HHS Public Access

Author manuscript

Immunol Rev. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

Immunol Rev. 2017 January ; 275(1): 245–261. doi:10.1111/imr.12514.

Complex Immune Correlates of Protection in HIV-1 Vaccine Efficacy Trials

Georgia D. Tomaras[1] and **Stanley A. Plotkin**[2,3]

[1]Duke Human Vaccine Institute, Departments of Surgery, Immunology, Molecular Genetics and Microbiology

[2]Vaxconsult, Doylestown, Pennsylvania, USA

[3]University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

Summary

Development of an efficacious HIV-1 vaccine is a major priority for improving human health worldwide. Vaccine mediated protection against human pathogens can be achieved through elicitation of protective innate, humoral, and cellular responses. Identification of specific immune responses responsible for pathogen protection enables vaccine development and provides insights into host defenses against pathogens and the immunological mechanisms that most effectively fight infection. Defining immunological correlates of transmission risk in preclinical and clinical HIV-1 vaccine trials has moved the HIV-1 vaccine development field forward and directed new candidate vaccine development. Immune correlate studies are providing novel hypotheses about immunological mechanisms that may be responsible for preventing HIV-1 acquisition. Recent results from HIV-1 immune correlates work has demonstrated that there are multiple types of immune responses that together, comprise an immune correlate—thus implicating polyfunctional immune control of HIV-1 transmission. An in depth understanding of these complex immunological mechanisms of protection against HIV-1 will accelerate the development of an efficacious HIV-1 vaccine.

Keywords

vaccination; immunity; correlate of decreased HIV-1 risk; correlates of protection; humoral; cellular; antibodies; HIV; complex immune correlate; sieve analysis

Introduction

Vaccines elicit a myriad of innate, cellular and humoral responses that do not all contribute to protective immunity. Elucidating the specific immune responses that are responsible for protection is a major effort in vaccine development for HIV-1, *Plasmodium falciparum (malaria)*, dengue virus (DENV), *M.tuberculosis*, *Norovirus*, Ebola Virus (EBOV), and Zika virus (ZIKV) where there are no currently licensed efficacious vaccines. Mechanistic

Correspondence to: Georgia D. Tomaras Duke Human Vaccine Institute 2 Genome Ct., Duke University Durham, NC 27710, USA gdt@duke.edu.

immune correlates of protection (CoP) are largely undefined for many pathogens, but efforts by immunologists and vaccinologists have resulted in improved knowledge of what constitutes protective immunity for many of these infectious pathogens. Identification of specific immune correlates will have immense scientific, economic and societal benefit by speeding up implementation of licensed vaccines that improve human health. Even definition of non-mechanistic correlates help the choice of effective vaccines. Efforts toward identifying CoP will also serve to catalyze basic research that can provide deeper insights on the interaction of the human immune system with pathogens *in vivo*. This review will discuss recent advances in the identification of immune CoP for HIV-1, with a focus on antibody correlates, in the context of what is already known for other licensed vaccines for other pathogens.

Immunological Correlates of Protection

A CoP is an immune biomarker that measures response to vaccination that predicts the level of vaccine efficacy for a given clinical outcome (1). The CoP can be a mechanistic or non-mechanistic correlate and either one can be useful as surrogate endpoints. However, a mechanistic correlate provides critical insights for designing studies that can improve upon a partially efficacious vaccine regimen as well as generate testable hypotheses about mechanisms of immune protection (2). Immune correlates may be complex (3) in that there may be multiple immune responses that correlate with protection. These immune responses may also depend on the type of vaccine and population characteristics such as route of transmission, host genetics, and pre-existing immunity. To date, correlates of HIV-1 risk involving multiple immune responses have been identified for the RV144 HIV-1 vaccine efficacy trial. However, these are not yet accepted to be CoP since they have not been confirmed in another vaccine efficacy trial. Follow-up vaccine efficacy studies are planned to determine if the RV144 correlates of HIV-1 risk are confirmed mechanistic CoPs. Data reported on the involvement of multiple immune responses, host genetics and virus diversity in HIV-1 correlates of risk, as well as the the likely influence of the mode of transmission, vaccine regimen, and the differences in regional virus diversity compared to vaccine insert, all coalesce toward the concept that the mechanistic CoP for HIV-1 vaccine efficacy will be complex.

Correlates of protection have been noted for currently licensed vaccines (1, 4–6) including Anthrax, Varicella, Diphtheria, Hepatitis A, Hepatitis B, Haemophilus Influenza, Human Papilloma Virus, Japanese Encephalitis, Measles, Meningococcal, Mumps, Pertussis, Pneumococcal, Polio, Rabies, Rotavirus, Rubella, Shingles, Smallpox, Tetanus, Typhoid Fever, Varicella, Yellow Fever, and Zoster (Table 1). The immune responses that are correlated with protection for licensed vaccines include binding antibody responses, functional antibody responses (i.e. neutralization/pathogen inhibition, opsonophagocytosis) and cellular immunity (CD4+ T cell, lymphoproliferation). CoPs for nearly all of the currently licensed vaccines are associated with antibody responses, with the exception of BCG, Zoster and Malaria that all act through T cell responses, with antibodies also being involved for Malaria vaccines. Approximately half of the vaccines utilize binding antibody levels as the CoP, predominantly because they are easier to measure than the functional responses. The other half utilize functional antibody responses as the measured CoP (1).

Immune correlates for the pneumococcal vaccine include both binding and functional antibody correlates (i.e. opsonophagocytosis) (7–9). Binding antibody levels are utilized as correlates of protection for Hepatitis A (10), Hepatitis B (11), Hib polysaccharide/ conjugate (12, 13), Human Papillomavirus (14, 15), Measles (16), Rubella (17–19), Varicella (20, 21), and Zoster (22). Functional antibody correlates of protection are utilized as CoP with Diphtheria, Influenza (23–25), Japanese Encephalitis, mumps (26), polio (27), rabies, smallpox (28, 29), tetanus, and yellow fever (30), all with neutralization or pathogen/toxin inhibition assays. A phase III herpes zoster vaccine (Zostavax Efficacy and Safety Trial (ZEST) that demonstrated 70% vaccine efficacy (31) identified that the rise in antibody titers from baseline was a stronger correlate of protection than the antibody titers after vaccination (22). The antibody response to the Zoster vaccine therefore is a non-mechanistic correlate of protection. The finding that an elevation of the antibody response rather than an absolute value suggests that it may be a surrogate for other immune response(s). CD4+ T cell breadth is also induced post vaccination (32) and cell mediated immunity (*i.e.* CD4+ T cell, lymphoproliferation) is associated with reduced disease severity (33). Although there is no licensed vaccine for HIV-1, an immune correlates analysis of the RV144 vaccine efficacy trial put forth multiple immune responses in each of these categories (i.e. binding Ab, functional antibodies and cellular responses) (Table 1) that correlated with HIV-1 risk. Notably, only vaccines for Pneumococcus and HIV-1 have identified an antibody Fc mediated effector function as a correlate; however, this may be due to the lack of testing of this type of immune response in other vaccines since functional antibody responses that are non-neutralizing have been associated with a number of non-HIV infections and vaccination studies (reviewed in (34)). Moreover, although a number of licensed vaccines have neutralization as an immune correlate, this has not yet been identified for HIV-1 since no HIV-1 vaccine to date has elicited a broadly neutralizing antibody response.

Recent outbreaks of Ebola and Zika virus (35) highlight the continued need for identifying immune correlates of protection. Based on studies in animal models, neutralizing antibodies and cell mediated immunity have both been proposed as CoP for Ebola (36–39) and are the key measurements utilized to analyze vaccine candidates in humans (40). Analyzing immune correlates in animal models can provide important leads for testing in human clinical trials. In the case of Zika virus, recent non-human primate studies implicate neutralizing antibody responses as a vaccine elicited immune response that may protect (41). Moreover, new vaccines may not yet have a certain correlate of protection as is the case for the newly licensed vaccine for Dengue (CYD-TDV) (42), although antibodies may play a role. Recent evidence also suggests that the protection by the chimeric Dengue vaccine may be mediated by a complex correlate, since vaccine efficacy is different in people pre-exposed to dengue vs. those naïve. Moreover, other Dengue vaccines that also elicit CD8 T cell response may provide different CoPs. The RTS,S/AS01E vaccine for malaria, which targets the circumsporozoite (CS) protein of *Plasmodium falciparum* sporozoites, demonstrated vaccine efficacy against clinical malaria in 55.8% of children and in 31.3% of infants (43–45) with waning efficacy over time (46). There is an active examination of both antibody and cell mediated responses to CSP as correlates of immunity.

Defining immune correlates is dependent on having a range of immune responses such that differentiating protected and unprotected status is possible. An example of a vaccine where

no immune correlate could be identified due to the lack of sufficient infection cases after the vaccine was given is the quadrivalent Human papillomavirus (HPV) vaccine (targeting HPV types 6, 11, 16, 18) with 96 −100% efficacy (47). Since there were few cases in vaccinees, an immune correlate could not be identified. However, robust neutralizing antibody responses to all four-vaccine HPV types were elicited and studies in animal models demonstrate that antibody is the effector of immunity.

The licensed vaccines for which binding antibodies are used as the immune correlate may reflect that either these measured responses are directly responsible for a functional response or that due to the simplicity and reproducibility of the assay, these antibody measurements serve as an excellent surrogate for more complex underlying immunological mechanisms. The efficacy of many of the licensed vaccines, as for example hepatitis B, may depend on the increase in specific immunity from an anamnestic response that is elicited upon pathogen exposure. For HIV-1, a vaccine that relies heavily on an anamnestic response would not be efficacious since this delayed immunity would occur too late to prevent HIV-1 integration into the host genome, an event that establishes HIV-1 infection. Thus, there is only a window of opportunity before the establishment of the latent pool of HIV-1 infected CD4+ T cells by which an effective HIV-1 vaccine can act (48). However, a rhesus CMV vector for SIV antigens has aborted SIV infection of macaques, showing that effector T cells can prevent establishment of a viral reservoir (49).

HIV-1 Vaccine Efficacy Trials

A major goal for HIV-1 vaccine development is to identify immune correlates of decreased transmission risk with the ultimate goal of identifying an immune CoP. HIV-1 vaccine efficacy trials have yielded a number of correlates of transmission risk; however, a correlate of protection for an HIV-1 vaccine has been proposed but not yet confirmed. In order to be recognized as a “correlate of protection” against HIV-1, the identified correlate of decreased risk must be tested and proven to correlate with decreased HIV-1 acquisition in another clinical trial. The ideal outcome is to be able to reproduce the immune correlate of HIV-1 vaccine efficacy in multiple geographic locations and risk groups. The RV144 trial was the only trial, out of six HIV-1 efficacy studies performed to date, that showed some level of vaccine efficacy at 31.2% (50, 51). Advances in understanding immune correlates of risk for HIV-1 involve evaluation of vaccine elicited immune responses, underlying host genetics, and HIV-1 virus sieve analyses. Intersections among these different approaches may generate hypotheses for further testing of potential mechanism(s) of protective immunity (51).

The first two HIV-1 vaccine efficacy trials (Vax003 (52), Vax004 (53)) were designed to induce and evaluate antibody responses and both ultimately showed no vaccine efficacy. Notably, the subsequent two HIV-1 vaccine efficacy trials were designed specifically to induce T cell responses; however, these vaccine regimens also did not result in vaccine efficacy. The fifth vaccine efficacy trial, RV144, did show efficacy and was capable of eliciting both cellular and humoral responses to the HIV-1 envelope. The sixth trial, HVTN505, which lacked vaccine efficacy was designed to primarily induce cellular immunity, but also elicited antibody responses. The four HIV-1 vaccine efficacy trials that

elicited antibody responses differed in strategy. VAX003 and VAX004 both tested a protein only immunogen strategy although with different clades of immunogens (Clades B/E vs. Clades B/B) and different risk populations (injection drug users vs. men who have sex with men (MSM)). RV144 and HVTN 505 were both prime/boost regimens but with different vector primes (ALVAC vs Ad5) and also in different risk populations (community based in Thailand vs. MSM in the US). The differences in immunogen design, route of HIV-1 transmission, and diversity of circulating HIV-1 strains are considerable and all may have contributed to the efficacy outcomes of the trials.

The only two vaccine trials, Step (HVTN 502) Phase IIb, and Phambili (HVTN 503) Phase IIb (54, 55), that did not contain the HIV-1 envelope were specifically designed to evaluated the capacity of HIV-1 specific T cell responses to reduce viral load in breakthrough infections. The vaccine regimen in Step and Phambili consisted of a replication defective adenovirus serotype 5 (Ad5) vector with clade B HIV-1 genes (*gag, pol nef*) (MRKAd5) given in three injections in men who have sex with men (MSM) (Step) and high risk heterosexual men and woman (Step, Phambili) in North and South America, Australia, Caribbean, and South Africa. Unexpectedly, there was a higher risk of HIV-1 acquisition in the vaccine arm compared to placebo in the Step trial prompting a discontinuation of vaccination in the Phambili study and early unblinding. As a result, there was an urgent need to understand immune responses to different vaccine vectors and potential mechanisms of increased HIV-1 risk. Although antigen specific T cell responses (54, 56, 57) were generated, there was also an increased rate of HIV-1 infection that was associated with being uncircumcised and having pre-existing Ad5 antibodies (reviewed in Gray *et al.*(58)). Follow-up studies reported a waning of the increased infection risk for the uncircumcised and Ad5 seropositive men over time (59).

Due to the outcome of the Step and Phambili trials, the phase IIb HVTN505 efficacy trial was redesigned to test the DNA/ Ad5 vector (Clade A, B, C, *Env*, Clade B *Gag/Pol*) in MSM and transgender (TG) participants that met the additional enrollment criteria of being Ad5 seronegative and circumcised. Nevertheless, efficacy futility was determined at the first interim analysis after full enrollment (60).

Immune Correlates of Decreased HIV-1 Transmission Risk in VAX004 and HVTN 505

Immune correlates of increased HIV-1 risk were identified for VAX004, RV144 and HVTN 505 (Table 2); however, the most scientific weight is given to immune correlates of decreased HIV-1 risk identified from RV144 since that was the only trial showing vaccine efficacy. One of the goals of follow-up studies of all efficacy trials is to examine potentially protective responses that are subdominant in the vaccine population but may have contributed to preventing acquisition or disease progression. Importantly, it is the heterogeneity of vaccine-elicited immune responses that allows identification of immune responses associated with HIV-1 risk and virus control. Thus, searching for immune correlates in large-scale efficacy trials and comparative immunogenicity studies in humans enable benchmarking current vaccine candidates against vaccines with known correlates of risk.

In Vax004, higher neutralizing antibodies (nAbs) to an easy to neutralize virus and antibody dependent cellular virus inhibition (ADCVI) correlated with decreased HIV-1 risk in the vaccinees (61). Vaccine induced antibodies could inhibit HIV-1 *in vitro* via FcR bearing effector cells through antibody dependent cellular virus inhibition (ADCVI)(62). Although neutralizing antibodies to viruses that are more difficult to neutralize (Tier 2, a phenotype of transmitted/founder viruses) were induced at low levels, they were not sufficient to correlate with vaccine efficacy (63) and suggest that a higher level and increased breadth of neutralizing antibodies would be needed for protection.

Although shown to lack overall vaccine efficacy (60), immune correlates analyses are underway for HVTN 505 to determine whether any cellular or humoral immune responses correlated with decreased risk of infection or decreased vaccine efficacy. HVTN 505 elicited particularly low levels of HIV-1 IgG V1V2 antibodies (60) supporting in a negative fashion the RV144 finding that higher levels correlated with decreased risk of HIV-1 infection. Analyses of the cellular response and a virus sieve analysis was performed to examine correlations with HIV-1 risk. Surprisingly, despite the lack of overall vaccine efficacy, there was both a cellular immune correlate of decreased HIV-1 risk and a significant sieve effect on the virus. Analyses of the antibody responses correlated with decreased HIV-1 risk are currently being examined (Fong, Gilbert, Tomaras, *et al.*). Polyfunctional CD8+ T cell responses significantly correlated with decreased HIV-1 risk (Frahm, McElrath *et al.* HIV Research for Prevention (R4P) meeting, Chicago, Illinois 2016) and efforts are underway to examine the capacity of these CD8+ T cells to mediate virus inhibition as previously reported for this vaccine regimen (64, 65). A genetic sequence analysis of the viruses transmitted in the vaccinees compared to the placebos (*i.e.* virus sieve analysis) examined if there were significant differences in virus sequences that could have resulted from vaccine-induced immune responses able to partially block HIV-1 acquisition or alter HIV evolution post-acquisition. Significant vaccine/placebo differences in the Env-gp120 sequence were identified in the HVTN 505 sieve analysis (66). Results of sieve analyses from all HIV-1 vaccine efficacy trials can generate testable hypotheses for mechanisms of protective immunity.

Vaccine Immune Correlates of Virus Control Post Infection in Step/HVTN 502

Follow-up studies of HIV-1 vaccinees that become infected, “breakthrough infections”, can also provide insights on the type and level of vaccine immunity that may control virus infection when acquisition does occur. Despite the lack of overall vaccine efficacy in the Step study there were subdominant vaccine-induced HIV-1 specific T cell responses with antiviral immunity in a subpopulation of vaccinees (67–73). Total T cell breadth and total magnitude of the vaccine elicited response before infection significantly correlated with lower mean viral load (74). A virus sieve analysis in Step/HVTN 502 identified significant sequence divergence of the infecting virus compared to the vaccine indicating selection at specific sites in the virus due to potential vaccine induced T cell specific immune pressure (67). However, the specific T cell responses measured in the trial did not correspond with the sieve findings. Host genetic associations were also identified in this study in that vaccinees with the HLA alleles (B*27, B*57, B*58:01), known to be associated with HIV-1 control, had lower viral load (69). The kinetics of the immune response also may have played a key

role in that the CD8+ T cell immunity that correlated with decreased virus load after breakthrough infection may have been related to the interval between vaccination and the time the vaccinees became infected (74).

RV144 Primary Immune Correlates

The RV144 vaccine efficacy trial remains the only study to date that demonstrated statistically significant vaccine efficacy (31%). A coordinated effort by a team of international scientists evaluated a wide range of vaccine elicited humoral and cellular responses. For the primary assessment of the immune correlates of risk,152 measurements were evaluated and specific hypotheses about immune correlates were tested with six final measurements chosen out of the 17 different types of immune assays. These downselected measurements (plasma Env IgA, Env IgG Avidity, HIV-1 neutralizing antibodies (nAbs), V1V2 IgG and Env specific CD4+ T cells) were selected for maximal statistical power since they met the criteria of assay reproducibility, sufficient dynamic range, and representation of unique immunological space hypothesized to be important for protection from HIV-1 acquisition. Of these six measurements, two primary immune correlates of risk (75) were identified. IgG antibodies to the variable 1/variable 2 (V1/V2) regions of the HIV-1 glycoprotein envelope correlated with decreased risk of HIV-1 infection (odds ratio 0.57, p= 0.02, Q = 0.08) (75, 76) and plasma Env IgA to specific envelope glycoproteins correlated with decreased vaccine efficacy (odds ratio 1.54, p =0.03, q =0.08) (75, 77) (Table 3).

V1V2 IgG Immune Correlate of Decreased HIV-1 Risk

The V2 loop of the HIV-1 envelope glycoprotein is a highly variable sequence that forms part of the CCR5 coreceptor binding site of the envelope. This region of the HIV-1 envelope was also reported to contain a putative α4β7 mucosal homing integrin binding site for HIV-1 infection, although binding to α4β7 was found not to be essential for infection by transmitted/founder viruses (78, 79). IgG responses to the V2 region of significantly correlated with decreased risk of infection (75, 76, 80–82). An intensive blinded, follow-up correlates study was planned to test whether the V1V2 HIV-1 IgG immune correlate of decreased HIV-1 risk could be confirmed. Since RV144 was the first vaccine trial to show efficacy, and follow-up trials were planned to test if V1V2 IgG was a correlate of protection in other settings, it was important to test whether the result could be reproduced and how robust the result was by testing with new assays and reagents. Zolla-Pazner *et al.*(81) reported that the V1V2 IgG correlate of decreased HIV-1 risk was confirmed and highly reproducible in different laboratories, with different assays and reagents (Table 4). The V1V2 sequence reagents were expanded to include cross-clade antigens and a “cross-reactivity” score also correlated with decreased risk of infection. The breadth of the V1V2 IgG response (81, 83, 84) significantly correlated with decreased HIV-1 infection risk (OR= 0.56/0.58, p value = 0.0073, 0.0015) (81). That the vaccine induced V2 antibodies specific for AE sequences representing circulating strains in the population as well as having evidence of breadth across multiple strains may be important for protection. Later, using the original data but with another analytical method, the V1V2 IgG correlate of decreased HIV-1 risk was also confirmed (82), indicating that the analytical result was robust to different methods as well. Thus, the approaches utilized in the analysis of the immune

correlates of HIV-1 risk may serve as a model for immune correlates analyses of other vaccine trials (HIV-1 and non-HIV-1 trials).

The V2 region is highly variable in length due to insertions and deletions along with differences in glycosylation across HIV-1 sequences. These variations will be a challenge for HIV-1 vaccines that aim to build on the V1V2 IgG correlate of decreased HIV-1 risk. HIV-1 virus sequences in HIV-1 vaccine recipients and placebos were analyzed within the V2 region to determine if there was evidence of immune pressure. This sieve analysis suggested that HIV strains matching the vaccine sequence with a lysine at position 169 (K169) in the V2 domain were less likely to be present in infected vaccinees suggesting immune pressure at this region (85). Moreover, the analysis of the B cell repertoire and the generation of mAbs that target the V2 region demonstrated that these V2 specific antibodies could mediate ADCC, neutralization and virus capture (86–88).

One question that arose with the identification of V1V2 specific antibodies as a correlate of decreased HIV-1 risk was whether the immunogen had a unique structure that enabled elicitation of certain specificities of antibodies. Both protein immunogens in RV144 were modified by an N-terminal 11-amino-acid deletion (Δ11) and addition of a herpes simplex virus (HSV) gD protein-derived tag (gD) to enable efficient expression and immunoaffinity purification of the protein for production (89, 90). The impact of this modification on gp120 expression, antigenicity and immunogenicity was determined in a follow-up study with plasma from RV144 vaccinees and rhesus macaque immunization study that compared the unmodified with the modified version of the envelope glycoprotein (91). The deletion, with or without the gD sequence, enhanced the antigenicity to the conformational C1 and V1/V2 regions on the A244 gp120 HIV-1 envelope glycoprotein. Thus, a unique aspect of the RV144 vaccine immunogen was the antigenicity of the A244 gp120 protein that exposed the V2 loop in a conformation that could be recognized by both linear and conformational V1V2-glycan broadly neutralizing antibodies and exposure of the conformational C1 region, a target for ADCC mediating antibodies (86, 91). The antigenicity of the vaccine immunogen likely contributes to the induction of V2 IgG antibodies compared to other vaccine immunogens (91, 92). However, the N-terminal deletion had little impact on the antigenicity of the B.MN gp120 (subtype B protein immunogen in RV144), AE.92TH023 gp120 (subtype AE sequence in the canarypox vector prime for RV144), and C.1086 gp120 (subtype C protein immunogen in current South African HIV-1 vaccine trials) envelope glycoproteins, since the folding, stability and conformational homogeneity of the immunogen is also influenced by the rest of the envelope sequence. For future HIV-1 vaccine trials, these data indicate that it is critical to evaluate protein immunogen conformation and antigenicity for suitability as vaccine immunogens that have the desired properties for eliciting potentially protective immune responses.

Envelope specific IgG3 Immune Correlate of Decreased Transmission Risk

Envelope-specific IgG3 responses correlated with decreased HIV-1 risk (76) and were associated with antibody Fc-mediated Ab function (76, 93). Antibody subclasses have distinct effector profiles due to their ability to differentially bind FcR and bind complement. Additionally, the longer hinge region in IgG3 enables more flexibility in recognition of

antigen on the Fab end. IgG3 antibodies have been associated with immune-mediated pathogen control for long-term control of Plasmodium falciparum and CHIKV neutralization (94–96). Notably, a number of HIV-1 bNAbs are of IgG3 origin (97) (Williams, Haynes *et al.* submitted) and the subclass is associated with increased neutralization activity, complement binding and ADCC (98). Recently, a comparison study of matched Env IgG1 and IgG3 antibodies showed that Env IgG3 mediated higher phagocytosis than Env IgG1 (99). Antibody-mediated phagocytosis of HIV-1 virions is an antiviral function that depends on both antibody specificity and isotype/subclass. Virion phagocytosis may be a mechanism by which IgG1 and IgG3 non-bNAbs in RV144 contributed to HIV-1 vaccine efficacy. These findings have important implications for harnessing antibody effector functions for HIV-1 vaccine design and passive immunotherapy for HIV-1 clearance at the portal of entry. Notably, in engineering bispecific antibodies for enhanced neutralization capacity, the IgG3 hinge was utilized to increase Fab domain flexibility for heterobivalent binding to improve neutralization (100).

Envelope IgA Immune Correlate of Decreased Vaccine Efficacy

Immunoglobulin A (IgA) is an important part of host defense by preventing transfer of pathogens across mucosal surfaces. Since mucosal samples were not collected in the RV144 trial, only circulating IgA could be examined in the immune correlates analysis. Surprisingly, HIV-1 Env IgA breadth correlated with decreased vaccine efficacy (75). Among the antigens comprising the breadth score, three IgA measurements independently correlated with vaccine efficacy. Plasma IgA to the constant 1 (C1) region of the envelope glycoprotein and two clade A envelope glycoproteins significantly correlated with decreased vaccine efficacy (odds ratio= 1.69, p-value <0.001) (75, 77). The secondary analysis demonstrated that in the presence of low vaccine-elicited IgA responses, both ADCC or neutralizing antibody responses correlated with decreased risk of infection. The ADCC responses were predominantly to the C1-C2 conformational region of gp120 (88, 101), although other epitope specificities were induced (i.e. V2, V3) that mediate ADCC (86). Follow-up studies demonstrated that IgA antibodies elicited in RV144 could compete with the function of the ADCC-mediating IgG responses targeting overlapping epitopes within the conformational C1-C2 region [5]. Moreover, the IgA/IgG ratio for some HIV-1 envelope glycoproteins correlates with infection risk (OR= 1.58 to 1.79, p-value= <0.01 to <0.001) (77). At the time, this type of interference of IgG function was only previously reported for host immunity to bacteria, the regulation of autoantibodies and ADCC activity of EBV-infected target cells in nasopharyngeal cancer (102–105). HIV-1 Env IgA interference with IgG mediated effector function was subsequently confirmed in an HIV-1 infection cohort study (106).

This work highlights the potential role of antibody interactions in a polyclonal mix of vaccine-induced antibodies. Thus, vaccine-induced polyclonal immune response may either be beneficial (107) as an antiviral response or detrimental (108) to the host vaccine immune response. Vaccine-induced epitope specific antibody responses can differentially engage cellular Fc receptors leading to diverse outcomes for effector functions dependent on the ratios of different antibodies induced by vaccination. It is important to note however that the plasma Env IgA response may not be a mechanistic correlate of increased HIV-1 risk but

rather a surrogate for an as yet unidentified mechanism leading to decreased vaccine efficacy. Mucosal specimens were not available for the RV144 studies, but future studies aim to evaluate the specificities and functions of mucosal IgA responses. Systemic and mucosal IgA differ in their subclass distribution and form. IgA1 is predominant in serum/plasma and is monomeric; whereas IgA2 is higher in mucosal secretions and is predominantly dimeric or polymeric with secretory component (109). These differences will significantly impact their effector function. Protection observed with IgA antibodies in the rhesus macaque model (110) and Identification of antiviral properties of vaccine elicited IgA responses (111) support efforts to further understand the potentially protective role of IgA for HIV −1 vaccines

Polyfunctional CD4+ T cell Correlate of Decreased HIV Transmission Risk

A follow-up immune correlates analysis identified that polyfunctional CD4 T cell responses correlated with decreased infection risk (112). This additional immune correlate of decreased HIV-1 risk was revealed with a novel unbiased analytical method based on a Bayesian hierarchical framework called “combinatorial polyfunctionality analysis of antigen specific T-cell subsets” (COMPASS), that can robustly evaluate complex T cell responses. Two polyfunctional antigen specific T cell subsets significantly correlated with decreased HIV-1 infection risk: one subset expressed CD40L, IL-2, IL-4, IFN-γ and TNF-α (OR= 0.58, p =0.006, q=0.05), while the second subset expressed CD40L, IL-2 and IL-4 (OR= 0.62, P =0.01, q=0.06). Notably, IL-4 and CD40L, molecules involved in T-B cell interactions were involved in both subsets that correlated with decreased infection risk. These findings indicate that the quality (i.e. the polyfunctional nature) of T cell responses is likely to play a key role in protective HIV-1 immunity and need to be evaluated independently of the magnitude of antigen specific T cell responses.

Complex Correlate of HIV-1 Transmission Risk in RV144

Multiple components of the immune system can act on pathogens along different stages of entry into the host. There are potentially multiple protective immune responses acting either sequentially or in concert. Analyses of the immune correlates of risk of RV144 have highlighted this complexity more than any other currently licensed vaccine regimen. HIV-1 vaccines induce a broad repertoire of antibody specificities and diverse subsets of antigen specific responses, each with different antiviral mechanisms and potencies. Notably, the RV144 correlates analysis identified antibody specificities and functions that can interact as part of the polyclonal vaccine induced response (50, 75, 76). In addition to V2, conformational C1 and V3 epitopes were part of the vaccine-induced ADCC response (88). Antibody synergy (113), additivity (114) and interference (77, 93) for ADCC and/or virion capture were all part of the polyclonal RV144 vaccine-induced response indicating that antibody Fc-FcR interactions may contribute to protective immunity.

The nature and characterization of the antibody responses that were identified to correlate with HIV-1 risk support the idea that the correlate of HIV-1 risk for the RV144 vaccine regimen is a complex correlate (3). Notably, supporting data from host genetics (115, 116) and virus sieve analysis (85) add a deeper layer of support to the primary immune correlates. The immune correlates of risk in RV144, although statistically associated with decreased

risk of HIV-1 infection, have not been proven to be mechanistically responsible for protection nor indicative that this immune measurement can be utilized to predict the protective efficacy of another vaccine regimen. The number of interactions among immune measurements in RV144 and that the primary V1V2 IgG antibody measurement was also present in VAX003 that lacked vaccine efficacy suggest that the magnitude of the V1V2 IgG response by itself is unlikely to signify an immune correlate that by itself can benchmark further vaccine designs.

The potential role of multiple immune measurements acting in concert to prevent HIV-1 acquisition was evaluated through interaction models of the six primary immune measurements in the original study of RV144 (75), analysis of T cell subsets by Bayesian statistics (112), and a computational approach to examine interactions among all immune measurements (82) (Table 3). Analytical methods for evaluation of immune correlates for vaccine trials have utilized a variety of analytical and statistical methods. These methods include hypothesis driven analyses using statistical testing and unbiased approaches utilizing Bayesian statistics and machine learning techniques. A combination of these different strategies has proven to be informative. In particular, a Bayesian approach was able to identify cellular subsets that were not selected a priori and statistical interaction models provided new insights into antibody interactions that may contribute to vaccine efficacy. Machine learning approaches provided insights on the complexity of the immune correlates as well as confirming the correlates findings from the hypothesis-driven approach. Going forward, replication of an immune correlate in an independent trial will be critical. Given the intricacies of the immune system and the expansion of immunological assays to probe host immunity these different approaches are complementary and will be needed across pathogens for the identifying correlates of immunity.

Immunological Mechanisms of Protection

Mode of HIV-1 Transmission

HIV-1 can be transmitted by sexual contact (i.e. heterosexual and homosexual), Intravenous needles (IV), and mother to child (MTCT). Despite being relatively inefficient, the vast majority of transmissions worldwide is through heterosexual contact. Vaccine designs are aimed to prevent transmission at the mucosal surface, and thus work to understand the event surrounding the mucosal bottleneck could inform vaccine design (117, 118). Understanding the sequence of events that must occur during HIV-1 transmission can provide a roadmap for the types of immunological defenses that would be needed to block infection. For genital mucosal transmission, HIV-1 virus particles must traverse the mucus layer and epithelial cells to infect target cells either in the lamina propria or in the bloodstream. Thus, vaccine elicited immunity can establish a blockade with antibodies that can bind virus to generate immune complexes that can aggregate and/or neutralize to be readily cleared. Additionally, vaccine elicited antibodies can engage effector cells present at the portal of entry to clear virus particles and/or infected cells. Preventing transmission through direct blood exposure through (*i.e.* intravenous drug use) may require different protective immunological mechanisms due to the different portal of entry. The number and characteristics of the transmitted viruses will also influence what is needed for protective immunity. Compared to

heterosexual transmission, there is a higher multiplicity of infection in men who have sex with men (119) and in injection drug users (120). Thus, immune correlates of protection for a given population may depend on the predominant risk category.

Impact of HIV-1 Genetic Diversity on Immune Correlates

Consideration of the genetic variability of HIV-1 for improved HIV-1 vaccine design is complex (121). The HIV-1 envelope protein sequence varies over time and globally impacting the immune specificities needed to prevent HIV-1 transmission (122). This growing diversity makes it difficult to match vaccine immunogens with current circulating strains and to be able to cover the global diversity. Strategies such as using consensus or ancestor sequences to minimize the diversity (121, 123, 124) have been tested in preclinical studies and are currently being tested in Phase I human clinical trials to expand the coverage across diverse isolates for both cellular and humoral immunity. The age of the epidemic varies in different regions around the world which is reflected in the overall diversity of the currently circulating viruses. For example, the clade AE viruses circulating in Thailand are less divergent from each other than the clade C viruses circulating in South Africa. This diversity makes it more difficult to match the HIV-1 sequence in the vaccine to the circulating virus isolates that are responsible for transmission in a Clade C endemic population than for subtype AE. Given the time from vaccine immunogen design to implementation of a Phase III efficacy study, the disparity in sequence between the vaccine and the target population will just become larger. In the case of the RV144 vaccine regimen, the relatedness of the vaccine strain to the circulating sequences at the time of vaccination may have contributed to the vaccine efficacy. This will be difficult to replicate in other vaccine regimens due to the widening virus diversity. Notably, the similarity of the gp120 vaccine sequence protein boosts (1086, TV-1) and the circulating strains in South Africa was calculated to be 8% more distant from each other than between the RV144 vaccine regimen and circulating CRF01_AE viruses in Thailand (125), which may make it more difficult to achieve vaccine efficacy in South Africa compared to Thailand. However, the overarching goals is to identify and implement vaccine strategies that elicit immune responses to conserved regions on the virus to overcome virus diversity and result in broad protective immunity.

Host Genetics and HIV-1 Vaccine Responses

Innate and adaptive immunity to pathogens and vaccination can be influenced at the individual level by host genetics. For HIV-1 infection, individual HLA alleles, killer cell immunoglobulin-like receptors (KIRs) and chemokine coreceptor polymorphisms are the primary genetic determinants shown to influence HIV-1 disease (126). Emerging data for HIV-1 vaccines indicate that host genetics can significantly impact vaccine efficacy. Understanding the mechanism of these genetic linkages with vaccine efficacy may enable a better understanding of mechanisms of antiviral immunity ultimately leading to improved vaccine designs for global coverage across diverse populations.

The multi-faceted functional properties of HIV-1 antibodies are influenced by the antibody specificity for infectious virions and/or infected cells, antibody affinity for specific Fc receptors, local inflammation at the portal of entry and also host genetics that may modulate

antibody interactions with FcR on effector cells. VAX004, a gp120 protein boost (Clade B/alum) vaccine efficacy trial in MSM and high-risk women reported that the presence of an Fcγ receptor IIIa genotype (VV genotype) associated with increased rate of HIV-1 infection in low risk (but not high risk) vaccinees (127). Thus, within one efficacy trial, there were antibody Fc mediated functional antibodies (ADCVI) that correlated inversely with infection rate (62); however there was also an FcR host genetic association with increased risk of infection in low risk vaccinees. Further work is needed to determine if this genetic association holds in other HIV-1 efficacy studies.

In follow-up studies to the Step trial (54, 56), it was reported that there was selection at specific sites in the breakthrough viruses indicating potential vaccine induced T cell specific immune pressure (67). The MHC class I HLA alleles (B*27, B*57, B*58:01) are known to associate with natural control of HIV-1 replication and it was found that Step vaccinees with these MHC class I alleles did show robust $CD8^+$ T cell activity (70) and lower viral loads post infection (69). However, the tested HIV-1 antigen specific T cell responses were not associated with lower risk of infection in the vaccine recipients,

For the RV144 efficacy trial, host genetic associations with HIV-1 infection risk have also been identified. In these recent studies, the biological mechanism is unclear, but they raise hypotheses that can be tested in further studies. In RV144, vaccine efficacy against viruses with a lysine at position 169 was higher in those with the HLA A*02 allele (128). In the A*02 (−) subgroup, there was a direct correlation with plasma C1 specific HIV-1 envelope IgA and HIV-1 infection rate. The mechanism by which the A*02 allele associates with the antibody response is unknown, but raises hypotheses that can be addressed in follow-up studies. In a subsequent study, Li *et al.* (116) demonstrated that vaccine efficacy for viruses with a lysine at position 169 was higher in vaccinees with a CT or TT SNP in FcγRIIC with 91% VE (116). These data support a role for understanding antibody Fc- FcR interactions in vaccine-induced immune responses. Notably, there are reported genetic differences in FcR in South Africans compared to Thais (129), so it will be important to continue to evaluate the interaction of host genetics with vaccine induced immunity in upcoming efficacy trials. In the Lassauniere *et al.* (129) study, black South Africans did not have the haplotypes associated with increased surface expression of FcγRIIb and FcγRIIIa, nor the FcγRIIC haplotype that correlated with decreased HIV-1 risk in the RV144 HIV-1 vaccine trial. Moreover, there are differences among Africans and Caucasians as well as among different African populations for FcγR allele distribution and linkage disequilibrium. A third follow-up study on the association of host genetics and RV144 vaccine efficacy examined HLA class II genotype association with HIV-1 antibody levels and HIV-1 acquisition. The rationale is that HLA class II-restricted CD4+ T cells are involved in antibody production. Thomas *et al.*(130) found that DQB1*06 had a significant interaction with plasma Env IgA responses, such that DQB1*06 had a significant effect on HIV-1 infection among the high IgA responders. IgG antibody responses to the HIV-1 envelope glycoprotein at positions 120–204 were associated with decreased risk of HIV-1 acquisition (*i.e.* increased vaccine efficacy) in the presence of DPB1*13. These finding indicate that host genetics related to HLA class II genotype impacted the protective efficacy of the RV144 trial. Further testing of these HLA genotypes and association with HIV-1 acquisition in upcoming vaccine efficacy trials will be important.

Systems Biology Analysis of Immune Correlates of Transmission Risk

Analyses across biological systems and across vaccine trials (systems vaccinology) provides a way to understand a biological system by interrogating multiple measurements and examining their interdependence. One goal is to utilize systems vaccinology to identify a universal signature for protective immunity through meta-analyses that compare signatures across vaccine studies. Gene expression analyses in different cell populations post vaccination have been analyzed to better understand the mechanisms of immune correlates of protection. A systems approach evaluated the innate immune response to a non-efficacious HIV vaccine and to yellow fever vaccine and found differences in gene expression related to inflammation, IFN response, myeloid cell trafficking and lymphocyte specific transcripts (131), suggesting that identifying immune signatures early after vaccination may inform selection of vaccine candidates that elicit innate pathways leading to a protective response. Additionally, data generated from vaccines against SIV, HIV-1, yellowfever, influenza, Hepatitis B have resulted in the identification of genes that correlate with antibody responses (132–134). Recently, multivariate approaches, based on non-hypothesis driven, or unbiased, approaches of the immune response to the partially efficacious RV144 vaccine have resulted in or confirmed HIV-1 correlates of risk (82, 112). Lin *et al.*(112) newly identified two T cell subsets, a five-function and three-function subset that correlate with decreased risk of HIV-1 infection. The five function subset matched the significance of the primary V1V2 IgG correlate of decreased HIV-1 risk (p-value = 0.006, q-value = 0.05). These correlates of decreased HIV-1 risk were not previously identified in the RV144 analysis (75) and were the first to highlight a role of unbiased approaches to the identification of HIV-1 vaccine correlates that complement the hypothesis driven approaches. Chung *et al.* (82) explored both a systems serology approach to examine relationships among multiple immune measurements in different vaccine regimens compared to RV144 and a machine learning approach to evaluate HIV-1 correlates of risk on the original data from RV144. The first approach examined six different antibody effector functions with two cell types: Natural Killer Cells and Monocytes across four different vaccine studies. Confirming earlier reports (76, 93), the analysis by LASSO (135) (the least absolute shrinkage and selection operator) and PLSDA (partial least-squares discriminant analysis) (136, 137) also identified that HIV-1 envelope IgG3 uniquely distinguished the efficacious vaccine RV144 regimen from the non-efficacious VAX003 vaccine regimen. In a correlation network analysis using non-case control samples, the antibody effector functions of antibody dependent cellular cytotoxicity (ADCC), antibody dependent cell mediated phagocytosis (ADCP), and antibody dependent complement deposition (ADCD) were tethered to Env IgG1 and IgG3 responses. Since the Env IgG3 was in lower abundance than IgG1 and strongly correlated with the IgG1 response, it was hypothesized that the IgG3 responses is a surrogate for a population of highly functional IgG1 responses that are acting together to prevent transmission. Notably, the IgG V1V2 responder profile was linked to ADCP, ADCC and antibody dependent NK degranulation. The IgA response that correlated with increased HIV risk (decreased vaccine efficacy) (75, 77) was linked to binding to the FcR inhibitory receptor, FCGR2B consistent with a role for FcR binding being a crticial player in immune correlates of risk (77). PLSDA and correlation networks analyses importantly confirmed the original correlates of HIV-1 risk: V1V2 IgG and Env IgA. Although the systems serology approach did not identify new correlates of risk, these

analyses provide a powerful and insightful portrait of the complex dimensions of antibody immunity that must work together to contribute to protective immunity. A similar systems approach combined with gene expression pathway analysis was applied to understanding the correlates of delayed SIV acquisition in a canarypox prime, protein boost in nonhuman primates (134). The systems biology analysis in Vaccari *et al*. identified differences in the two adjuvants (Alum, MF-59) linked to immune responses associated with delayed acquisition (i.e. Env dependent mucosal innate lymphoid cells (ILCs) producing IL-17, mucosal IgG V2, and RAS pathway). The implementation of these systems wide analyses across immune measurements, gene expression pathways, and across vaccine trials will accelerate the path toward understanding the complex dimensions of protective immunity for HIV-1 vaccines, as well as for other persistent pathogens.

Balancing Protective Immunity

HIV-1 vaccines generally induce a diverse array of immune responses in response to the same vaccine regimen (i.e. multiple specificities with different kinetics, levels and functions of cellular and humoral immunity) within individuals, and as well, among individuals in the population. The factors that underpin this diversity of immune responses are likely due to differences in host genetics, influence of pre-existing immune repertoires among individuals, gender, age, body mass index, and/or other unknown factors. Moreover, within individuals, the diverse vaccine-induced immune responses could interact to potentiate or interfere with antiviral immunity or could act together to inhibit HIV-1 acquisition at various locations at the portal of entry. Thus, an improved understanding of how to balance vaccine-induced HIV-1 immunity in favor of protective immunity and away from responses that favor HIV-1 replication or interfere with the protective immune responses is critical for advancing HIV-1 vaccine science.

Immunogenicity to vaccines can involve both potentially protective and potentially harmful immune responses. Several studies have highlighted the delicate balance of immunity and are instructive for further vaccine development (reviewed in (138, 139). Some immune responses may tip the balance toward increased pathology or decreased vaccine efficacy rather than protection. Two of the six HIV-1 efficacy trials, Step (54, 59) and Phambili (55, 57, 140), conducted to date were halted due to increased risk of HIV-1 infection and suggest that some immune responses may need to be avoided. Follow-up studies of the Phambili trial identified that there was a statistically significant increased risk of infection in long term follow-up that could not be attributed to changes in risk behavior after un-blinding (55). The follow-up meta analysis of the HIV-1 vaccine efficacy study (HVTN 505: DNA prime, Ad5 boost regimen in Ad5 seronegative, circumcised men) (60, 141) did not find evidence of increased risk of infection. Thus, not all Ad5 containing vaccine regimens have resulted in increased HIV-1 risk. A role for understanding immune activation in the context of increased infection was noted as a key goal for HIV-1 vaccine design and evaluation at a 2013 National Institutes of Health summit (138).

HIV-1 is unique from other pathogens targeted by currently licensed vaccines in that CD4+ T cells, the key modulators of T cell help for mature and protective adaptive responses are also the primary cellular target for HIV-1 infection. Thus, vaccine regimens that serve to

elicit HIV-1 specific CD4+ T cells may also promote a cellular environment conducive to infection, rather than only eliciting a cell population that contributes to protective immunity. Effective HIV-1 vaccine strategies are likely to induce some level of $CD4^+$ T cell activation; however, substantive overall HIV-1 specific immunity may tip the scale in favor of the host toward protection from HIV-1 acquisition(138). Thus, the Step study provides information on how vaccination may lead to increased HIV-1 acquisition and also how the same vaccine can induce effective immune responses (i.e. that impact virus replication). These findings support the notion that it will be important to determine how a vaccine regimen can induce potentially protective immune responses in some vaccinees but in contrast lead to increased acquisition at the overall population level. Understanding how to harness effective immunity induced by vaccination while eliminating the consequence of increased acquisition is likely to be key in tipping the balance in favor of the human host and away from an advantage for HIV-1.

The Next HIV-1 Efficacy Trials

Modeling the impact of HIV-1 vaccine efficacy on new infections has been studied for a number of scenarios and suggests that even moderately efficacious vaccine regimens can have a significant impact on controlling the number of new HIV-1 infections. One example is a modeling study of the impact of an HIV vaccine with 40% efficacy that estimated a dramatic 52% reduction in new infections when high risk populations were vaccinated (142).

The global burden of HIV-1/AIDS is especially concentrated and rising in sub-Saharan Africa (143). HIV-1 vaccine efficacy studies designed to build upon the correlates of decreased HIV-1 risk in RV144 are planned using clade A/E immunogens with improved adjuvants in Thailand and also with clade C inserts to be tested in clade C endemic regions. A Phase I program, HVTN 100, is evaluating a canarpox pox, protein boost vaccine regimen with clade C inserts to build upon the type of immunization utilized in RV144. This vaccine regimen differs from that of RV144 in two major ways: 1) it utilizes different HIV-1 genetic sequences replacing with Clade C sequences and 2) a different adjuvant. The rationale for these changes was to match the clade to the region that the efficacy trial would be tested in (clade C) and to try to improve upon the durability of the immune response by replacing alum with a more potent clinically licensed adjuvant, MF59. A Phase 2–3 efficacy trial, HVTN 702, is planned in South Africa by the Pox Protein Public Private Partnership (P5) to build upon the correlates results of the RV144 trial and based on the HVTN 100 poxvirus prime, protein boost regimen (144). A separate approach leading to planned efficacy trials are planned with replication-incompetent Ad26 Mosaic with a modified vaccinia Ankara (MVA) mosaic vector and Clade C recombinant protein boost that builds both upon the success of RV144 trial and protection studies in non-human primates (145, 146).

Additionally, studies of passively infused broadly neutralizing antibodies are planned for efficacy trials. Passive infusion of VRC01 bnAb (147), that targets the CD4bs, is being tested in an HIV-1 efficacy trial to determine if a bNAb can prevent transmission in humans. Although not a vaccine, these types of trials will provide a critical proof of concept as to whether a bNAb, when present before infection can protect against acquisition, and, if so, in what concentrations and specificities are necessary (148). Exciting developments in antibody

engineering for improved neutralization potency and breadth, durability and effector function will further enrich understanding of what constitutes protective immunity (149). These results will help guide and focus current vaccine studies that aim to induce these types of antibodies.

Immune Measurements

The human immune system is a complex and interconnected network of tissues, organs and molecules that traverse the three-dimensional space of the human body. The route of transmission by HIV-1 is through genital/anal sexual transmission and intravenous drug use and the protective mechanisms for each route of transmission may differ. The sampling of immune responses that correspond to protection have been limited to cells and antibodies circulating in the peripheral blood, predominantly due to cost and efficiency of trial design and immune evaluations. Surrogates of humoral and cellular responses residing in lymph node and genital mucosa are sought after, but have not been identified. The genital mucosa is a primary portal of entry for HIV-1 acquisition, and yet few of the immune responses evaluated in clinical trials focus on mucosal immunity. Similarly even in immune correlates analyses, there are limitations on what immune responses are tested in a correlates analyses due to inherent assay limitations, specimen volumes and the requirement of minimizing test variables to maintain statistical power. In the HIV field, functional assays for either antibody mediated or cell mediated antiviral activity are complex and were in their infancy when the preceding HIV vaccine efficacy trials were evaluated. Due to the findings from the RV144 trial that highlight the potential importance of antibody Fc-mediated effector functions and different CD4 T cell polyfunctional responses there is now more in depth work on mechanisms of humoral and cellular immunity elicited by vaccination.

Genital mucosal Immunity

An emerging focus on improving the assay technologies to evaluate mucosal immunity is likely to lead to robust immune measurements that can be utilized to evaluate immune correlates of protection in upcoming efficacy trials. Mucosal specimens were not collected in many of the previous efficacy trials, in part due to the concern of disrupting the genital mucosa in a way that would compromise the integrity of the mucosal barrier leading to transmission. In one efficacy trial, mucosal secretions were evaluated and although plasma IgA to the HIV-1 gp120 envelope glycoprotein was detected, there was no HIV-1 specific cervicovaginal IgA (150). However, ongoing studies of vaccine candidates continue to measure mucosal and systemic immunity to understand potential differences and identify surrogates of mucosal immunity that can be sampled routinely or non-invasively for evaluating correlates of protection.

Immune Response Durability

Maintenance of vaccine-elicited immune responses is critical for vaccine efficacy. Antibody responses elicited by several vaccines such as measles have the longest durability whereas responses to tetanus and diphtheria were comparably shorter (151). HIV-1 antibody responses are markedly shorter and thus pose a unique obstacle to overcome for effective vaccine design. Due to the decay in HIV-1 vaccine induced immune responses post vaccination, the time point at which vaccine efficacy is determined can substantially impact

the reported level of efficacy. RV144 had a higher level of vaccine efficacy (60.5%) through 12 months post initial vaccination (152) that declined to 31.2% (75) at the 42 months follow-up time pre-specified for reporting vaccine efficacy. For RV144, this higher vaccine efficacy corresponds to a time when most immune responses were higher (i.e. Env IgG / IgG3 and ADCC responses (76)) suggesting that a focus on improved vaccine regimens and adjuvants that can increase the durability of HIV-1 immune responses post vaccination is central to raising the level of HIV-1 vaccine efficacy in future trials.

Conclusions

Although existing prevention strategies, including the scale up of ART with strategies like pre-exposure prophylaxis (PreP) (153–155), antiretroviral treatment for prevention (156), implementation of male circumcision (157, 158), and topical microbicides (159) in highly endemic areas, have proven successful in large scale trials, the number of new infections has not substantially diminished (143). There was a peak of new infections in 1997 at 3.3 million that dropped to 2.5 million in 2005 and has stayed at this number for over the last ten years (143). This is in contrast to HIV deaths that have substantially declined over this time. However, in 2015 there were still 6000 new HIV-1 infections per day. Thus, there is an urgent need to understand the immunological mechanisms that can lead to an efficacious vaccine. Further HIV-1 vaccine efficacy trials will serve to test novel promising vaccine concepts as well as build upon the hypotheses generated from the RV144 trial. If vaccine efficacy is obtained for any of these planned trials, immune correlates analyses will be able to build upon the findings from RV144 to and answer whether there are different immune correlates for different vaccine regimens or whether a common immune correlate of protection, either simple or complex, can be obtained for HIV-1 vaccines. In the meantime, additional evaluation of a myriad of new phase 1/2 HIV-1 vaccine trials will further understanding the role that different vaccine modalities (DNA, vector, protein) and adjuvants play in shaping immunity.

Long-term follow-up of vaccinees at high risk of infection will continue to be important to understand the lasting impact of HIV-1 vaccine immunity. Further studies to interrogate the potential mechanisms for increased risk of infection seen in the one HIV-1 vaccine efficacy trials to date will be key for understanding how vaccines can modulate immunity toward preventing HIV-1 acquisition. One goal going forward is to understand how to modulate the vaccine induced immune response toward protective immune responses as the dominant response within individuals and across the population. Particularly important will be to understand if in some vaccinees there are subdominant protective immune responses that could be expanded and preferentially and specifically induced by the newly emerging immunogen design strategies. The pre-existing immune repertoire within an individual may shape vaccine-induced immunity (160) and ultimately impact the overall efficacy results at the population level. Development of improved vaccine immunogens in the form of stabilized HIV-1 envelope trimers that are engineered to expose vulnerable regions in the HIV-1 envelope for bnAb development (161), development of improved selection of envelope sequences to include in the immunogen either in the form of computationally derived mosaic envelopes and well defined envelope sequences known to drive bnAb

development *in vivo* or capable of binding to germline B cells will also be critical for advancing toward an efficacious HIV-1 vaccine and identifying correlates of protection.

An improved understanding of the underlying basic mechanisms for anti-viral immunity elicited by HIV-1 vaccines is needed in order to avert increased infections in further HIV-1 vaccine trials and also to improve upon the 31.2% efficacy observed to date. This includes understanding the mechanisms of protection by V1V2 IgG antibodies (75) and the CD4+ T cell polyfunctional responses (112) that significantly correlated with decreased HIV-1 infection risk. These basic insights are needed to implement methods for evaluating promising vaccine candidates early on in phase I studies for their potential to induce elicit protective immunity. Importantly, work toward translating the most effective vaccine concepts from animal models to humans, including the induction of HLA-E and Class II restricted CD8+ T cell responses (49) could fundamentally change the nature of immune correlates of protection for an HIV-1 vaccine.

Acknowledgments

Funding support was provided by the National Institutes of Health (NIH/NIAID/ DAIDS) HIV Vaccine Trials Network Laboratory Center (AI068618), P01 A1120756-01A1, Duke Center for AIDS Research (AI064518) and the Duke Interdisciplinary Research Training Program in HIV/AIDS (T32 AI007392) and the Bill & Melinda Gates Foundation grants OPP1068333 and OPP1151372.

References

1. Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol. 2010; 17(7): 1055–1065. [PubMed: 20463105]
2. Plotkin SA, Gilbert PB. Nomenclature for immune correlates of protection after vaccination. Clin Infect Dis. 2012; 54(11):1615–1617. [PubMed: 22437237]
3. Plotkin SA. Complex correlates of protection after vaccination. Clin Infect Dis. 2013; 56(10):1458–1465. [PubMed: 23386629]
4. Plotkin SA. Vaccines: correlates of vaccine-induced immunity. Clin Infect Dis. 2008; 47(3):401–409. [PubMed: 18558875]
5. Iwasaki A. Exploiting Mucosal Immunity for Antiviral Vaccines. Annu Rev Immunol. 2016; 34:575–608. [PubMed: 27168245]
6. Thakur A, Pedersen LE, Jungersen G. Immune markers and correlates of protection for vaccine induced immune responses. Vaccine. 2012; 30(33):4907–4920. [PubMed: 22658928]
7. Johnson SE, Rubin L, Romero-Steiner S, Dykes JK, Pais LB, Rizvi A, et al. Correlation of opsonophagocytosis and passive protection assays using human anticapsular antibodies in an infant mouse model of bacteremia for Streptococcus pneumoniae. The Journal of infectious diseases. 1999; 180(1):133–140. [PubMed: 10353871]
8. Vitharsson G, Jonsdottir I, Jonsson S, Valdimarsson H. Opsonization and antibodies to capsular and cell wall polysaccharides of Streptococcus pneumoniae. The Journal of infectious diseases. 1994; 170(3):592–599. [PubMed: 8077716]
9. Schuerman L, Wysocki J, Tejedor JC, Knuf M, Kim KH, Poolman J. Prediction of pneumococcal conjugate vaccine effectiveness against invasive pneumococcal disease using opsonophagocytic activity and antibody concentrations determined by enzyme-linked immunosorbent assay with 22F adsorption. Clin Vaccine Immunol. 2011; 18(12):2161–2167. [PubMed: 21994351]
10. Nalin DR, Kuter BJ, Brown L, Patterson C, Calandra GB, Werzberger A, et al. Worldwide experience with the CR326F-derived inactivated hepatitis A virus vaccine in pediatric and adult populations: an overview. J Hepatol. 1993; 18(Suppl 2):S51–S55. [PubMed: 8182275]
11. Jack AD, Hall AJ, Maine N, Mendy M, Whittle HC. What level of hepatitis B antibody is protective? The Journal of infectious diseases. 1999; 179(2):489–492. [PubMed: 9878036]

12. Kayhty H, Peltola H, Karanko V, Makela PH. The protective level of serum antibodies to the capsular polysaccharide of Haemophilus influenzae type b. The Journal of infectious diseases. 1983; 147(6):1100. [PubMed: 6602191]
13. Kayhty H. Difficulties in establishing a serological correlate of protection after immunization with Haemophilus influenzae conjugate vaccines. Biologicals. 1994; 22(4):397–402. [PubMed: 7779368]
14. Frazer I. Correlating immunity with protection for HPV infection. Int J Infect Dis. 2007; 11(Suppl 2):S10–S16. [PubMed: 18162240]
15. Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, Andrade RP, et al. Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. Vaccine. 2006; 24(27–28):5571–5583. [PubMed: 16753240]
16. Chen RT, Markowitz LE, Albrecht P, Stewart JA, Mofenson LM, Preblud SR, et al. Measles antibody: reevaluation of protective titers. The Journal of infectious diseases. 1990; 162(5):1036–1042. [PubMed: 2230231]
17. Matter L, Kogelschatz K, Germann D. Serum levels of rubella virus antibodies indicating immunity: response to vaccination of subjects with low or undetectable antibody concentrations. The Journal of infectious diseases. 1997; 175(4):749–755. [PubMed: 9086126]
18. Skendzel LP. Rubella immunity. Defining the level of protective antibody. Am J Clin Pathol. 1996; 106(2):170–174. [PubMed: 8712168]
19. Plotkin SA. Rubella vaccine. The Journal of infectious diseases. 1995; 171(6):1690–1692. [PubMed: 7769321]
20. White CJ, Kuter BJ, Ngai A, Hildebrand CS, Isganitis KL, Patterson CM, et al. Modified cases of chickenpox after varicella vaccination: correlation of protection with antibody response. Pediatr Infect Dis J. 1992; 11(1):19–23. [PubMed: 1312704]
21. Clements DA, Armstrong CB, Ursano AM, Moggio MM, Walter EB, Wilfert CM. Over five-year follow-up of Oka/Merck varicella vaccine recipients in 465 infants and adolescents. Pediatr Infect Dis J. 1995; 14(10):874–879. [PubMed: 8584315]
22. Gilbert PB, Gabriel EE, Miao X, Li X, Su SC, Parrino J, et al. Fold rise in antibody titers by measured by glycoprotein-based enzyme-linked immunosorbent assay is an excellent correlate of protection for a herpes zoster vaccine, demonstrated via the vaccine efficacy curve. The Journal of infectious diseases. 2014; 210(10):1573–1581. [PubMed: 24823623]
23. Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg (Lond). 1972; 70(4):767–777. [PubMed: 4509641]
24. Black S, Nicolay U, Vesikari T, Knuf M, Del Giudice G, Della Cioppa G, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. Pediatr Infect Dis J. 2011; 30(12):1081–1085. [PubMed: 21983214]
25. Coudeville L, Bailleux F, Riche B, Megas F, Andre P, Ecochard R. Relationship between haemagglutination-inhibiting antibody titres and clinical protection against influenza: development and application of a bayesian random-effects model. BMC Med Res Methodol. 2010; 10:18. [PubMed: 20210985]
26. Weibel RE, Buynak EB, McLean AA, Hilleman MR. Long-term follow-up for immunity after monovalent or combined live measles, mumps, and rubella virus vaccines. Pediatrics. 1975; 56(3): 380–387. [PubMed: 1161394]
27. Faden H, Modlin JF, Thoms ML, McBean AM, Ferdon MB, Ogra PL. Comparative evaluation of immunization with live attenuated and enhanced-potency inactivated trivalent poliovirus vaccines in childhood: systemic and local immune responses. The Journal of infectious diseases. 1990; 162(6):1291–1297. [PubMed: 2172403]
28. Mack TM, Noble J Jr, Thomas DB. A prospective study of serum antibody and protection against smallpox. Am J Trop Med Hyg. 1972; 21(2):214–218. [PubMed: 5061278]
29. Sarkar JK, Mitra AC, Mukherjee MK. The minimum protective level of antibodies in smallpox. Bull World Health Organ. 1975; 52(3):307–311. [PubMed: 1084801]
30. Monath TP, Nichols R, Archambault WT, Moore L, Marchesani R, Tian J, et al. Comparative safety and immunogenicity of two yellow fever 17D vaccines (ARILVAX and YF-VAX) in a phase

III multicenter, double-blind clinical trial. Am J Trop Med Hyg. 2002; 66(5):533–541. [PubMed: 12201587]

31. Schmader KE, Levin MJ, Gnann JW Jr, McNeil SA, Vesikari T, Betts RF, et al. Efficacy, safety, and tolerability of herpes zoster vaccine in persons aged 50–59 years. Clin Infect Dis. 2012; 54(7): 922–928. [PubMed: 22291101]
32. Laing KJ, Russell RM, Dong L, Schmid DS, Stern M, Magaret A, et al. Zoster Vaccination Increases the Breadth of CD4+ T Cells Responsive to Varicella Zoster Virus. The Journal of infectious diseases. 2015; 212(7):1022–1031. [PubMed: 25784732]
33. Weinberg A, Zhang JH, Oxman MN, Johnson GR, Hayward AR, Caulfield MJ, et al. Varicella-zoster virus-specific immune responses to herpes zoster in elderly participants in a trial of a clinically effective zoster vaccine. The Journal of infectious diseases. 2009; 200(7):1068–1077. [PubMed: 19712037]
34. Excler JL, Ake J, Robb ML, Kim JH, Plotkin SA. Nonneutralizing functional antibodies: a new "old" paradigm for HIV vaccines. Clin Vaccine Immunol. 2014; 21(8):1023–1036. [PubMed: 24920599]
35. Haug CJ, Kieny MP, Murgue B. The Zika Challenge. The New England journal of medicine. 2016; 374(19):1801–1803. [PubMed: 27028782]
36. Wilson JA, Hevey M, Bakken R, Guest S, Bray M, Schmaljohn AL, et al. Epitopes involved in antibody-mediated protection from Ebola virus. Science. 2000; 287(5458):1664–1666. [PubMed: 10698744]
37. Shedlock DJ, Bailey MA, Popernack PM, Cunningham JM, Burton DR, Sullivan NJ. Antibody-mediated neutralization of Ebola virus can occur by two distinct mechanisms. Virology. 2010; 401(2):228–235. [PubMed: 20304456]
38. Sullivan NJ, Martin JE, Graham BS, Nabel GJ. Correlates of protective immunity for Ebola vaccines: implications for regulatory approval by the animal rule. Nat Rev Microbiol. 2009; 7(5): 393–400. [PubMed: 19369954]
39. Sullivan NJ, Hensley L, Asiedu C, Geisbert TW, Stanley D, Johnson J, et al. CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. Nature medicine. 2011; 17(9):1128–1131.
40. Regules JA, Beigel JH, Paolino KM, Voell J, Castellano AR, Munoz P, et al. A Recombinant Vesicular Stomatitis Virus Ebola Vaccine - Preliminary Report. The New England journal of medicine. 2015
41. Abbink P, Larocca RA, De La Barrera RA, Bricault CA, Moseley ET, Boyd M, et al. Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. Science. 2016
42. Vannice KS, Durbin A, Hombach J. Status of vaccine research and development of vaccines for dengue. Vaccine. 2016; 34(26):2934–2938. [PubMed: 26973072]
43. Agnandji ST, Lell B, Soulanoudjingar SS, Fernandes JF, Abossolo BP, Conzelmann C, et al. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. N Engl J Med. 2011; 365(20):1863–1875. [PubMed: 22007715]
44. Rts SCTP. Efficacy and safety of the RTS,S/AS01 malaria vaccine during 18 months after vaccination: a phase 3 randomized, controlled trial in children and young infants at 11 African sites. PLoS medicine. 2014; 11(7):e1001685. [PubMed: 25072396]
45. Rts SCTP, Agnandji ST, Lell B, Fernandes JF, Abossolo BP, Methogo BG, et al. A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. N Engl J Med. 2012; 367(24):2284–2295. [PubMed: 23136909]
46. Rts SCTP. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. Lancet. 2015; 386(9988):31–45. [PubMed: 25913272]
47. Joura EA, Kjaer SK, Wheeler CM, Sigurdsson K, Iversen OE, Hernandez-Avila M, et al. HPV antibody levels and clinical efficacy following administration of a prophylactic quadrivalent HPV vaccine. Vaccine. 2008; 26(52):6844–6851. [PubMed: 18930097]
48. Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. Proceedings of the

National Academy of Sciences of the United States of America. 1998; 95(15):8869–8873. [PubMed: 9671771]

49. Hansen SG, Sacha JB, Hughes CM, Ford JC, Burwitz BJ, Scholz I, et al. Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. Science. 2013; 340(6135):1237874. [PubMed: 23704576]
50. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. The New England journal of medicine. 2009; 361(23):2209–2220. [PubMed: 19843557]
51. Tomaras GD, Haynes BF. Advancing Toward HIV-1 Vaccine Efficacy through the Intersections of Immune Correlates. Vaccines (Basel). 2014; 2(1):15–35. [PubMed: 24932411]
52. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F, et al. 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):1661–1671. [PubMed: 17109337]
53. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF, et al. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. The Journal of infectious diseases. 2005; 191(5):654–665. [PubMed: 15688278]
54. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. 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):1881–1893. [PubMed: 19012954]
55. Gray GE, Moodie Z, Metch B, Gilbert PB, Bekker LG, Churchyard G, et al. 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):388–396. [PubMed: 24560541]
56. McElrath MJ, De Rosa SC, Moodie Z, Dubey S, Kierstead L, Janes H, et al. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. Lancet. 2008; 372(9653):1894–1905. [PubMed: 19012957]
57. Gray GE, Allen M, Moodie Z, Churchyard G, Bekker LG, Nchabeleng M, et al. 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):507–515. [PubMed: 21570355]
58. Gray G, Buchbinder S, Duerr A. Overview of STEP and Phambili trial results: two phase IIb test-of-concept studies investigating the efficacy of MRK adenovirus type 5 gag/pol/nef subtype B HIV vaccine. Current opinion in HIV and AIDS. 2010; 5(5):357–361. [PubMed: 20978374]
59. Duerr A, Huang Y, Buchbinder S, Coombs RW, Sanchez J, del Rio C, et al. Extended follow-up confirms early vaccine-enhanced risk of HIV acquisition and demonstrates waning effect over time among participants in a randomized trial of recombinant adenovirus HIV vaccine (Step Study). The Journal of infectious diseases. 2012; 206(2):258–266. [PubMed: 22561365]
60. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, Grove D, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. The New England journal of medicine. 2013; 369(22): 2083–2092. [PubMed: 24099601]
61. Gilbert PB, Peterson ML, Follmann D, Hudgens MG, Francis DP, Gurwith M, et al. 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):666–677. [PubMed: 15688279]
62. Forthal DN, Gilbert PB, Landucci G, Phan T. Recombinant gp120 vaccine-induced antibodies inhibit clinical strains of HIV-1 in the presence of Fc receptor-bearing effector cells and correlate inversely with HIV infection rate. Journal of immunology. 2007; 178(10):6596–6603.
63. Gilbert P, Wang M, Wrin T, Petropoulos C, Gurwith M, Sinangil F, et al. Magnitude and breadth of a nonprotective neutralizing antibody response in an efficacy trial of a candidate HIV-1 gp120 vaccine. The Journal of infectious diseases. 2010; 202(4):595–605. [PubMed: 20608874]
64. Freel SA, Lamoreaux L, Chattopadhyay PK, Saunders K, Zarkowsky D, Overman RG, et al. Phenotypic and functional profile of HIV-inhibitory CD8 T cells elicited by natural infection and

heterologous prime/boost vaccination. Journal of virology. 2010; 84(10):4998–5006. [PubMed: 20200250]

65. Hayes PJ, Cox JH, Coleman AR, Fernandez N, Bergin PJ, Kopycinski JT, et al. Adenovirus-based HIV-1 vaccine candidates tested in efficacy trials elicit CD8+ T cells with limited breadth of HIV-1 inhibition. Aids. 2016; 30(11):1703–1712. [PubMed: 27088318]
66. Rolland, M., DeCamp, A., Hall, BM., Tovanabutra, S., McElrath, MJ., Hammer, S., et al. HVTN 505 Breakthrough sequences showed HIV vaccine-associated differences in Env-gp120. Conference on Retroviruses and Opportunistic Infections (CROI)2015; 2015.
67. Rolland M, Tovanabutra S, deCamp AC, Frahm N, Gilbert PB, Sanders-Buell E, et al. Genetic impact of vaccination on breakthrough HIV-1 sequences from the STEP trial. Nature medicine. 2011; 17(3):366–371.
68. Hertz T, Ahmed H, Friedrich DP, Casimiro DR, Self SG, Corey L, et al. HIV-1 vaccine-induced T-cell responses cluster in epitope hotspots that differ from those induced in natural infection with HIV-1. PLoS pathogens. 2013; 9(6):e1003404. [PubMed: 23818843]
69. Fitzgerald DW, Janes H, Robertson M, Coombs R, Frank I, Gilbert P, et al. An Ad5-vectored HIV-1 vaccine elicits cell-mediated immunity but does not affect disease progression in HIV-1-infected male subjects: results from a randomized placebo-controlled trial (the Step study). The Journal of infectious diseases. 2011; 203(6):765–772. [PubMed: 21343146]
70. Migueles SA, Rood JE, Berkley AM, Guo T, Mendoza D, Patamawenu A, et al. Trivalent adenovirus type 5 HIV recombinant vaccine primes for modest cytotoxic capacity that is greatest in humans with protective HLA class I alleles. PLoS pathogens. 2011; 7(2):e1002002. [PubMed: 21383976]
71. Janes H, Friedrich DP, Krambrink A, Smith RJ, Kallas E, Horton H, et al. Vaccine-induced Gag-specific T cells are associated with reduced viremia after HIV infection. The Journal of infectious diseases. 2013
72. Pollara J, Hart L, Brewer F, Pickeral J, Packard BZ, Hoxie JA, et al. High-throughput quantitative analysis of HIV-1 and SIV-specific ADCC-mediating antibody responses. Cytometry Part A : the journal of the International Society for Analytical Cytology. 2011; 79(8):603–612. [PubMed: 21735545]
73. Tomaras G, Haynes BF. Advancing Toward HIV-1 Efficacy through the Intersections of Immune Correlates. Vaccines. 2014; 2(1):15–35. [PubMed: 24932411]
74. Janes H, Friedrich DP, Krambrink A, Smith RJ, Kallas EG, Horton H, et al. Vaccine-induced gag-specific T cells are associated with reduced viremia after HIV-1 infection. The Journal of infectious diseases. 2013; 208(8):1231–1239. [PubMed: 23878319]
75. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. The New England journal of medicine. 2012; 366(14):1275–1286. [PubMed: 22475592]
76. Yates NL, Liao HX, Fong Y, deCamp A, Vandergrift NA, Williams WT, et al. 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.
77. Tomaras GD, Ferrari G, Shen X, Alam SM, Liao HX, Pollara J, et al. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110(22): 9019–9024. [PubMed: 23661056]
78. Arthos J, Cicala C, Martinelli E, Macleod K, Van Ryk D, Wei D, et al. HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. Nature immunology. 2008; 9(3):301–309. [PubMed: 18264102]
79. Parrish NF, Wilen CB, Banks LB, Iyer SS, Pfaff JM, Salazar-Gonzalez JF, et al. Transmitted/founder and chronic subtype C HIV-1 use CD4 and CCR5 receptors with equal efficiency and are not inhibited by blocking the integrin alpha4beta7. PLoS pathogens. 2012; 8(5):e1002686. [PubMed: 22693444]
80. Gottardo R, Bailer RT, Korber BT, Gnanakaran S, Phillips J, Shen X, et al. 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. [PubMed: 24086607]

81. Zolla-Pazner S, deCamp A, Gilbert PB, Williams C, Yates NL, Williams WT, et al. Vaccine-induced IgG antibodies to V1V2 regions of multiple HIV-1 subtypes correlate with decreased risk of HIV-1 infection. PloS one. 2014; 9(2):e87572. [PubMed: 24504509]
82. Chung AW, Kumar MP, Arnold KB, Yu WH, Schoen MK, Dunphy LJ, et al. Dissecting Polyclonal Vaccine-Induced Humoral Immunity against HIV Using Systems Serology. Cell. 2015; 163(4): 988–998. [PubMed: 26544943]
83. Karasavvas N, Billings E, Rao M, Williams C, Zolla-Pazner S, Bailer RT, et al. The Thai Phase III HIV Type 1 Vaccine trial (RV144) regimen induces antibodies that target conserved regions within the V2 loop of gp120. AIDS research and human retroviruses. 2012; 28(11):1444–1457. [PubMed: 23035746]
84. Zolla-Pazner S, deCamp AC, Cardozo T, Karasavvas N, Gottardo R, Williams C, et al. Analysis of V2 antibody responses induced in vaccinees in the ALVAC/AIDSVAX HIV-1 vaccine efficacy trial. PloS one. 2013; 8(1):e53629. [PubMed: 23349725]
85. Rolland M, Edlefsen PT, Larsen BB, Tovanabutra S, Sanders-Buell E, Hertz T, et al. Increased HIV-1 vaccine efficacy against viruses with genetic signatures in Env V2. Nature. 2012; 490(7420):417–420. [PubMed: 22960785]
86. Liao HX, Bonsignori M, Alam SM, McLellan JS, Tomaras GD, Moody MA, et al. Vaccine induction of antibodies against a structurally heterogeneous site of immune pressure within HIV-1 envelope protein variable regions 1 and 2. Immunity. 2013; 38(1):176–186. [PubMed: 23313589]
87. Liu P, Yates NL, Shen X, Bonsignori M, Moody MA, Liao HX, et al. Infectious virion capture by HIV-1 gp120-specific IgG from RV144 vaccinees. Journal of virology. 2013; 87(14):7828–7836. [PubMed: 23658446]
88. Bonsignori M, Pollara J, Moody MA, Alpert MD, Chen X, Hwang KK, et al. Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. Journal of virology. 2012; 86(21):11521–11532. [PubMed: 22896626]
89. Berman PW. Development of bivalent rgp120 vaccines to prevent HIV type 1 infection. AIDS research and human retroviruses. 1998; 14 Suppl 3:S277–S289. [PubMed: 9814956]
90. Lasky LA, Groopman JE, Fennie CW, Benz PM, Capon DJ, Dowbenko DJ, et al. Neutralization of the AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. Science. 1986; 233(4760):209–212. [PubMed: 3014647]
91. Alam SM, Liao HX, Tomaras GD, Bonsignori M, Tsao CY, Hwang KK, et al. Antigenicity and immunogenicity of RV144 vaccine AIDSVAX clade E envelope immunogen is enhanced by a gp120 N-terminal deletion. Journal of virology. 2013; 87(3):1554–1568. [PubMed: 23175357]
92. Shen X, Duffy R, Howington R, Cope A, Sadagopal S, Park H, et al. Vaccine-Induced Linear Epitope-Specific Antibodies to Simian Immunodeficiency Virus SIVmac239 Envelope Are Distinct from Those Induced to the Human Immunodeficiency Virus Type 1 Envelope in Nonhuman Primates. Journal of virology. 2015; 89(16):8643–8650. [PubMed: 26018159]
93. Chung AW, Ghebremichael M, Robinson H, Brown E, Choi I, Lane S, et al. Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX003 vaccines. Science translational medicine. 2014; 6(228):228ra38.
94. Versiani FG, Almeida ME, Melo GC, Versiani FO, Orlandi PP, Mariuba LA, et al. High levels of IgG3 anti ICB2-5 in Plasmodium vivax-infected individuals who did not develop symptoms. Malaria journal. 2013; 12:294. [PubMed: 23977965]
95. Roussilhon C, Oeuvray C, Muller-Graf C, Tall A, Rogier C, Trape JF, et al. Long-term clinical protection from falciparum malaria is strongly associated with IgG3 antibodies to merozoite surface protein 3. PLoS medicine. 2007; 4(11):e320. [PubMed: 18001147]
96. Kam YW, Simarmata D, Chow A, Her Z, Teng TS, Ong EK, et al. 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):1147–1154. [PubMed: 22389226]
97. McElrath MJ, Haynes BF. Induction of immunity to human immunodeficiency virus type-1 by vaccination. Immunity. 2010; 33(4):542–554. [PubMed: 21029964]

98. Scharf O, Golding H, King LR, Eller N, Frazier D, Golding B, et al. Immunoglobulin G3 from polyclonal human immunodeficiency virus (HIV) immune globulin is more potent than other subclasses in neutralizing HIV type 1. Journal of virology. 2001; 75(14):6558–6565. [PubMed: 11413323]

99. Tay MZ, Liu P, Williams LD, McRaven MD, Sawant S, Gurley TC, et al. Antibody-Mediated Internalization of Infectious HIV-1 Virions Differs among Antibody Isotypes and Subclasses. PLoS pathogens. 2016; 12(8):e1005817. [PubMed: 27579713]

100. Bournazos S, Gazumyan A, Seaman MS, Nussenzweig MC, Ravetch JV. Bispecific Anti-HIV-1 Antibodies with Enhanced Breadth and Potency. Cell. 2016; 165(7):1609–1620. [PubMed: 27315478]

101. Ferrari G, Pollara J, Kozink D, Harms T, Drinker M, Freel S, et al. An HIV-1 gp120 envelope human monoclonal antibody that recognizes a C1 conformational epitope mediates potent antibody-dependent cellular cytotoxicity (ADCC) activity and defines a common ADCC epitope in human HIV-1 serum. Journal of virology. 2011; 85(14):7029–7036. [PubMed: 21543485]

102. Wilton JM. Suppression by IgA of IgG-mediated phagocytosis by human polymorphonuclear leucocytes. Clin Exp Immunol. 1978; 34(3):423–428. [PubMed: 369752]

103. Griffiss JM, Goroff DK. IgA blocks IgM and IgG-initiated immune lysis by separate molecular mechanisms. Journal of immunology. 1983; 130(6):2882–2885.

104. Quan CP, Watanabe S, Forestier F, Bouvet JP. Human amniotic IgA inhibits natural IgG autoantibodies of maternal or unrelated origin. Eur J Immunol. 1998; 28(12):4001–4009. [PubMed: 9862336]

105. Mathew GD, Qualtiere LF, Neel HB 3rd, Pearson GR. IgA antibody, antibody-dependent cellular cytotoxicity and prognosis in patients with nasopharyngeal carcinoma. Int J Cancer. 1981; 27(2): 175–180. [PubMed: 6169655]

106. Ruiz MJ, Ghiglione Y, Falivene J, Laufer N, Holgado MP, Socias ME, et al. Env-Specific IgA from Viremic HIV-Infected Subjects Compromises Antibody-Dependent Cellular Cytotoxicity. Journal of virology. 2016; 90(2):670–681. [PubMed: 26491172]

107. Pollara J, Bonsignori M, Moody MA, Liu P, Alam SM, Hwang KK, et al. HIV-1 vaccine-induced C1 and V2 Env-specific antibodies synergize for increased antiviral activities. Journal of virology. 2014

108. Amos JD, Wilks AB, Fouda GG, Smith SD, Colvin L, Mahlokozera T, et al. Lack of B cell dysfunction is associated with functional, gp120-dominant antibody responses in breast milk of simian immunodeficiency virus-infected African green monkeys. Journal of virology. 2013; 87(20):11121–11134. [PubMed: 23926338]

109. Woof JM, Mestecky J. Mucosal immunoglobulins. Immunol Rev. 2005; 206:64–82. [PubMed: 16048542]

110. Watkins JD, Sholukh AM, Mukhtar MM, Siddappa NB, Lakhashe SK, Kim M, et al. Anti-HIV IgA isotypes: differential virion capture and inhibition of transcytosis are linked to prevention of mucosal R5 SHIV transmission. Aids. 2013; 27(9):F13–F20. [PubMed: 23775002]

111. Stieh DJ, King DF, Klein K, Liu P, Shen X, Hwang KK, et al. Aggregate complexes of HIV-1 induced by multimeric antibodies. Retrovirology. 2014; 11:78. [PubMed: 25274446]

112. Lin L, Finak G, Ushey K, Seshadri C, Hawn TR, Frahm N, et al. COMPASS identifies T-cell subsets correlated with clinical outcomes. Nat Biotechnol. 2015; 33(6):610–616. [PubMed: 26006008]

113. Pollara J, Bonsignori M, Moody MA, Liu P, Alam SM, Hwang KK, et al. HIV-1 Vaccine-Induced C1 and V2 Env-Specific Antibodies Synergize for Increased Antiviral Activities. Journal of virology. 2014; 88(14):7715–7726. [PubMed: 24807721]

114. Liu P, Williams LD, Shen X, Bonsignori M, Vandergrift NA, Overman RG, et al. Capacity for infectious HIV-1 virion capture differs by envelope antibody specificity. Journal of virology. 2014; 88(9):5165–5170. [PubMed: 24554654]

115. Prentice HA, Tomaras GD, Geraghty DE, Apps R, Fong Y, Ehrenberg PK, et al. HLA class II genes modulate vaccine-induced antibody responses to affect HIV-1 acquisition. Science translational medicine. 2015; 7(296):296ra112.

116. Li SS, Gilbert PB, Tomaras GD, Kijak G, Ferrari G, Thomas R, et al. FCGR2C polymorphisms associate with HIV-1 vaccine protection in RV144 trial. The Journal of clinical investigation. 2014; 124(9):3879–3890. [PubMed: 25105367]

117. Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, et al. 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):7552–7557. [PubMed: 18490657]

118. Joseph SB, Swanstrom R, Kashuba AD, Cohen MS. Bottlenecks in HIV-1 transmission: insights from the study of founder viruses. Nat Rev Microbiol. 2015; 13(7):414–425. [PubMed: 26052661]

119. Li H, Bar KJ, Wang S, Decker JM, Chen Y, Sun C, et al. High Multiplicity Infection by HIV-1 in Men Who Have Sex with Men. PLoS pathogens. 2010; 6(5):e1000890. [PubMed: 20485520]

120. Sterrett S, Learn GH, Edlefsen PT, Haynes BF, Hahn BH, Shaw GM, et al. Low Multiplicity of HIV-1 Infection and No Vaccine Enhancement in VAX003 Injection Drug Users. Open Forum Infect Dis. 2014; 1(2) ofu056.

121. Gaschen B, Taylor J, Yusim K, Foley B, Gao F, Lang D, et al. Diversity considerations in HIV-1 vaccine selection. Science. 2002; 296(5577):2354–2360. [PubMed: 12089434]

122. Hraber P, Korber BT, Lapedes AS, Bailer RT, Seaman MS, Gao H, et al. Impact of clade, geography, and age of the epidemic on HIV-1 neutralization by antibodies. Journal of virology. 2014; 88(21):12623–12643. [PubMed: 25142591]

123. Gao F, Korber BT, Weaver E, Liao HX, Hahn BH, Haynes BF. Centralized immunogens as a vaccine strategy to overcome HIV-1 diversity. Expert Rev Vaccines. 2004; 3(4 Suppl):S161–S168. [PubMed: 15285714]

124. Haynes BF, Shaw GM, Korber B, Kelsoe G, Sodroski J, Hahn BH, et al. HIV-Host Interactions: Implications for Vaccine Design. Cell host & microbe. 2016; 19(3):292–303. [PubMed: 26922989]

125. Rademeyer C, Korber B, Seaman MS, Giorgi EE, Thebus R, Robles A, et al. Features of Recently Transmitted HIV-1 Clade C Viruses that Impact Antibody Recognition: Implications for Active and Passive Immunization. PLoS pathogens. 2016; 12(7):e1005742. [PubMed: 27434311]

126. Fellay J, Shianna KV, Telenti A, Goldstein DB. Host genetics and HIV-1: the final phase? PLoS pathogens. 2010; 6(10):e1001033. [PubMed: 20976252]

127. Forthal DN, Gabriel EE, Wang A, Landucci G, Phan TB. Association of Fcgamma receptor IIIa genotype with the rate of HIV infection after gp120 vaccination. Blood. 2012; 120(14):2836–2842. [PubMed: 22915639]

128. Gartland AJ, Li S, McNevin J, Tomaras GD, Gottardo R, Janes H, et al. Analysis of HLAA*02 association with vaccine efficacy in the RV144 HIV-1 vaccine trial. Journal of virology. 2014

129. Lassauniere R, Tiemessen CT. Variability at the FCGR locus: characterization in Black South Africans and evidence for ethnic variation in and out of Africa. Genes Immun. 2016; 17(2):93–104. [PubMed: 26673965]

130. Thomas, R. AIDS Vaccine 2013. Spain: Barcelona; 2013. HLA Class II Genes Interact with the Immune Correlates from the RV144 Vaccine Efficacy Trial and Impact HIV-1 Acquisition.

131. Zak DE, Andersen-Nissen E, Peterson ER, Sato A, Hamilton MK, Borgerding J, et al. Merck Ad5/HIV induces broad innate immune activation that predicts CD8(+) T-cell responses but is attenuated by preexisting Ad5 immunity. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109(50):E3503–E3512. [PubMed: 23151505]

132. Fourati S, Cristescu R, Loboda A, Talla A, Filali A, Railkar R, et al. Pre-vaccination inflammation and B-cell signalling predict age-related hyporesponse to hepatitis B vaccination. Nat Commun. 2016; 7:10369. [PubMed: 26742691]

133. Muyanja E, Ssemaganda A, Ngauv P, Cubas R, Perrin H, Srinivasan D, et al. Immune activation alters cellular and humoral responses to yellow fever 17D vaccine. The Journal of clinical investigation. 2014; 124(7):3147–3158. [PubMed: 24911151]

134. Vaccari M, Gordon SN, Fourati S, Schifanella L, Liyanage NP, Cameron M, et al. Adjuvant-dependent innate and adaptive immune signatures of risk of SIVmac251 acquisition. Nature medicine. 2016; 22(7):762–770.

135. Tibshirani R. The lasso method for variable selection in the Cox model. Stat Med. 1997; 16(4): 385–395. [PubMed: 9044528]
136. Arnold KB, Burgener A, Birse K, Romas L, Dunphy LJ, Shahabi K, et al. Increased levels of inflammatory cytokines in the female reproductive tract are associated with altered expression of proteases, mucosal barrier proteins, and an influx of HIV-susceptible target cells. Mucosal immunology. 2016; 9(1):194–205. [PubMed: 26104913]
137. Archary D, Seaton KE, Passmore JS, Werner L, Deal A, Dunphy LJ, et al. Distinct genital tract HIV-specific antibody profiles associated with tenofovir gel. Mucosal immunology. 2016; 9(3): 821–833. [PubMed: 26813340]
138. Fauci AS, Marovich MA, Dieffenbach CW, Hunter E, Buchbinder SP. Immunology. Immune activation with HIV vaccines. Science. 2014; 344(6179):49–51. [PubMed: 24700849]
139. Lewis GK, DeVico AL, Gallo RC. Antibody persistence and T-cell balance: two key factors confronting HIV vaccine development. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111(44):15614–15621. [PubMed: 25349379]
140. Gray GE, Metch B, Churchyard G, Mlisana K, Nchabeleng M, Allen M, et al. Does participation in an HIV vaccine efficacy trial affect risk behaviour in South Africa? Vaccine. 2013; 31(16): 2089–2096. [PubMed: 23370155]
141. Gilbert, P. AIDS Vaccine. Spain: Barcelona; Oct. Met-analysis of Ad5-vector HIV vaccine trials to assess the vaccine effect on HIV acquisition; p. 7-10.PL04.062013
142. Fonseca MG, Forsythe S, Menezes A, Vuthoori S, Possas C, Veloso VG, et al. Modeling HIV vaccines in Brazil: assessing the impact of a future HIV vaccine on reducing new infections, mortality and number of people receiving ARV. PloS one. 2010; 5(7):e11736. [PubMed: 20668523]
143. Collaborators GH, Wang H, Wolock TM, Carter A, Nguyen G, Kyu HH, et al. Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980–2015: the Global Burden of Disease Study 2015. Lancet HIV. 2016; 3(8):e361–e387. [PubMed: 27470028]
144. Gray GE, Laher F, Lazarus E, Ensoli B, Corey L. Approaches to preventative and therapeutic HIV vaccines. Curr Opin Virol. 2016; 17:104–109. [PubMed: 26985884]
145. Barouch DH, Alter G, Broge T, Linde C, Ackerman ME, Brown EP, et al. HIV-1 vaccines. Protective efficacy of adenovirus/protein vaccines against SIV challenges in rhesus monkeys. Science. 2015; 349(6245):320–324. [PubMed: 26138104]
146. Barouch DH, Stephenson KE, Borducchi EN, Smith K, Stanley K, McNally AG, et al. Protective efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. Cell. 2013; 155(3):531–539. [PubMed: 24243013]
147. Ledgerwood JE, Coates EE, Yamshchikov G, Saunders JG, Holman L, Enama ME, et al. Safety, pharmacokinetics and neutralization of the broadly neutralizing HIV-1 human monoclonal antibody VRC01 in healthy adults. Clin Exp Immunol. 2015; 182(3):289–301. [PubMed: 26332605]
148. Halper-Stromberg A, Nussenzweig MC. Towards HIV-1 remission: potential roles for broadly neutralizing antibodies. The Journal of clinical investigation. 2016; 126(2):415–423. [PubMed: 26752643]
149. Hua CK, Ackerman ME. Engineering broadly neutralizing antibodies for HIV prevention and therapy. Adv Drug Deliv Rev. 2016; 103:157–173. [PubMed: 26827912]
150. Schneider JA, Alam SA, Ackers M, Parekh B, Chen HY, Graham P, et al. Mucosal HIV-binding antibody and neutralizing activity in high-risk HIV-uninfected female participants in a trial of HIV-vaccine efficacy. The Journal of infectious diseases. 2007; 196(11):1637–1644. [PubMed: 18008247]
151. Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. The New England journal of medicine. 2007; 357(19):1903–1915. [PubMed: 17989383]
152. Robb ML, Rerks-Ngarm S, Nitayaphan S, Pitisuttithum P, Kaewkungwal J, Kunasol P, et al. Risk behaviour and time as covariates for efficacy of the HIV vaccine regimen ALVAC-HIV (vCP1521) and AIDSVAX B/E: a post-hoc analysis of the Thai phase 3 efficacy trial RV 144. The Lancet Infectious diseases. 2012; 12(7):531–537. [PubMed: 22652344]

153. Murnane PM, Celum C, Mugo N, Campbell JD, Donnell D, Bukusi E, et al. Efficacy of preexposure prophylaxis for HIV-1 prevention among high-risk heterosexuals: subgroup analyses from a randomized trial. Aids. 2013; 27(13):2155–2160. [PubMed: 24384592]

154. Van Damme L, Corneli A, Ahmed K, Agot K, Lombaard J, Kapiga S, et al. Preexposure prophylaxis for HIV infection among African women. The New England journal of medicine. 2012; 367(5):411–422. [PubMed: 22784040]

155. Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. The New England journal of medicine. 2010; 363(27):2587–2599. [PubMed: 21091279]

156. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. The New England journal of medicine. 2011; 365(6):493–505. [PubMed: 21767103]

157. Bailey RC, Moses S, Parker CB, Agot K, Maclean I, Krieger JN, et al. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. Lancet. 2007; 369(9562):643–656. [PubMed: 17321310]

158. Auvert B, Taljaard D, Lagarde E, Sobngwi-Tambekou J, Sitta R, Puren A. Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. PLoS medicine. 2005; 2(11):e298. [PubMed: 16231970]

159. Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science. 2010; 329(5996):1168–1174. [PubMed: 20643915]

160. Williams WB, Liao HX, Moody MA, Kepler TB, Alam SM, Gao F, et al. HIV-1 VACCINES. Diversion of HIV-1 vaccine-induced immunity by gp41-microbiota cross-reactive antibodies. Science. 2015; 349(6249):aab1253. [PubMed: 26229114]

161. de Taeye SW, Moore JP, Sanders RW. HIV-1 Envelope Trimer Design and Immunization Strategies To Induce Broadly Neutralizing Antibodies. Trends Immunol. 2016; 37(3):221–232. [PubMed: 26869204]

162. Gilbert PB, McKeague IW, Sun Y. The 2-sample problem for failure rates depending on a continuous mark: an application to vaccine efficacy. Biostatistics. 2008; 9(2):263–276. [PubMed: 17704528]

163. Gilbert PB, Wu C, Jobes DV. Genome scanning tests for comparing amino acid sequences between groups. Biometrics. 2008; 64(1):198–207. [PubMed: 17608781]

164. Zolla-Pazner S, Edlefsen PT, Rolland M, Kong XP, deCamp A, Gottardo R, et al. Vaccine-induced Human Antibodies Specific for the Third Variable Region of HIV-1 gp120 Impose Immune Pressure on Infecting Viruses. EBioMedicine. 2014; 1(1):37–45. [PubMed: 25599085]

Table 1

Types of Immune Responses Associated/Correlated with Protection /Infection Risk.

Immune Response	Vaccine
Antibody Binding	Hepatitis A, Hepatitis B, Human Papilloma Virus, Measles, Pertussis, Rubella, Varicella, Zoster*, HibPolysaccharides/Conjugate, Lyme disease, Tick borne encephalitis, Pneumococcus*, *HIV-1**
Antibody Function	**Neutralization or Pathogen/Toxin inhibition** Anthrax, Diphtheria, Influenza, Japanese Encephalitis, Measles, Meningococcal, Mumps, Polio, Rabies, Smallpox, Tetanus, Yellow Fever **Opsonophagocytosis** Pneumococcus*, **ADCC** *HIV-1**
Cellular Response	**CD4 T cell, Lymphoproliferation** Zoster*, BCG **CD4+ T Cell polyfunctionality** *HIV-1**

Categories of identified immune correlates for licensed vaccines, identified correlates of risk for HIV-1 are also shown.

* Both antibody and cellular correlates, *Italics*- not licensed vaccines.

Table 2

HIV-1 Vaccine Efficacy Trials: Immune Correlates of Risk.

Vaccine Regimen	Location/ Risk Population	Overall Vaccine Efficacy	Increased Risk of Infection	Immune Correlates of Decreased Vaccine Efficacy[2]	Immune Correlates of Decreased HIV Risk	Immune Correlates of Immune Control Post Infection	Virus Sieve	Host Genetic Correlates
VAX003 (Phase III) Protein/ Alum (CRF01_A E/Clade B Env) (52)	Thailand/ Injection Drug Users	No Efficacy	No	No	No (52)	No	No[3]	*n/d*
VAX004 (Phase III) Protein/ Alum (Clade B Envs)(53)	USA/ MSM/ High Risk Women	No Efficacy	No	No	**Yes** ADCVI, CD4 Blocking, Tier 1 NAb	*n/d*	No (162, 163)	**Yes** Fcγ receptor IIIa genotype (VV genotype)(127)
STEP HVTN502 (Phase IIb) Ad5 Vector (Clade B Gag/Pol Nef) (54)	North and South America, Australia, Caribbean/M SM and High Risk Heterosexual Men and Women	No Efficacy (Efficacy futility determined at first interim analysis after full enrollment)	**Yes**	*n/d*	No	**Yes** T cell breadth /magnitude, Lower VL	**Yes(67)**	**Yes** HLA alleles (B*27, B*57, B*58:01), Lower viral load
Phambili HVTN503 (Phase IIb) Ad5 Vector (Clade B Gag/Pol Nef) (57)	South Africa/ Heterosexual men and women	No Efficacy[1]	*n/d*	*n/d*	*n/d*	*n/d*	*n/d*	*n/d*
RV144 (Phase III) ALVAC vector (Clade B Gag/Pro + CRF01_ A/E Env)+ Protein/Alum (CRF01_A E/B	Thailand/ Community[2]	**31.2% Efficacy**	No	**Yes** Plasma Env IgA (75, 77)	**Yes** V1V2 IgG, Linear V2, V1V2 IgG3, Interactions (ADCC, Avidity, Tier 1 NAb, IgA) , CD4+ T cell Polyfunction, Cytokines(75,	*n/d*	**Yes**(85, 164)	**Yes** HLA A*02 allele (128): FcγRIIC −118l allele (116): DQB1*06 (115)

Vaccine Regimen	Location/ Risk Population	Overall Vaccine Efficacy	Increased Risk of Infection	Immune Correlates of Decreased Vaccine Efficacy[2]	Immune Correlates of Decreased HIV Risk	Immune Correlates of Immune Control Post Infection	Virus Sieve	Host Genetic Correlates
Env)(50)					76, 80, 81, 112)			
HVTN505 (Phase IIb) DNA/Ad5 (Clade A,B,C *Env Clade BGag/Pol*)(60)	USA/ MSM and TG, Ad5 sero-negative, Circumcised	No Efficacy (Efficacy futility determined at first interim analysis after full enrollment)	No	No	**Yes** CD8+ T-cell Polyfunction[4]	*n/d*	**Yes** (66)	*n/d*

The six HIV-1 vaccine efficacy studies are listed alongside their corresponding outcomes for vaccine efficacy, immune correlates of risk, and associations of vaccine efficacy with virus sieve analysis and host genetics. Findings with positive outcomes for vaccine efficacy are shaded in blue and findings with negative outcomes for vaccine efficacy are shaded in gray. MSM = Men who have sex with Men; TG = transgender; ADCVI = antibody-dependent, cell-mediated virus inhibition; Tier 1 NAb = neutralizing antibodies that target easy to neutralize viruses (i.e. not circulating transmitted/founder viruses); V= Valine, FcγRIIIa is encoded by alleles that confer either a phenylalanine (F) or valine (V) at amino acid position 158

[1] Vaccinations discontinued: unblinded early based on STEP result.

[2] No increased risk of infection compared to the placebo group

[3] No significant virus sieve that correlated with acquisition (120). An atypical genetic sieve in the V2 region was identified but also did not correlate with acquisition.

[4] Frahm N, McElrath MJ *et al.* 2016 R4P meeting, Chicago, Il.

Table 3

Complex Immune Correlates of Decreased HIV-1 Risk.

Immune and/or Host Genetic Interactions		
Interactions	Low IgA/ ADCC (75, 77) Low IgA/ nAb (75) Low IgA/ IgG Env Avidity (75) IgG3/ ADCC (76) IgG3/IgG1 (82)	IgG, IgG3, nAb, Avidity and FcγRIIC SNP (116) IgA/ HLA A*02 allele (128) IgA/ HLA II DQB1*06 (115) IgG/ HLA II DPB1*13 (115) V3 IgG Env /Low IgA/ Low nAb (80)
V2 Env Immune Correlate		
V1V2 IgG	V1V2 IgG3 (76) A244 gp120 (75)	Linear V2 AE hotspot peptide microarray (80) V1V2 IgG Breadth(81)
IgA Immune Correlate		
IgA Env	IgA Env Score (75) IgA A. OOMSA gp140 CF (75) IgA. A1 Congp140 (75)	IgA C1 (75) IgA Non-Vaccine Strains (75) IgA/IgG ratio (75, 77)
Cellular Correlates		
Cytokine Score from Env Stimulated PBMC	Cytokine response (IL-10, IL-13) from Env stimulated PBMC supernatants correlated (75)	Polyfunctional CD4+ T cell (CD40L, IL-2, IL-4, IFN-γ and TNF-α) and (CD40L, IL-2 and IL-4) (112)

Multiple immune measurements, including interactions among immune responses and with host genetics, correlate with HIV-1 infection risk. The RV144 trial studied canarypox (ALVAC) with CRF01_AE Envelope as a priming immunogen, followed by a combination of ALVAC + 2 gp120s, one clade B (MN) and one subtype CRF01-AE, A244. Statistically significant results from the RV144 correlates analysis are listed.

Table 4

V1V2 IgG Cross-reactivity RV144 Vaccine Efficacy Trial that Correlates with Infection Risk (adapted from (81)).

A. Subtype	Antigen Name	IgG Binding ELISA Odds Ratio	P-Value	IgG Binding BAMA Odds Ratio	P-Value
AE	gp70.AE(92TH023)-V1V2	0.62	*p <0.05*	0.67	*p <0.01*
B	gp70. CaseA2- V1V2	0.62	*p < 0.05*	0.60	*p < 0.01*
C	gp70.C(97ZA012)-V1V2	0.56	*p <0.005*	0.55	*p < 0.001*
	tags.C(1086)-V1V2	0.53	*p < 0.005*	0.62	*p < 0.005*
Multiple	Cross-reactivity[1]	0.58	*p < 0.01*	0.56	*p < 0.005*

B. HIV-1 Envelope V2 Sequences	
gp70.AE(92TH023)-V1V2	CSFNMTTELRDKKQKVHALFYKLDIVPIEDNTSSS.E.YRLINC
gp70. CaseA2- V1V2	CSFNITTSIRDKVQKEYALFYKLDIVPI.DNPKNST.N.YRLISC
gp70.C(97ZA012)-V1V2	CSFNTTTEIRDKKQQGYALFYRPDIVLLKENRNNSNNSEYILINC
tags.C(1086)-V1V2	CSFKATTELKDKKHKVHALFYKLDVVPL.NGNSSSSGE.YRLINC

A. The results of a blinded repeat of the RV144 case control study by two different laboratories (Zolla-Pazner, New York University and Tomaras, Duke University Medical School), with different methods (ELISA, BAMA) with an array of cross-clade V1V2 antigens produced in three different laboratories (A. Pinter, Rutgers; H. Liao/B. Haynes, Duke; and G. Nabel, Vaccine Research Center). Results shown are the IgA adjusted odds ratio of HIV-1 infection risk from plasma IgG binding at week 26 at the peak immunogenicity time point in RV144. The cross-reactivity score was determined by a panel of cross-clade V1V2 antigens as previously described (81). B. The V2 sequences of the subtypes A, B and C envelopes assessed in the case control study.