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Modeling HIV vaccine trials of the future

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Abstract

Purpose of review—Models of implementation of known-effective interventions for HIV prevention indicate that an efficacious vaccine to prevent HIV infection would be critical for controlling the HIV pandemic. Key issues in the design of future HIV vaccine trials are (1) How to develop reliable immunological correlates of vaccine efficacy, (2) How to down-select candidate vaccine regimens into efficacy trials, and (3) How to learn about vaccine efficacy in the context of the evolving HIV prevention landscape.

Recent findings—Whereas in the past phase-I/II HIV vaccine trials have addressed (1) and (2) using a small set of immunological assays and readouts, recently they have used a battery of assays with highly multivariate readouts. In addition, systems vaccinology studies of other pathogens measuring PBMC transcriptomics and other immunological features pre- and post-first vaccination are demonstrating value, for example providing discoveries that pre-immunization and early post-immunization cell population markers can predict influenza-specific antibody titer that is a correlate of vaccine protection. The HIV prevention landscape continues to evolve, and the design and analysis of vaccine trials is evolving alongside, to accommodate increasingly dynamic and regional standards of HIV prevention.

Summary—Development of interpretable and robust functional assays, in addition to the associated bioinformatics and statistical analytic tools, are needed to improve the assessment of correlates of protection in efficacy trials and the down-selection of candidate vaccine regimens into efficacy trials. Moreover, high-priority trials should integrate systems vaccinology, including analysis of pre-vaccination and early post-vaccination markers.

Keywords

Clinical trials; HIV prevention; Immune correlates of vaccine efficacy; Statistical learning; Systems vaccinology

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Introduction

The development of an efficacious HIV vaccine remains a top priority. This effort has been hindered by, among other obstacles, a lack of knowledge of immunological correlates of vaccine efficacy and of the optimal methods and criteria for down-selecting vaccine regimens into efficacy trials. Below we discuss recently developed statistical approaches and tools that can be applied to help overcome these obstacles. We also discuss how systems vaccinology has been recently applied in vaccine trials and consider the potential of this approach to improve HIV vaccine trial design and analysis. Finally, we discuss the implications of new HIV prevention modalities and standards on HIV vaccine trial design, including recent discussions about how to accommodate these modalities in HIV vaccine efficacy trials.

Assessment of correlates of vaccine efficacy

The identification of immune correlates of protection (CoPs) is key for developing efficacious HIV vaccines [1]. CoPs are immunological biomarkers measured after vaccination that are statistically correlated with vaccine efficacy (VE) to prevent HIV infection. Validated CoPs can be used to improve vaccine design and/or accelerate vaccine testing. Some CoPs are mechanistic correlates causally responsible for a vaccine's protective effect, whereas others are non-mechanistic [1]. Both types of correlates can accelerate vaccine development by e.g. helping screen candidates for efficacy based on early immunogenicity studies. The VE modification or framework assesses CoPs by estimating VE for each of many subgroups of vaccine recipients defined by the level of their immune response to vaccination. In this fashion, it examines how the immune response modifies VE [2–5]. The most useful CoP is a strong effect modifier such that VE is zero for vaccine recipients with negative/absent immune response and VE is near 100% for vaccine recipients with response above a threshold. The major challenge of the VE modification approach is that it requires estimation of how the risk of the clinical endpoint for a placebo recipient depends on an unmeasured variable– the immune response to the vaccine that the individual would have had, if, counter to fact, s/he had been assigned to receive the vaccine. Consequently, statistical methods for implementing the VE modification framework incorporate techniques for “filling in” the counterfactual immune responses of placebo recipients. Follmann (2006) proposed two techniques: 1) using baseline immunogenicity predictors (BIPs) correlated with the immune biomarkers of interest to predict their missing values; and 2) vaccination of placebo recipients who remain HIV-1 uninfected at the end of follow-up, called close-out placebo vaccination (CPV), and subsequent measurement of their immune responses to vaccination [3] (illustrated in Figure 1). Various statistical methods using these techniques have been developed for evaluating a single CoP [2, 6] and for addressing various issues including CoP combinations [7], biomarker sampling design optimization [6], and prediction of temporal VE waning [8]. In varicella zoster vaccine (VZV) research, fold-rise in VZV antibody titer has been validated as a CoP using the BIP approach [4]. Development of predictive BIPs has been fruitful; e.g. using systems vaccinology Tsang and colleagues developed a model for predicting antibody titers post-influenza vaccination based on cell population frequencies [9]. Statistical methods have also

been developed for evaluating CoPs using BIPs and CPV in nonhuman primate (NHP) repeated low-dose challenge studies [10, 11].

Since past HIV efficacy trials have not used CPV and have lacked a strongly predictive BIP, research has focused on identifying correlates of risk (CoRs) as potential CoPs. CoRs are vaccine-induced immune response biomarkers associated with subsequent HIV infection and can be evaluated among vaccine recipients in a standard efficacy trial design [1]. While a CoR may merely represent an immune response that is associated with pathogen exposure or natural susceptibility to infection, and does not necessarily associate with VE, CoR analysis is an important pre-requisite for CoP identification. To date, the RV144 trial is the only HIV vaccine trial to have demonstrated partial VE against HIV infection [12]. To generate hypotheses about the CoPs and mCoPs of the RV144 vaccine regimen, a case-control study identified several CoRs (e.g. [13, 14**, 15]), including a CD4+ T-cell polyfunctionality score [16]. These CoRs require additional validation as CoPs [17**] in upcoming efficacy trials. These trials are planning to include CoP assessment using both CPV and BIP design techniques.

Down-selection of vaccine regimens

Phase-IIb and III HIV vaccine trials are large, operationally challenging, and costly. To mitigate these challenges, the HVTN has pursued HIV vaccine trials incorporating concurrent vaccine regimens [18]. However, a limited number of vaccine regimens can be evaluated simultaneously, making it essential to rigorously down-select the most promising vaccine regimens based on phase-I/IIa and NHP trials.

Huang et al. [19**] described a two-step down-selection framework: i) filter each regimen individually based on screening criteria, and then ii) compare head-to-head all regimens passing (i). The filtering step can be based on several criteria. For example, it may require better immunogenicity relative to the placebo arm and to the RV144 regimen, and meeting pre-specified benchmarks for designated core immune endpoints deemed essential for a promising vaccine regimen. The second step applies a rank/filter/selection algorithm that i) sequentially evaluates regimens based on a summary score integrating multiple immune responses [20], and ii) conducts multiple comparisons of individual endpoints and selects regimens with non-redundant immune profiles so that each selected regimen is superior to the others with respect to some immune endpoints. Unsupervised learning techniques also contribute to down-selection by graphically displaying similarities in multivariate immune response patterns[19**].

A critical consideration in down-selection is which immune endpoints should be compared between regimens. Huang et al. [19**] recommended using both knowledge-based and comprehensive approaches for this choice. It is certainly desirable to advance regimens that perform well based on validated RV144 CoRs. However, a more unbiased assessment based on immune markers not correlating with risk in RV144 should also be employed. NHP data can also contribute to down-selection by providing direct comparisons of VE among candidate vaccine regimens and by providing assessments of VE correlates. In addition, the transport formula proposed by Gilbert and Huang [21**] can be used to integrate phase-I/IIa

immunogenicity data with VE-by-immune response curve information from previous efficacy trials to predict overall VE in the setting of a new efficacy trial, providing complementary information.

Systems vaccinology

Established CoPs for licensed vaccines are ‘single measure’ univariable assay readouts applied to a samples drawn from vaccine recipients at approximate peak immunogenicity after the vaccination series [1, 22, 23]. As discussed above, CoP analyses in HIV VE trials have expanded beyond univariable peak readouts to several-dimensional multivariable readouts (e.g., six readouts at 2 weeks post last vaccination in the RV144 primary case-control analysis [15]). For future HIV vaccine trials this ‘moderate-dimensional peak readout’ assessment merits continued use given its potential to identify simple CoPs; however, CoP analyses should also expand systems vaccinology, which assesses high-dimensional readouts at timepoints shortly after first vaccination. The field of systems vaccinology has emerged with advances in high-throughput technologies that allow the properties and abundances of intracellular molecules to be measured. This field combines ‘omics’ technologies with bioinformatics and statistical modeling to predict and understand immune responses to vaccination in a holistic manner, with the objective of identifying better CoPs [24–27].

Systems vaccinology holds promise to build better CoPs for HIV vaccines in at least three ways. First, a reliable CoP could be potentially measured as soon as hours after the first vaccination. Such a validated surrogate endpoint [28, 29] would be highly practical in allowing inferences about VE from small trials with only a few weeks of follow-up. While no such early time-point CoP for a licensed vaccine has been established, some vaccine fields are approaching this reality. For example, changes in plasmablast frequencies pre-vaccination to day 7 post-vaccination and accompanying transcript changes in plasma cell-associated genes have been shown to be highly predictive of influenza-specific antibody titer fold-rise, an accepted CoP for influenza disease [22]. Second, a signature derived from the high-dimensional readouts could potentially predict VE better than a lower-dimensional multivariable readout. Third, systems vaccinology may provide insights into mechanistic CoPs, generating hypotheses that could be tested in future trials and accelerating iterative vaccine improvement. CoPs based on blood samples are most useful, since their statistical evaluation within VE trials requires samples from key timepoints from all vaccine recipients. However, systems vaccinology analysis of mucosal immunological features and their association with blood responses is also important when evaluating mechanistic CoPs.

Systems vaccinology studies for yellow fever [30–32], influenza [9, 33–35], and pneumococcal [35] vaccines have yielded insights with application to HIV vaccine trials, including: (1) it is critical to use biologically interpretable and statistically reproducible phenotypes for correlating with omics measurements [24–27]; (2) valuable biomarkers derived from omics measurements that well-predict phenotypes typically have low intra-vaccine recipient temporal variability [9, 36**] pre-vaccination, broad inter-vaccine recipient variability post-vaccination, and low measurement error [37*]; and (3) both unsupervised and supervised statistical learning techniques are needed to develop predictive

signatures. For HIV VE trials, the most important phenotype [factor (1)] may be whether a vaccine recipient acquires HIV infection; however, the limited numbers of infections and lack of data on HIV [38] limit statistical power. Relevant phenotypes that may be studied with greater power are leading putative CoPs generated from RV144, e.g., HIV Env V2-directed cross-reactivity scores [39]. Lesson (2) suggests the value of studying blood samples at multiple timepoints pre-vaccination [9], to screen out markers that are temporally unstable within individuals. Lesson (2) also suggests the value of pilot/screening statistical analyses of post-vaccination pilot samples internal or external to the VE trial to quantify the dynamic ranges and degrees of measurement error of immune response biomarkers [39, 40]. For lesson (3), the sample sizes available over the next several years limit the statistical power of fully supervised statistical learning analyses that directly build classifiers of HIV infection from the high-dimensional input features measured from omics technologies. Therefore, unsupervised clustering and module analyses [35] are critical for initially reducing the dimensionality of the input feature set prior to supervised clustering/case-control analysis. Gilbert et al. [37*] suggested an approach to power calculations for systems vaccinology-derived biomarker CoR analyses in VE trials that focuses on an ordinal biomarker with three levels, with the bottom and top levels defined by clusters/signatures from high-dimensional omics readouts. Table 1 summarizes some of the advantages and disadvantages of single measure, moderate dimension, and systems vaccinology marker approaches to developing CoRs and CoPs.

One systems vaccinology study of HIV vaccine trials has been published. Chung et al. [41**] measured six Fc-effector functions and 58 biophysical features of antibodies two weeks post last vaccination for 30 recipients each of four HIV vaccines, two with no VE [42–44], the RV144 vaccine with estimated VE = 31% [12], and a prototype Ad26 vector Env vaccine not yet evaluated for VE [45]. Their hierarchical and supervised clustering analyses showed that 15 of the 64 features explained most of the differences among the four vaccines. Using correlation network analysis, they revealed linkages between antibody functions and IgG1 features, including an IgG1 V1V2-driven antibody-dependent cellular phagocytosis response. In addition, their supervised clustering analysis of the case-control data from RV144 generated hypotheses that certain antibody features were associated with VE. This research illustrates that a confluence of (1) higher dimensional immunological measurements with (2) bioinformatics and (3) statistical inferences can provide a more thorough basis for down-selecting candidate HIV vaccines into efficacy trials and dissecting immune correlates in efficacy trials. Rigorous statistical inference is especially important for ensuring that valid answers about interpretable scientific questions are provided under transparent and plausible assumptions.

Accounting for the evolving HIV prevention landscape

The evolving standards of practice for HIV prevention impact the design and analysis of HIV vaccine efficacy trials. Typically, participants are provided with state-of-the-art HIV prevention management; these guidelines change as new tools become available and as regional/country-specific prevention standards shift. For example, a vaginal ring containing dapivirine was recently shown to be partially efficacious in preventing HIV acquisition among women [46, 47]. Similarly, male circumcision [48, 49] and oral anti-retroviral (ARV)

use as pre-exposure prophylaxis (PrEP) have been found to be partially efficacious at preventing HIV acquisition in populations such as MSM, discordant couples, IDUs, heterosexual men, and some populations of women [50–54]. Topical and systemic PrEP have shown more variable efficacy in women in sub-Saharan Africa than in MSM [50–54]. This variation has been attributed to both behavioral (e.g. lack of adherence [55]) and biological (e.g. genital inflammation [56]) factors. Importantly, product adherence has been correlated with effectiveness [57]. Indeed, recent follow-up analysis of the Partners in Prevention Trial yielded estimates of highly-adherent-PrEP efficacy that are greater than in the original MITT analysis [58]; this is consistent with other estimates of highly-adherent-PrEP efficacy [59].

Both the CDC and the WHO have released guidelines regarding oral PrEP use in high-risk populations [60–62]. These recommendations are being evaluated by governments and regulatory agencies where the cost-effectiveness of oral PrEP must compete against other health measures (e.g. government-funded health care or prevention methods such as test-and-treat). Thus, access to oral PrEP is expected to vary across and within countries.

These issues have implications for HIV vaccine science [63–65]. Trialists must incorporate PrEP into the design and analysis, taking into account participants using PrEP at enrollment and those that may initiate or discontinue PrEP during the trial [66**]. Moreover, attitudes towards PrEP and PrEP availability are expected to vary over time [67]. Given the mixed PrEP efficacy results across populations, the expected variation in PrEP use, and the different states of product availability and licensure – oral PrEP is currently approved in only nine countries [68], and vaginal rings are not yet licensed – vaccine efficacy (VE) may be heterogeneous and dynamic.

It is essential to assess PrEP use among trial participants to enable accurate interpretation of VE. Such assessment can help interpret the placebo-group HIV incidence and/or inform on potential modification of VE by PrEP use at acquisition. The best method and frequency for data collection is still unclear, given PrEP uptake uncertainty and anticipated changes in PrEP uptake throughout the trial. Additionally, current assays for detecting drug levels have limited accuracy for determining PrEP use at the critical time of HIV infection [69].

Two recently designed efficacy trials illustrate the HVTN's approach regarding PrEP. HVTN 704/HPTN 085 and HVTN 703/HPTN 081 are ongoing phase-IIb trials of the VRC01 broadly neutralizing monoclonal antibody for the prevention of HIV infection, enrolling 2700 MSM and transgender women (TGW) in North+South America and 1500 sub-Saharan African women, respectively. Interest in, and usage of PrEP at enrollment, do not affect eligibility. Participants are randomized to 10 µg/ml VRC01, 30 µg/ml VRC01, or placebo, administered every 8 weeks for 10 infusions. Participants are provided standard-of-care for HIV prevention, including risk reduction counseling and referral for free oral PrEP via service providers at separate sites for the Americas trial. In the sub-Saharan African trial, risk reduction counselling includes PrEP information. Sites can prescribe PrEP where it is licensed but not yet publicly available, or refer participants to nearby demonstration projects. Sample size calculations allow up to 30–50% of the person-years at risk to be under PrEP use, assuming 90% PrEP efficacy. ARV levels are measured using cross-sectional sampling

of specimens for a subset of trial participants. By quantifying drug concentrations in stored specimens, detectable and inferred effective PrEP use on the sampling dates can be ascertained [70, 71], allowing estimation of the percentage of person-years at risk for HIV infection during detectable PrEP use and during inferred effective PrEP use. Although PrEP has been postulated to affect the timing of HIV infection diagnosis, this appears unlikely [72].

Conclusion

The development of efficacious HIV vaccines will be greatly facilitated by the identification and validation of correlates of vaccine efficacy and by efficient down-selection of candidate vaccine regimens into efficacy trials. Much progress has been made in the past year with respect to statistical and bioinformatics tools that can be applied to these two goals. Systems vaccinology has also just begun to be applied to HIV vaccine trials, demonstrating the potential contributions that this field can make to HIV vaccine research. Lessons have also been learned regarding accommodation of PrEP use in efficacy trials that can be applied to future trials.

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Key points

- Upcoming efforts to identify immunological correlates of HIV vaccine efficacy should leverage both the baseline immunogenicity predictors (BIP) and close-out placebo vaccination (CPV) design components
- Statistical frameworks and approaches have been developed to facilitate rigorous down-selection of the most promising HIV vaccine regimens based on data from early-phase safety and immunogenicity trials
- Systems-based approaches combining high dimensional immunological data, bioinformatics, and rigorous statistical inference can aid in candidate HIV vaccine down-selection and in evaluating immune correlates of vaccine efficacy in efficacy trials
- The design and analysis of HIV vaccine trials must evolve to accommodate new prevention strategies and tools, in addition to changes in regional-specific standards of prevention

Figure 1.

Schematic representation of the baseline immunogenicity predictor (BIP) and closeout placebo vaccination (CPV) vaccine efficacy trial design techniques. $S(V)$ is a participant's immune response if assigned to receive vaccine, which can be measured only among vaccine recipients in the standard trial follow-up period [$S(V)$ is denoted as S for observed immune response]. In placebo recipients the immune response to vaccination $S(V)$ is a counterfactual and not observable. With CPV, participants in the placebo arm that are HIV negative (HIV-) at the end of the standard trial follow-up period (closeout) can be vaccinated and their immune response S_c subsequently measured and used to substitute for their unobserved $S(V)$ values.

Table 1

Attributes of single measure, moderate dimension and systems vaccinology approaches to evaluating immune response biomarkers as correlates of risk (CoRs) and/or protection (CoPs).

	Single Marker	Moderate Dimension (Several Markers)	Systems Vaccinology (Many Markers)
Suitable for discovery or validation of CoRs/CoPs?	Validation	Discovery and Validation	Discovery
Role of specific hypotheses regarding mechanisms by which vaccine protects	Important motivation but not absolutely necessary give a CoP can be non-mechanistic	Important motivation but not absolutely necessary give a CoP can be non-mechanistic	Not required given the discovery objective
Number of distinct CoR/CoP hypotheses that can be investigated	One	Several	Many
Timing of marker measurements	Typically shortly after immunizations, at approximate peak immunogenicity, to measure adaptive immune responses	Typically shortly after immunizations, at approximate peak immunogenicity, to measure adaptive immune responses	Within hours or days following vaccination, to measure innate immune responses
Objective for timing of immune response measurement	Based on previous longitudinal time point studies, typically aim to measure approximate peak response	Based on previous longitudinal time point studies, typically aim to measure approximate peak response	The study of response kinetics is an objective; pilot data are needed to identify time windows of gene expression
Suitable for discovering a multi-marker CoR/CoP molecular signature?	No	Secondary analyses can be used to discover low-dimensional marker signatures	Yes, suitable for discovering both low- and high-dimensional marker signatures
Multiplicity of statistical testing	Issue is avoided	A minor issue	A major issue, although statistical methodology exists to deal with it
Correlation among immune response biomarkers	Is not relevant	Is relevant and is taken into account in the analysis, except for univariate analysis of the markers	Is relevant and is taken into account in the analysis
Resource requirements to conduct in standard vaccine efficacy trial	Requires storing samples at baseline and at approximate peak response for all vaccine recipients	Requires storing samples at baseline and at approximate peak response for all vaccine recipients	Requires storing samples from additional time points for all vaccine recipients (e.g., two baseline samples, day 3, day 7)