine, as well as with the previously described high dose, low dose, or no dose of the Clade C Env gp140 subunit vaccine in Alum. The MVA vector vaccine was given at a dosage of 10^8 plaque forming units/0.5mL. There was also a 7th test group that was only boosted with the Clade C Env gp140 high dose, in addition to the placebo group that only received 0.9% saline at each vaccination. After administering each vaccination (in which the participants and clinicians weren't aware which group each participant was in - double-blind), a blood serum sample was subsequently taken from each individual four weeks later (or 6 months after the final vaccination) in order to determine the immune

responses elicited (of which, ADCP and IgG production will be discussed.) The same vaccine groups were then administered to 72 rhesus macaques (12 in each group), minus the low dose option for gp140 subunit booster, which were then challenged with SHIV to determine the protective effects of each of the vaccine groups against SHIV infection as a model of protection for each vaccine regimen in humans (Barouch et al., 2018). Having now discussed the methods that Barouch et al. used, it's important to take a brief look at what Barouch et al. found from this overarching study.

From the APPROACH study, Barouch et al. noticed that the inclusion and dosage of the gp140 subunit booster did seem to increase the titers of total Abs (measured by ELISA) produced that targeted Clade C gp140. Furthermore, the inclusion of a viral vector vaccine booster also seemed to increase total titers of Ab's specific to Clade C gp140. The caveat with these observations, however, is the fact that Barouch et al. didn't conduct any formal statistical analyses (particularly t-tests) between the titer values of overall Ab's produced against the Clade C gp140. Therefore, Barouch et al. were merely capable of only looking at apparent, non-statistically significant trends in total Ab production. This was also the case when Barouch et al. were using ELISAs to look at the relative quantities of specifically IgGs produced against Clade A, B, and C gp140 antigens by each regimen, when looking at which IgG subtypes were most prevalent against Clade C gp140, and when looking at which regimen produced the most ADCP activity. Therefore, there was no evidence provided to demonstrate that the null hypothesis (in this case that two groups being compared had the same titer of Abs produced, same titers of total IgG Abs produced, same IgG subtypes produced, or same amount of ADCP induced) could be disproven. In fact, the only statistically significant data provided by Barouch et al., was a linear regression graph that demonstrated (with an r of 0.6957 and a p-value of $<0.0001$) that the ADCP activity and the Clade C specific Ab titers (which were shown - though without statistical significance - to be primarily IgG1 and IgG3) were strongly correlated. From this piece of statistically significant data, however, and despite the lack of statistical significance in the ELISAs, it could still be argued that Barouch et al.'s vaccine regimens did accomplish the goal of eliciting the currently-understood immune correlates of protection against HIV-1 infection (IgG Ab production with accompanying Fc-mediated effector functions). For example, it could be reasoned that the missing p-values, for the differences between the titers of Abs produced and ADCP observed, isn't harmful to the impact of the overarching study, due to one particularly important finding from the concurrent rhesus monkey study. Since Barouch et al. were able to conduct the same experiment in rhesus monkeys (though at a smaller sample size of 72), they were able to directly see how well the different vaccine regimens protected against viral infection by attempting to infect the rhesus macaques with SHIV (a very similar virus to HIV-1) six separate times. What they found, was that out of the 12 macaques injected with the Ad26 prime-Ad26/gp140 boost (Ad26/Ad26 HD gp140) regimen, and subsequently challenged with SHIV, 8 (67%) of them remained uninfected after the sixth challenge (p-value = 0.007). This was higher than the second most effective regimen (the Ad26 prime-MVA/gp140 boost regimen). It was then from this data, along

with the fact that the immune responses were similar in the monkeys compared to in the human participants, and that Ad26/Ad26 HD gp140 regimen appeared to be among the most immunogenic within the aforementioned ELISAs, that Barouch et al. determined the Ad26/Ad26 HD gp140 regimen to be the most optimal for further clinical testing in a phase 2 trial. Thus, the lack of statistical significances in the ELISAs seems to be overshadowed by the findings of the concurrent macaque study that demonstrated the effectiveness of the Ad26/Ad26 HD gp140 regimen against actual viral challenge - though, of course, it still isn't entirely known how accurate the macaque model represents humans (Barouch et al., 2018). Thus, with this evaluation of this overarching study's results completed, it should be asked whether or not this study supports the idea of advancing an Ad26 viral vector/gp140 subunit vaccine regimen into further clinical trials.

As seems obvious based on the preceding paragraph, especially considering the fact that Barouch et al. determined that the Ad26/Ad26 HD gp140 was appropriate for phase 2 clinical testing, it does appear that Barouch et al.'s study helps to answer whether or not an Ad26vectored/gp140 subunit vaccine regimen can induce sufficient immune correlates of protection to warrant further trial testing - particularly by answering that, yes, it can. Specifically, this study demonstrated that an Ad26-vectored/gp140 subunit regimen can induce immune correlates in the first place; it also showed that one of those regimens is also effective in providing protection to macaques (one of the closest animal models to humans for HIV-1 infection) against a very similar virus to HIV-1. Therefore, the selected, optimal regimen from this study demonstrates all of the currently predicted hallmarks of a potentially successful universal HIV-1 vaccine (including, very importantly, being safe and well tolerated - thereby fulfilling the key goal of passing initial safety assessments of a phase 1/2a trial). There are, however, important questions still left unanswered from this study. For example, there is still the uncertainty as to whether or not it's appropriate to predict the protective ability of a vaccine regimen in humans based upon the protective ability of that same vaccine in nonhuman primates (Barouch et al., 2019). Another question that this study also doesn't answer is whether it's the quantity or the quality of the IgG1/IgG3 and/or Fc-mediated effector function responses that matters more in preventing the acquisition of HIV-1. If it's the quality of these immune correlates that matter more than quantity of correlates produced, then predicting whether or not an HIV-1 vaccine is effective in an individual may have more to do with underlying genetics, such as SNPs (single nucleotide polymorphisms) in Fc-receptor genes (Su et al., 2019), then it does with the titers of IgG Abs or magnitudes of Fc-mediated effector functions produced in clinical trials. Another question that this study leaves open-ended, is what effects increasing the valency of the viral vector would have on the immunogenicity of the vaccine. Fortunately, however, it is this question that is answered in the following study.

The second study that will be discussed in this review, was published in late 2020 and details the observations/data collected from another phase 1/2a vaccine trial (called TRAVERSE) conducted by Baden et al. Interestingly, Dr. Dan H. Barouch, the lead investigator from the previous APPROACH study, is also credited as being one of the

authors of this TRAVERSE study as well. Also like before, is the fact that the explicit goal of this study was to further determine what Ad26 viral vector-based vaccine regimen was optimal for progressing into later-stage, phase 2 clinical trials. What's different, however, about the goals of this TRAVERSE study - and what makes this study, at the same time, intertwined with the

APPROACH study - is that Baden et al. were essentially picking up their research where the Barouch-led team left off. For example, Baden et al. start their study already presuming that the Ad26/Ad26 HD gp140 regimen (the "winner" from the APPROACH study) was the best, most-promising regimen at the time of this TRAVERSE study. Yet, despite the Ad26/Ad26 HD gp140 regimen's promise, Baden et al, for this APPROACH study, wanted to test a brand new replication-incompetent, tetravalent Ad26 vector vaccine (Ad26.Mos4.HIV - now containing an additional mosaic Env antigen-carrying Ad26 vector) against the Ad26 vector vaccine (Ad26.Mos.HIV) that was in the APPROACH study's Ad26/Ad26 HD gp140 regimen. This new

Mosaic Env antigen, called "Mosaic 2 Env" was designed to provide better coverage of Clade C HIV-1 strains in addition to the primarily Clade B HIV-1 strains covered by the Ad26.Mos.HIV vaccine's single "Mosaic 1 Env" antigen (Baden et al., 2020; Barouch et al., 2018). Specifically,

Baden et al. wanted to compare the safety and (more critical to this review) the

immunogenicity of the new tetravalent vector against the older trivalent vector. With these goals in mind, it would be appropriate to briefly look at how Baden et al. set about accomplishing their study to further answer whether or not an Ad26 viral vector-based vaccine regimen can elicit sufficient immune correlates of protection against HIV-1 to warrant its further testing in clinical trials.

Similar to Barouch et al.'s study, Baden et al., set their TRAVERSE study up as a randomized, parallel-group, placebo-controlled, double-blind study. Participants for this study were also required to be HIV-1-uninfected, as well as between the ages of 18 and 50 (ensuring that no immune responses measured were from pre-existing infection, as well as ensuring that differences in variables between the APPROACH and TRAVERSE studies were reduced.) Also, Baden et al., like Barouch et al., did "mask" both the participants and clinicians towards which group a particular participant was in during the study. Unlike previously, however, Baden et al. used only 198 participants from either 11 different medical centers in the United States or from one medical center in Rwanda. Thus, the sample group and variation in participants' ethnicities/possible living conditions (no uniform distribution of ethnicities was mentioned for the United States participants) were significantly smaller, though Baden et al. did ensure to stratify the participants based on the two regions, ensuring a similar proportion of both region's participants were randomly assigned to each group - of which there were only three in this TRAVERSE study. The three groups were the "tetravalent" group (110 people), the "trivalent" group (55 people), and the placebo group

(33 people). In terms of the regimens/doses of vaccines utilized, these were identical to the Ad26/Ad26 HD gp140 regimen from the APPROACH study, except for the fact that the tetravalent group had the new tetravalent Ad26.Mos4.HIV vaccine, but the trivalent group had the trivalent Ad26.Mos.HIV vaccine. As such, both the tetravalent and trivalent vaccines were given at a dose of 5x10^10 vp/0.5mL, and the gp140 subunit booster vaccines were given at the same dose as the APPROACH study - 250μg of gp140 with 0.425mg of Alum adjuvant. Likewise, the placebo group got 0.9% saline solution. In terms of the injection schedule, two Ad26 vector priming vaccines (either tetravalent or trivalent) were given at weeks 0 (beginning) and 12. Then at weeks 24 and 48, the boosting Ad26 vector vaccines were also accompanied by the gp140 booster vaccines. Of course, the placebo group followed the same injection schedule but with the saline vaccines. Finally, blood serum was taken from each of the participants 4 weeks after the second, third and fourth vaccinations (as well as 6 months after the fourth vaccination) in order to calculate the humoral and cellular immune responses elicited. (Baden et al., 2020; Barouch et al., 2018). Now, after discussing how Baden et al. conducted their research, it is important to discuss the results of Baden et al.'s TRAVERSE study.

The overall discovery of the TRAVERSE study was that the vaccine regimen that included the tetravalent Ad26 vaccine (Ad26.Mos4.HIV) appeared significantly more immunogenic than the vaccine regimen with only the trivalent Ad26 vaccine (Ad26.Mos.HIV).

First, as determined by ELISA, in overall IgG Ab production (specific to a Clade A, a Clade B, and two Clade C gp140 antigens), the tetravalent group demonstrated higher titers than the trivalent group at each of the four serum draws. For this study, unlike in the APPROACH study, statistical analyses were conducted between the titer values on the total IgG ELISAs, thereby demonstrating that the higher titers produced in response to the tetravalent vaccine were statistically significant ($p<0.05$). Specifically, at both the tetravalent and trivalent-based vaccine regimens' most immunogenic (serum draw four weeks after fourth vaccination) the tetravalent group produced, on average, around three-fold higher titers of total IgG Abs then the trivalent group did. Additionally, when looking at longer-term total IgG titers, the tetravalent group demonstrated more than two-fold higher IgG titers than the trivalent group. Another really important finding from this study, was that when Baden et al. ran three BAMAs (Binding Antibody Multiplex Assays) - one with 9 gp140 variants as antigens, one with 20 gp120 variants as antigens, and one with 16 gp70 V1V2 variants as antigens - the tetravalent vaccine demonstrated significantly higher binding breadth (post-third vaccination, post-fourth vaccination, and post 6 months after fourth vaccination) compared to the trivalent vaccine ($p<0.0001$). This finding is critical, since one of the needed characteristics of a universal, prophylactic HIV-1 vaccine would be an ability to elicit immune responses that are able to recognize (and build immunological memory against) an increasingly broader spectrum of possible HIV-1 antigen variants. Additionally, when analyzing the relative titers of specifically IgG1 and IgG3 Abs produced between the groups, the tetravalent vaccine elicited significantly higher titers, at all tested serum draws,

compared to the trivalent vaccine ($p<0.05$). This TRAVERSE study, however, did produce a few concerning results (Baden et al., 2020).

What is rather disconcerting, is the fact that, although the tetravalent vaccine induced more IgG3 and IgG1 Abs, IgG3s were only detectable in 66-67% of participants, and no IgG1 response rates were provided. Further, slightly disconcerting, findings had to do with the elicited ADCP and ADCC responses. In terms of ADCP responses, the tetravalent group could induce significantly higher phagocytosis scores than the trivalent vaccine, but only against Clade A gp140 and Clade C gp140 - not against Clade B gp140. Additionally, although the ADCC response rates and ADCC percent killing did appear higher from the tetravalent vaccine, no statistical significance was achieved here. Also, despite the seemingly improved ADCC response rates of the tetravalent vaccine versus the trivalent vaccine, the induced ADCC response rates by the tetravalent vaccine were still no higher than 52%. Thus, although the tetravalent vaccine does begin to show significant improvements upon the trivalent vaccine in terms of eliciting important immune correlates of protection (particularly ADCP and IgG1/3), these correlates were by no means elicited uniformly among all the participants in the study (Baden et al., 2020). Thus, from Baden et al.'s findings, it must be asked whether or not this TRAVERSE study supports the idea of advancing an Ad26 viral vector/gp140 subunit vaccine regimen for HIV-1 into further clinical trials.

Although Baden et al. did make a few concerning discoveries, it seems as though this TRAVERSE study further answers the question as to whether or not an Ad26-vectored/gp140 subunit regimen should be advanced into phase 2 clinical trials, by providing evidence that it should. From the APPROACH study, Barouch et al. had already determined that the older Ad26.Mos.HIV/gp140 subunit vaccine regimen should be, based on its protectiveness in macaques' models, advanced to phase 2 clinical trials. Thus, since the newer

Ad26.Mos4.HIV/gp140 subunit regimen elicits even greater total IgG Ab production - as well as, more specifically, greater IgG3 and IgG1 Ab production - in addition to a significantly improved Ab ability for recognition/binding to a larger spectrum of HIV-1 antigen variants, it seems as though the new tetravalent-based regimen should be advanced to phase 2 clinical trials as well - which is exactly what Baden et al. concluded at the end of this TRAVERSE study. This decision is supported by the fact that the new Ad26.Mos4.HIV vaccine was deemed just as safe and tolerable as the earlier Ad26.Mos.HIV vaccine. Thus, utilizing the newer tetravalent-based vaccine regimen would likely not prove harmful for recipients of the vaccine in the future - though it should be acknowledged that this TRAVERSE study did use both a smaller and less diverse sample size of participants. Additionally, although IgG3 Abs were not elicited in all patients by the tetravalent-based vaccine regimen, the response rates were certainly improved over the trivalent-based vaccine regimen (66% vs 46% at both vaccine regimen's peak immunogenicity), which is necessary for advancing the development of a potentially useful, prophylactic vaccine

regimen. Furthermore, though ADCC % killing values weren't significantly higher in response to the tetravalent vaccine regimen, the ADCP scores were significantly higher against some tested antigens. This discrepancy, however, should - perhaps - be an expected result. Additional studies into the contributions of ADCP and ADCC in combating HIV-1 infection have suggested that ADCP contributes significantly more to fighting the virus than ADCC (Sips et al., 2016). Thus, there is precedence then, while still acknowledging the lack of significance in ADCC % killings, to focus more heavily on the improvements in ADCP scores. If this viewpoint is presumed, then the tetravalent regimen demonstrates an even more impressive improvement upon the trivalent regimen (since Clade C-specific and Clade A-specific ADCP responses were significantly higher in the tetravalent group). What's truly fascinating, however, is the fact that not only did Baden et al. provide evidence that the Ad26.Mos4.HIV vaccine would be more effective in eliciting immune correlates of protection than the Ad26.Mos.HIV vaccine, it seems as though the researchers in charge of the ongoing Imbokodo study (a phase 2 trial) have replaced the trivalent Ad26.Mos.HIV vaccine with the newer, tetravalent Ad26.Mos4.HIV vaccine. At the end of the APPROACH study, Barouch et al. mention that the Imbokodo study was being initiated based on state-of-the-art, at the time, Ad26.Mos.HIV vaccine. However, when reading over the end of the TRAVERSE study, Baden et al. mention that the ongoing Imbokodo study is utilizing the newer Ad26.Mos4.HIV vaccine

(Baden et al., 2020; Barouch et al., 2018). Upon further examination of this seeming contradiction, one finds on *ClinicalTrials.gov* that the Ad26.Mos.HIV vaccine is not mentioned in the intervention/treatment section. In fact, only the Ad26.Mos4.HIV vaccine is mentioned alongside the gp140 subunit vaccine (Janssen Vaccines & Prevention B.V., 2020). Therefore, it seems as though Barouch et al. replaced the Ad26.Mos.HIV vaccine with the Ad26.Mos4.HIV vaccine shortly after discovering the improved capabilities of the new tetravalent vaccine during the TRAVERSE trial. Evidently then, Baden et al. found the Ad26.Mos4.HIV/gp140 subunit regimen so convincing that they were willing to replace their older, successful vaccine with it in not just the phase 2 Imbokodo study, but also initiate another phase 2 clinical study called MOSAICO using the tetravalent vaccine exclusively (Baden et al., 2020). The TRAVERSE study, however, still doesn't answer a few key questions.

First, the TRAVERSE study, like the APPROACH study, doesn't elucidate whether or not the rhesus macaque model with SHIV challenge is a reliable basis for predicting the protection of a particular HIV-1 vaccine regimen in humans. In addition to this, this TRAVERSE also doesn't answer whether it is the quantity of Fc-mediated effector functions and Fc-mediating Abs that are the best predictors of protection against HIV-1 acquisition, or if it is more-so the quality (increased cooperation/correlation) of Fc-mediated effector functions that best predict protection against acquiring HIV-1. Hopefully, with the conclusion of the MOSAICO and

Imbokodo studies, these - and other important questions - will be at least partially answered.

# Conclusion

After evaluating the APPROACH and TRAVERSE studies, particular pieces of data must be reiterated that demonstrate the advancement of an Ad26-vectored/gp140 subunit HIV-1 vaccine regimen, past phase 1/2a testing, is warranted. In doing this, the APPROACH and TRAVERSE trials clearly answer the question of this paper, put forth at the beginning of this analysis section, asking whether or not an Ad26-vector/gp140 subunit vaccine regimen could adequately elicit immune correlates of protection against HIV-1 to warrant further testing past phase 1/2a clinical trials. First, the APPROACH study (as the first phase 1/2a trial in which a non-prototype, Ad26-vectored was tested vaccine was tested) helped to demonstrate that an Ad26 vector-based prophylactic vaccine regimen can be safe, while also eliciting key immune correlates of protection that warrant its further investigation into phase 2 clinical trials. More specifically, however, the APPROACH study demonstrated that the most optimal regimen is a regimen incorporating two Ad26-vectored priming vaccinations, along with Ad26-vectored and gp140 subunit (high dose) booster vaccinations. Though the validity of the current infection model is still uncertain, this decision on the optimal regimen was determined via the protection of the regimen in rhesus macaques (Barouch et al., 2018). Then, following the APPROACH study, the TRAVERSE study demonstrated that the successful vaccine regimen from the APPROACH study could be further improved by increasing the valency of the Ad26-vectored component of the APPROACH regimen. Specifically, the new tetravalent component elicited increased magnitudes of key immune correlates of infection, further cementing the idea of advancing an Ad26 vector-based regimen - based on the knowledge that inclusion of additional mosaic antigens appears to reliably increase the breadth and quantity of immune correlates of protection, yet without increasing the side-effects of the vaccination (Baden et al., 2020). In addition to these aforementioned, critical pieces of evidence, remaining, unanswered questions were also touched on in the analysis section. Therefore, a brief discussion on future research that should occur to answer these, and other questions, seems appropriate.

There are a couple of avenues that should be investigated based on some of the shortcomings of the APPROACH and TRAVERSE studies. First, based on the fact that the accuracy of the macaque model for HIV-1 infection in humans is still uncertain (Barouch et al., 2018), it is crucial that scientists elucidate how accurately the protective efficacy of an Ad26 vector/subunit HIV-1 vaccine in humans can be predicted based on that same vaccine's protection against infection in rhesus macaques. Fortunately, the ongoing Imbokodo and MOSAICO trials, which are aimed at testing the protective efficacy of TRAVERSE's tetravalent regimen in humans, will likely provide critical data for helping to answer this issue. Once the results from the Imbokodo and MOSAICO trials are published, the protective efficacy of the utilized regimen could be readily compared to the protective efficacy of that same regimen in rhesus monkeys. Another avenue of research that should be undertaken is regarding the uncertainty of whether the quantity or quality of the elicited immune correlates of protection is more vital in fighting HIV-1 infection. It appears that the TRAVERSE study was more concerned with the quantity aspect of the immune correlates rather than the quality - paying particular attention towards the fact that the tetravalent regimen was eliciting higher titers of Fc mediating Abs and higher ADCP scores (Baden et al., 2020). However, focusing entirely on the quantity of elicited immune correlates may negatively affect the overall vaccine development process - especially if future research discovers that it is the greater coordination of elicited immune responses that is more critical than greater quantities/concentrations of immune correlates. If a vaccine candidate is capable of both eliciting greater magnitudes of immune correlates, as well as greater (more effective) cooperation between the elicited immune correlates, then a more favorable middle-ground would be reached in satisfying both the quantity and quality aspects of the elicited immune correlates of protection. One way that researchers could emphasize the quality aspect more, is by checking correlation coefficient values between immune correlates (such as between ADCP and ADCC) that are elicited during future trials, rather than simply looking at the magnitudes (scores and titers) of immune correlates of protection. Finally, one last avenue of further research that should be explored would investigate whether or not repeatedly using an Ad26 vector builds up a sufficient immune memory against the vector to negatively impact its ability to deliver its transgenic cargo. As discussed in the introduction, previous research has demonstrated that, even in the presence of an Ad26-specific immune response, Ad26 vectors are still successful in eliciting effective immune responses against the immunogen expressed by the Ad26 vector (Baden et al., 2013). What is unclear, however, is how long the Ad26 vector is capable of effectively delivering its cargo in the presence of increasing Ad26-specific immune responses. If, for example, the prophylactic HIV-1 vaccine regimen would need to become more of a yearly vaccination (similar to how the influenza vaccine currently is), there is no reliable data to strongly suggest that the Ad26 vector-based vaccine would remain effective for long. Thus, research into this subject could have beneficial long-term implications.

# Bibliography

Ackerman, M. E., Mikhailova, A., Brown, E. P., Dowell, K. G., Walker, B. D., Bailey-Kellogg, C., Suscovich, T. J., & Alter, G. (2016). Polyfunctional HIV-Specific Antibody Responses Are Associated with Spontaneous HIV Control. *PLoS pathogens*, *12*(1), e1005315. https://doi.org/10.1371/journal.ppat.1005315

Altfeld, M. & Gale, M. (2015). Innate immunity against HIV-1 infection. *Nature Immunology, 16(6),* 554-562. https://doi.org/10.1038/ni.3157

Baden, L. R., Walsh, S. R., Seamen, M. S., Tucker, R. P., Krause, K. H., Patel, A., Johnson, J. A., Kleinjan, J., Yanosick, K. E., Perry, J., Zablowsky, E., Abbink, P., Peter, L., Iampietro, M. J.,
Cheung, A., Pau, M. G., Weijtens, M., Goudsmit, J., Swann, E., Wolff, M., Loblein, H., Dolin, R., & Barouch D. H. (2013). First-in-Human Evaluation of the Safety and Immunogenicity of a Recombinant Adenovirus Serotype 26 HIV-1 Env Vaccine (IPCAVD 001). *The Journal of Infectious Diseases, 207(2),* 240-247. https://doi.org/10.1093/infdis/jis670

Baden, L. R.; Stieh, D.J.; Sarnecki, M.; Walsh, S. R.; Tomaras, G. D.; Kublin, J. G.; McElrath, M. J.; Alter, G.; Ferrari, G.; Montefiori, D.; Mann, P.; Nijs, S.; Callewaert, K.; Goepfert, P.; Edupuganti, S.; Karita, E.; Langedijk, J. P.; Wegmann, F.; Corey, L.; Pau M. G.; Barouch, D. H.; Schuitemaker, H.; Tomaka, F. (2020). Safety and immunogenicity of two heterologous HIV vaccine regimens in healthy, HIV-uninfected adults (TRAVERSE): a randomised, parallel-group, placebo-controlled, double-blind, phase 1/2a study. *The Lancet, 7(10),* 688-698. https://doi.org/10.1016/S2352-3018(20)30229-0

Barouch, D. H., Stephenson, K. E., Borducchi, E. N., Smith, K., Stanley, K., McNally, A. G., Liu, J., Abbink, P., Maxfield, L. F., Seaman, M. S., Dugast, A. S., Alter, G., Ferguson, M., Li, W., Earl, P. L., Moss, B., Giorgi, E. E., Szinger, J. J., Eller, L. A., Billings, E. A., … Michael, N. L. (2013). Protective efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. *Cell*, *155*(3), 531–539. https://doi.org/10.1016/j.cell.2013.09.061

Barouch, D. H.; Tomaka, F. L.; Wegmann, F.; Stieh, D. J.; Alter, G.; Robb, M. L.; Michael, N. L.; Peter, L.; Nkolola, J. P.; Borducchi, E. N.; Chandrashekar, A.; Jeton, D.; Stephenson, K. E.;
Li, W.; Koerber, B.; Tomaras, G. D.; Montefiori, D. C.; Gray, G.; Frahm, N.; … Schuitemaker, H. (2018). Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomized, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19). *The Lancet, 392(10143),* 232-243. https://doi.org/10.1016/S0140-6736(18)31364-3

Baum, L. L., Cassutt, K. J., Knigge, K., Khattri, R., Margolick, J., Rinaldo, C., Kleeberger, C. A., Nishanian, P., Henrard, D. R., & Phair, J. (1996). HIV-1 gp120-specific

antibodydependent cell-mediated cytotoxicity correlates with rate of disease progression.
*Journal of immunology (Baltimore, Md. : 1950)*, *157*(5), 2168–2173.

Cavrois, M., Neidleman, J., Kreisberg, J., Greene, W. (2007). In Vitro Derived Dendritic Cells *trans*-infect CD4 T Cells Primarily with Surface-Bound HIV-1 Virions. *PLoS Pathogens,*
*3(1).* https://dx.doi.org/10.1371%2Fjournal.ppat.0030004

Centers for Disease Control and Prevention. (n.d.) *What Body Fluids Transmit HIV?.* Body Fluids

That Transmit HIV | HIV Transmission | HIV Basics | HIV/AIDS | CDC

Corti, D., Langedijk, J. P., Hinz, A., Seaman, M. S., Vanzetta, F., Fernandez-Rodriguez, B. M., Silacci, C., Pinna, D., Jarrossay, D., Balla-Jhagjhoorsingh, S., Willems, B., Zekveld, M. J., Dreja, H., O'Sullivan, E., Pade, C., Orkin, C., Jeffs, S. A., Montefiori, D. C., Davis, D., Weissenhorn, W., … Lanzavecchia, A. (2010). Analysis of memory B cell responses and isolation of novel monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals. *PloS one*, *5*(1), e8805.
https://doi.org/10.1371/journal.pone.0008805

Custers, J., Kim, D., Leyssen, M., Gurwith, M., Tomaka, F., Robertson, J., Heijnen, E., Condit, R., Shukarev, G., Heerwegh, D., van Heesbeen, R., Shuitemaker, H., Douoguih, M., Evans, E., Smith, E. R., Chen, R. T. (2020). Vaccines based on replication incompetent Ad26 viral vectors: Standardized template with key considerations for risk/benefit assessment.
*Vaccine.* https://doi.org/10.1016/j.vaccine.2020.09.018.

Doitsh, G., & Greene, W. C. (2016). Dissecting How CD4 T Cells Are Lost During HIV Infection. *Cell host & microbe*, *19*(3), 280–291.
https://doi.org/10.1016/j.chom.2016.02.012

Engelman, A. & Cherepanov, P. (2012). The structural biology of HIV-1: mechanistic and therapeutic insights. *Nature Review Microbiology* 10, 279–290.
https://doi.org/10.1038/nrmicro2747

Fischer, W., Perkins, S., Theiler, J., Bhattacharya, T., Yusim, K., Funkhouser, R., Kuiken, C., Haynes, B., Letvin, N. L., Walker, B. D., Hahn, B. H., & Korber, B. T. (2007). Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1 variants. *Nature medicine*, *13*(1), 100–106. https://doi.org/10.1038/nm1461

Fox, J. & Fidler, S. (2010). Sexual transmission of HIV-1. *Antiviral Research, 85(1),* 276-285.
https://doi.org/10.1016/j.antiviral.2009.10.012

Gonzalez, S. M., Aguilar-Jimenez, W., Su, R. C., & Rugeles, M. T. (2019). Mucosa: Key Interactions Determining Sexual Transmission of the HIV Infection. *Frontiers in immunology*, *10*, 144. https://doi.org/10.3389/fimmu.2019.00144

Haynes, B. F., Gilbert, P. B., McElrath, M. J., Zolla-Pazner, S., Tomaras, G. D., Alam, S. M., Evans, D. T., Montefiori, D. C., Karnasuta, C., Sutthent, R., Liao, H. X., DeVico, A. L.,

Lewis, G. K., Williams, C., Pinter, A., Fong, Y., Janes, H., DeCamp, A., Huang, Y., Rao, M., … Kim, J. H.
(2012). Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *The New England journal of medicine*, *366*(14), 1275–1286.
https://doi.org/10.1056/NEJMoa1113425

Janssen Vaccines & Prevention B.V. (2020). *A Study to Assess the Efficacy of a Heterologous Prime/Boost Vaccine Regimen of Ad26.Mos4.HIV and Aluminum Phosphate-Adjuvanted Clade C gp140 in Preventing Human Immunodeficiency Virus (HIV) -1 Infection in Women in Sub-Saharan Africa.* (Clinicaltrials.gov Identifier: NCT03060629). Retrieved from A Study to Assess the Efficacy of a Heterologous Prime/Boost Vaccine Regimen of
Ad26.Mos4.HIV and Aluminum Phosphate-Adjuvanted Clade C gp140 in Preventing Human Immunodeficiency Virus (HIV) -1 Infection in Women in Sub-Saharan Africa - Full Text View - ClinicalTrials.gov

Justiz Vaillant, A. A., & Naik, R. (2020). HIV-1 Associated Opportunistic Infections. In *StatPearls*. StatPearls Publishing.

Kim, J. H., Excler, J. L., & Michael, N. L. (2015). Lessons from the RV144 Thai phase III HIV-1 vaccine trial and the search for correlates of protection. *Annual review of medicine*, *66*,
423–437. https://doi.org/10.1146/annurev-med-052912-123749

Kovacs, J. M., Noeldeke, E., Jiwon Ha, H., Peng, H., Rits-Volloch, S., Harrison, S. C., Chen, B. (2014). Stable, uncleaved HIV-1 envelope glycoprotein gp140 forms a tightly folded trimer with a native-like structure. *Proceedings of the National Academy of Sciences of the United States of America, 111(52),* 18542-18547.
https://doi.org/10.1073/pnas.1422269112

Lambotte, O., Ferrari, G., Moog, C., Yates, N. L., Liao, H. X., Parks, R. J., Hicks, C. B., Owzar, K., Tomaras, G. D., Montefiori, D. C., Haynes, B. F., & Delfraissy, J. F. (2009). Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. *AIDS (London, England)*, *23*(8), 897–906.
https://doi.org/10.1097/QAD.0b013e328329f97d

Li, G., & De Clercq, E. (2016). HIV Genome-Wide Protein Associations: a Review of 30 Years of Research. *Microbiology and molecular biology reviews : MMBR*, *80*(3), 679–731.
https://doi.org/10.1128/MMBR.00065-15

Mohan, T., Bhatnagar, S., Gupta, D. L., & Rao, D. N. (2014). Current understanding of HIV-1 and T-cell adaptive immunity: progress to date. *Microbial pathogenesis*, *73*, 60–69.
https://doi.org/10.1016/j.micpath.2014.06.003

Moris, A., Nobile, C., Buseyne, F., Porrot, F., Abastado, J. P., & Schwartz, O. (2004). DC-SIGN promotes exogenous MHC-I-restricted HIV-1 antigen presentation. *Blood*, *103*(7), 2648–
2654. https://doi.org/10.1182/blood-2003-07-2532

National Institutes of Health. (2020, December 10). *Pre-Exposure Prophylaxis (PrEP) to Reduce*

*HIV Risk.* Pre-exposure Prophylaxis (PrEP) to Reduce HIV Risk | NIH: National Institute of
Allergy and Infectious Diseases

Ng'uni, T., Chasara, C., & Ndhlovu, Z. M. (2020). Major Scientific Hurdles in HIV Vaccine Development: Historical Perspective and Future Directions. *Frontiers in immunology*, *11*,
590780. https://doi.org/10.3389/fimmu.2020.590780

Picchio, G. R., Gulizia, R. J., Wehrly, K., Chesebro, B., Mosier, D. E. (1998). The Cell Tropism of Human Immunodeficiency Virus Type 1 Determines the Kinetics of Plasma Viremia in

SCID Mice Reconstituted with Human Peripheral Blood Leukocytes. *Journal of Virology,*

*72(3),* 2002-2009. https://doi.org/10.1128/JVI.72.3.2002-2009.1998

Pitisuttithum, P. & Marovich, M. A. (2020). Prophylactic HIV vaccine: vaccine regimens in clinical trials and potential challenges. *Taylor Francis Online, 19(2),* 133-142.

https://doi.org/10.1080/14760584.2020.1718497

Rappocciolo, G., Piazza, P., Fuller, C. L., Reinhart, T. A., Watkins, S. C., Rowe, D. T., Jais, M., Gupta, P., & Rinaldo, C. R. (2006). DC-SIGN on B lymphocytes is required for transmission of HIV-1 to T lymphocytes. *PLoS pathogens*, *2*(7), e70. https://doi.org/10.1371/journal.ppat.0020070

Rauch, S., Jasny, E., Schmidt, K. E., & Petsch, B. (2018). New Vaccine Technologies to Combat

Outbreak Situations. *Frontiers in immunology*, *9*, 1963.

https://doi.org/10.3389/fimmu.2018.01963

Shaw, G. M., & Hunter, E. (2012). HIV transmission. *Cold Spring Harbor Perspectives in*

*Medicine*, *2*(11), https://dx.doi.org/10.1101%2Fcshperspect.a006965

Siliciano, R. F. & Greene, W. C. (2011). HIV Latency. *Cold Spring Harbor Perspectives in Medicine,*

*1(1).* https://dx.doi.org/10.1101%2Fcshperspect.a007096

Sips, M., Krykbaeva, M., Diefenbach, T. J., Ghebremichael, M., Bowman, B. A., Dugast, A. S.,

Boesch, A. W., Streeck, H., Kwon, D. S., Ackerman, M. E., Suscovich, T. J., Brouckaert,

P., Schacker, T. W., & Alter, G. (2016). Fc receptor-mediated phagocytosis in tissues

as a potent mechanism for preventive and therapeutic HIV vaccine strategies.

*Mucosal immunology*, *9*(6), 1584–1595. https://doi.org/10.1038/mi.2016.12

Spicer, B. A., Conroy, P. J., Law, R., Voskoboinik, I., & Whisstock, J. C. (2017). Perforin-A key (shaped) weapon in the immunological arsenal. *Seminars in cell & developmental biology*, *72*, 117–123. https://doi.org/10.1016/j.semcdb.2017.07.033

Su, B., Dispinseri, S., Iannone, V., Zhang, T., Wu, H., Carapito, R., Bahram, S., Scarlatti, G., & Moog, C. (2019). Update on Fc-Mediated Antibody Functions Against HIV-1 Beyond Neutralization. *Frontiers in Immunology*, *10*, 2968. https://doi.org/10.3389/fimmu.2019.02968

Tay, M. Z., Wiehe, K., & Pollara, J. (2019). Antibody-Dependent Cellular Phagocytosis in Antiviral Immune Responses. *Frontiers in immunology*, *10*, 332. https://doi.org/10.3389/fimmu.2019.00332

*The Global HIV/AIDS Epidemic*. (2020, November 25). HIV. Retrieved February 25th, 2021, from Global Statistics | HIV.gov.

Vrba, S. M., Kirk, N. M., Brisse, M. E., Liang, Y., & Ly, H. (2020). Development and Applications of Viral Vectored Vaccines to Combat Zoonotic and Emerging Public Health Threats. *Vaccines*, *8*(4), 680. https://doi.org/10.3390/vaccines8040680

Zolla-Pazner, S. & Gilbert, P. B. (2019). Revisiting the Correlate of Reduced HIV Infection Risk in the Rv144 Vaccine Trial. *Journal of Virology, 93(17).* https://doi.org/10.1128/JVI.0062919