Baseline host determinants of robust human HIV-1 vaccine-induced immune responses: A meta-analysis of 26 vaccine regimens

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Summary

Background The identification of baseline host determinants that associate with robust HIV-1 vaccine-induced immune responses could aid HIV-1 vaccine development. We aimed to assess both the collective and relative performance of baseline characteristics in classifying individual participants in nine different Phase 1-2 HIV-1 vaccine clinical trials (26 vaccine regimens, conducted in Africa and in the Americas) as High HIV-1 vaccine responders.

Methods This was a meta-analysis of individual participant data, with studies chosen based on participant-level (vs. study-level summary) data availability within the HIV-1 Vaccine Trials Network. We assessed the performance of 25 baseline characteristics (demographics, safety haematological measurements, vital signs, assay background

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measurements) and estimated the relative importance of each characteristic in classifying 831 participants as High (defined as within the top 25th percentile among positive responders or above the assay upper limit of quantification) versus Non-High responders. Immune response outcomes included HIV-1-specific serum IgG binding antibodies and Env-specific CD4+ T-cell responses assessed two weeks post-last dose, all measured at central HVTN laboratories. Three variable importance approaches based on SuperLearner ensemble machine learning were considered.

Findings Overall, 30.1%, 50.5%, 36.2%, and 13.9% of participants were categorized as High responders for gp120 IgG, gp140 IgG, gp41 IgG, and Env-specific CD4+ T-cell vaccine-induced responses, respectively. When including all baseline characteristics, moderate performance was achieved for the classification of High responder status for the binding antibody responses, with cross-validated areas under the ROC curve (CV-AUC) of 0.72 (95% CI: 0.68, 0.76) for gp120 IgG, 0.73 (0.69, 0.76) for gp140 IgG, and 0.67 (95% CI: 0.63, 0.72) for gp41 IgG. In contrast, the collection of all baseline characteristics yielded little improvement over chance for predicting High Env-specific CD4+ T-cell responses [CV-AUC: 0.53 (0.48, 0.58)]. While estimated variable importance patterns differed across the three approaches, female sex assigned at birth, lower height, and higher total white blood cell count emerged as significant predictors of High responder status across multiple immune response outcomes using Approach 1. Of these three baseline variables, total white blood cell count ranked highly across all three approaches for predicting vaccine-induced gp41 and gp140 High responder status.

Interpretation The identified features should be studied further in pursuit of intervention strategies to improve vaccine responses and may be adjusted for in analyses of immune response data to enhance statistical power.

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Keywords: Baseline characteristics; SuperLearner; Variable importance measurements; Vaccine response heterogeneity; Antibody; CD4+ T cell

Research in Context

Evidence before this study

For many licensed vaccines, baseline immune signatures and/or other participant characteristics have been found to associate with vaccine immunogenicity and/or efficacy. However, for HIV-1 vaccines, little is known about whether such "baseline predictors" exist. We queried PubMed on Oct 26, 2021 using various combinations of the search terms "baseline", "demographic", "haematological", "safety", "predict", "predictor", "HIV-1 vaccine", "response", "immune response", "vaccine-induced", "immunogenicity", "adaptive response", and "high response". Our search yielded one study investigating whether and how baseline demographic variables and immune responses measured two weeks post vaccination predicted response rate and/or magnitude of HIV-1 vaccine-induced cellular and humoral responses measured at six months post last vaccination. In that study, baseline demographic variables contributed little to no predictive information. We also found a study reporting that certain baseline variables (sex, BMI, and heavy drinking) each significantly predicted HIV-1 specific cellular responses induced by a recombinant adenovirus HIV-1 vaccine, and another reporting that sex and BMI were each associated with Env-specific cellular responses induced by DNA plasmid HIV-1 vaccines.

Added value of this study

To our knowledge, this is the largest meta-analysis of HIV-1 vaccine trials to evaluate the relationship of a comprehensive set of baseline features with antibody and cellular vaccine-induced immune responses. In addition, this study contributes to the additional assessment of commonly measured demographic, metabolic, and haematological parameters to enable resolution of their broad value in understanding vaccine response heterogeneity, and identifies sex, body height, and total white blood cell count as worthy of additional investigation in future research. Lastly, because the absolute magnitudes of immune responses are often vaccine regimen-dependent, this study identified host determinants of robust immune responses irrespective of the vaccine regimen, by first defining High responder status

within each vaccine regimen before combining data across regimens.

Implications of all the available evidence

Variations in immune responses to vaccines among individuals including those considered healthy are frequently observed but poorly understood. This study resolves the association of baseline parameters with exceptionally high responses to HIV vaccines, providing justification to further study these parameters including sex, body height, and total white blood cell count in the context of HIV and other vaccines, and dissect the underlying mechanisms whereby they may promote enhanced vaccine responses.

Introduction

Vaccine-induced immune responses are generally heterogeneous across individuals and across populations.[1–4] For instance, the immune response continuum induced by a given vaccine may range from no response ("non-responders") all the way to "high-responders" – individuals with exceptional cellular and humoral responses. This large degree of inter-individual immune response heterogeneity, even among immunocompetent individuals, has been observed with many licensed vaccines, including Covid-19,[5] influenza,[6] dengue,[7] and hepatitis B, as well as in HIV-1 vaccine trials.[8] Understanding the basis of this heterogeneity is important in the context of vaccine development because once protective immune responses are identified through immune correlates analyses,[9–11] devising approaches for enhancing the response rates or magnitudes of these responses in low- or non-responders could help improve vaccine efficacy. Moreover, understanding of the baseline characteristics associated with high response could help identify targets that could be modulated before vaccination to improve vaccine response,[3,12] where possible, or alternatively to customize vaccination strategies via, for example, using different adjuvants,[13,14] reducing intervals between boosts or including additional boosts.[15]

There are many categories of both vaccine- and host-specific factors that can influence the immune response to vaccination. Zimmermann and Curtis[1] provide comprehensive references and outline a number of host-specific categories, including intrinsic (e.g. genetics, age), perinatal (e.g. gestational age, birth weight), extrinsic (e.g. pre-existing immunity, gut microbiota), environmental (e.g. geographic location), behavioural (e.g. smoking status, exercise), and nutritional (e.g. body mass index). Vaccine nonresponse as measured by antibody levels has also been estimated to occur in 2-10% of all healthy individuals[16]; this phenomenon is particularly well documented for the hepatitis B vaccine.[17] There is also a growing body of evidence to support that baseline immune state, i.e. transcriptomic and/or cellular signatures, can predict vaccine-induced antibody and/or cellular immune responses,[3] with some molecular signatures associated with antibody response potentially shared across different vaccines.[18]

Clinical laboratory measurements [e.g., alanine aminotransferase (ALT), creatinine, platelets, white blood cell count, mean corpuscular volume, haematocrit, and haemoglobin] are often obtained at enrolment in vaccine trials to verify that individuals fulfil the trial's inclusion criteria related to being in good general health. These measurements provide an overall picture at gross resolution of metabolic, inflammatory, and haematological processes and may thus be associated with vaccine response – as populations with co-morbidities, who often show reduced vaccine response,[1] often have clinical laboratory measurements different from an average healthy individual. It is unknown whether these clinical laboratory measurements that are in the normal range could be used to predict low or high HIV vaccine response in healthy individuals, akin to simple clinical risk scores that have been used to identify non-responders in the context of other vaccines.[19]

Despite significant recent advances in HIV-1 basic research,[20–22] a safe and effective vaccine to protect against HIV-1 acquisition remains lacking. Of the seven phase 3 HIV-1 vaccine efficacy trials that have been conducted to date,[23–29] only the RV144 trial of a pox-protein clade AE/B alum-adjuvanted regimen demonstrated protection, albeit modest, against HIV-1 acquisition.[28] Vaccinations in HVTN 702 (a Phase 2b/3 trial that tested a clade C-adapted version of the RV144 regimen in South Africa) were stopped early for non-efficacy[29] and HVTN 705 (a Phase 2b trial that tested a "mosaic" regimen in Sub-Saharan Africa) did not meet predefined efficacy criteria for longer follow-up and was recently stopped.[30] However, even in the face of these disappointing results, progress has been made towards identifying immune correlates[31]: Initially, IgG antibodies against the HIV-1 envelope (Env) V1V2 region were found to be inversely correlated with HIV-1 infection risk and plasma IgA antibodies against HIV-1 envelope were found to be positively correlated with HIV-1 infection risk[32]; subsequent studies expanded on these findings and provided potential mechanistic insights.[33–35] Later analyses also identified Env-specific CD4+ T-cell polyfunctionality[36] and IgG3 antibodies against the HIV-1 envelope V1V2 region[37] as inverse correlates of risk. Substantial heterogeneity was seen across trial participants with respect to immune responses linked to protection, with Zhao and Fiore-Gartland et al.[8] identifying distinct participant subgroups with 1) high responses across all variables, 2) nonresponse across all variables, 3) a broad anti-V1V2 antibody response but no CD4+ T-cell response, and 4) a broad anti-V1V2 antibody response as well as a robust CD4+ T-cell response. As potential explanations underlying this heterogeneity, we and others have reported that host factors such as immunogenetics,[38–41] body mass index (BMI),[42,43] and sex[42,43] influence

immune responses to HIV-1 vaccination and/or vaccine efficacy. However, it is unknown whether there is a baseline characteristic that predicts immunogenicity generally across HIV-1 vaccines, as we found in another study of seven preventive HIV-1 vaccine regimens that baseline demographic information had little to no capacity to improve prediction of immune responses measured six months post last vaccination.[44]

In this meta-analysis of nine clinical trials of 26 HIV-1 vaccine regimens, we assessed the collective performance of 25 baseline characteristics, as well as the relative importance of each characteristic to classify individual vaccine recipients as a "high responder" in terms of serum HIV-1 Env-specific IgG binding antibodies or Env-specific CD4+ T-cells as assessed two weeks post-last dose to a given HIV-1 vaccine regimen. This knowledge is expected to inform strategies to maximize response rates to future candidate HIV-1 vaccines; perhaps *a priori* knowledge of expected response rate for an individual or population may allow customization of vaccination strategy or pre-vaccination interventions to promote robust responses. This knowledge may also be used to improve statistical power in the analyses of immune responses by controlling for the identified host determinants.

Methods

Study cohorts and vaccine regimens

This one-stage meta-analysis included individual participant data of 26 HIV-1 vaccine regimens from nine Phase 1-2 clinical trials conducted by the HIV-1 Vaccine Trials Network (HVTN) in 2008-2018, at study sites in Peru, Tanzania, the United States, South Africa, and Zambia. A total of 1,111 participants were enrolled in these trials, with 838 per-protocol participants who received all scheduled vaccinations and had immune response data available for the analyses (Figure 1; Supplementary Methods). All trial participants were HIV-1-negative, healthy adults not considered to be at high risk for HIV-1 acquisition. Details of each trial have been previously described: HVTN 073/SAAVI 102 (NCT00574600),[45] HVTN 086/SAAVI 103 (NCT01418235),[46] HVTN 094 (NCT01571960),[47] HVTN 097 (NCT02109354),[48] HVTN 098 (NCT02431767),[49] HVTN 100 (NCT02404311),[50] HVTN 105 (NCT02207920),[51] HVTN 111 (NCT02997969),[52] and HVTN 205 (NCT00820846).[53] These trials were selected based on their stimulation of HIV-1 Env specific antibody responses and data availability, and represent a variety of immunogen platforms, including DNA plasmid, viral vector [Modified Vaccinia virus Ankara (MVA), canarypox (ALVAC)], and HIV-1 Env protein.

Laboratory methods

Binding antibody multiplex assay (BAMA). Serum HIV-1-specific IgG responses (serum at a 1:50 dilution; primary detection: Mouse Anti-Human IgG Fc-Biot, SouthernBiotech, catalog number 9042-08; followed by Streptavidin-PE, BDPharmingen, catalog number 554061) against 3 antigens: Con 6 gp120/B (Duke Human Vaccine Institute Protein Production Facility, Plasmid ID: HV1300454), ConS gp140 CFI (Duke Human Vaccine Institute Protein Production Facility, Plasmid ID: HV1300111_avi) and gp41 (ImmunoDx, Catalog number 1091) were measured on a Bio-Plex instrument (Bio-Rad) using a standardized custom HIV-1 Luminex assay[54] in serum samples collected at baseline (pre-vaccination) and 2 weeks after the last dose of each vaccine regimen. The readout was background-subtracted mean fluorescence intensity (MFI), where background refers to a plate level control (i.e., a blank well run on each plate). The positive control was purified polyclonal IgG from people living with HIV-1 (HIVIG, NIH AIDS Reagent Program, Catalog number 3957) using a 10-point standard curve (4PL fit). The negative controls were NHS (HIV-1 seronegative human sera) and blank beads.

Samples were declared to have positive responses if they met three conditions: (1) the MFI minus Blank values were greater than or equal to the antigen-specific cut-off at the 1:50 dilution level (based on the average + 3 standard deviations of at least 60 seronegative plasma samples or at least 100 MFI), (2) the MFI minus Blank values were greater than 3 times the baseline (day 0) MFI minus Blank values, and (3) the MFI values were greater than 3 times the baseline MFI values.

Intracellular cytokine staining (ICS). HIV-1-specific CD4+ and CD8+ T-cell responses were measured by a validated flow cytometry assay similar to what was previously described[32,36,51,55–59] in PBMC samples collected at two weeks post the last dose of each vaccine regimen. Table S1 provides detailed information (manufacturer, catalog number, clone) for the antibodies used in the ICS assays. Previously cryopreserved PBMC were stimulated with synthetic HIV-1 Envelope peptide pools. As a negative control run in duplicate, cells were incubated with DMSO, the diluent for the peptide pools. As a positive control, cells were stimulated with a polyclonal stimulant, staphylococcal enterotoxin B (SEB).

Samples were declared to have a positive response based on a one-sided Fisher's exact test of whether the frequency of IL-2 and/or IFN-γ-producing cells in the peptide-stimulated well was equal to that in the negative control wells. A multiplicity adjustment was made to the individual peptide-pool-specific p-values using the discrete Bonferroni-Holm adjustment method. If the adjusted p-value was $\leq$ 10e-5, the response to the peptide pool for the T-cell subset was considered positive. If any Env peptide pool was positive for a T-cell subset, then the overall Env-specific response for that T-cell subset was considered positive. Due to the sparseness of

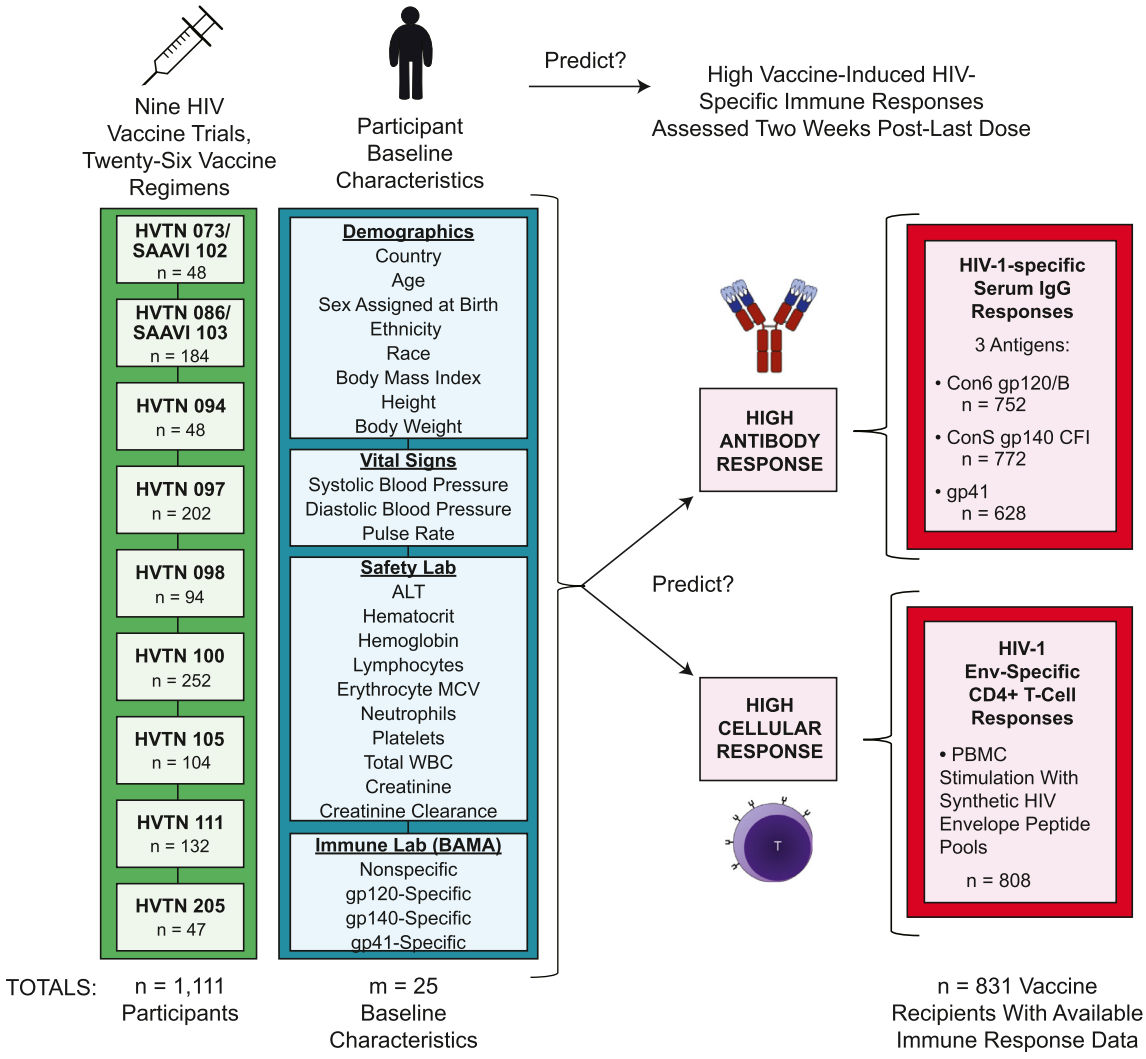

Figure 1. Analysis schema. The nine HIV-1 vaccine trials (covering 26 vaccine regimens) included in the analysis are shown in the green box. Numbers are total trial participants (vaccine and placebo recipients). The 25 participant baseline characteristics used to predict High vaccine-induced HIV-1-specific immune responses are shown in the blue box. The HIV-1-specific immune responses measured at 2 weeks post the last vaccination are shown in the pink boxes. Numbers in the pink boxes are vaccine recipients with available immune assay data for each immune response, after excluding participants from vaccine regimens with no responders.

responses, CD8+ T-cell data were not considered in the analyses presented here. Further details on the response definitions and statistical criteria for analysis are given in Supplementary Methods.

Definitions of high responders

For each immune response measured at 2 weeks post last vaccination, participants were categorized as either a "High responder" or a "Non-High responder." For IgG binding antibody responses against HIV-1-specific antigens (outcomes labelled as 'gp120', 'gp140' and 'gp41'), a participant was defined as a High responder if the participant's background-subtracted MFI value against the particular antigen was within the top 25th percentile among positive responders for a given vaccine regimen or $\geq$ 22,000 MFI (the upper limit of quantification at 1:50 sample dilution). In addition, for outcomes labelled as 'gp140 and gp120' and 'gp140 or gp120', a participant was defined as a High responder if they were a High responder to both 'gp140' and 'gp120, and to either 'gp140' or 'gp120', respectively. The first combination outcome was included to assess whether some baseline characteristics might predict High responder status more broadly, i.e. across multiple antigens. For HIV-1 specific CD4+ T-cell responses ('CD4'), a participant was defined as a High responder if the background-subtracted percentage of CD4+ T-cells

secreting IL-2 and/or IFN-γ was within the top 25th percentile among positive responders for a given vaccine regimen. Participants that did not meet the High responder criterion for a given immune response variable, including non-responders, were categorized as "Non-High" for that particular immune response.

Based on a comment from a reviewer, we also repeated the analyses using an alternative definition of a "High responder". For IgG binding antibody responses against HIV-1-specific antigens, a participant was classified as a "High Responder – 20%tile" if their background-subtracted MFI value against the particular antigen was within the top 20th percentile for a given vaccine regimen or $\geq$ 22,000 MFI (the upper limit of quantification at 1:50 sample dilution). A participant was classified as a "Low Responder – 20%tile" if their background-subtracted MFI value against the particular antigen was within the lowest 20th percentile for a given vaccine regimen, or if they were not a positive response (see definition of positive response under *Binding antibody multiplex assay (BAMA) in Laboratory Methods)*. According to this definition, more than 20% of the individuals could be in the High Responder or in the Low Responder category if there are more than 20% with responses $\geq$ 22,000 MFI or more than 20% with a non-positive response, respectively, The middle approximately 60% of data were removed from this analysis. These definitions were applied similarly for CD4+ T-cell responses, except that background-subtracted percentage of CD4+ T-cells secreting IL-2 and/or IFN-γ was used in place of MFI value.

Baseline host characteristics

Twenty-five baseline characteristics measured prior to initiating the HIV-1 vaccine regimen were considered as potential predictors of the immune responses. Each characteristic was classified into one of four overall categories: demographics (m=8) [age (years), sex assigned at birth (female or male), ethnicity (Hispanic or Other), race (Black, White, or Other), body mass index (BMI, kg/m^2), height (cm), body weight (kg), country (Africa or Americas)]; vital signs (m=3) [systolic blood pressure (mm Hg), diastolic blood pressure (mm Hg), pulse (beats/min)]; safety haematologicallab (m=10) [alanine aminotransferase (ALT, U/L), haematocrit (%), haemoglobin (g/dL), lymphocytes (/µL), neutrophils (/µL), platelets (/nL), total white blood cell (/nL), mean corpuscular volume (MCV, fL/red cell), creatinine (mg/dL), creatinine clearance (mL/min)]; and background binding antibody (m=4) [baseline HIV-1-specific antibody responses measured in pre-vaccination serum samples, capturing the background levels in the BAMA assays and possible non-specific reactivity,[60] as an indication of the participant's general immune state (background gp120, background gp140, background gp41): difference in MFI between the HIV-1-specific gp120, gp140 or gp41 antigen beads-added wells with and without the tested serum sample and baseline non-specific binding antibody responses (background blank): difference in MFI between the blank-bead wells (no HIV-1 antigens) with and without the tested serum samples] (Figure 1). Missing values (<18%) in baseline predictors were imputed by median within each region (Americas vs. Africa).

Statistical methods

Machine learning models. All 25 baseline host characteristics were assessed as predictors of High responder status by building convex ensemble models using regression stacking,[61,62] also known as Super Learning,[63] with the SuperLearner R package.[63,64] SuperLearner is an algorithm that uses cross-validation to estimate the performance of multiple machine learning models, or the same model with different parameter settings. It then creates an optimal weighted average of those models, aka an "ensemble" that may use all or only a subset of the models, using the test data performance. This approach has been proven to be asymptotically as accurate as the best possible prediction algorithm that is tested.[65,66] We used SuperLearner to estimate the optimal convex weights to combine candidate learning algorithms with the aim of minimizing the negative log-likelihood loss (for dichotomous outcomes) via an internal 10-fold cross-validation. The candidate learning algorithms included a benchmark learner using the frequency of the outcome (SL.mean, R base function), as well as random forests[67] (SL.randomForest, Version 4.6-14), lasso[68] with logistic link (SL.glmnet, Version 4.1-2), stepwise-selected generalized linear models with logistic link (SL.step, R base function) and generalized additive models[69] (SL.gam, Version 1.20). The candidate learners and the Super Learner were evaluated via an external 10-fold cross-validation (CV) to guard against overfitting. The cross-validated area under the receiver operating characteristics curve (CV-AUC) and the cross-validated prediction accuracy (CV-Accuracy) were used as prediction metrics. Wald-type 95% confidence intervals about CV-AUC were computed using influence function-based standard error estimates.[70]

Identification of important baseline predictors. For each immune response outcome, the heterogeneity index I^2 was calculated for each baseline predictor in terms of the proportion of the variance in their association with the High responder status that is due to heterogeneity across the 9 clinical trials.[71] Because no vaccine regimens were tested in more than one country, Country was included as a confounding variable to adjust for in all analyses but not considered in the ranking of predictors. Three approaches were used to obtain variable importance estimates for each baseline predictor. Approach 1 used causal inference statistics and was

based on the average "treatment effect" (ATE) as implemented in the varimpact R package (Version 1.3.0-9005).[72] Each predictor was analysed using targeted minimum loss-based estimation (TMLE)[73] as though it were a treatment, with all other variables serving as adjustment variables via SuperLearner (Version 2.0-28). This involves determining the two levels of a given variable (discretized into 6 levels for continuous variables) that result in the largest estimated absolute risk difference when that variable is set to "treatment" (high level) or "control" (low level). For binary variables, this is a true average treatment effect, where treatment corresponds to presence of the variable of interest. Then the statistical significance of the estimated ATE in terms of a marginal, adjusted risk difference in the probability of being a High responder comparing universal application of one treatment vs. the other determined the variable importance ranking. The results provide raw *p*-values as well as *p*-values adjusted for false discovery rate using the Benjamini-Hochberg[74] procedure. For continuous variables, the risk difference represents the estimated increase in the probability of High responder status when moving from a lower quantile level to a higher quantile level of the variable that resulted in the largest difference in the estimated probability of being a High responder. For each immune response outcome, risk difference estimates and associated *p*-values were only reported for baseline predictors that exhibited consistent directionality of effects across the 10-fold cross-validation datasets.

Approaches 2 and 3 focused on identification of association and were based on differential prediction performance as implemented in the vimp R package (Version 2.2.5).[75] The variable importance of each baseline predictor was defined as the increase in CV-AUC (Approach 2) or CV-accuracy (Approach 3) when adding each specific baseline predictor to the other baseline predictors relative to considering only the other baseline predictors in the SuperLearner estimation procedure. A 10-fold cross-validation procedure was used in all three Approaches. Specifically, the dataset was first split into 10 folds. Using each fold in turn as held-out test set, we applied the three approaches to determine the variable importance measures based on the appropriate SuperLearner for each baseline predictor. For Approach 1, a new SuperLearner using the "treatment variable" was fit on the training data and the treatment effect was evaluated on the held-out data; for both Approaches 2 and 3, two SuperLearners were fit, one using all variables and another using all variables except the predictor of interest and the difference in AUC (Approach 2) and prediction accuracy (Approach 3) was evaluated on the held-out data. The variable importance measurements from the 10 folds were then averaged, resulting in a 10-fold cross-validated estimates of variable importance measurements for each baseline predictor. All analyses were performed using R 4.1.0.

Ethics

The trials were approved by the following Institutional Review Boards (IRBs)/Ethics Committees (ECs): **HVTN 073/SAAVI 102** – Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg (No. 071109), Partners Human Research Committee (No. 2007P002556/BWH), University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (035/2008), Fenway Community Health IRB (No. 410727), University of Rochester Research Subjects Review Board (No. RSRB00027762); **HVTN 086/SAAVI 103** – Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg (No. 100907), University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (No. 536/2010), University of Kwazulu-Natal Research Office: Biomedical Research Ethics Administration (No. BFC206/010); **HVTN 094** – University of Alabama at Birmingham Office of the Institutional Review Board for Human Use (No. IRB-120216009), University of Rochester Research Subjects Review Board (No. RSRB00041139), Partners Human Research Committee (No. 2012P000374/MGH), University of California San Francisco Human Research Protection Program Committee on Human Research (No. 11-08211); **HVTN 097** –Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg (No. AUR1-3-122), Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg (No. 121107), University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (No. 139/2013); **HVTN 098** – University of Rochester Research Subjects Review Board (No. STUDY00000732), Fred Hutchinson Cancer Research Center IRB (No. 8362), Emory University IRB (No. IRB00080777), Vanderbilt Human Research Protections Program (No. 150585); **HVTN 100** – South African Medical Research Council Human Research Ethics Committee (No. EC013-9/2014), Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg (No. 140705B), University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (No. 443/2014), University of Kwazulu-Natal Research Office Biomedical Research Ethics Administration (No. BFC301/14), Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg (No. 140705B); **HVTN 105** – Columbia Human Research Protection Office IRB (No. IRB-AAAN6603), Fred Hutchinson Cancer Research Center IRB (No. 8267), Vanderbilt University IRB (140828), University of California San Francisco Human Research Protection Program Committee on Human Research (No. 14-13672), University of Pennsylvania IRB (No. 820330), University of Rochester Research Subjects Review Board (RSRB00052283); **HVTN 111** – University of Zambia Biomedical Research Ethics Committee (No. 009-11-

14), University of North Carolina at Chapel Hill Office of Research Ethics Biomedical IRB (No. 14-3138), Mbeya Medical Research and Ethics Committee (No. SZEC-2439/R.E/V.1/03), Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg (No. 150212), South African Medical Research Council Human Research Ethics Committee (No. EC011-3/2015), Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg (No. 150212); **HVTN 205** – University of Alabama at Birmingham Office of the Institutional Review Board for Human Use (No. IRB-081120005), Partners Human Research Committee (No. 2008P002441/PHS), Emory University IRB (No. IRB00015382), Fenway Community Health IRB (No. 410814), Fred Hutchinson Cancer Research Center IRB (No. 6893), Vanderbilt University IRB (No. 081244), Impacta Comite Institucional de Bioetica (No. 0245-2013-CE), University of California San Francisco Human Research Protection Program Committee on Human Research (No. 10-02374), Impacta Comite Institucional de Bioetica (No. 0227-2013-CE), Columbia University Medical Center IRB (No. IRB-AAAD7235), New York Blood Center IRB (No. 517-08), University of Rochester Research Subjects Review Board (No. RSRB00026307). Informed consent was obtained from all participants.

Role of funders

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Dr Yunda Huang (corresponding author) had full access to all the data in the study and with Dr James Kobie (corresponding author) had final responsibility for the decision to submit for publication.

Results

Distribution of baseline predictors

Twenty-five baseline characteristics including demographics, vital signs, safety haematological measurements and immune assay background measurements were considered as potential predictors of High responder status of six immune response outcomes. Table S2 provides the baseline predictor distributions separately for each outcome. The median age was 24 years old in this analysis cohort, with near equal representation of females and males as well as of individuals in Africa and the Americas. All other baseline characteristics possess a reasonable dynamic range, including the four background binding antibody variables.

Distribution of High responders within each vaccine regimen

It is anticipated that an effective HIV vaccine will require optimal induction of HIV Envelope-antibody and T cell responses. To enable analysis across the trials, consistently assessed vaccine-induced HIV-1 specific immune responses were considered, including plasma binding IgG specific for consensus gp120, gp140, and gp41 components of HIV Env, as well as CD4+ T cell response to HIV Env peptide pools. For each of these four immune responses, the distributions of the observed immune marker measurements according to High/Non-High responder status are shown in Figure 2 and according to the positive response status, based on which the High responder status is defined, are shown in Figure S1. Table S3 additionally provides High/Non-High responder counts and percentages within each vaccine regimen, along with geographical information for the sites in which each trial was conducted. While the High HIV-1 specific CD4+ T-cell responses were generally uniformly distributed across vaccine regimens (ranging from 3–26% High responses within each vaccine regimen), greater variability was seen in the distribution of High antibody IgG response magnitudes across vaccine regimens. For gp140 IgG responses, for instance, the percentages of High responders ranged from 7–100% across regimens, with particularly percentages of High responders in all regimens in the HVTN 111 trial (96–100%) and in some regimens in the HVTN 086 trial (T1: 97% High responders, T4: 87% High responders). For gp120 IgG responses, the percentages of High responders in each regimen ranged from 8–76%; the range was widest for gp41 IgG responses, with percentages of High responders within each regimen ranging from 1–100%. This variation can be explained by differences in vaccine platforms and gp41-containing immunogens across regimens (Supplementary Methods) and was intentionally embraced so that findings from our analyses would potentially be applicable to diverse vaccine regimens inducing a wide range of immune responses. Such variation potentially also increased the statistical power to identify predictors of High responder status.

Relationship of response status between immune response parameters

The treatment-pooled frequencies of High and Non-High responders (of those with available baseline predictor data) are shown in Figure 3a. Overall, 30.1%, 50.5%, 36.2%, and 13.9% of participants were categorized as High for gp120 IgG, gp140 IgG, gp41 IgG, and Env-specific CD4+ responses, respectively. While 54.2% of participants were categorized as High for either gp140 IgG or gp120 IgG, only 27.6% were categorized as High for both gp140 IgG and gp120 IgG.

When including all participants, only slight agreement was seen in High responder status for a given individual across pairs of immune response variables, with six of the seven kappa values ranging from 0.06 to 0.20 (Table S4). This finding implies that a given participant with e.g. a High Env-specific CD4+ T-cell

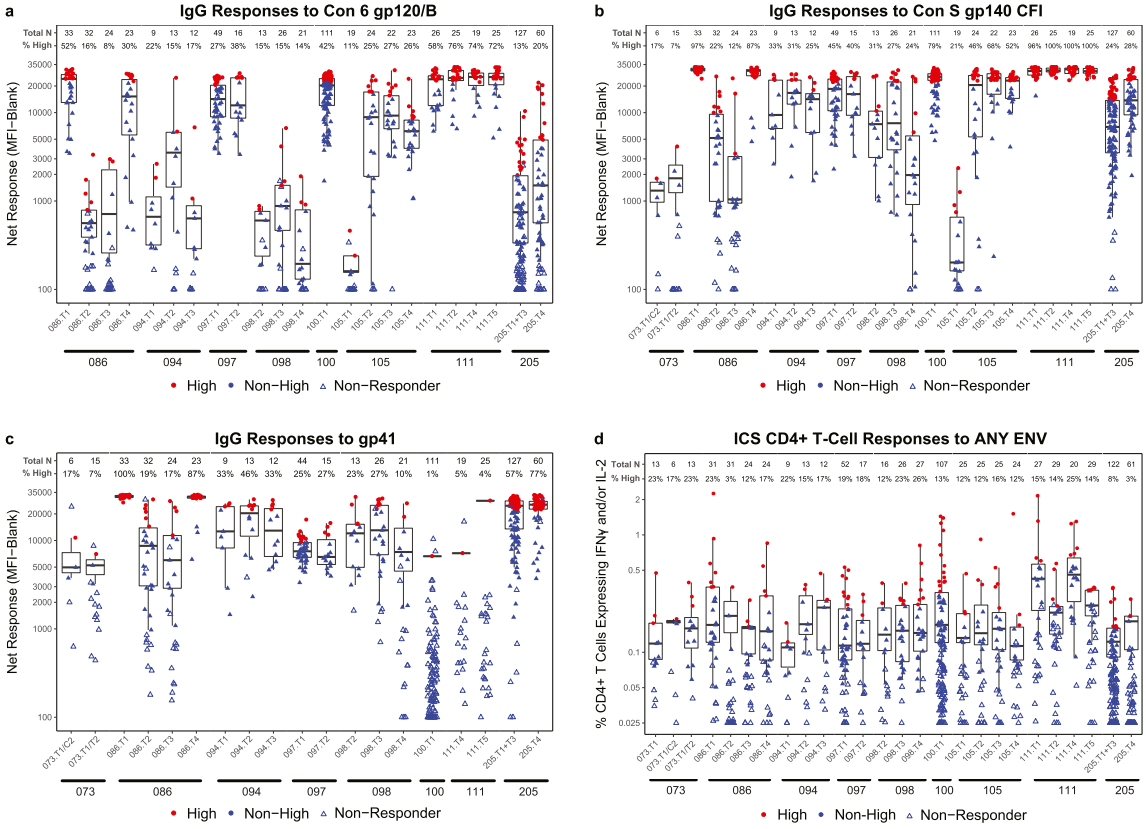

Figure 2. Distributions of observed immune marker measurements by High/Non-High responder status, for each of the four assessed HIV-1 specific adaptive responses. a) Serum IgG BAMA responses to Con6 gp120/B (n = 752), b) Serum IgG BAMA responses to Con S gp140 CFI (n = 772), c) Serum IgG BAMA responses to gp41 (n = 628), and d) IFN-γ and/or IL-2 CD4+ T-cell responses to ANY ENV (n = 808). The top and bottom of each boxplot indicate the interquartile range; whiskers extend along the range among the positive responders. Red dots indicate High responders. Blue filled triangles and blue open triangles both indicate Non-High responders. Blue open triangles indicate the Non-Responder subset of the Non-High responders. Definitions of High vs. Non-High, Responder vs. Non-Responder are provided in Methods.

response likely did not also have a High IgG binding antibody response against any of the antigens examined. The pair of gp120 IgG and gp140 IgG was an exception, with moderate agreement in High responder status (kappa = 0.47).

When restricting to High responders, the magnitude of responses showed a significant ($p < 0.05$) correlation between the four immune outcomes (Figure 3b). Moderate-to-high correlation was observed between gp120 and gp140 IgG binding antibody response magnitude (rho = 0.76, $p < 0.001$, t-distribution) and between gp140 and gp41 response magnitude (rho = 0.68, $p < 0.001$, t-distribution). Env-specific CD4+ T-cell response magnitude showed moderate correlation with gp120 IgG binding antibody response magnitude (rho = 0.54, $p < 0.001$, t-distribution) and lower correlation with gp41 IgG binding antibody response magnitude (rho = 0.42, $p < 0.05$, t-distribution). When BAMA responses were truncated at 22,000 (the upper limit of quantification of the assay), each correlation decreased somewhat, with gp41 IgG binding antibody response magnitude not correlating significantly with any of the other immune responses assessed yet all other correlations remaining significant (Figure S2).

Identification of baseline predictors of High responder status using machine learning supervised analysis

Using SuperLearner, all 25 baseline characteristics were assessed as predictors of High responder status. The median heterogeneity index I^2 across all 25 baseline characteristics was generally low with 0.17 for gp120 IgG, 0 for both gp140 and gp41 with the maximum I^2 being less than 0.7, suggesting supportive evidence for proceeding with the meta-analysis. Based on the collection of all 25 covariates, moderately successful prediction of whether a participant was a gp120 IgG High responder was achieved, with a CV-AUC (95% CI) of 0.72 (0.68, 0.76). Results were similar for gp140 IgG, with a CV-AUC of 0.73 (0.69, 0.76); prediction of gp41 IgG High responder status was slightly less successful,

a

Assay	Antigen	Response category	Count	%
ICS (CD4+)	ANY ENV	High	112	13.9%
		Non-High	696	86.1%
BAMA (IgG)	Con6 gp120/B	High	226	30.1%
		Non-High	526	70.0%
	ConS gp140 CFI	High	390	50.5%
		Non-High	382	49.5%
	gp41	High	227	36.2%
		Non-High	401	63.9%
	gp140 + gp120	High	207	27.6%
		Non-High	544	72.4%
	gp140 or gp120	High	407	54.2%
		Non-High	344	45.8%

b

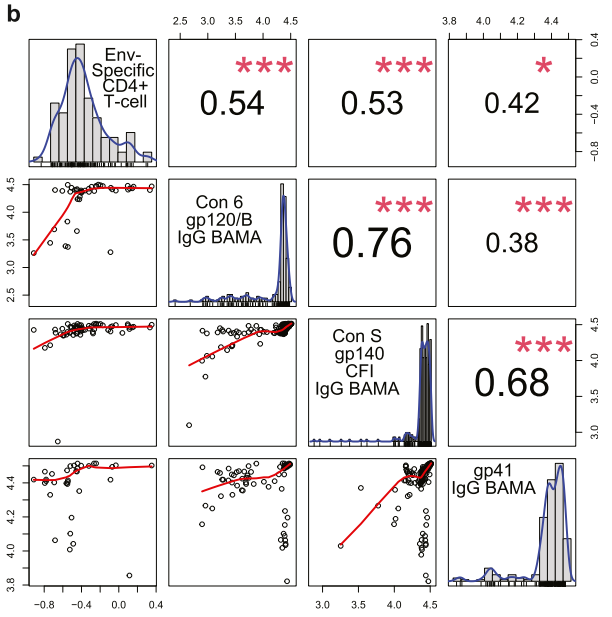

Figure 3. a) Total High/Non-High responder counts for the different immune responses assessed; b) Spearman correlation matrix of log-transformed immune responses among High responders (n = 831). BAMA IgG responses above the upper limit of quantification of the assay (22,000) were not truncated at 22,000. The correlation coefficient and the significance level (based on the t-distribution) are shown in the upper diagonal: ***: p-value < 0.001; **: 0.001 ≤ p-value < 0.01; *: 0.01 ≤ p-value < 0.05. In (a), participants who have missing values for any of the covariates used in the superlearner analysis were excluded.

with a CV-AUC (95% CI) of 0.67 (0.63, 0.72). In contrast, even when using all 25 baseline characteristics, prediction of High Env-specific CD4+ T-cell responder status was essentially no better than that achieved by random chance, with CV-AUC (95% CI) of 0.53 (0.48, 0.58). This finding suggests that a low-to-moderate level of information is contained in the baseline characteristics in predicting High responder status of each immune response variable, with the exception of Env-specific CD4+ T-cell response.

For the antibody outcomes, we next drilled down using the three approaches detailed in Methods to investigate which individual baseline characteristics contributed the most information towards predicting High responder status. We first present the results for each approach separately, and then describe the similarities and differences across the three approaches. Using Approach 1, the absolute risk difference estimates obtained for each baseline characteristic are shown in Figure 4a, risk difference estimates are given in Table S5 and Table S6. In general, more baseline characteristics were significant predictors (adjusted p-value < 0.05, TMLE) of High gp140 IgG responder status and of High gp41 IgG responder status (7 and 5 predictors, respectively) than of High gp120 IgG responder status (2 predictors). The most significant predictor of any immune response using Approach 1 was total white blood cell count, where the estimated risk difference was 0.36 (95% CI: 0.31, 0.41; adjusted p-value < 0.001, TMLE) for gp41, with higher total white blood cell count corresponding to greater probability of being a High responder. This result can be interpreted as an increase of 0.36 in the probability of being a High gp41 IgG responder when moving from a lower quantile level to a higher quantile level of the six-quantized baseline total white blood cell count that resulted in the largest probability difference of being a High gp41 IgG responder. Total white blood cell count was also a significant predictor of High gp140 IgG responder status, with an estimated risk difference of 0.14 (0.08, 0.19; adjusted p-value < 0.001, TMLE). Other notable predictors of High gp41 IgG responder status were haemoglobin and creatinine clearance, with estimated risk differences of −0.22 (−0.32, −0.13; adjusted p-value < 0.001, TMLE) and −0.22 (−0.34, −0.10; adjusted p-value < 0.001, TMLE), respectively. For gp120 IgG, the most significant predictor of High responder status was height, with an estimated risk difference of −0.20 (−0.30, −0.10; adjusted p-value < 0.001, TMLE); for gp140 IgG, the most significant predictor of High responder status was systolic blood pressure, with an estimated risk difference of 0.21 (0.12, 0.29; adjusted p-value < 0.001, TMLE). Based on the inability of the cumulative baseline characteristics to achieve any measurable improvement in prediction of High CD4+ T-cell responder status above random chance, we do not report the contributions of individual baseline characteristics to CD4+ T-cell prediction in this or any of the subsequent analyses.

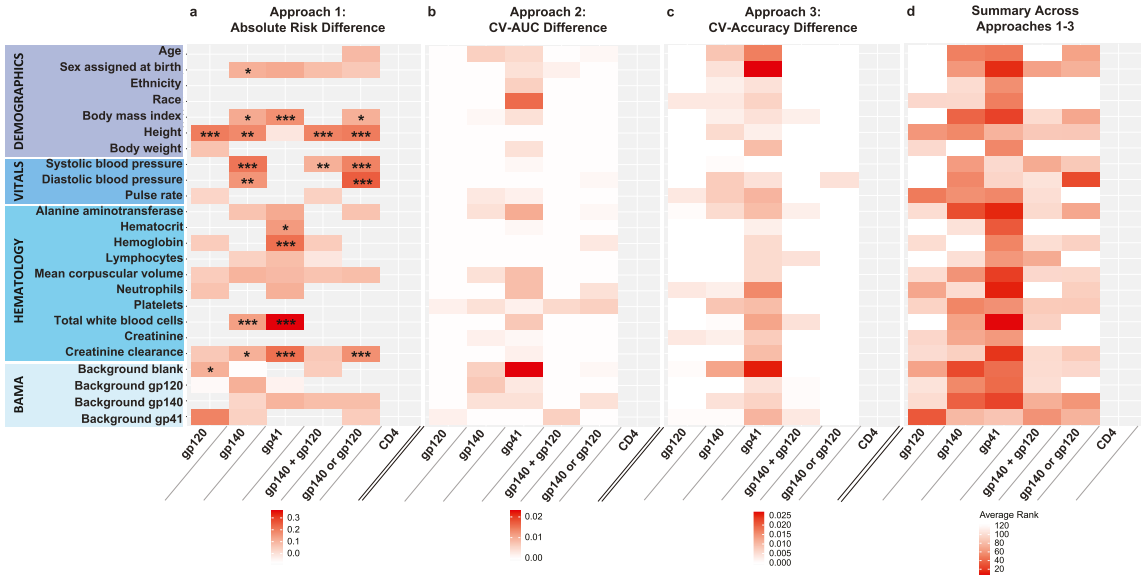

Figure 4. Study-pooled prediction of High responder status by baseline variable. a-c) Heatmaps of estimated a) absolute risk difference (Approach 1), b) cross-validated (CV)-AUC difference (Approach 2), and c) CV-accuracy difference (Approach 3) for each baseline variable, where darker red cell color indicates greater variable importance. ***: adjusted *p*-value <0.001; **: adjusted *p*-value < 0.01; *: adjusted *p*-value < 0.05 with Wald-test *p*-values calculated based on TMLE standard errors and adjusted by the false discovery rate method. In B and C, the difference estimates represent the difference achieved by additionally including each individual baseline characteristic, as compared to including the full set of baseline characteristics minus the baseline characteristic under consideration, in the SuperLearner estimation procedure. Based on the inability of the cumulative baseline characteristics to achieve any measurable improvement in prediction of High CD4+ T-cell responder status above random chance, the individual characteristic results are not shown for CD4+ T cells. d) Summary variable importance measurements across Approaches 1, 2, and 3 of baseline variable prediction of High responder status for each of the immune response outcomes. For each Approach, the baseline predictors were ordered in terms of most to least predictive pooling over all outcomes in rank 1-125, with most predictive given the smallest rank number. Each cell in this heatmap is color-coded according to the average of each baseline predictor's rank across each of the Approaches, i.e. Cell color determined by: [rank by Approach 1 for predicting High responder status of immune response X + rank by Approach 2 for predicting High responder status of immune response X + rank by Approach 3 for predicting High responder status of immune response X]/3. Darker red cell color indicates a higher rank across the three approaches. gp120 IgG: *n* = 752; gp140 IgG: *n* = 772; gp41 IgG: *n* = 628; CD4: *n* = 808; gp140 IgG + gp120 IgG: *n* = 751; gp140 IgG or gp120 IgG: *n* = 751.

Using Approach 2, the improvements in CV-AUC achieved by additionally including each individual baseline characteristic, as compared to including the full set of baseline characteristics minus the baseline characteristic under consideration, in the SuperLearner estimation procedure are shown in Figure 4b, Table S7, and Table S8. Similar to the Approach 1 results, individual baseline characteristics had the best ability to predict High gp41 IgG responder status. The most predictive baseline characteristic was background blank, with an estimated CV-AUC difference of 0.02 (95% CI: 0.01, 0.04). As background blank is the extent of background binding of the participant's plasma IgG to uncoated assay beads it may represent the presence of low-affinity poly-reactive IgG. No individual baseline variable appeared to have utility in predicting High gp120 responder status, and performance was also poor for prediction of High gp140 responder status (top baseline variable: background gp120, with an estimated CV-AUC difference of 0.01 (0.00, 0.02).

Using Approach 3, the improvements in CV-accuracy achieved by additionally including each individual baseline characteristic, as compared to including the full set of baseline characteristics minus the baseline characteristic under consideration, in the SuperLearner estimation procedure are shown in Figure 4c, Table S9, and Table S10. The results largely mirrored those of Approach 2 in that individual baseline characteristics had the best ability to predict High gp41 IgG responder status, with few or no baseline variables appearing to have utility in predicting High gp120 IgG responder status. Consistent with the Approach 2 results, background blank was a top predictor of High gp41 IgG responder status (estimated CV-accuracy difference 0.03; 95% CI: 0.01, 0.05); in Approach 3, background blank was additionally the top predictor of High gp140 IgG responder status (estimated CV-accuracy difference 0.01; 95% CI: 0.00, 0.03). Female sex assigned at birth was also a top predictor of High gp41 IgG responder status (estimated CV-accuracy difference 0.03; 95% CI: 0.01, 0.05) in Approach 3.

a

	gp120 IgG			gp140 IgG			gp41 IgG			gp140 IgG and gp120 IgG			gp140 IgG or gp120 IgG		
	RD (95% CI)	P val.	Adj P val.	RD (95% CI)	P val.	Adj P val.	RD (95% CI)	P val.	Adj P val.	RD (95% CI)	P val.	Adj P val.	RD (95% CI)	P val.	Adj P val.
Sex (F vs. M)				0.11 (0.02, 0.21)	1.05E-02	2.88E-02	0.12 (0.01, 0.24)	2.04E-02	5.11E-02	0.08 (0.01, 0.15)	1.81E-02	8.61E-02	0.06 (-0.03, 0.16)	9.89E-02	2.07E-01
Height	-0.20 (-0.30, -0.10)	5.60E-05	2.94E-04	-0.18 (-0.28, -0.08)	1.95E-04	1.07E-03	-0.01 (-0.11, 0.09)	6.06E-01	8.07E-01	-0.20 (-0.30, -0.10)	7.80E-05	7.41E-04	-0.20 (-0.28, -0.12)	4.00E-07	4.50E-06
Hemoglobin	-0.05 (-0.14, 0.04)	1.30E-01	2.73E-01				-0.22 (-0.32, -0.13)	2.70E-06	1.82E-05	-0.05 (-0.13, 0.04)	1.40E-01	3.02E-01			
MCV	-0.05 (-0.14, 0.04)	1.23E-01	2.73E-01	-0.10 (-0.19, -0.00)	2.17E-02	5.30E-02	-0.09 (-0.22, 0.04)	9.00E-02	1.50E-01	-0.07 (-0.16, 0.02)	5.25E-02	2.00E-01	-0.08 (0.17, 0.02)	5.60E-02	1.43E-01
Total WBC				0.14 (0.08, 0.19)	2.00E-07	2.70E-06	0.36 (0.31, 0.41)	0.00E+00	0.00E+00						
Background blank	0.11 (0.02, 0.20)	7.11E-03	2.98E-02	0.07 (-0.04, 0.18)	8.94E-01	1.00E+00				0.05 (-0.04, 0.15)	1.43E-01	3.02E-01			
Background gp140				0.03 (-0.12, 0.18)	3.38E-01	5.31E-01	0.11 (-0.04, 0.25)	7.46E-02	1.43E-01	0.08 (-0.06, 0.22)	1.31E-01	3.02E-01	0.08 (-0.07, 0.24)	1.37E-01	2.63E-01
Background gp41	0.19 (0.11, 0.26)	3.00E-07	2.10E-06	0.04 (-0.08, 0.15)	2.59E-01	4.38E-01							0.05 (-0.05, 0.15)	1.77E-01	3.14E-01

b

gp140 High responder status	Assigned Female Sex at Birth (column %)	Assigned Male Sex at Birth (column %)
High	194 (55.7%)	196 (46.2%)
Non-High	154 (44.3%)	228 (53.8%)

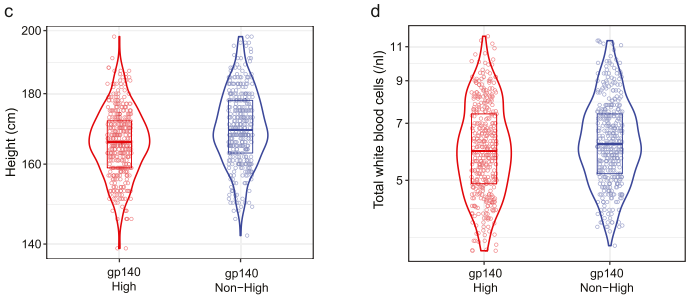

Figure 5. Baseline variables with consistent directionality of effect on High responder status across multiple antibody outcomes. a) Risk difference, P value (Wald-test based on TMLE standard errors), and false-discovery-rate-adjusted P values for the eight baseline variables with consistent effect directionality across at least two immune response outcomes. Blank cells indicate inconsistency of effect directionality across the 10 folds of cross-validation datasets for a given antibody outcome. b) Cross-classification of gp140 High responder status (High, Non-High) x (Assigned female sex at birth, Assigned male sex at birth). c, d) Violin plots of study-pooled distributions of c) height (cm) and d) total white blood cell count (/nl) displayed according to gp140 IgG binding antibody High or Non-High responder status. The top and bottom of each boxplot within the violins indicate the interquartile range; the horizontal line is the median. gp120 IgG: n = 752; gp140 IgG: n = 772; gp41 IgG: n = 628; CD4: n = 808; gp140 IgG + gp120 IgG: n = 751; gp140 IgG or gp120 IgG: n = 751. MCV, mean corpuscular volume. WBC, white blood cell.

Comparing across approaches, visual inspection of the heatmaps in Figure 4a-4c (Approaches 1, 2, and 3, respectively) revealed that more individual baseline features were identified as important for predicting High gp41 IgG response (vs. the other antibody responses), with several haematological features having at least modest variable importance across the three approaches. As an alternative to visual inspection, we ranked the prediction performance measurements of all 125 combinations across the 25 baseline characteristics and five antibody outcomes and computed the sum of the average rank across the three approaches ("rank-based" method). The results are shown in Figure 4d. Both total white blood cell count and sex assigned at birth were ranked highly across the three approaches for predicting High gp41 responder status.

We also repeated the same analyses using an alternative definition of High Responder status, detailed in Methods. Figure S3 shows an analogous version of Figure 4, except based on predicting "High Responder – 20%tile" vs. "Low Responder – 20%tile" status. Results were generally similar to those obtained above and are further discussed in the Supplementary Text.

We next sought baseline variables with consistent effect directionality across multiple antibody outcomes. Figure 5a shows the risk differences (from Approach 1), p-values, and adjusted p-values for the eight baseline variables (sex, height, haemoglobin, mean corpuscular volume, total white blood cell count, background blank, background gp140, and background gp41) that had consistent directionality of effect within all the cross-validation folds of a given antibody outcome, as well as across at least two of the antibody outcomes. Of these, three (sex, height, and total white blood cell count) also had a significant association with at least two of the outcomes (unadjusted p-$value < 0.05$). Specifically, female sex, shorter height, and higher total white blood cell count were each associated with High responder status across multiple antibody outcomes (female sex: gp140 IgG, gp41 IgG, gp140 IgG + gp120 IgG; shorter height: gp120 IgG, gp140 IgG, gp140 IgG + gp120 IgG, gp140 or gp120 IgG; higher total white blood cell count: gp140 IgG, gp41 IgG).

For female sex predicting High responder status, only the gp140 IgG outcome had a significant association after multiplicity adjustment (adjusted p-value= 0.029, TMLE). For total white blood cell count, the gp140 IgG and gp41 IgG outcomes had a significant association after multiplicity adjustment (adjusted p-value < 0.001, TMLE). For shorter height predicting High responder status, significant associations were seen for the gp120, gp140, 'gp140 and gp120', and 'gp140 or gp120' outcomes after multiplicity adjustment (all adjusted p-values < 0.01, TMLE). Figure 5b shows a cross-tabulation of gp140 High responder status by sex assigned at birth, where a greater percentage of High responders (55.7%) was female compared to male (46.2%). Figure 5c and 5d show the study-pooled

distributions of observed height and total white blood cell count, respectively, according to gp140 IgG binding antibody High or Non-High responder status, without adjusting for other covariates.

Discussion

Access to individual-level data in the HIV Vaccine Trials Network affords the opportunity to investigate host determinants of vaccine-induced immune responses in this meta-analysis of 26 vaccine regimens. In order to gain a comprehensive understanding of the predictive value of the baseline characteristics, three different approaches were applied to identify and rank 25 potential predictors. These approaches identified differing lists of significant predictors, with a relatively small overlap. Such a variation in patterns of estimated variable importance across the three approaches was evident and can be explained by several factors. The first is that the target of inference – in other words, the underlying statistical parameter of interest – is different across the three approaches. Approach 1 considers an analogue of the average treatment effect comparing high versus low levels of a variable, while Approaches 2 and 3 consider the difference in population prediction potential when including vs excluding a variable. Though Approaches 2 and 3 are conceptually similar, AUC and accuracy provide different summaries of prediction performance. These differences in the target of inference are similar to those encountered when using linear versus logistic regression for binary outcomes, where an effect may be deemed statistically significant on the scale of the risk difference but not the odds ratio (or vice versa). Thus, in this context the three approaches provide complementary information about the importance of the baseline predictors. A second factor that might explain the differing variable importance patterns is that the estimated prediction performance of all variables is modest. This constrains the impact that any one variable can have over the others.

Specifically, we found that body height was inversely associated with the High responder status of both gp140 and gp41 responses. As the gp140 subunit of Env includes much of the gp41 subunit, commonalities in their associations are not unexpected. There are limited studies describing an association of body height and vaccine response. Krams et al. reported a non-linear relationship in the magnitude of Hepatitis B vaccine-induced antibody responses in young men, with a positive relationship up to a height of 185 cm, but an inverse association in those men taller than 185 cm.[76] Based on their data, body weight could be inversely associated with a dichotomized High responder status of the antibody responses, consistent with our finding for HIV vaccine-induced immune responses. In addition, in young children, an inverse association of body height for age z-scores and immune parameters including B cells has been reported.[77] Pawlowski et al. reported there was no association between body height and influenza vaccine response in a study of 96 males and 97 female young adults; however, the study was likely underpowered to observe this relationship given its small effect.[78] Note that our analysis does not aim to identify causal relationships between baseline characteristics and High responder status; rather, our results indicate body height should be further studied as it may reflect genetic or environmental influences that impact immune response.

It is important to consider that these HIV vaccine trial participants were generally healthy individuals that met pre-defined inclusion and exclusion criteria to participate in the trials, including baseline measures such as total white blood cells, haemoglobin, creatine clearance, and blood pressure that were within normal ranges. Haemoglobin emerged from Approach 1 with an inverse association with gp41 IgG and gp120 IgG High response statuses, although the association was only statistically significant for gp41 response. While clinically defined low haemoglobin is indicative of anaemia and iron deficiency, which has been associated with lower vaccine responses,[79–81] it is not yet known whether haemoglobin levels in lower-normal ranges have implications on vaccine responses. The high and consistent ranking of white blood cell (WBC) across the three approaches ("rank-based" method) as important for predicting High gp41 IgG response and High gp140 response is notable. We are not aware of baseline WBC count in healthy individuals being previously reported to correlate with vaccine-induced antibody response. In this study increased WBC count, although still within normal range, may be an indicator of generally healthy individuals that have a minor subclinical infection and/or modestly increased systemic immune activation. Increased immune activation such as in patients with autoimmune diseases that are not receiving substantially immunosuppressive medications has been associated with increased IgG responses to other vaccines.[82–84] The association observed in this study may be a subtler manifestation of this. Female sex assigned at birth was also highly ranked across the three approaches as an important predictor for High responder status of the antibody outcomes, except for gp120 response. Consistent with our findings, increased antibody responses to various vaccines in females have been previously reported.[85–88]

Increased HIV Env specific plasma IgG in HIV negative individuals has been previously reported, particularly in some individuals with frequent HIV exposure.[89–92] Though not statistically significant based on Approach 1, similar trends are seen across the three approaches. One potential explanation is that elevated baseline HIV-specific plasma IgG may also be the consequence of antibodies that are developed in response to microbiome exposure and cross-react with

HIV Env and can further expand following HIV vaccination or HIV infection, as has been described for gp41.[93,94] Further, gp41 reactivity is well described among pre-existing poly-reactive low-affinity antibodies in HIV negative individuals[93,95] and is consistent with increased background blank IgG being predictive of gp41 IgG response in Approach 2 and 3. It can be speculated that increased baseline gp140 plasma IgG is a surrogate for increased frequency of memory B cells that may be able to respond to the HIV vaccination, contributing to the pool of antibody producing cells that develop following the vaccination. Given the cross-reactive nature of some gp41 antibodies and the finding that more individual baseline features were identified for prediction of High gp41 response compared to gp120 and gp140 High response, more in-depth assessment of the gp41 antibodies is warranted.

As no baseline characteristic was found to strongly associate with High responder status across all the approaches used, baseline characteristics may have relatively weak universal (i.e. across many platforms) predictive power for HIV-1 vaccines. A previous analysis of 10 HVTN trials of candidate HIV-1 plasmid DNA vaccines reported that female sex and low BMI were each associated with a higher HIV-specific CD4+ T-cell response rate,[43] and a study of the phase 2b Phambili (HVTN 503) HIV-1 vaccine efficacy trial in South Africa of the Merck (MRK)-Adenovirus type-5 (Ad5) HIV-1 clade B trivalent HIV vaccine reported that female sex significantly predicted a positive clade C-specific IFN-γ T-cell response, whereas having an overweight/obese BMI or being a heavy drinker significantly predicted clade C-specific IFN-γ T-cell nonresponse.[42] Given that the entire collection of baseline characteristics provided little to no improvement over random chance for predicting High Env-specific CD4+ T-cell response in the current study, we were unable to investigate the relative importance of each characteristic to such prediction; it is possible that the previously identified findings are vaccine regimen-specific.

It would be of high scientific interest to determine whether the findings described here also hold for other non-HIV vaccines, for example, the COVID-19 vaccines that have been found efficacious against symptomatic SARS-CoV-2 infection. Immune marker data that could be used to answer this question are still being generated in the COVID-19 vaccine trials using validated and consistent assays. In the meantime, our group is planning such an investigation for the COVID-19 vaccines, with the analyses described in the current manuscript serving as an important basis and reference. A further interesting scientific question is whether there exists a "universal" baseline signature (or variables) that are associated with high vaccine response. However, to answer this question, data from many more vaccines would need to be included in the analysis. (As an example, Fourati et al. integrated data from 28 studies of 13 different vaccines to investigate the generalizability of whether a pre-vaccination immune state is associated with vaccine-induced antibody response[96]).

A limitation of our study is that due to the lack of availability on individual-level data from other sources and the availability of relatively large amount of data on a diverse set of HIV vaccine regimens from the HVTN, we restricted our scope to the trials conducted within the HVTN, i.e. we did not perform a systematic review. Thus, it is possible that some HIV-1 vaccine trials were excluded from our analysis. Another limitation of our study is that discrepant vaccine regimens were included; however, this diversity may also be considered a strength as it allows identification of universal baseline predictors. Other strengths of our study include the large number of HIV-1 vaccine regimens (26) and trials (nine); the use of validated and consistent immune assays (performed at HVTN centralized laboratories), thus minimizing cross-laboratory variation; and the application of three different approaches, one based on a causal inference framework and two focused on identification of association, to obtain variable importance estimates for each baseline predictor. The results of our study suggest that baseline features including those obtained from routine metabolic and haematological measures that are frequently monitored in clinical trials and as part of standard of care may be informative in evaluating vaccine response disparity. They also suggest that concerns about "population heterogeneity" are misguided and that trials should seek to enroll as diverse a population as possible. These identified features may be considered in different types of analyses of immune responses to improve statistical power or efficiency, including 1) as confounding factors to adjust for in the comparisons of immune responses between vaccine regimens or study populations, and 2) as baseline immune predictors in the analysis of immune responses as correlates of risk of HIV-1 acquisition or correlates of protection (e.g.,[97]). Findings of our study warrant additional evaluation of these identified features for prediction of response to additional vaccines either in the setting of clinical trials or retrospective analysis of standard of care vaccine responses. Further resolution of vaccine response disparity, including those individuals that have exceptional antibody and T cell responses, is likely to identify mechanisms that can be targeted by novel adjuvants or interventions to improve vaccine responses for all.

Contributors

Literature search: Y.H., L.N.C., J.T., J.K. Study concept and design: Y.H., L.R.M., P.M., E.V., J.T. and J.K. Data generation and collection: K.E.S., S.D.R., J.H., K.W.C., M.J.M., G.D.T. Data management and analysis: Y.H., Y. Z., A.K.R., B.D.W. Investigation: G.E.G., G.C., S.P.B., S. E., L.-G.B., M.C.K., M.C.H., P.A.G. Data interpretation: Y.H., Y.Z., L.N.C., B.D.W., J.T., J.K. Verification of the

underlying data: Y.H., Y.Z. Writing – original draft: Y. H., L.N.C., J.K. Writing – review & editing: all authors. All authors read and approved the final version of the manuscript.

Data sharing statement

Upon acceptance, the data underlying the findings of this manuscript will be made publicly available at the public-facing HVTN website (https://atlas.scharp.org/).

Declaration of interests

Y.H. declares contract payments to her institution from the World Health Organization to conduct statistical analysis work (outside the scope of the current work; related to COVID-19 vaccines) and plan to submit the results for publication, within the past 36 months, as well as service to WHV as an SMB (payment directly to her) within the past 36 months. K.E.S. and J.H. declare coverage of travel expenses for attendance at the HVTN Full Group Meeting in 2022 (reimbursement by the HVTN); K.E.S. additionally declares payment of a registration fee for virtual seminar attendance directly to Keystone Symposium by the HVTN. S.D.R. declares contracts awarded to his institution from Battelle and from Janssen, and grants awarded to his institution from the Gates Medical Research Institute and from the Paul G. Allen Family Foundation, within the past 36 months. P.A.G. declares consulting fees received from Johnson and Johnson within the past 36 months, as well as a patent for a COVID-19 monoclonal antibody that is not yet clinically available. K.W.C. declares funding from the Bill and Melinda Gates Foundation in the form of payments to her institution within the last 36 months. B.D.W. declares grant payments made to his institution within the past 36 months from the following entities: Centers for Disease Control and Prevention, Patient-Centered Outcomes Research Institute, National Institute of Mental Health, National Institute of Allergy and Infectious Diseases, National Cancer Institute, and the National Institute of General Medical Studies; an honorarium for delivering an invited webinar to the American Statistical Association: Statistical Learning and Data Science Section; travel support through a grant from the National Institute of Mental Health; and retirement accounts invested in mutual funds operated by Vanguard and by TIAA (he does not choose individual stocks). L.-G.B. declares payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Gilead Sciences, Janssen, and Merck PTY LTD within the past 36 months, as well as participation on a Data Safety Monitoring Board or Advisory Board for the PrEPVAC study within the past 36 months. G.D.T. declares consulting fees received from Axon and from Janssen (not related to this work), serving as an Advisory Board member for the NIH VRC, UNC CFAR, and Johns Hopkins (not related to this work), and serving as a scientific review member for Gilead (not related to this work), all within the past 36 months, as well as travel as part of research funding (more than 3 years prior, with funding paid to her institution). J.T. declares consulting fees from Vaccitech within the past 36 months.

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Supplementary materials

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