JVI Accepts, published online ahead of print on 14 May 2014
J. Virol. doi:10.1128/JVI.01164-14

Analysis of HLA A*02 association with vaccine efficacy in the RV144 HIV-1 vaccine trial

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Running title: Analysis of HLA A*02 and vaccine efficacy in RV144

Abstract

The RV144 HIV-1 vaccine trial demonstrated partial efficacy of 31% against HIV-1 infection. Studies into possible correlates of protection found that antibodies specific to the V1/V2 region of *envelope* correlated inversely with infection risk and that viruses isolated from trial participants contained genetic signatures of vaccine-induced pressure in the V1/V2 region. We explored the hypothesis that the genetic signatures in V1/V2 could be partly attributed to selection by vaccine primed T cells. We performed a T-cell based sieve analysis of breakthrough viruses in the RV144 trial and found evidence of predicted HLA binding escape that was greater in vaccine versus placebo recipients. The predicted escape depended on class I HLA A*02 and A*11 restricted epitopes in the MN-strain rgp120 vaccine immunogen. Though we hypothesized that this was indicative of post-acquisition selection pressure, we also found that vaccine efficacy (VE) was greater in $A*02^+$ compared to $A*02^-$ participants (VE=54% vs. 3%, p=0.05). Vaccine efficacy against viruses with a lysine residue at site 169, important to antibody binding and implicated in vaccine-induced immune pressure, was also greater in $A*02^+$ participants (VE=74% vs. 15%, p=0.02). Additionally, a reanalysis of vaccine-induced immune responses focused on those that were shown to correlate with infection risk, suggested that the humoral response may have differed in $A*02^+$ participants. These exploratory and hypothesis-generating analyses indicate there may be an association between a class I HLA allele and vaccine efficacy, highlighting the importance of considering HLA alleles and host immune genetics in HIV vaccine trials.

Impact

The RV144 trial was the first to show efficacy against HIV-1 infection. Subsequently, much effort has been directed towards understanding the mechanisms of protection, including this T-cell based sieve analysis which compared the genetic sequences of viruses isolated from infected vaccine and placebo recipients. Though we hypothesized that the observed sieve effect indicated post-acquisition T-cell selection, we also found that vaccine efficacy was greater for participants who expressed HLA A*02, an allele implicated in the sieve analysis. Though HLA alleles have been associated with disease progression and viral load in HIV-1 infection, these data are the first to suggest the association of a class I HLA allele and vaccine efficacy. While these statistical analyses do not provide mechanistic evidence of protection in RV144, they generate testable hypotheses for the HIV vaccine community and they highlight the importance of assessing the impact of host immune genetics in vaccine-induced immunity and protection.

Introduction

Vaccines are the most cost effective form of public health intervention and have greatly reduced the global burden of infectious disease (1). While many licensed vaccines are highly effective and have led to dramatic reductions in disease, such as those for smallpox, measles and polio, others, like the seasonal influenza vaccine and the BCG tuberculosis vaccine offer only partial or heterogeneous protection (2, 3). The mechanisms underlying heterogeneous efficacy are often challenging to identify, though they may be partially due to variability in the host immune response to vaccination (4), which can vary with individual characteristics, such as age, gender, and major histocompatibility complex (MHC) group (5–9). Trials of HIV-1 vaccines have elicited heterogeneous immune responses including the Step trial, in which prior immunity to adenovirus decreased innate and HIV-specific cellular immune responses to the adenovirus vectored vaccine (10–12) and the VaxGen Vax004 trial in which race was associated with vaccine-induced neutralizing antibody responses (13). Most recently the RV144 trial of a canarypox vector prime (ALVAC-HIV) and bivalent rgp120 boost (AIDSVAX B/E) vaccine regimen was the first trial to show partial efficacy in reducing the risk of HIV-1 infections (14) and several studies have followed these results attempting to understand the mechanisms of partial and potentially heterogeneous protection (15–25). Specifically, Haynes *et al*. (15) designed a study intended to identify the immune correlates of risk (CoR) of infection, by comparing the rate of HIV-1 infection over time among vaccinated subgroups defined by their levels of vaccine-induced immune responses. The study identified two V2-specific immune response variables that significantly inversely correlated with risk of infection: (1) levels of IgG binding to a gp70-scaffolded V1/V2 antigen and (2) levels of IgG binding to a linear V2 peptide "hotspot". These findings together with more recent studies of V2-specific antibodies isolated from trial participants (18, 22, 23), suggest that vaccine-induced immune responses to the V1/V2 region of envelope may have had antiviral functions and predicted the efficacy observed in the trial.

Informed by these findings, which implied that the vaccine elicited immune pressure on the V1/V2 region, Rolland and Edlefsen *et al*. (17) conducted a V1/V2 focused sieve analysis of the envelope sequences of viruses that infected RV144 participants. The sieve analysis –a statistical comparison of the viruses isolated from HIV infected trial participants in the vaccine versus

placebo groups– showed genetic differences relative to the vaccine sequences at sites 169 and 181 in the V2 loop (HXB2 numbering). This was consistent with the hypothesis that vaccine-induced antibodies targeting the V1/V2 region selectively blocked specific genetic variants from infection. However, as with any sieve analysis, the immune mechanism applying pressure could not be discerned from the viral sequences alone, nor could it eliminate the possibility that the sieve effect was induced by post-acquisition pressure that was present prior to viral sequencing (26, 27). Given the growing evidence that CD8+ and CD4+ T-cell induced viral escape can happen in the acute phase of infection (28–32), additional, and not necessarily mutually exclusive hypotheses, could be that cytolytic T cells selectively targeted HIV-1 prior to the establishment of infection or that, after infection, they induced viral escape mutations in vaccine epitopes prior to viral sequencing. These hypotheses are analogous to those proposed in the analysis of the Step HIV vaccine trial (26), in which sieve effects were attributed mainly to selective pressure from T cells. Though the CD8+ T-cell response rate was low overall in the RV144 trial, the envelope-specific CD4+ T-cell response rate was 63% (14, 33, 34) and there is preliminary data suggesting that the RV144 vaccine regimen may have induced envelope-specific responses in CD8+ T cells of the mucosa (21).

Therefore, we conducted a CD4+ and CD8+ T-cell based sieve analysis focused on the V1/V2 region of the envelope protein (HXB2 143 – 185), hypothesizing that the sieve effects identified in this region could be partially attributed to selective pressure from cytotoxic T cells. Specifically, we tested for a sieve effect associated with “HLA binding escape”, as predicted using computational HLA-peptide binding algorithms (35–37). We defined binding escape as any variation in HIV peptide sequences from infected participants, relative to predicted vaccine epitopes, that substantially reduced the HLA binding affinity. Our analysis identified A*02 and A*11 restricted CD8+ T-cell epitopes that were found only in the subtype B (MN) component of the protein boost, in which the number of predicted viral HLA binding escapes was greater in vaccine compared to placebo recipients. Although we hypothesized that such an effect was indicative of post-acquisition selection pressure, we also found that VE was significantly increased in participants carrying the class I HLA allele A*02. While associations of HLA genotype with vaccine-induced immune responses have been previously noted (38–42), this is the first report of a potential association with vaccine efficacy. We also found evidence

suggesting that the HLA A*02 allele may have modulated two vaccine-induced antibody responses and their associations with infection risk in the trial. Taken together, the results of this exploratory statistical analysis generate the hypothesis that HLA A*02 may have played a role in the partial efficacy that was observed in RV144. Importantly, they suggest that additional studies of T-cell responses to the subtype B protein component of the RV144 vaccine should be conducted in both blood and mucosal samples.

Materials and Methods

Ethics Statement

The RV144 protocol was approved by the Institutional Review Boards of the Ministry of Public Health, the Royal Thai Army, Mahidol University, and the US Army Medical Research and Materiel Command (ClinicalTrials.gov number, NCT00223080) (14). Written informed consent was obtained from all participants.

Study design and vaccine sequences

Details about the cohort of volunteers enrolled in the RV144 HIV-1 vaccine trial and the subsequent case-control immune correlates study are presented by Rerks-Ngarm *et al*. (14) and Haynes *et al*. (15) in the respective primary manuscripts. Briefly, the vaccine regimen consisted of four injections (at 0, 1, 3 and 6 months) of the canarypox vector ALVAC-HIV [vCP1521], which expresses gp120 of CRF01_AE (92TH023), and two injections (at 3 and 6 months) with AIDSVAX B/E, which is composed of two gp120 proteins truncated at the amino terminus (start at amino-acid 42): MN (subtype B) and CM244 (CRF01_AE). Following the same approach to screening amino-acid sites taken by Rolland and Edlefsen *et al*. (17), we conducted a focused analysis on the alignable portions of the V1/V2 region from amino-acid residues 143 – 185 (HXB2 numbering).

HIV-1 sequencing

Viral genomes from infected RV144 participants were reported previously (17, 26). Briefly, from each participant we obtained 2-14 (median 10) near-full-length-genome sequences from plasma specimens collected at the time of HIV-1 diagnosis, typically between 1 and 6 months after infection. Intrasubject sequence diversity was low with an average of 1.08±0.02 unique

amino acid residues per person at each site within the V1/V2 region. At any given site at least 80% of participants had a single amino acid residue in all of their breakthrough sequences. Therefore, we limited our analysis to one representative sequence per individual, using the sequence with the smallest Hamming distance to the subject's consensus sequence. The dataset analyzed here contained sequences from 109 infected participants (all CRF01_AE strains), after excluding participants who were infected with non-CRF01 AE viruses and one participant who was determined to be infected by another RV144 trial participant.

T-cell based sieve analysis

A sieve effect can be understood as a statistical difference between the genetic sequences of viruses that infected vaccine compared to placebo recipients in a treatment-randomized, double-blind placebo-controlled vaccine trial (44). Here we describe a "Binding Escape Count" sieve method, a T-cell based sieve analysis method that estimates the number of predicted HLA binding escapes in the amino acid sequences of viruses that infected trial participants. Escapes were identified by comparing the vaccine immunogen sequences to the sequences of viruses isolated from trial participants. For this comparison we used all three vaccine immunogen sequences independently: 92TH023 in ALVAC-HIV (vCP1521) and (CM244, MN) in the bivalent AIDSVAX B/E rgp120 boost, where 92TH023 and CM244 are CRF01_AE and MN is subtype B. Analysis was restricted to the V1/V2 region of envelope protein (HXB2 positions 143-185).

To count binding escapes in the viral sequence from a single participant, we first predicted likely vaccine-induced T-cell epitopes using a computational predictor of either class I HLA binding to 9-mer peptides (ADT, adaptive double threading) (35, 45) or class II HLA binding to 15-mer peptides (NetMHCIIpan) (37). Using the vaccine sequence and the participant's HLA-A, B and DRB alleles, we identified as putative epitopes any peptide that was predicted to bind to one of their HLA alleles with affinity (i.e., half-maximal inhibitory concentration, IC_{50}) less than a specific "binder" threshold. We then considered the sequence of the corresponding peptide in the aligned sequence of the virus isolated from the participant. If changes in the amino acids within an epitope weakened the binding affinity beyond the "escape" threshold, then it was counted as

an escape. The number of binding escapes was summed for each participant and the resulting sums were compared between the vaccine and placebo groups using a t-statistic. Within some predicted epitopes a viral isolate may differ from the vaccine immunogen at several residues while another isolate may only differ at a single residue, however the determination of which isolate is counted as an escape is based on the change in the predicted HLA binding affinity which considers all residues within the epitope. For this reason the use of the term "escape" may differ from its typical usage to refer to single point mutations that result in abrogation of a T cell epitope.

The Binding Escape Count T-cell based sieve method has two adjustable parameters: (1) a binder threshold and (2) an escape threshold. Typically, peptide-HLA interactions are classified according to two binding thresholds as either "strong" (<50 nM) or "weak" (50-500 nM) binders (36). However, since binding affinity is a dynamic process and is only one of several factors that determine the set of T-cell responses in a given individual, we considered binding thresholds that spanned a biologically relevant range (50 - 1100 nM). Each binding threshold determines the set of epitopes that are used for comparing the vaccine sequence to the participants' viral sequences, with lower IC_{50} values corresponding to stronger binding affinities and fewer predicted epitopes. Similarly, we considered a range of thresholds in defining HLA binding escape (IC_{50} = 500 - 8000 nM). Each escape threshold defines non-binding peptides. We tested for a sieve effect over the entire range of parameters and used an absolute maximum t-statistic test to calculate an overall p-value. Conceptually, rather than testing if there was a sieve effect using *each* pair of binder and escape thresholds, the test asks if there is *any* pair of thresholds that yield a significant sieve effect. To calculate the p-value for this test we permuted participant treatment labels and with each permuted data set we computed the absolute maximum t-statistic over the range of all pairs of binding and escape thresholds. By repeating this procedure 20,000 times, we obtained a distribution of t-statistics under the null hypothesis that the vaccine had no effect. The overall p-value was defined as the fraction of permuted datasets in which the absolute maximum t-statistic was greater (corresponding to a stronger sieve effect) than the one observed in the actual (unpermuted) data set. The results of this sieve method, including predicted epitopes and binding escapes, are presented using the optimal binder and escape thresholds that led to the maximum t-

222 statistic, but the reported p-value is always the overall p-value that accounts for multiple testing
223 over the spectrum of thresholds.
224
225 The "site-specific" sieve methods employed in Rolland and Edlefsen *et al*. (17) differ from the
226 Binding Escape Count method. The site-specific sieve methods analyze the distribution of
227 residues at individual amino acid sites one at a time, and count all mutations relative to the
228 vaccine residues. In contrast, the Binding Escape Count T-cell based sieve method tests whether
229 the observed genetic differences can be attributed to vaccine-induced T-cell immune responses
230 by assessing short peptides. This method is sensitive only to mutations in predicted T-cell
231 epitopes that alter HLA binding affinity. Moreover, each vaccine epitope is only considered
232 within the subset of participants who carry the restricting HLA allele. Due to these differences it
233 is possible that a T-cell driven sieve effect that is missed by a site-specific method could be
234 identified by the Binding Escape Count method. It is also possible that sieve effects mediated by
235 other immune responses could be detected by a site-specific method and yet not found to be
236 attributable to T-cell selection by this method. Importantly, a sieve effect may be the result of
237 multiple mechanisms of selection, and neither this method nor the site-specific sieve analysis
238 method can rule out any single mechanism of immune pressure.
239
240 *HLA genotyping of volunteers*
241 High-resolution typing of class I HLA-A, -B, and –C loci was performed on samples obtained
242 from all the infected cases in the trial and the uninfected control participants selected for the
243 case-control immune correlates study (15). Typing was performed using both DNA sequence-
244 based typing (SBT) and the sequence-specific oligonucleotide probe (SSOP) method, with fully
245 concordant results. SBT was carried out by PCR amplification and sequencing of exons 2 and 3,
246 with ambiguous types being resolved to four digits (46) using the dbMHC SBT interpretation
247 interface (http://www.ncbi.nlm.nih.gov/projects/gv/mhc/). SSOP was conducted using the
248 LABType SSO Class I HD system (One Lambda, Canoga Park, CA), which is based on Luminex
249 xMAP technology, and results were interpreted using the accompanying HLA Fusion 2.0.0
250 software. HLA types are reported according to the IMGT/HLA nomenclature (version 3.7.0,
251 http://www.ebi.ac.uk/imgt/hla/ambig.html). The HLA haplotype analysis was completed using
252 the HLA I and HLA II genotypes of uninfected RV144 volunteers and the PyPop software

253 package (47). The T-cell based sieve analysis uses the four-digit HLA types; however,
254 subsequent associations with VE and immune measurements were studied using two-digit HLA
255 types to increase statistical power.
256
257 *Vaccine efficacy and immune correlates of risk of HIV infection*
258 Overall vaccine efficacy and viral genotype-specific vaccine efficacy were estimated in HLA
259 A*02$^{+/-}$ subgroups using the "case-only" method (48), which uses data only from the infected
260 subjects. The case-only method is valid only if the trial is randomized and has a rare study
261 outcome, and the RV144 trial meets both criteria. The method is optimally powerful (adding
262 host-genetic information from uninfected vaccine recipients would not increase statistical power)
263 and cost-effective because only data from infected cases are needed. Immune correlates of risk
264 were assessed in HLA^{+} and HLA^{-} vaccinated subgroups using the same method used in the
265 original report of RV144 immune correlates (15)− logistic regression with subject weighting to
266 account for the two-phase sampling case-control design. To test if each response variable was
267 differentially correlated with infection risk in A*02^{+} versus A*02^{-} vaccinated subgroups an A*02
268 interaction variable was included in the regression model. Immune measurements in the IgA-
269 ConsensusA and IgA-C1 Biotin assays were treated as positive or negative responses, and
270 because of small numbers of vaccine recipients in some HLA × immune response × infection
271 status strata, we used Zelen's exact test of homogeneity of odds ratios (49) to determine whether
272 the response variable was differentially correlated with infection risk in A*02^{+} versus A*02^{-}
273 vaccine recipients. This test gives a *p*-value for the null-hypothesis that the odds ratio of
274 infection risk for a positive vs. negative immune response is identical in the A*02$^{+/-}$ vaccinated
275 subgroups.
276
277 *Experimental validation of the A*02-KMQKEYALL epitope*
278 The A*02-KMQKEYALL epitope was validated using PBMC samples from subjects in a long
279 term non-progressor HIV-1 infected cohort (43). The subjects were recruited and enrolled at the
280 U.S. NIH-sponsored HIV Vaccine Trials Units. The appropriate Institutional Review Boards
281 approved the studies, and volunteers provided written consent. Details of the experiments are
282 included in the Appendix. Briefly, we measured functional T-cell responses in cryopreserved
283 peripheral blood mononuclear cells (PBMCs) using an interferon-gamma ELISpot assay. We

used 15-mer and 9-mer peptides from the V1/V2 region of envelope including variants from the vaccine and breakthrough strains (**Table A2, A3**).

MHC Purification and Peptide-Binding Assays

Purification of MHC molecules by affinity chromatography has been detailed elsewhere (50). Briefly, EBV transformed homozygous cell lines or single MHC allele transfected 721.221 or C1R lines are utilized as sources of HLA class I MHC molecules. HLA molecules are purified from cell pellet lysates by repeated passage over Protein A Sepharose beads conjugated with the W6/32 (anti-HLA A, B, C) antibody. In some cases, HLA-A molecules may be separated from HLA-B and -C molecules by pre-passage over a B1.23.2 (anti-HLA B, C and some A) column. Protein purity, concentration, and the effectiveness of depletion steps are monitored by SDS-PAGE and BCA assay.

Assays to quantitatively measure peptide binding to class I MHC molecules are based on the inhibition of binding of a high affinity radiolabeled peptide to purified MHC molecules, and are performed essentially as detailed elsewhere (50–52). Briefly, 0.1-1 nM of radiolabeled peptide is co-incubated at room temperature with 1 μM to 1 nM of purified MHC in the presence of a cocktail of protease inhibitors and 1 μM β2-microglobulin. Following a two-day incubation, MHC bound radioactivity is determined by capturing MHC/peptide complexes on W6/32 (anti-class I) antibody coated Lumitrac 600 plates (Greiner Bio-one, Frickenhausen, Germany), and measuring bound cpm using the TopCount (Packard Instrument Co., Meriden, CT) microscintillation counter. In the case of competitive assays, the concentration of peptide yielding 50% inhibition of the binding of the radiolabeled peptide is calculated. Under the conditions utilized, where [label]<[MHC] and $IC_{50} \geq$ [MHC], the measured IC_{50} values are reasonable approximations of the true Kd values (53, 54). Each competitor peptide is tested at six different concentrations covering a 100,000-fold dose range, and in three or more independent experiments. As a positive control, the unlabeled version of the radiolabeled probe is also tested in each experiment.

Results

T-cell based sieve analysis finds more HLA binding escapes in viruses isolated from vaccine than placebo recipients

To address the hypothesis that the sieve effects previously identified in the V1/V2 region of envelope (17) could be attributed to vaccine-induced selective pressure from cytolytic T cells (28–32), we performed a CD4+ and CD8+ T-cell based sieve analysis of the V1/V2 region (HXB2 143 – 185) of viruses that were isolated from infected trial participants. This statistical method compares the sequences of the vaccine immunogens to those of the isolated viruses and identifies amino acid differences in predicted vaccine epitopes that abrogate HLA binding. In some cases a viral isolate may differ from the vaccine immunogen at several residues within an epitope and the determination of whether these differences constitute a "binding escape" depends on the effect of these differences on the predicted HLA binding affinity, relative to that of the vaccine immunogen. A sieve effect was detected if the distributions of these viral "binding escapes" indicated significantly greater numbers in viruses isolated from vaccine compared to placebo recipients. In this analysis we considered the sequences of each of the three vaccine immunogens independently, including the ALVAC-HIV (vCP1521) 92TH023 CRF01_AE sequence and the AIDSVAX B/E protein boost with CM244 CRF01_AE and MN subtype B sequences (see *Methods* for details).

Of the three vaccine immunogens that were tested, only epitopes in the MN protein boost showed evidence of viral binding escape that was greater in vaccine compared to placebo recipients (p-value = 0.018). Though the sieve analysis was a test for a vaccine treatment effect summing over the whole V1/V2 region, once the effect was detected the epitopes and HLA alleles driving the effect were examined. Based on the HLA alleles that were present in infected RV144 participants there were 12 predicted CD8+ T-cell epitopes in the MN sequence V1/V2 region (**Fig. 1A, Table A1**). These epitopes, all characterized by HLA-peptide binding affinities less than 80 nM, were evenly distributed among the treatment groups (**Fig. 1B**) with 91% of vaccine and 83% of placebo recipients having at least one predicted epitope (p = 0.37). Only three of these epitopes, restricted by alleles expressed by 72% of vaccine and 65% of placebo recipients, were associated with viral HLA binding escape (**Fig. 1C**): A*11-GTIKGGEMK (HXB2 147-155), A*11-TSIGDKMQK (163-171) and A*02-KMQKEYALL (168-176). The number of HLA binding escapes detected in the corresponding viral 9-mers was significantly

higher in vaccine recipients (**Fig. 1D**) (average escapes per participant of 0.72 in vaccine vs. 0.29 in placebo; p-value = 0.018). This evidence of a T-cell based sieve effect is specific to epitopes that were predicted in the sequence of the MN-strain rgp120 immunogen. Due to differences in the sequences (**Fig 1A**) of the 92TH023- and CM244- compared to the MN-strain immunogen, some peptides are predicted epitopes in only the MN immunogen. These differences account for the lack of evidence for a sieve effect when considering the CRF01_AE immunogens (92TH023 p-value = 0.28; CM244 p-value = 0.25). Though 89% of the infections in the trial were CRF01_AE, it is possible that an MN-derived epitope may have applied cross-reactive pressure on the viruses (see *Discussion* for details).We performed an identical analysis using the class II HLA alleles of participants to test for binding escape in predicted CD4+ T-cell epitopes. Though vaccine peptides in V1/V2 were predicted to bind many class II alleles carried by RV144 participants (**Fig. A1**), we did not detect a difference in the number of predicted binding escapes in vaccine versus placebo recipients.

*HLA A*02 allele modifies overall vaccine efficacy*

We hypothesized that if the predicted HLA binding escapes in the V1/V2 region were indicative of vaccine-induced anamnestic responses, then vaccine efficacy (VE) should not have been different in the subgroups of participants with and without each of the HLA alleles implicated in the sieve analysis. To test this hypothesis we first estimated VE in $A*11^{+}$ and $A*11^{-}$ participants and found no difference ("Case-only" method (48) testing A*11 and VE interaction, p = 0.45; **Fig. 2A**; **Table 1**). For this reason we did not further consider the effects of A*11 in this study. We then estimated VE in the $A*02^{+/-}$ subgroups and found that in participants with A*02 estimated VE was 54% (p = 0.006 for non-zero VE; 95% CI 21-73%), which was higher than the estimated overall VE (31%) and significantly higher than that in participants without the A*02 allele (3%; p = 0.90 for non-zero VE; $A*02^{+/-}$ interaction-p = 0.050) (**Fig. 2A**; **Table 1**). These estimates of VE were based on the 125 infections in the modified intent-to-treat (mITT) cohort, whereas the sieve analysis was based on a subset of 109 CRF01_AE infections. We recomputed VE in $A*02^{+/-}$ subgroups within this subset of infections, thereby estimating the VE against CRF01_AE viruses. The results were similar to the overall results: VE for $A*02^{+}$ participants was 60% (CI 26% - 78%, p = 0.004), which was significantly greater than for $A*02^{-}$ participants in which VE was 3% (CI -62% - 43%, p = 0.90, $A*02^{+/-}$ interaction-p = 0.034). Together these

findings establish A*02 as a borderline significant effect modifier of VE and suggest that the A*02 allele and the predicted A*02 vaccine epitope in V2 may be related to protective vaccine-induced immune responses.

*HLA A*02 modifies genotype-specific vaccine efficacy at site 169 in the V2 loop*

Previously, Rolland and Edlefsen *et al*. (17) reported a sieve effect in the sequences of viruses that were isolated from infected RV144 participants. Specifically, sieve effects were identified at two positions, 169 and 181 (HX2B numbering), in the V2 loop of envelope. The effect at site 169 was evidenced by a relative enrichment of the amino-acid lysine (K) in viruses that infected placebo compared to vaccine recipients. The 92TH023 and CM244 vaccine sequences also contain K at site 169, while the MN sequence has methionine (M). Though the majority of infections were type CRF01_AE and none of the viral isolates had M at site 169 and since we observed a T-cell based sieve effect that depended on the A*02 epitope KMQKEYALL (168-176), in which position 169 was an anchor residue, we hypothesized that the K169 sieve effect was related to the A*02 epitope and would be stronger in the $A*02^{+}$ subgroup. To test this, we computed genotype-specific VE against viruses matching K169 in the $A*02^{+/-}$ subgroups. While the overall VE against viruses matching K169 was 48% (17), we found that in the $A*02^{+}$ participants VE against K169 viruses was 74% (p = 0.001 for non-zero VE; 95% CI 44−88%) (**Fig. 2A**, **Table 2**), and was significantly higher than in $A*02^{-}$ participants, estimated at 15% (p = 0.56 for non-zero VE; 95% CI -49−52%; $A*02^{+/-}$ interaction-p = 0.01). These estimates of genotype-specific VE fundamentally depend on a difference in the number of viruses matching K169 in vaccine versus placebo recipients (70% vs. 86% respectively; **Fig. 2B**). While this difference was evident in the $A*02^{+}$ subgroup with 53% vs. 84% matching the K169 genotype, the vaccine and placebo recipients in the $A*02^{-}$ subgroup were more similar^{-} (79% vs. 90%). This finding suggested that immune responses in $A*02^{+}$ vaccine recipients – compared to all other study participants – preferentially blocked infection by or selected against viruses matching the 92TH023 and CM244 vaccine sequences at position 169.

Since Rolland and Edlefsen *et al*. (17) also reported a sieve effect at position 181, we repeated the above analysis based on the virus genotypes at position 181. For the entire cohort, VE against viruses not matching the ALVAC vaccine at position 181 (X181) was 78% (p = 0.0028 for non-

zero VE, **Fig. 2C**) while the VE against matched viruses (I181) was 17% (p = 0.38 for non-zero VE). Our analyses showed that while VE against X181 viruses in A*02^{+} participants was 90% (p = 0.028 for non-zero VE; 95% CI 22−99%), it was not significantly different from VE against the same X181 viruses in A*02^{-} participants (63%; p = 0.15 for non-zero VE; with A*02$^{+/-}$ interaction-p = 0.29). Therefore there was no statistical evidence that the A*02 allele modified genotype-specific VE against X181 viruses.

*Experimental validation of the A*02-KMQKEYALL epitope*

The T-cell based sieve analysis identified differential HLA binding escape in a predicted A*02-KMQKEYALL epitope. However, the HIV LANL epitope database (55) does not list any epitopes in the V2 region. To experimentally confirm the predicted A*02 CD8+ T-cell epitope we first conducted HLA binding experiments to test the binding of the KMQKEYALL peptide to HLA A*02. We found that the epitope binds strongly to A*0203 (IC_{50} 12 nM), A*2402 (IC_{50} 21 nM) and less strongly to A*0201 (IC_{50} 719 nM) (**Table A2**). We then assayed PBMC from 18 HIV-1 infected individuals from a long-term non-progressor cohort who were A*02^{+} (see Appendix for details). Using ELISpot and ICS assays we identified one A*0201^{+} individual (A*02G1, 03G1, B*08,15 Cw*04,07) that had a CD8+ T-cell response to the KMQKEYALL peptide (magnitude of 118 SFC per million). Taken together these data show that the KMQKEYALL peptide binds to HLA A*02 alleles and can elicit functional CD8+ responses in an HIV-infected individual.

*HLA A*02 modifies immune correlates of risk of HIV infection*

The statistical association between the HLA A*02 allele, overall VE and K169-genotype specific VE led us to question whether A*02 could also have a role in modifying the associations of vaccine-induced immune responses with risk of infection. Therefore, we assessed A*02 as an effect modifier of the immune correlates of infection risk that were previously identified (15). Due to the small number of infected vaccine recipients who carry A*02 (19 individuals) and the large number of possible immune measurements to study, we preserved statistical power by restricting analyses to six immune measurements that were identified as correlates of risk in the previous analyses: (1) Env V1/V2 IgG antibody binding, (2) IgA binding to a panel of Env isolates (M-B gD), (3) IgA binding to consensus A Env gp140, (4) IgA antibody binding to

gp120-C1, (5) V2 hotspot peptide microarray, and (6) PBMC Luminex cytokine score (see Appendix for details). For each of these measurements we tested if the presence of A*02 significantly modified the previously reported correlation with risk of infection.

The V2 hotspot immune variable, an average of the magnitude of antibody responses to overlapping V2-peptides from 6 HIV-1 subtypes in a peptide microarray, was previously found to correlate inversely with risk of infection in vaccine recipients; we found no evidence that the presence of A*02 modified this effect. Following this we performed a focused analysis that considered only responses to the two subtype B peptides that contained a variant of the predicted A*02-KMQKEYALL epitope. We found that responses to one peptide, TSIRDKVQKEYALFY (positions 163-182 HXB2 numbering), were directly correlated with risk of infection in vaccine recipients lacking A*02 (OR of infection per SD = 1.92; 95% CI 1.07 – 3.45; p = 0.030), but were not correlated in vaccine recipients that expressed the A*02 allele (OR of infection per SD = 0.84; 95% CI 0.42 – 1.70; p = 0.63; $A*02^{+/-}$ interaction p-value = 0.060) (**Fig. 3A**, **Table 4**). This analysis considered the peptide microarray measurement as a continuous quantitative variable. The finding was also reproduced in an analysis that categorized responses into high, medium and low tertiles of vaccine recipient responses, similar to the categorical analysis in Haynes *et al.* (15). Medium compared to low responses were not significantly associated with risk of infection in either of the $A*02^{+/-}$ subgroups (**Table 4**). However, high compared to low levels of antibodies were associated with increased risk of infection in the $A*02^{-}$ subgroup (OR for high vs. low response = 6.04; 95% CI 1.80 – 20.3; p = 0.0036), but not in the $A*02^{+}$ subgroup (OR for high vs. low response = 0.66; 95% CI 0.15 – 2.96; p = 0.59) (**Table 4**). A p-value for the hypothesis test that these categorized responses were significantly different in the $A*02^{+/-}$ vaccine recipients were not computed due to the low number of responders in each category.

We also found a borderline significant direct correlation between IgA-C1 binding and infection risk is only evident in the $A*02^{-}$ subjects ($A*02^{+/-}$ interaction p-value = 0.13), with OR = 6.01 (p = 0.0005; 95% CI 2.19 – 16.5) for $A*02^{-}$ subjects and OR = 1.26 (p=0.75; 95% CI 0.31 – 5.15) for $A*02^{+}$ subjects (**Fig. 3B**, **Table 4**). These results suggest that the increased infection risk of vaccine recipients with positive IgA-C1 binding levels may have only been present in vaccine

recipients who lacked the A*02 allele (56). In tests of the other four immune measurements that were previously identified as correlates of risk, we found no statistical evidence that either the correlation with risk of infection or the immune response was different in the $A*02^+$ and $A*02^-$ vaccine recipient subgroups.

*HLA A*02 modifies vaccine-induced V2-specific immune response*

Whereas an assessment of A*02 as a possible modifier of immune CoRs involves comparing infected and uninfected vaccine recipients, it is also important to assess A*02 as a possible modifier of vaccine-induced immune responses, regardless of their impact on infection risk. To address this we tested if immune responses in each of the assays tested above were significantly different in $A*02^+$ versus $A*02^-$ uninfected vaccine recipients. We found that in the peptide microarray the antibody response to the subtype B peptide TSIRDKVQKEYALFY (positions 163-182 HXB2 numbering) was significantly greater in $A*02^+$ (median = -0.039 normalized intensity) versus $A*02^-$ (median = -0.107 normalized intensity) uninfected vaccine recipients ($p = 0.010$, Wilcoxon rank-sum test), indicating that the presence of A*02 may have modulated the vaccine-induced immune response, albeit the magnitude of the effect is small. There were no other significant differences found between the $A*02^{+/-}$ subgroups in the five additional assays that were tested (see Appendix).

Discussion

We performed a novel sieve analysis of viruses isolated from infected RV144 participants focused on the V1/V2 region of envelope to assess whether the sieve effects observed in this region could be partially attributed to HLA binding escape under the selective pressure of vaccine-primed T cells. We found that the amino acid residues of the viruses differed from those of the MN-strain rgp120 vaccine immunogen in a way that substantially decreased the predicted HLA binding affinity of three predicted CD8+ T-cell vaccine epitopes. These differences were significantly more common in viruses isolated from vaccine compared to placebo recipients, providing evidence of a T-cell based sieve effect. This effect implicated two HLA class I alleles, one of which was found to be associated with greater vaccine efficacy in the trial.

Previously, the effects of HLA –and more generally host genetics– on the efficacy of vaccines have not been well studied, in part due to the cost of HLA typing and to the difficulty in identifying associations within the highly diverse set of HLA haplotypes (estimated at $>10^{13}$ unique alleles (4)). Despite these challenges, studies of influenza, measles/mumps/rubella (MMR) and hepatitis B vaccination have identified indirect links between both class I and class II host-HLA alleles and putatively protective antibody responses to vaccination ((38), (40, 41), (39) respectively). While the mechanisms of these associations are not well understood, they may reflect a combination of known (57) and/or unknown links between class I and class II HLA alleles and humoral immunity.

Our analysis found evidence of T-cell mediated escape in the V1/V2 region of HIV envelope. However, CD8+ T-cell response rates of RV144 vaccine recipients were low overall; depending on the sample time point and the assay that was used, 12−63% of vaccine recipients had a T-cell response to Env peptides and 25% had a response to V2 peptides, but these responses were predominantly CD4+ T-cell mediated(14, 33, 34). Furthermore, there are no $CD8^+$ V1/V2 T-cell epitopes listed in the LANL HIV epitope database (55). Despite these facts, there are several findings that provide rationale for studying T-cell epitopes in this region: (1) The variable loops of HIV envelope are epitope "hotspots" containing numerous CD4+ and CD8+ T-cell epitopes, likely due to the availability of the loops for antigen processing (58, 59); (2) A cathepsin-D cleavage site was identified at L176-Y177 (HXB2 numbering) that is highly conserved across subtypes (60); (3) Proteosomal degradation of the vaccine-strain of envelope protein (92TH023) yielded several fragments, one of which, DKKQKVHALF (HXB2 167-176) (61), contained a homolog of the MN-strain A*02 restricted epitope KMQKEYALL that was implicated in our analysis and (4) Preliminary data suggest that the RV144 vaccine regimen elicited higher $CD8^+$ T-cell response rates in the sigmoid mucosa compared to those found in peripheral blood (21). Finally, though the observation of robust T-cell responses would seem to be a necessary requirement for CTL mediated escape, in Step trial participants, despite high response rates overall (12), none of the observed T-cell responses correlated with the genetic signatures identified in the sieve analysis (26, 62).

In preliminary experiments to validate the predicted A*02 epitope KMQKEYALL, we showed that the peptide binds two A*02 alleles that were expressed by RV144 trial participants and that the peptide can elicit a CD8+ T-cell response in an HIV-1 infected A*0201^{+} individual. We note that all of the T-cell stimulation assays conducted with RV144 samples used CRF01_AE derived peptides (92TH023) which matched the vaccine insert, but not the subtype B (MN) protein boost. It may therefore be important to study CD8+ T-cell responses to the V1/V2 region using subtype B (MN) peptides and/or mucosal samples in assays that are sensitive enough to detect potentially low magnitude responses.

The T-cell based sieve analysis investigated the hypothesis that T cells induced viral escape after acquisition, yet we also found that both overall and genotype-specific vaccine efficacies were significantly increased in participants who carried the HLA A*02 allele implicated in our analysis. While these statistical associations do not imply that A*02 was a direct cause of greater vaccine efficacy in the trial, since it could be a marker of an unknown causal factor, they generate the hypothesis that A*02 contributed to greater vaccine efficacy. To consider this hypothesis we entertain two possibilities: (1) that A*02 restricted CTLs offered some degree of protection in the trial and/or (2) that the A*02 allele acted via a possibly undescribed CTL-independent mechanism. Recent studies of non-human primates have demonstrated prolonged CD8+ T-cell mediated control of HIV replication (63), have identified lymph node T-cell responses as an immune correlate of risk in a trial of a live attenuated SIV vaccine (64) and have shown that a SIV cytomegalovirus vector vaccine can induce T cell responses that correlate with control and clearance of a SIV challenge infection (65, 66). Though it was antibody and not T-cell responses that correlated most significantly with infection risk in the RV144 trial (15), it is possible that lymphnode or mucosal T-cell responses, previously thought to be important in SIV vaccines (64, 67), were also correlated. Therefore, it may be that A*02 restricted CD8+ T cells played a direct role in increasing vaccine efficacy.

Alternatively, we considered the possibility that HLA A*02 played a role in the efficacy of the vaccine that was independent of CD8+ T cells. To date there are numerous documented associations of HLA alleles with disease outcomes, many whose mechanisms have not yet been fully described. For example, in HIV-1 infection, specific HLA alleles have been implicated in

561 the control of viral load and disease progression (43, 68–75). In dengue-related disease (76) and
562 HTLV-1 infection (77) HLA has been associated with disease severity and progression. As noted
563 above, there are also several documented examples of HLA alleles impacting vaccine-induced
564 immune responses to influenza, hepatitis B and MMR vaccination (38, 39, 41, 78). Though there
565 are no canonical pathways allowing for a MHC class I allele to influence antibody production, a
566 recent study describes one possible mechanism. The study proposes a model for MHC class I
567 antigen cross presentation operating in activated immune cells through MHC-I epitope
568 recognition of exogenous polypeptides (79). The model predicts that MHC-I recognition of
569 epitopes within an extended polypeptide chain facilitates exogenous antigen uptake and
570 processing through a lysosomal, TAP independent, antigen presenting pathway. It is intriguing to
571 posit that class II epitopes within the same polypeptide chain could be presented after class I
572 directed antigen acquisition, thereby facilitating CD4+ T-cell responses to the region and also
573 potentially modulating humoral responses. Based on this model we hypothesize that the MN
574 protein immunogen may have been internalized via an HLA-F dependent A*02-specific
575 mechanism and subsequently presented by MHC-I and/or MHC-II. This provides a CTL-
576 independent mechanism for the modulation of the immune response in $A*02^{+}$ participants.
577 Further studies must be completed to determine if the mechanism identified by Goodridge *et al*.
578 was involved in the immune response to the RV144 vaccine.
579
580 The possibility that HLA A*02 modulated the antibody response to vaccination is consistent
581 with our finding that antibodies specific to the subtype B V2-peptide TSIRDKVQKEYALFY
582 were significantly greater in vaccine recipients expressing A*02. Furthermore, two borderline
583 significant findings support the hypothesis that A*02 may have modified immune correlates of
584 risk. First, we found that IgA-C1 antibodies, which were previously found to correlate directly
585 with risk in the trial overall, were a significant correlate in participants without A*02. This
586 suggests that the IgA antibodies, which were associated with greater risk of infection overall,
587 may not have had the same association in $A*02^{+}$ participants. Second, we found that the binding
588 of vaccine-induced antibodies to a subtype B peptide that encompasses site 169 and the A*02-
589 KMQKEYALL epitope was significantly greater in $A*02^{+}$ compared to $A*02^{-}$ vaccine recipients
590 and that there was a direct correlation with risk only in participants lacking A*02. Previously it
591 was shown that high levels of V2-specific antibodies were associated with decreased risk of

592 infection in the cohort overall (15), therefore it seems inconsistent that low-levels of V2-specific
593 antibodies were associated with decreased risk in A*02$^-$ participants. One hypothesis to account
594 for this inconsistency is that the antibodies specific to this subtype B peptide are different from
595 those that correlate indirectly with risk overall and therefore their effects may also differ. It is
596 also possible that higher levels of antibodies binding this subtype B peptide are associated with
597 decreased risk of infection within the A*02$^+$ subgroup, but that the low number of A*02$^+$
598 infected participants reduced the statistical power needed to detect such an effect. Both
599 possibilities implicate the A*02 allele in an immune modulatory role offering a potential link
600 between one of the genetic signatures found in the V2-region of breakthrough viruses, the V2-
601 specific antibodies elicited by the vaccine and the overall efficacy of the vaccine.
602
603 Understanding the interactions between the cellular and humoral immune responses upon
604 vaccination is especially important in the RV144 trial since the vaccine regimen combined the
605 ALVAC-HIV vector as a prime with the AIDSVAX B/E rgp120 as a boost. Though the
606 ALVAC-HIV vector expressing a CRF01_AE strain of envelope and a clade B strain of gag and
607 pol had previously been shown to induce T-cell responses (34) and the subtype B and
608 CRF01_AE AIDSVAX proteins had been shown to induce antibody responses (80), negative
609 results from a phase II trial of combined ALVAC and AIDSVAX B/B failed to trigger an
610 efficacy trial (81) and trials of AIDSVAX B/B and AIDSVAX B/E products alone did not show
611 efficacy (80, 82). Some researchers have speculated about the importance of the subtype-
612 mismatched MN-strain rgp120 included in the boost (16). Several recent findings seem to
613 suggest that the V2-antibodies that correlated inversely with risk may have been cross-reactive:
614 (1) the IgG V1/V2 CoR reported in RV144 by Haynes *et al*. (15) was based on an assay using a
615 subtype B V1/V2 scaffold antigen mismatched to the predominant circulating HIV-1 subtype, (2)
616 the IgG V1/V2 CoR has been recapitulated using V1/V2 scaffolds from multiple different
617 subtypes (16, 24) and (3) the V2 hotspot immune variable, another CoR in RV144, was the
618 average antibody response to peptides from 6 HIV-1 Group M subtypes (15). One surprising
619 aspect of our T-cell based sieve analysis, is that the sieve effect was driven by epitopes predicted
620 in the MN (B) and not the 92TH023 (E) nor the CM244 (E) sequences. Since 89% of the viruses
621 isolated in the trial were CRF01_AE, the vaccine-induced T-cell responses driving the sieve
622 effect would have needed to be cross-reactive, despite the fact that the MN vaccine sequence

differed from each viral isolate at 3, 4 or 5 amino acid residues within the KMQKEYALL epitope. The T-cell based sieve method described here considered all residues within each potential epitope when predicting changes in the HLA binding affinity, effectively estimating the cross-reactivity of each potential viral epitope with the predicted vaccine epitope. Though often a single mutation can mediate viral escape, it was recently demonstrated that Pichinde virus (PV) and lymphocytic choriomenigitis virus (LCMV) encode two epitopes sharing 6 of 8 amino acids and induce distinct cross-reactive T-cell repertoires (83). Another study showed that an Epstein-Barr virus epitope cross-reacts with an influenza epitope despite sharing only 3 of 9 amino acid residues (84). In the V2 region of envelope, though the extreme sequence variability would seem to make cross-reactivity unlikely, there is evidence of structural conservation (85). Given these previous reports and our results, we hypothesize that the MN strain protein boost may have played a role in developing cross-reactive immune responses.

In considering the role of the HLA A*02 allele in the RV144 trial, the results could be attributed to underlying associations with other genes or other HLA alleles that are genetically linked to the A*02 allele. To partially address this possibility we performed a genetic analysis to identify HLA alleles in linkage disequilibrium with the A*02 allele (47) (**Table A4**). The analysis revealed an HLA haplotype A*0207:B*4601:Cw*0102 that is common in the RV144 cohort (estimated prevalence of 14%) and has been previously documented as one of the most common haplotypes in ethnic northeast Thais (86). We estimated VE for each of several subgroups of participants who carried one or all of the alleles within this haplotype (**Table A5**). We found no conclusive evidence that VE was restricted to this haplotype or to any other HLA allele. However, because this haplotype spans the HLA-A, B, C loci and is common in RV144 participants it remains possible that the A*02 associations we describe could be related to other unobserved genes that are included in the haplotype. In addition, given that the finding of differential VE in $A*02^{+}$ versus $A*02^{-}$ individuals is of borderline significance (p=0.050), it is possible that this finding is a false-positive result. Moreover, a comprehensive scan assessing all HLA alleles as modifiers of vaccine efficacy yielded no significant findings, due to the need to correct for the multiple hypothesis tests. Nevertheless, focusing the main analyses on A*02 and A*11 was justified by the fact that predicted vaccine-induced T cell pressure was only predicted in these HLA defined subgroups.

654

655 Follow-up analyses and ongoing experiments using samples from the RV144 study are providing
656 in depth information on the immune responses induced by RV144 vaccination. Both
657 experimental and exploratory statistical analyses, like this one, can generate hypotheses that will
658 inform the design of future vaccine trials. Our findings emphasize the importance of considering
659 HLA genotypes, and more generally host genetics in vaccine clinical trials and their potential
660 role in the complex web of humoral and cellular immune responses in vaccine-induced viral
661 immunity.

662

663 **Acknowledgments**

664 The authors thank the study participants, investigators, and sponsors of the RV144 Thai trial,
665 including the U.S. Military HIV Research Program (MHRP); U.S. Army Medical Research and
666 Materiel Command; NIAID; U.S. and Thai Components, Armed Forces Research Institute of
667 Medical Science; Ministry of Public Health, Thailand; Mahidol University; SanofiPasteur; and
668 Global Solutions for Infectious Diseases. Funding: NIH NIAID R37 AI054165-11 (PBG),
669 NIH/NIAID UM1 AI06861 (MJM) and NIH K25 AI087397-01 career award (TH). The authors
670 have no competing interests with this research.

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673 **References**

674 1. **Plotkin S, Orenstein W, Offit P**. 2008. Vaccines, 5th Ed. Elsevier Inc.

675 2. **Osterholm MT, Kelley NS, Sommer A, Belongia EA**. 2012. Efficacy and effectiveness
676 of influenza vaccines: a systematic review and meta-analysis. Lancet Infect. Dis. **12**:36–
677 44.

678 3. **Fine PE**. 1995. Variation in protection by BCG: implications of and for heterologous
679 immunity. Lancet **346**:1339–45.

680 4. **Poland GA, Kennedy RB, Ovsyannikova IG**. 2011. Vaccinomics and Personalized
681 Vaccinology: Is Science Leading Us Toward a New Path of Directed Vaccine
682 Development and Discovery? PLoS Pathog. **7**:e1002344.

683 5. **Plotkin SA**. 2010. Correlates of protection induced by vaccination. Clin. Vaccine
684 Immunol. **17**:1055–65.

6. **Haralambieva IH, Ovsyannikova IG, Kennedy RB, Larrabee BR, Shane Pankratz V, Poland G a**. 2013. Race and sex-based differences in cytokine immune responses to smallpox vaccine in healthy individuals. Hum. Immunol. **74**:1263–6.

7. **Haralambieva IH, Ovsyannikova IG, Umlauf BJ, Vierkant R a, Shane Pankratz V, Jacobson RM, Poland G a**. 2011. Genetic polymorphisms in host antiviral genes: associations with humoral and cellular immunity to measles vaccine. Vaccine **29**:8988–97.

8. **Haralambieva IH, Oberg AL, Dhiman N, Ovsyannikova IG, Kennedy RB, Grill DE, Jacobson RM, Poland G a**. 2012. High-dimensional gene expression profiling studies in high and low responders to primary smallpox vaccination. J. Infect. Dis. **206**:1512–20.

9. **Kennedy RB, Ovsyannikova IG, Pankratz VS, Haralambieva IH, Vierkant R a, Jacobson RM, Poland G a**. 2012. Genome-wide genetic associations with IFNγ response to smallpox vaccine. Hum. Genet. **131**:1433–51.

10. **Frahm N, DeCamp AC, Friedrich DP, Carter DK, Defawe OD, Kublin JG, Casimiro DR, Duerr A, Robertson MN, Buchbinder SP, Huang Y, Spies GA, De Rosa SC, McElrath MJ**. 2012. Human adenovirus-specific T cells modulate HIV-specific T cell responses to an Ad5-vectored HIV-1 vaccine. J. Clin. Invest. **122**:359–67.

11. **Zak DE, Andersen-Nissen E, Peterson ER, Sato A, Hamilton MK, Borgerding J, Krishnamurty AT, Chang JT, Adams DJ, Hensley TR, Salter AI, Morgan C a, Duerr AC, De Rosa SC, Aderem A, McElrath MJ**. 2012. Merck Ad5/HIV induces broad innate immune activation that predicts CD8+ T-cell responses but is attenuated by preexisting Ad5 immunity. Proc. Natl. Acad. Sci. U. S. A. 1–10.

12. **McElrath MJ, De Rosa SC, Moodie Z, Dubey S, Kierstead L, Janes H, Defawe OD, Carter DK, Hural J, Akondy R, Buchbinder SP, Robertson MN, Mehrotra D V, Self SG, Corey L, Shiver JW, Casimiro DR**. 2008. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. Lancet **372**:1894–905.

13. **Montefiori DC, Metch B, McElrath MJ, Self S, Weinhold KJ, Corey L**. 2004. Demographic factors that influence the neutralizing antibody response in recipients of recombinant HIV-1 gp120 vaccines. J. Infect. Dis. **190**:1962–9.

14. **Rerks-Ngarm S, Pitisuttithum P**. 2009. Vaccination with ALVAC and ADISVAX to Prevent HIV-1 Infection in Thailand. N. Engl. J. Med. **361**:2209–2220.

15. **Haynes BF, Gilbert PB, McElrath M, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, Liao H-X, DeVico AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang Y, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Bailer RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen X, Koup RA, Pitisuttithum P, Kaewkungwal J, Nitayaphan S, Rerks-**

Ngarm S, Michael NL, Kim JH, Alam M. 2012. Immune-Correlates Analysis of an HIV-1 Vaccine Efficacy Trial. N. Engl. J. Med. **366**:1275–1286.

16. **Zolla-Pazner S, Decamp AC, Cardozo T, Karasavvas N, Gottardo R, Williams C, Morris DE, Tomaras G, Rao M, Billings E, Berman P, Shen X, Andrews C, O'Connell RJ, Ngauy V, Nitayaphan S, de Souza M, Korber B, Koup R, Bailer RT, Mascola JR, Pinter A, Montefiori D, Haynes BF, Robb ML, Rerks-Ngarm S, Michael NL, Gilbert PB, Kim JH**. 2013. Analysis of V2 Antibody Responses Induced in Vaccinees in the ALVAC/AIDSVAX HIV-1 Vaccine Efficacy Trial. PLoS One **8**:e53629.

17. **Rolland M, Edlefsen PT, Larsen BB, Tovanabutra S, Sanders-Buell E, Hertz T, Decamp AC, Carrico C, Menis S, Magaret C a, Ahmed H, Juraska M, Chen L, Konopa P, Nariya S, Stoddard JN, Wong K, Zhao H, Deng W, Maust BS, Bose M, Howell S, Bates A, Lazzaro M, O'Sullivan A, Lei E, Bradfield A, Ibitamuno G, Assawadarachai V, O'Connell RJ, Desouza MS, Nitayaphan S, Rerks-Ngarm S, Robb ML, McLellan JS, Georgiev I, Kwong PD, Carlson JM, Michael NL, Schief WR, Gilbert PB, Mullins JI, Kim JH**. 2012. Increased HIV-1 vaccine efficacy against viruses with genetic signatures in Env V2. Nature 1–5.

18. **Karasavvas N, Billings E, Rao M, Williams C, Zolla-Pazner S, Bailer RT, Koup R a, Madnote S, Arworn D, Shen X, Tomaras GD, Currier JR, Jiang M, Magaret C, Andrews C, Gottardo R, Gilbert P, Cardozo TJ, Rerks-Ngarm S, Nitayaphan S, Pitisuttithum P, Kaewkungwal J, Paris R, Greene K, Gao H, Gurunathan S, Tartaglia J, Sinangil F, Korber BT, Montefiori DC, Mascola JR, Robb ML, Haynes BF, Ngauy V, Michael NL, Kim JH, de Souza For The Moph Taveg Collaboration MS**. 2012. The Thai Phase III HIV Type 1 Vaccine Trial (RV144) Regimen Induces Antibodies That Target Conserved Regions Within the V2 Loop of gp120. AIDS Res. Hum. Retroviruses **28**:1444–1457.

19. **De Souza MS, Ratto-Kim S, Chuenarom W, Schuetz A, Chantakulkij S, Nuntapinit B, Valencia-Micolta A, Thelian D, Nitayaphan S, Pitisuttithum P, Paris RM, Kaewkungwal J, Michael NL, Rerks-Ngarm S, Mathieson B, Marovich M, Currier JR, Kim JH**. 2012. The Thai phase III trial (RV144) vaccine regimen induces T cell responses that preferentially target epitopes within the V2 region of HIV-1 envelope. J. Immunol. **188**:5166–76.

20. **Montefiori DDC, Karnasuta C, Huang Y, Ahmed H, Gilbert P, de Souza MS, McLinden R, Tovanabutra S, Laurence-Chenine A, Sanders-Buell E, Moody MA, Bonsignori M, Ochsenbauer C, Kappes J, Tang H, Greene K, Gao H, LaBranche CC, Andrews C, Polonis VR, Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Self SG, Berman PW, Francis D, Sinangil F, Lee C, Tartaglia J, Robb ML, Haynes BF, Michael NL, Kim JH**. 2012. Magnitude and Breadth of the Neutralizing Antibody Response in the RV144 and Vax003 HIV-1 Vaccine Efficacy Trials. J. Infect. **206**:431–41.

21. **Schuetz A, Huangngern Y, de Souza M, Sukhumvittaya S, Jongrakthaitae S, Rerknimitr R, Saengtawan P, Ananworanich J, Vasan S, Ratto-Kim S, Pitisuttithum P, Michael N, O'Connell R, Ngauy V, Rerks-Ngarm S, Kim J**. 2013. Evaluation of Peripheral and Mucosal Cellular Immune Responses induced by Late Boost Strategies in HIV-negative Participants prior enrolled in RV144AIDS Vaccine.

22. **Liu P, Yates NL, Shen X, Bonsignori M, Moody MA, Liao H-X, Fong Y, Alam SM, Overman RG, Denny T, Ferrari G, Ochsenbauer C, Kappes JC, Polonis V, Pitisuttithum P, Kaewkungwal J, Nitayaphan S, Rerks-Ngarm S, Montefiori DC, Gilbert P, Michael NL, Kim JH, Haynes BF, Tomaras GD**. 2013. Infectious Virion Capture by HIV-1 gp120 Specific IgG from RV144 Vaccinees. J. Virol. **1**.

23. **Liao H-X, Bonsignori M, Alam SM, McLellan JS, Tomaras GD, Moody MA, Kozink DM, Hwang K-K, Chen X, Tsao C-Y, Liu P, Lu X, Parks RJ, Montefiori DC, Ferrari G, Pollara J, Rao M, Peachman KK, Santra S, Letvin NL, Karasavvas N, Yang Z-Y, Dai K, Pancera M, Gorman J, Wiehe K, Nicely NI, Rerks-Ngarm S, Nitayaphan S, Kaewkungwal J, Pitisuttithum P, Tartaglia J, Sinangil F, Kim JH, Michael NL, Kepler TB, Kwong PD, Mascola JR, Nabel GJ, Pinter A, Zolla-Pazner S, Haynes BF**. 2013. Vaccine Induction of Antibodies against a Structurally Heterogeneous Site of Immune Pressure within HIV-1 Envelope Protein Variable Regions 1 and 2. Immunity 1–11.

24. **Zolla-Pazner S, Decamp A, Gilbert PB, Williams C, Yates NL, Williams WT, Howington R, Fong Y, Morris DE, Soderberg K a, Irene C, Reichman C, Pinter A, Parks R, Pitisuttithum P, Kaewkungwal J, Rerks-Ngarm S, Nitayaphan S, Andrews C, O'Connell RJ, Yang Z-Y, Nabel GJ, Kim JH, Michael NL, Montefiori DC, Liao H-X, Haynes BF, Tomaras GD**. 2014. Vaccine-Induced IgG Antibodies to V1V2 Regions of Multiple HIV-1 Subtypes Correlate with Decreased Risk of HIV-1 Infection. PLoS One **9**:e87572.

25. **Yates NL, Liao H-X, Fong Y, deCamp a., Vandergrift N a., Williams WT, Alam SM, Ferrari G, Yang Z -y., Seaton KE, Berman PW, Alpert MD, Evans DT, O'Connell RJ, Francis D, Sinangil F, Lee C, Nitayaphan S, Rerks-Ngarm S, Kaewkungwal J, Pitisuttithum P, Tartaglia J, Pinter a., Zolla-Pazner S, Gilbert PB, Nabel GJ, Michael NL, Kim JH, Montefiori DC, Haynes BF, Tomaras GD**. 2014. Vaccine-Induced Env V1-V2 IgG3 Correlates with Lower HIV-1 Infection Risk and Declines Soon After Vaccination. Sci. Transl. Med. **6**:228ra39–228ra39.

26. **Rolland M, Tovanabutra S, DeCamp AC, Frahm N, Gilbert PB, Sanders-Buell E, Heath L, Magaret C a, Bose M, Bradfield A, O'Sullivan A, Crossler J, Jones T, Nau M, Wong K, Zhao H, Raugi DN, Sorensen S, Stoddard JN, Maust BS, Deng W, Hural J, Dubey S, Michael NL, Shiver J, Corey L, Li F, Self SG, Kim J, Buchbinder S, Casimiro DR, Robertson MN, Duerr A, McElrath MJ, McCutchan FE, Mullins JI**. 2011. Genetic impact of vaccination on breakthrough HIV-1 sequences from the STEP trial. Nat. Med. **17**:366–71.

27. **Sun Y, Gilbert PB**. 2012. Estimation of Stratified Mark-Specific Proportional Hazards Models with Missing Marks. Scand. Stat. Theory Appl. **39**:34–52.

28. **Henn MR, Boutwell CL, Charlebois P, Lennon NJ, Power KA, Macalalad AR, Berlin AM, Malboeuf CM, Ryan EM, Gnerre S, Zody MC, Erlich RL, Green LM, Berical A, Wang Y, Casali M, Streeck H, Bloom AK, Dudek T, Tully D, Newman R, Axten KL, Gladden AD, Battis L, Kemper M, Zeng Q, Shea TP, Gujja S, Zedlack C, Gasser O, Brander C, Hess C, Günthard HF, Brumme ZL, Brumme CJ, Bazner S, Rychert J, Tinsley JP, Mayer KH, Rosenberg E, Pereyra F, Levin JZ, Young SK, Jessen H, Altfeld M, Birren BW, Walker BD, Allen TM**. 2012. Whole Genome Deep Sequencing of HIV-1 Reveals the Impact of Early Minor Variants Upon Immune Recognition During Acute Infection. PLoS Pathog. **8**:e1002529.

29. **Goonetilleke N, Liu MKP, Salazar-Gonzalez JF, Ferrari G, Giorgi E, Ganusov V V, Keele BF, Learn GH, Turnbull EL, Salazar MG, Weinhold KJ, Moore S, Letvin N, Haynes BF, Cohen MS, Hraber P, Bhattacharya T, Borrow P, Perelson AS, Hahn BH, Shaw GM, Korber BT, McMichael AJ**. 2009. The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. J. Exp. Med. **206**:1253–72.

30. **Ferrari G, Korber B, Goonetilleke N, Liu MKP, Turnbull EL, Salazar-Gonzalez JF, Hawkins N, Self S, Watson S, Betts MR, Gay C, McGhee K, Pellegrino P, Williams I, Tomaras GD, Haynes BF, Gray CM, Borrow P, Roederer M, McMichael AJ, Weinhold KJ**. 2011. Relationship between functional profile of HIV-1 specific CD8 T cells and epitope variability with the selection of escape mutants in acute HIV-1 infection. PLoS Pathog. **7**:e1001273.

31. **Soghoian DZ, Jessen H, Flanders M, Sierra-Davidson K, Cutler S, Pertel T, Ranasinghe S, Lindqvist M, Davis I, Lane K, Rychert J, Rosenberg ES, Piechocka-Trocha A, Brass AL, Brenchley JM, Walker BD, Streeck H**. 2012. HIV-specific cytolytic CD4 T cell responses during acute HIV infection predict disease outcome. Sci. Transl. Med. **4**:123ra25.

32. **Herbeck JT, Rolland M, Liu Y, McLaughlin S, McNevin J, Zhao H, Wong K, Stoddard JN, Raugi D, Sorensen S, Genowati I, Birditt B, McKay A, Diem K, Maust BS, Deng W, Collier AC, Stekler JD, McElrath MJ, Mullins JI**. 2011. Demographic processes affect HIV-1 evolution in primary infection before the onset of selective processes. J. Virol. **85**:7523–34.

33. **De Souza MS, Ratto-Kim S, Chuenarom W, Schuetz A, Chantakulkij S, Nuntapinit B, Valencia-Micolta A, Thelian D, Nitayaphan S, Pitisuttithum P, Paris RM, Kaewkungwal J, Michael NL, Rerks-Ngarm S, Mathieson B, Marovich M, Currier JR, Kim JH**. 2012. The Thai phase III trial (RV144) vaccine regimen induces T cell responses that preferentially target epitopes within the V2 region of HIV-1 envelope. J. Immunol. **188**:5166–76.

839 34. **Nitayaphan S, Pitisuttithum P, Karnasuta C, Eamsila C, de Souza M, Morgan P,**
840 **Polonis V, Benenson M, VanCott T, Ratto-Kim S, Kim J, Thapinta D, Garner R,**
841 **Bussaratid V, Singharaj P, El-Habib R, Gurunathan S, Heyward W, Birx D, McNeil**
842 **J, Brown AE**. 2004. Safety and immunogenicity of an HIV subtype B and E prime-boost
843 vaccine combination in HIV-negative Thai adults. J. Infect. Dis. **190**:702–6.

844 35. **Jojic N, Reyes-Gomez M, Heckerman D, Kadie C, Schueler-Furman O**. 2006.
845 Learning MHC I--peptide binding. Bioinformatics **22**:e227–35.

846 36. **Nielsen M, Lundegaard C, Blicher T, Lamberth K, Harndahl M, Justesen S, Røder**
847 **G, Peters B, Sette A, Lund O, Buus S**. 2007. NetMHCpan, a method for quantitative
848 predictions of peptide binding to any HLA-A and -B locus protein of known sequence.
849 PLoS One **2**:e796.

850 37. **Nielsen M, Justesen S, Lund O, Lundegaard C, Buus S**. 2010. NetMHCIIpan-2.0 -
851 Improved pan-specific HLA-DR predictions using a novel concurrent alignment and
852 weight optimization training procedure. Immunome Res. **6**:9.

853 38. **Poland GA, Ovsyannikova I, Jacobson R**. 2008. Immunogenetics of seasonal influenza
854 vaccine response. Vaccine **26**.

855 39. **Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ, Dienstag**
856 **JL, Awdeh Z, Yunis EJ**. 1989. Genetic prediction of nonresponse to hepatitis B vaccine.
857 N. Engl. J. Med. **321**:708–12.

858 40. **Ovsyannikova IG, Jacobson RM, Poland GA**. 2004. Variation in vaccine response in
859 normal populations. Pharmacogenomics **5**:417–27.

860 41. **Ovsyannikova IG, Pankratz VS, Vierkant RA, Jacobson RM, Poland GA**. 2012.
861 Consistency of HLA associations between two independent measles vaccine cohorts: a
862 replication study. Vaccine **30**:2146–52.

863 42. **Ovsyannikova IG, Jacobson RM, Vierkant R a, O'Byrne MM, Poland G a**. 2009.
864 Replication of rubella vaccine population genetic studies: validation of HLA genotype and
865 humoral response associations. Vaccine **27**:6926–31.

866 43. **Pereyra F, Jia X, McLaren PJ, Telenti A, de Bakker PIW, Walker BD, Ripke S,**
867 **Brumme CJ, Pulit SL, Carrington M, Kadie CM, Carlson JM, Heckerman D,**
868 **Graham RR, Plenge RM, Deeks SG, Gianniny L, Crawford G, Sullivan J, Gonzalez**
869 **E, Davies L, Camargo A, Moore JM, Beattie N, Gupta S, Crenshaw A, Burtt NP,**
870 **Guiducci C, Gupta N, Gao X, Qi Y, Yuki Y, Piechocka-Trocha A, Cutrell E,**
871 **Rosenberg R, Moss KL, Lemay P, O'Leary J, Schaefer T, Verma P, Toth I, Block B,**
872 **Baker B, Rothchild A, Lian J, Proudfoot J, Alvino DML, Vine S, Addo MM, Allen**
873 **TM, Altfeld M, Henn MR, Le Gall S, Streeck H, Haas DW, Kuritzkes DR, Robbins**
874 **GK, Shafer RW, Gulick RM, Shikuma CM, Haubrich R, Riddler S, Sax PE, Daar**
875 **ES, Ribaudo HJ, Agan B, Agarwal S, Ahern RL, Allen BL, Altidor S, Altschuler EL,**

Ambardar S, Anastos K, Anderson B, Anderson V, Andrady U, Antoniskis D, Bangsberg D, Barbaro D, Barrie W, Bartczak J, Barton S, Basden P, Basgoz N, Bazner S, Bellos NC, Benson AM, Berger J, Bernard NF, Bernard AM, Birch C, Bodner SJ, Bolan RK, Boudreaux ET, Bradley M, Braun JF, Brndjar JE, Brown SJ, Brown K, Brown ST, Burack J, Bush LM, Cafaro V, Campbell O, Campbell J, Carlson RH, Carmichael JK, Casey KK, Cavacuiti C, Celestin G, Chambers ST, Chez N, Chirch LM, Cimoch PJ, Cohen D, Cohn LE, Conway B, Cooper D a, Cornelson B, Cox DT, Cristofano M V, Cuchural G, Czartoski JL, Dahman JM, Daly JS, Davis BT, Davis K, Davod SM, DeJesus E, Dietz C a, Dunham E, Dunn ME, Ellerin TB, Eron JJ, Fangman JJW, Farel CE, Ferlazzo H, Fidler S, Fleenor-Ford A, Frankel R, Freedberg K a, French NK, Fuchs JD, Fuller JD, Gaberman J, Gallant JE, Gandhi RT, Garcia E, Garmon D, Gathe JC, Gaultier CR, Gebre W, Gilman FD, Gilson I, Goepfert P a, Gottlieb MS, Goulston C, Groger RK, Gurley TD, Haber S, Hardwicke R, Hardy WD, Harrigan PR, Hawkins TN, Heath S, Hecht FM, Henry WK, Hladek M, Hoffman RP, Horton JM, Hsu RK, Huhn GD, Hunt P, Hupert MJ, Illeman ML, Jaeger H, Jellinger RM, John M, Johnson J a, Johnson KL, Johnson H, Johnson K, Joly J, Jordan WC, Kauffman C a, Khanlou H, Killian RK, Kim AY, Kim DD, Kinder C a, Kirchner JT, Kogelman L, Kojic EM, Korthuis PT, Kurisu W, Kwon DS, LaMar M, Lampiris H, Lanzafame M, Lederman MM, Lee DM, Lee JML, Lee MJ, Lee ETY, Lemoine J, Levy J a, Llibre JM, Liguori M a, Little SJ, Liu AY, Lopez AJ, Loutfy MR, Loy D, Mohammed DY, Man A, Mansour MK, Marconi VC, Markowitz M, Marques R, Martin JN, Martin HL, Mayer KH, McElrath MJ, McGhee T a, McGovern BH, McGowan K, McIntyre D, Mcleod GX, Menezes P, Mesa G, Metroka CE, Meyer-Olson D, Miller AO, Montgomery K, Mounzer KC, Nagami EH, Nagin I, Nahass RG, Nelson MO, Nielsen C, Norene DL, O'Connor DH, Ojikutu BO, Okulicz J, Oladehin OO, Oldfield EC, Olender S a, Ostrowski M, Owen WF, Pae E, Parsonnet J, Pavlatos AM, Perlmutter AM, Pierce MN, Pincus JM, Pisani L, Price LJ, Proia L, Prokesch RC, Pujet HC, Ramgopal M, Rathod A, Rausch M, Ravishankar J, Rhame FS, Richards CS, Richman DD, Rodes B, Rodriguez M, Rose RC, Rosenberg ES, Rosenthal D, Ross PE, Rubin DS, Rumbaugh E, Saenz L, Salvaggio MR, Sanchez WC, Sanjana VM, Santiago S, Schmidt W, Schuitemaker H, Sestak PM, Shalit P, Shay W, Shirvani VN, Silebi VI, Sizemore JM, Skolnik PR, Sokol-Anderson M, Sosman JM, Stabile P, Stapleton JT, Starrett S, Stein F, Stellbrink H-J, Sterman FL, Stone VE, Stone DR, Tambussi G, Taplitz R a, Tedaldi EM, Theisen W, Torres R, Tosiello L, Tremblay C, Tribble M a, Trinh PD, Tsao A, Ueda P, Vaccaro A, Valadas E, Vanig TJ, Vecino I, Vega VM, Veikley W, Wade BH, Walworth C, Wanidworanun C, Ward DJ, Warner D a, Weber RD, Webster D, Weis S, Wheeler D a, White DJ, Wilkins E, Winston A, Wlodaver CG, van't Wout A, Wright DP, Yang OO, Yurdin DL, Zabukovic BW, Zachary KC, Zeeman B, Zhao M. 2010. The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. Science **330**:1551–7.

44. **Edlefsen PT, Gilbert PB, Rolland M**. 2013. Sieve analysis in HIV-1 vaccine efficacy trials. Curr. Opin. HIV AIDS 1–5.

45. **Schueler-Furman O, Altuvia Y, Sette A, Margalit H**. 2000. Structure-based prediction of binding peptides to MHC class I molecules: application to a broad range of MHC alleles. Protein Sci. **9**:1838–46.

46. **Hurley CK, Mack SJ, Mickelson E, Marsh S, Tilanus MGJ, Gorodezky C, Wade JA, Müller C, Hartzman RJ, Baxter-Lowe LA, Raffoux C**. 2006. HLA typing and informatics, in immunobiology of the human MHC. 13th Int. Histocompat. Work. Protoc. 179–352.

47. **Lancaster a K, Single RM, Solberg OD, Nelson MP, Thomson G**. 2007. PyPop update--a software pipeline for large-scale multilocus population genomics. Tissue Antigens **69 Suppl 1**:192–7.

48. **Dai JY, Li SS, Gilbert PB**. 2013. Case-only method for cause-specific hazards models with application to assessing differential vaccine efficacy by viral and host genetics. Biostatistics 1–8.

49. **Zelen M**. 1971. The analysis of several 2× 2 contingency tables. Biometrika **58**:129–137.

50. **Sidney J, Southwood S**. 2001. Measurement of MHC/peptide interactions by gel filtration. Curr. Protoc. Immunol. 1–19.

51. **Sidney J, Assarsson E, Moore C, Ngo S, Pinilla C, Sette A, Peters B**. 2008. Quantitative peptide binding motifs for 19 human and mouse MHC class I molecules derived using positional scanning combinatorial peptide libraries. Immunome Res. **4**:2.

52. **Sidney J, Southwood S, Mann DL, Fernandez-Vina M a, Newman MJ, Sette a**. 2001. Majority of peptides binding HLA-A*0201 with high affinity crossreact with other A2-supertype molecules. Hum. Immunol. **62**:1200–16.

53. **Cheng Y, Prusoff WH**. 1973. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem. Pharmacol. **22**:3099–108.

54. **Gulukota K, Sidney J, Sette A, DeLisi C**. 1997. Two complementary methods for predicting peptides binding major histocompatibility complex molecules. J. Mol. Biol. **267**:1258–67.

55. **LANL**. 2012. Los Alamos National Laboratory HIV Immunology website.

56. **Tomaras GD, Ferrari G, Shen X, Alam SM, Liao H-X, Pollara J, Bonsignori M, Moody MA, Fong Y, Chen X, Poling B, Nicholson CO, Zhang R, Lu X, Parks R, Kaewkungwal J, Nitayaphan S, Pitisuttithum P, Rerks-Ngarm S, Gilbert PB, Kim JH, Michael NL, Montefiori DC, Haynes BF**. 2013. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. Proc. Natl. Acad. Sci. U. S. A.

954 57. **Crotty S**. 2011. Follicular helper CD4 T cells (TFH). Annu. Rev. Immunol. **29**:621–63.

955 58. **Surman S, Lockey TD, Slobod KS, Jones B, Riberdy JM, White SW, Doherty PC,**
956 **Hurwitz JL**. 2001. Localization of CD4+ T cell epitope hotspots to exposed strands of
957 HIV envelope glycoprotein suggests structural influences on antigen processing. Proc.
958 Natl. Acad. Sci. U. S. A. **98**:4587–92.

959 59. **Zhan X, Hurwitz JL, Brown S a, Slobod KS**. 2007. HIV-1 envelope T cell epitope
960 "hotspots " among mice and humans and among CD4+ and CD8+ T cell subpopulations.
961 AIDS Res. Hum. Retroviruses **23**:471–6.

962 60. **Yu B, Fonseca DP a J, O'Rourke SM, Berman PW**. 2010. Protease cleavage sites in
963 HIV-1 gp120 recognized by antigen processing enzymes are conserved and located at
964 receptor binding sites. J. Virol. **84**:1513–26.

965 61. **Steers NJ, Ratto-Kim S, de Souza MS, Currier JR, Kim JH, Michael NL, Alving CR,**
966 **Rao M**. 2012. HIV-1 envelope resistance to proteasomal cleavage: implications for
967 vaccine induced immune responses. PLoS One **7**:e42579.

968 62. **Janes H, Frahm N, DeCamp A, Rolland M, Gabriel E, Wolfson J, Hertz T, Kallas E,**
969 **Goepfert P, Friedrich DP, Corey L, Mullins JI, McElrath MJ, Gilbert P**. 2012.
970 MRKAd5 HIV-1 Gag/Pol/Nef vaccine-induced T-cell responses inadequately predict
971 distance of breakthrough HIV-1 sequences to the vaccine or viral load. PLoS One
972 **7**:e43396.

973 63. **Mudd PA, Martins MA, Ericsen AJ, Tully DC, Power KA, Bean AT, Piaskowski SM,**
974 **Duan L, Seese A, Gladden AD, Weisgrau KL, Furlott JR, Kim Y-I, Veloso de**
975 **Santana MG, Rakasz E, Iii SC, Wilson NA, Bonaldo MC, Galler R, Allison DB,**
976 **Piatak Jr M, Haase AT, Lifson JD, Allen TM, Watkins DI**. 2012. Vaccine-induced
977 CD8(+) T cells control AIDS virus replication. Nature.

978 64. **Fukazawa Y, Park H, Cameron MJ, Lefebvre F, Lum R, Coombes N, Mahyari E,**
979 **Hagen SI, Bae JY, Iii MDR, Swanson T, Legasse AW, Sylwester A, Hansen SG,**
980 **Smith AT, Stafova P, Shoemaker R, Li Y, Oswald K, Axthelm MK, McDermott A,**
981 **Ferrari G, Montefiori DC, Edlefsen PT, Piatak M, Lifson JD, Sékaly RP, Picker LJ**.
982 2012. Lymph node T cell responses predict the efficacy of live attenuated SIV vaccines.
983 Nat. Med. **18**.

984 65. **Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L,**
985 **Whizin N, Oswald K, Shoemaker R, Swanson T, Legasse AW, Chiuchiolo MJ, Parks**
986 **CL, Axthelm MK, Nelson J a, Jarvis M a, Piatak M, Lifson JD, Picker LJ**. 2011.
987 Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine.
988 Nature **473**:523–7.

989 66. **Hansen SG, Jr MP, Ventura AB, Hughes CM, Gilbride RM, Ford JC, Oswald K,**
990 **Shoemaker R, Li Y, Lewis MS, Gilliam AN, Xu G, Whizin N, Burwitz BJ, Planer SL,**

Turner JM, Legasse AW, Axthelm MK, Nelson J a., Früh K, Sacha JB, Estes JD, Keele BF, Edlefsen PT, Lifson JD, Picker LJ. 2013. Immune clearance of highly pathogenic SIV infection. Nature.

67. **Fuller DH, Rajakumar P, Che JW, Narendran A, Nyaundi J, Michael H, Yager EJ, Stagnar C, Wahlberg B, Taber R, Haynes JR, Cook FC, Ertl P, Tite J, Amedee AM, Murphey-Corb M**. 2012. Therapeutic DNA vaccine induces broad T cell responses in the gut and sustained protection from viral rebound and AIDS in SIV-infected rhesus macaques. PLoS One **7**:e33715.

68. **Honeyborne I, Prendergast A, Pereyra F, Leslie A, Crawford H, Payne R, Reddy S, Bishop K, Moodley E, Nair K, van der Stok M, McCarthy N, Rousseau CM, Addo M, Mullins JI, Brander C, Kiepiela P, Walker BD, Goulder PJR**. 2007. Control of human immunodeficiency virus type 1 is associated with HLA-B*13 and targeting of multiple gag-specific CD8+ T-cell epitopes. J. Virol. **81**:3667–72.

69. **Duda A, Lee-Turner L, Fox J, Robinson N, Dustan S, Kaye S, Fryer H, Carrington M, McClure M, McLean a R, Fidler S, Weber J, Phillips RE, Frater a J**. 2009. HLA-associated clinical progression correlates with epitope reversion rates in early human immunodeficiency virus infection. J. Virol. **83**:1228–39.

70. **Chen H, Ndhlovu ZM, Liu D, Porter LC, Fang JW, Darko S, Brockman M a, Miura T, Brumme ZL, Schneidewind A, Piechocka-Trocha A, Cesa KT, Sela J, Cung TD, Toth I, Pereyra F, Yu XG, Douek DC, Kaufmann DE, Allen TM, Walker BD**. 2012. TCR clonotypes modulate the protective effect of HLA class I molecules in HIV-1 infection. Nat. Immunol.

71. **Shea PR, Shianna K V, Carrington M, Goldstein DB**. 2012. Host Genetics of HIV Acquisition and Viral Control. Annu. Rev. Med. 1–15.

72. **Song H, Pavlicek JW, Cai F, Bhattacharya T, Li H, Iyer SS, Bar KJ, Decker JM, Goonetilleke N, Liu MKP, Berg A, Hora B, Drinker MS, Eudailey J, Pickeral J, Moody MA, Ferrari G, McMichael A, Perelson AS, Shaw GM, Hahn BH, Haynes BF, Gao F**. 2012. Impact of immune escape mutations on HIV-1 fitness in the context of the cognate transmitted/founder genome. Retrovirology **9**:89.

73. **Carrington M**. 1999. HLA and HIV-1: Heterozygote Advantage and B*35-Cw*04 Disadvantage. Science (80-.). **283**:1748–1752.

74. **Kaslow R, Carrington M, Apple R, Park L**. 1996. Influence of combinations of human major histocompatibility complex genes on the course of HIV–1 infection. Nat. Med.

75. **Kiepiela P, Ngumbela K, Thobakgale C, Ramduth D, Honeyborne I, Moodley E, Reddy S, de Pierres C, Mncube Z, Mkhwanazi N, Bishop K, van der Stok M, Nair K, Khan N, Crawford H, Payne R, Leslie A, Prado J, Prendergast A, Frater J, McCarthy N, Brander C, Learn GH, Nickle D, Rousseau C, Coovadia H, Mullins JI,**

1028 **Heckerman D, Walker BD, Goulder P**. 2007. CD8+ T-cell responses to different HIV
1029 proteins have discordant associations with viral load. Nat. Med. **13**:46–53.

1030 76. **Chaturvedi U, Nagar R, Shrivastava R**. 2006. Dengue and dengue haemorrhagic fever:
1031 implications of host genetics. FEMS Immunol. Med. Microbiol. **47**:155–66.

1032 77. **Macnamara A, Rowan A, Hilburn S, Kadolsky U, Fujiwara H, Suemori K,**
1033 **Yasukawa M, Taylor G, Bangham CRM, Asquith B**. 2010. HLA class I binding of
1034 HBZ determines outcome in HTLV-1 infection. PLoS Pathog. **6**:e1001117.

1035 78. **Ovsyannikova IG, Jacobson RM, Vierkant RA, Jacobsen SJ, Pankratz VS, Poland**
1036 **GA**. 2004. The contribution of HLA class I antigens in immune status following two
1037 doses of rubella vaccination. Hum. Immunol. **65**:1506–15.

1038 79. **Goodridge JP, Lee N, Burian A, Pyo C-W, Tykodi SS, Warren EH, Yee C, Riddell**
1039 **SR, Geraghty DE**. 2013. HLA-F and MHC-I open conformers cooperate in a MHC-I
1040 antigen cross-presentation pathway. J. Immunol. **191**:1567–77.

1041 80. **Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF**. 2005. Placebo-
1042 controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1
1043 infection. J. Infect. Dis. **191**:654–65.

1044 81. **Russell ND, Graham BS, Keefer MC, McElrath MJ, Self SG, Weinhold KJ,**
1045 **Montefiori DC, Ferrari G, Horton H, Tomaras GD, others**. 2007. Phase 2 study of an
1046 HIV-1 canarypox vaccine (vCP1452) alone and in combination with rgp120: negative
1047 results fail to trigger a phase 3 correlates trial. J. Acquir. Immune Defic. Syndr. **44**:203.

1048 82. **Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, Griensven F Van,**
1049 **Hu D, Tappero JW, Choopanya K, Vaccine B, Group E**. 2006. Efficacy Trial of a
1050 Bivalent Recombinant Glycoprotein 120 HIV-1 Vaccine among Injection Drug Users in
1051 Bangkok , Thailand. Vaccine.

1052 83. **Chen AT, Cornberg M, Gras S, Guillonneau C, Rossjohn J, Trees A, Emonet S, de la**
1053 **Torre JC, Welsh RM, Selin LK**. 2012. Loss of anti-viral immunity by infection with a
1054 virus encoding a cross-reactive pathogenic epitope. PLoS Pathog. **8**:e1002633.

1055 84. **Clute S, Watkin L, Cornberg M, Naumov Y, Sullivan J, Luzuriaga K, Welsh R, Selin**
1056 **L**. 2005. Cross-reactive influenza virus–specific CD8+ T cells contribute to
1057 lymphoproliferation in Epstein-Barr virus–associated infectious mononucleosis. J. Clin.
1058 Invest. **115**.

1059 85. **Zolla-Pazner S, Cardozo T**. 2010. Structure-function relationships of HIV-1 envelope
1060 sequence-variable regions refocus vaccine design. Nat. Rev. Immunol. **10**:527–35.

1061 86. **Romphruk A V, Kongmaroeng C, Klumkrathok K, Paupairoj C, Leelayuwat C**.
1062 2010. HLA class I and II alleles and haplotypes in ethnic Northeast Thais. Tissue Antigens
1063 **75**:701–11.

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Figure Legends

Figure 1. CD8+ T-cell associated sieve effect

A. Twelve 9-mer peptides of MN gp120 were identified as potential vaccine epitopes (horizontal bars and boxed residues) based on their predicted binding affinities (IC_{50}) with the HLA alleles of RV144 participants. Their locations in the amino-acid sequence of V1/V2 are shown relative to the MN-strain (subtype B) and the 92TH023-strain (CRF01_AE) gp120 sequences. Three of these 9-mers were identified as peptides in which HLA "binding escapes" were predicted in at least one infected participant (boxed amino acid residues) with the restricting allele appearing below each epitope. **B**. The percentage of infected participants with an allele that was predicted to bind each of the twelve potential epitopes is shown, with the epitope identified by its N-terminal starting amino acid. **C.** The percentage of each treatment group that had predicted binding escapes in the three 9-mer peptides within which any escape was predicted. These epitopes are indicated at the bottom of the figure with their starting position, designation, and predicted restricting allele. **D**. The two bars show the percentage of infected participants in each group whose viral isolate had either 0, 1 or 2 predicted binding escapes in the V1/V2 region.

Figure 2. Greater VE in A*02^{+} participants

A. Estimated VE in the RV144 trial for the entire cohort and also for participants with and without the HLA A*02 allele. Error bars indicate 95% confidence intervals. For each group along the *x*-axis significant p-values are given for the hypothesis test that VE = 0. The p-value shown between the A*02$^{+/-}$ subgroups indicates a significant difference in VE between the two groups. **B**. The percentage of infected participants whose isolated virus contained the vaccine matched lysine at position 169 is reported as a percentage of the treatment group and/or A*02$^{+/-}$ subgroup indicated along the *x*-axis. The pair of columns on the left refer to the entire cohort, whereas the next two sets divide the cohort into A*02$^{+/-}$ subgroups. **C**. Estimated genotype-specific VE against K169 and X181 viruses. Estimates are reported for the whole cohort and also for A*02$^{+/-}$ subgroups, as indicated on the *x*-axis. Error bars show the 95% confidence interval for each VE estimate.

Figure 3. HLA A*02 modifies vaccine-induced immune responses and correlates of infection risk

Vaccine induced immune responses were associated with the expression of HLA A*02. Assays were performed as part of the Case-Control immune correlates analysis (15) using serum samples obtained two weeks after the fourth and final vaccine injection (week 26). Vaccine recipients were further divided into $A*02^{+}$ (n = 15 infected, 97 uninfected) and $A*02^{-}$ (n = 26 infected, 108 uninfected) subgroups for analysis. **A**. Normalized intensity representative of antibody responses to the peptide TSIRDKVQKEYALFY (Env V2 positions 163-177 HXB2 numbering) in the peptide microarray assay are shown using boxplots. The extent of the box indicates the inter-quartile range with a line indicating the median and whiskers indicating the full range. Responses from all case-control participants were divided into high, medium and low tertiles with the gray box showing the medium responses. Each dot is a single response, its color indicating whether it is in the high (black), medium (gray) or low (white) tertile. The percent of responses in each tertile are indicated below the box plot. One outlier response in the $A*02^{+}$ uninfected subgroup (8.07) is not plotted. **B**. The IgA-C1 Biotin assay was analyzed as a binary variable with positive responders having a measurement > 0 log(MFI, median fluorescence intensity). The percent of positive responders in each subgroup is indicated below the box plot.

Tables

Table 1. HLA A*02 modifies VE

HLA type	No. of infections vaccine : placebo	Est. VE	95% CI	*p*-value (H_0: VE = 0)	A*02$^{+/-}$ *p*-value
Overall	51 : 74	31%	1% - 51%	0.04	-
A*02^{+}	19 : 41	54%	21% - 73%	0.006	0.050
A*02^{-}	32 : 33	3%	-58% - 40%	0.9	
A*11^{+}	29 : 37	22%	-27% - 52%	0.33	0.45
A*11^{-}	22 : 37	41%	-0.8% - 65%	0.053	

Table 2. HLA A*02 modifies genotype-specific VE against K169 viruses

HLA type	Site 169	No. of infections vaccine : placebo	Est. VE	95% CI	*p*-value (H_0: VE = 0)	A*02$^{+/-}$ *p*-value
Overall	Match (K)	30 : 57	48%	18% - 66%	0.0036	-
A*02^{+}		8 : 31	74%	44% - 88%	0.001	0.02
A*02^{-}		22 : 26	15%	-49% - 52%	0.56	
Overall	Mismatch (X)	14 : 9	-55%	-258% - NA	0.3	-
A*02^{+}		8 : 6	-33%	-284% - 54%	0.59	0.65
A*02^{-}		6 : 3	-100%	-700% - 50%	0.32	

Table 3. HLA A*02 does not modify genotype-specific VE against I181 or I181X viruses

HLA type	Site 181	No. of infections vaccine : placebo	Est. VE	95% CI	*p*-value (H_0: VE = 0)	A*02$^{+/-}$ *p*-value
Overall	Match (I)	40 : 48	17%	-26% - 45%	0.38	-
A*02^{+}		15 : 27	44%	-4% - 70%	0.068	0.08
A*02^{-}		25 : 21	-19%	-113% - 33%	0.56	
Overall	Mismatch (X)	4 : 18	78%	35% - 93%	0.0028	-
A*02^{+}		1 : 10	90%	22% - 99%	0.028	0.29
A*02^{-}		3 : 8	63%	-41% - 90%	0.15	

1124 **Table 4**. HLA A*02 modifies immune correlates of risk of HIV-1 infection

Immune variable	HLA type	Est. OR	95% CI	*p*-value (H_0: OR = 1)	A*02$^{+/-}$ *p*-value	A*02$^{+/-}$ *q*-value
IgA C1 Biotin* (positive vs. negative response)	Overall	3.15	1.48 – 6.71	0.003	-	-
	A*02^{+}	1.26	0.31 – 5.15	0.75	0.13	0.44
	A*02^{-}	6.01	2.19 – 16.5	0.0005		
Peptide microarray 163-TSIRDKVQKEYALFY (quantitative analysis**)	A*02^{+}	0.84	0.42 – 1.70	0.63	0.060	0.41
	A*02^{-}	1.92	1.07 – 3.45	0.030		
Peptide microarray 163-TSIRDKVQKEYALFY (med vs. low response***)	A*02^{+}	1.71	0.40 – 7.37	0.47	-	-
	A*02^{-}	1.63	0.48 – 5.56	0.44		
Peptide microarray 163-TSIRDKVQKEYALFY (high vs. low response***)	A*02^{+}	0.66	0.15 – 2.96	0.59	-	-
	A*02^{-}	6.04	1.80 – 20.3	0.0036		

1125 * OR is for vaccine recipients with a positive versus a negative response using the positivity criteria determined
1126 previously (Haynes *et al.,* 2012)
1127 ** OR is per standard deviation change in the assay measurement
1128 *** OR is for vaccine recipients with a high (or medium) response versus a low response

Figure 1. CD8+ T-cell associated sieve effect

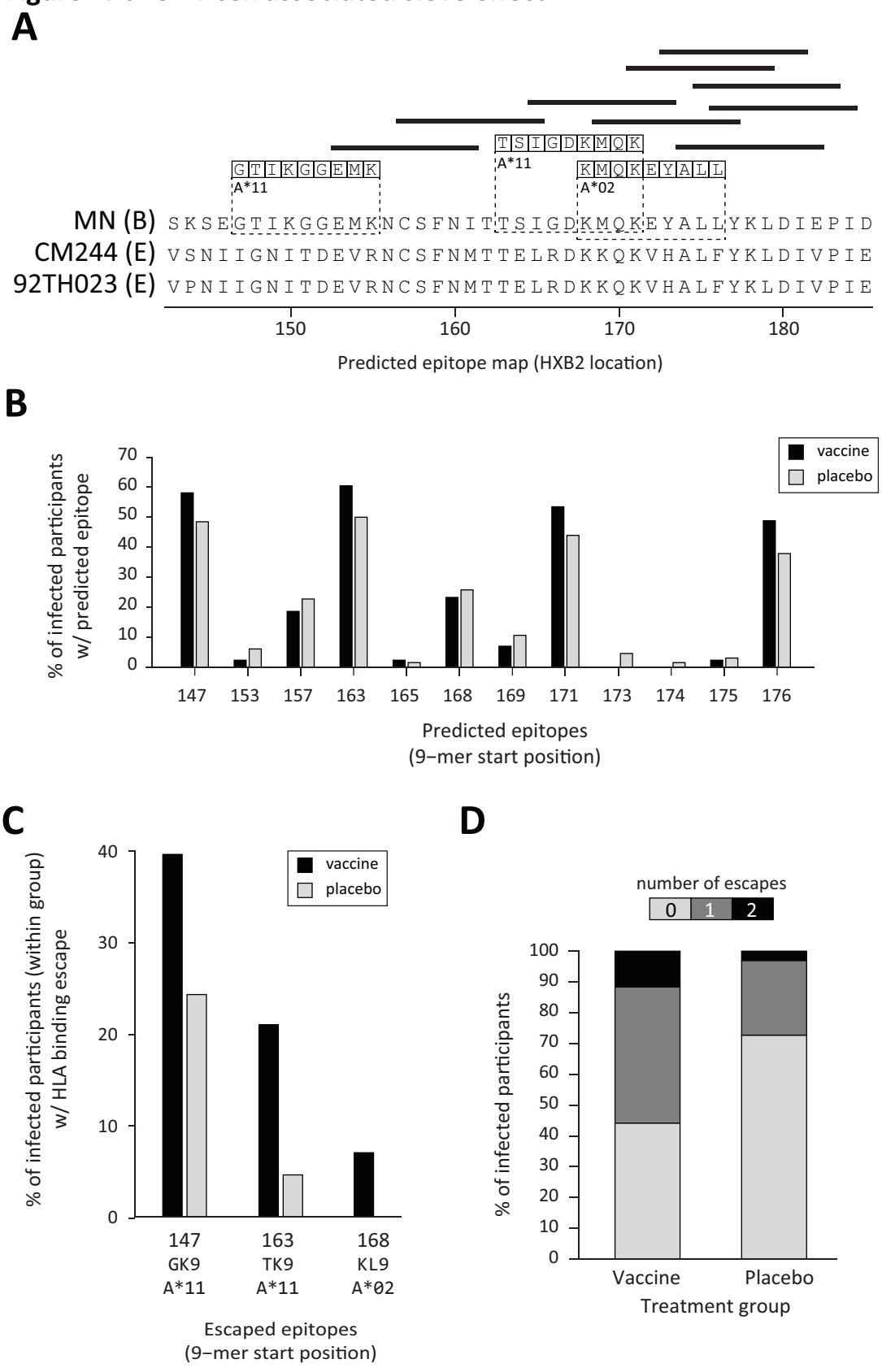

Figure 2. Greater VE in A*02+ participants

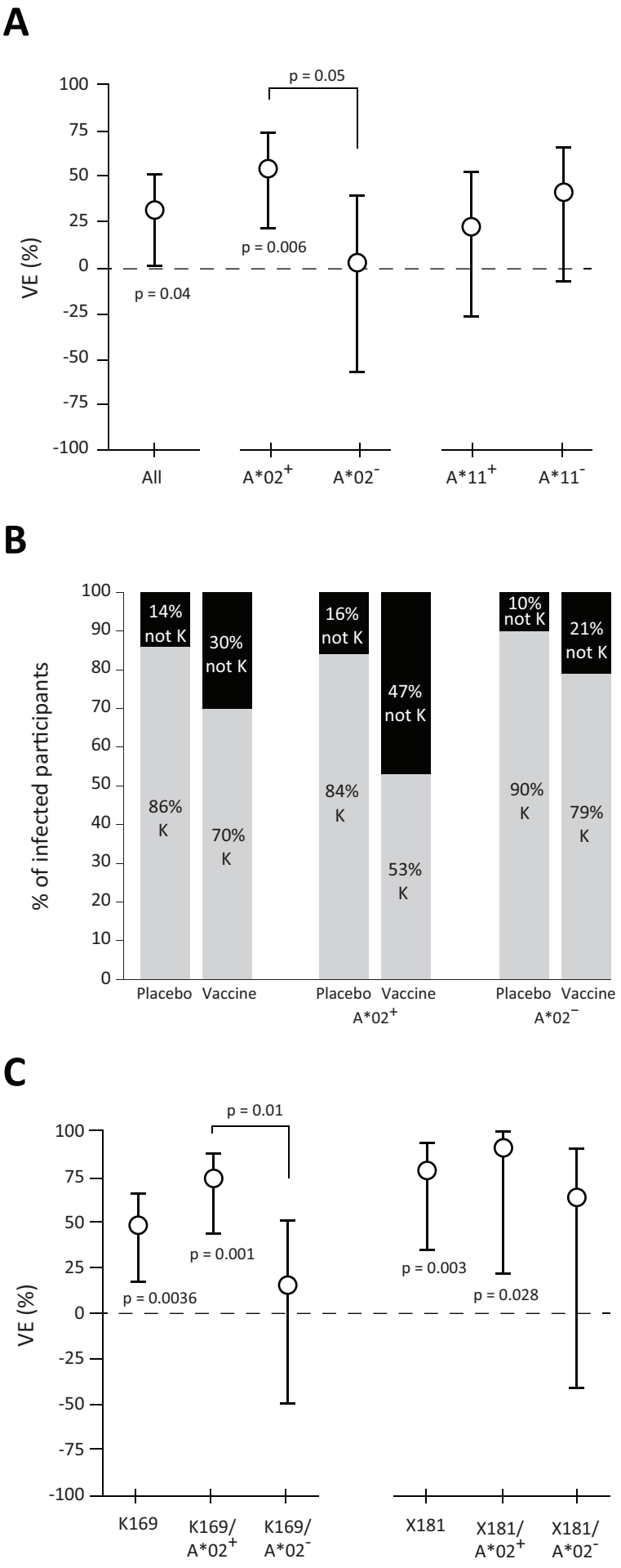

Figure 3. HLA A*02 modifies vaccine-induced immune responses and correlates of infection risk