

COMMENTARY



T cell-based strategies for HIV-1 vaccines

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ABSTRACT

Despite 30 years of effort, we do not have an effective HIV-1 vaccine. Over the past decade, the HIV-1 vaccine field has shifted emphasis toward antibody-based vaccine strategies, following a lack of efficacy in CD8+ T-cell-based vaccine trials. Several lines of evidence, however, suggest that improved CD8+ T-cell-directed strategies could benefit an HIV-1 vaccine. First, T-cell responses often correlate with good outcomes in non-human primate (NHP) challenge models. Second, subgroup studies of two no-efficacy human clinical vaccine trials found associations between CD8+ T-cell responses and protective effects. Finally, improved strategies can increase the breadth and potency of CD8+ T-cell responses, direct them toward preferred epitopes (that are highly conserved and/or associated with viral control), or both. Optimized CD8+ T-cell vaccine strategies are promising in both prophylactic and therapeutic settings. This commentary briefly outlines some encouraging findings from T-cell vaccine studies, and then directly compares key features of some T-cell vaccine candidates currently in the clinical pipeline.

ARTICLE HISTORY

Received 13 June 2019
Revised 19 August 2019
Accepted 5 September 2019

KEYWORDS

HIV vaccines; conserved region vaccines; mosaics; CD8+ T cells; immunotherapy

Introduction

Thirty-seven million people are infected with HIV, and AIDS continues to have devastating impacts throughout the globe.¹ Over the last two decades, improved access to treatment and prevention has led to substantial declines in the annual incidence of HIV-1 infections and AIDS-related deaths, but this has leveled off in recent years¹, and the ambitious goal of eliminating of AIDS by 2030 using currently available clinical strategies may be unattainable.² Vaccines have been the turning point for many epidemics,^{3,4} and an effective HIV-1 vaccine would be an invaluable addition to current prevention efforts. Engaging multiple arms of the immune response while targeting multiple epitopes may be the most efficient and effective path toward vaccine efficacy.



A case for a multicomponent HIV vaccine

The elicitation of broadly neutralizing antibodies (bNAbs)^{5–8} is often called the “holy grail”^{9,10} of HIV vaccine research, because bNAbs directly block infection of target cells, passive administration of bNAbs can protect against heterologous HIV infection,¹¹ and bNAbs can be a correlate of vaccine-elicited immune defense against other viruses.¹² HIV bNAb induction, however, is particularly challenging: bNAbs take years to develop in natural HIV infections, and they often have high levels of somatic mutation, insertions and deletions, and unusually long CDRH3 regions.^{13,14} Their target is the extraordinarily diverse Envelope protein, and their epitopes are frequently targeted in natural infections;¹⁴

consequently, bNAbs isolated from natural infections generally have limited cross-reactivity and dramatic differences in potency against heterologous viruses.¹⁵ Thus, even if vaccine elicitation of bNAbs is achieved, response to any one bNAb epitope is unlikely to provide universal sterilizing protection against circulating HIV-1.

In contrast, CD8+ T-cell-directed vaccines can target more conserved protein regions with greater cross-reactive potential and for which escape has greater fitness costs;^{16–25} moreover, vaccination can shift responses toward highly conserved epitopes that are infrequently presented in natural infection, which may be advantageous in a therapeutic setting.^{16,18,24,26} In addition, a new type of T-cell-based vaccine modality is being explored based on the observation that non-classical MHC-E restricted CD8+ T-cell responses elicited by SIV antigens delivered in a modified cytomegalovirus vector can clear SIV infections in over half of vaccinated macaques in an SIV challenge model.^{27,28}

The epidemiological success of an HIV-1 vaccine may ultimately depend both on protection from infection and on better immunological control of viremia among people with breakthrough infections (leading to improved clinical outcomes and reduced transmission). To achieve this, the integration of multiple beneficial vaccine approaches may be needed: bNAbs if they can be elicited; non-neutralizing antibodies, which were the correlate of protection in RV144, the only human clinical HIV-1 vaccine trial to show vaccine efficacy (a modest but statistically significant 31%^{29,30}); and CD8+ T cells. Each could contribute, and while vaccine strategies for cellular and humoral responses may need to be optimized independently,³¹ merging them may ultimately be required.

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T-cell vaccines

Vaccine-induced CD8⁺ T-cells responses associated with protective effects

Two HIV prevention vaccine trials with no efficacy nevertheless had intriguing T-cell response associations in follow-up subset analyses. The HIV Vaccine Trials Network 505 (HVTN505) trial used a DNA prime, recombinant adenovirus serotype 5 vector boost (DNA/rAd5) vaccine expressing a B-clade Gag, Pol, and Nef, and three gp145 Env proteins (clades A, B, and C).³² There was no reduction in HIV-1 acquisition or in viral load upon infection in the vaccine group,³³ but subset analysis showed a primary association between reduced infection risk and high-level Env-specific CD8⁺ T cell responses,³⁴ and, among low CD8⁺ T-cell responders, a further association with IgG responses.³⁵ The

HVTN505 vaccine, however, had a limited capacity to elicit broad T-cell responses. Only 56% of vaccinees made detectable CD8⁺ T-cell responses to Env, 28% to Gag, 10% each to Pol and Nef. The cross-reactive potential of these responses is unknown, as vaccine-matched peptides were used for response detection, but based on our analysis it is likely to be low. HVTN505 was conducted in the context of a B clade epidemic. We compared the vaccine proteins to B-clade sequences in the Los Alamos HIV database, assessing the coverage of all potential T-cell epitopes (PTEs), essentially all 9-amino acid length fragments, or “9-mers”.³⁶ PTEs in natural B-clade Gag or Pol sequences matched the vaccine 54% of the time. Even though 3 distinct natural gp145 Envs were incorporated into the HVTN505 vaccine cocktail³² (Figure 1), on average only 33% of the PTEs in a natural B-clade Env were matched by vaccine PTEs. Thus, given the specificity of effective CD8⁺ T-cell

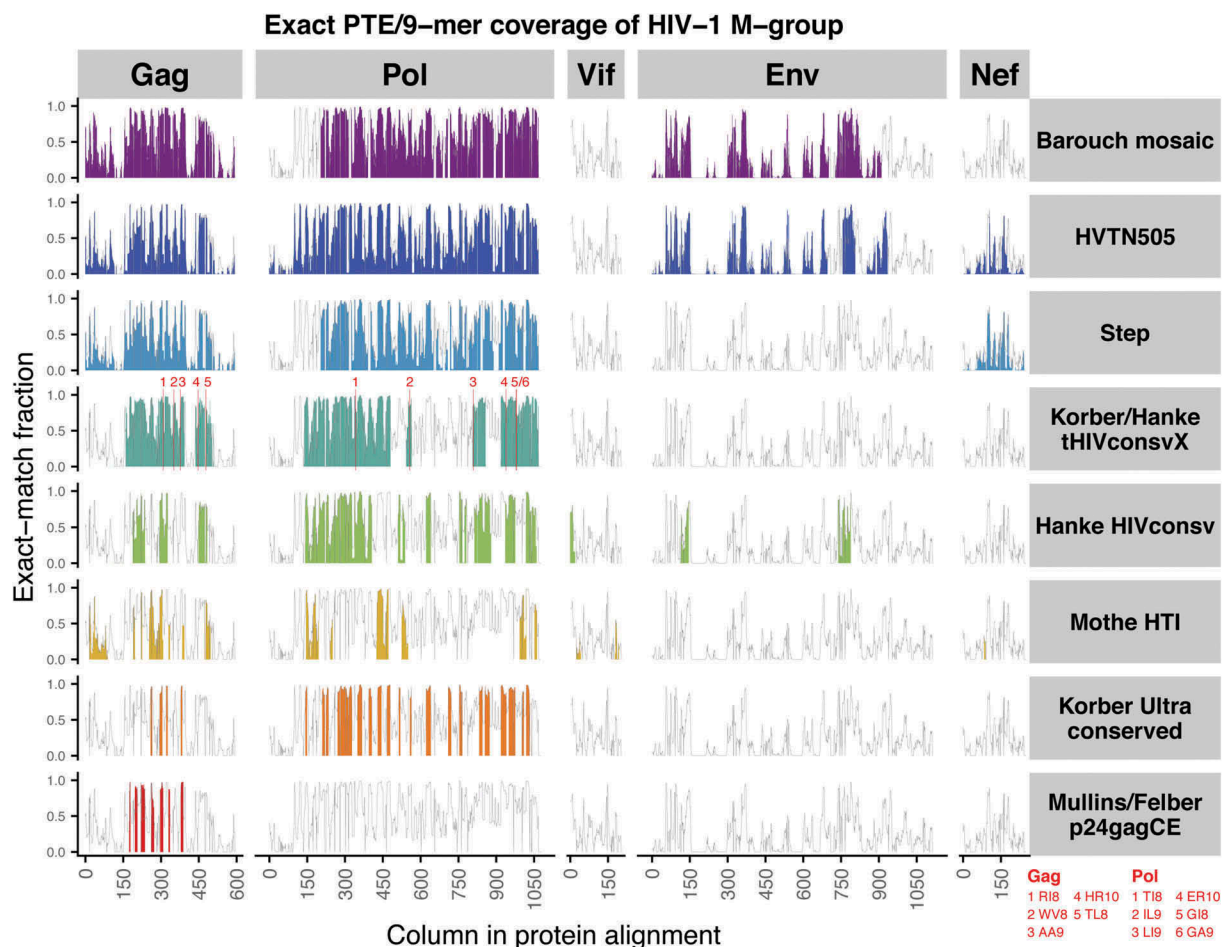


Figure 1. Exact 9-mer coverage of M group HIV by vaccine. For each graph, a position in the alignment marks the beginning of a 9-mer (a PTE); gray lines indicate the frequency in the HIV-1 M group alignment (see methods) of the most common form of 9-mer for sliding windows starting at positions 1–9, advancing to 2–10, then 3–11. For each 9-mer included in a vaccine, a colored bar indicates the summed frequencies of 9-mers that are present in (covered by) each vaccine. Brief descriptions of the vaccines: **Barouch mosaic**: two global mosaics for each of Gag and Pol, and Env;³⁷ these were the same inserts that were used in the MVA constructs in Barouch et al.³⁸ One of the Envs in the original vaccine³⁷ has been redesigned for stability and production reasons for use in the Adenovirus vector for the Imbokodo clinical trial.³⁸ **HVTN505**: natural HIV isolates: 1 each of Gag, Pol, and Nef, and 3 modified Envs (gp145). **Step 2008**: one Gag, Pol, and Nef protein, derived from natural HIV-1 B clade sequences.³⁹ **Korber/Hanke tHIVconsVX 2015**: 2 global mosaics spanning 6 highly conserved fragments of Gag and Pol.¹⁹ Red numbers mark 5 epitopes in Gag and 6 in Pol that are included in this conserved vaccine that was subsequently found to be strongly associated with viral control *in vitro* and *in vivo* (the key to these epitopes is at bottom right).^{40,41} **Hanke HIVconsV 2007**: 14 concatenated peptide fragments derived from different subtype consensus sequences (median length 40, range 27–130 amino acids (aa));¹⁷ **Mothe HTI 2015**: 16 fragments (median length 22.5, range 11–78 aa), concatenated with three joining alanine residues, based on regions that were preferentially targeted by individuals with low viral loads, published as European Patent EP2620446A1;²⁵ **Korber Ultra CE**: 40 highly conserved peptides (median length 18, range 14–22 aa), selected based on contiguous HIV-1 M group 9-mer coverage >80%, designed using the Epigraph tools,⁴² currently under study, and first published here; **Mullins p24 CE**: Two variants each, of 7 regions of Gag (median 18, range 12–24 aa), concatenated with 2–4 aa alanine-rich linkers.⁴³

responses,^{44,45} many of the observed CD8+ T-cell responses would have been immunologically silent against circulating viruses in the study population.

The Step vaccine trial tested the Merck rAd5 HIV-1 vector with genes encoding a natural B-clade Gag, Pol, and Nef protein. Although CD8+ T-cell responses were detected in 73% of vaccinees, the vaccine was not associated with decreased infection risk;^{46,47} instead, there was an unexpected increase in acquisition of HIV-1 among uncircumcised and/or Ad5-seropositive vaccinated men observed in a post-hoc subgroup analysis.⁴⁸ Similar findings were obtained in a second study conducted in South Africa.^{49,50} Still, there were indications that this vaccine imposed immune pressure at transmission: viruses isolated from vaccinees were genetically further from the vaccine antigens than viruses from the placebo group,^{51,52} particularly in Gag.⁵¹ Follow-up analyses of those infected found that reduced viral loads were associated with vaccine-induced CD8+ T-cell responses, with the lowest viral set points among those who made three or more responses to Gag.⁵³ However, only 5 of 72 (7%) people had 3 or more Gag responses, and only 37% had *any* detectable Gag CD8+ T cell responses.⁵³ As with effective antiretroviral therapy,⁵⁴ three or more responses with distinct targets may be important in terms of limiting selection for resistance.

Thus, despite the lack of overall protection in these human trials, the highest levels of CD8+ T-cell response were associated with protection from infection^{34,35} or reductions in viral load.⁵¹ Vaccines with improved antigen design and delivery strategies can elicit higher numbers of responses^{38,55} with greater cross-reactive potential.^{17,19,21,24,25,56} Furthermore, NHP SIV challenge studies have repeatedly found that CD8+ T-cell responses, particularly those targeting Gag, directly correlate with better viral control and survival.^{57–63}

Vaccine antigen designs to elicit enhanced CD8+ T-cell immune and humoral responses

The diversity of HIV is daunting: aligned HIV protein sequences vary in ~10–40% of amino acid positions,⁶⁴ and even the relatively conserved proteins (Gag and Pol) are highly variable at the epitope level. Both B-cell epitopes (often discontinuous, but spatially proximal) and T-cell epitopes (which are linear) directly involve ~9–12 amino acids (aa), and even single amino acid changes can confer relative or complete resistance. Various strategies have been employed to improve CD8+ T-cell vaccine antigens. Contenders include (i) full-length mosaic proteins, designed to better cover viral epitope diversity than natural strains and to maximize the number of epitopes presented by many different HLAs by using full proteins (note that the mere number of T-cell epitopes targeted is often correlated with a beneficial effect in NHPs^{36–38,55,58}); (ii) concatenation of large conserved protein regions that still retain high numbers of PTEs, while minimizing unnatural junctions and excluding variable epitopes;^{17,19,21} and (iii) multiple short peptides intended to more narrowly focus responses on preferred epitopes.^{20,25,43,65,66}

Figures 1–3 provide direct comparisons of vaccine candidates to illustrate differences in design strategy and in diversity coverage. Figure 1 illustrates the frequency of the most common 9-mer starting in each position in HIV database M group

(global) protein alignments as a measure of PTE diversity, highlighting the regions included in different vaccines and indicating the level of coverage provided by vaccine cocktails. Figure 2(a) reorders the maps in Figure 1, from best-conserved PTEs to least, to enable direct comparisons of vaccine lengths and overall levels of diversity coverage. Figure 2(b) provides an indication of how many PTEs are matched between the vaccine candidates and typical natural HIV strains, and includes immunological summaries of the proteomic region covered by each vaccine, in terms of inclusion of PTEs, known CTL epitopes, and the diversity of HLAs presenting those epitopes, all on a mean-per-natural-sequence basis. Figure 3 provides regional coverage comparisons between vaccine candidates across just Gag, as it is possibly the most critical target for protective T-cell responses against HIV-1, and the only protein targeted by all eight vaccine candidates discussed here (Figure 1). Colored bars show which of two vaccines has greater PTE coverage in the alignment. It is noteworthy that the Barouch mosaic Gag has substantial coverage advantages over the HVTN505 and Step immunogens, and is essentially identical or superior in coverage throughout the overlapping regions relative to all the other vaccines except for tHIVconsvX, which has slight coverage advantages in a few positions.

Mosaics are small sets of vaccine antigens that are computationally designed, using a genetic algorithm, to increase the presentation of common PTEs relative to natural proteins. A mosaic vaccine is a “cocktail” containing several complementary sequences that, in combination, provide nearly optimal coverage of PTEs circulating in a target population for a given cocktail size.³⁶ To achieve this, large sets of candidate mosaic proteins are generated from natural sequence sets via cycles of *in silico* recombination, and several (typically 2 or 3) of these candidates are selected from among these recombined sequences such that in combination they maximize PTE coverage. This selection criterion naturally tends to minimize PTE redundancy within a vaccine cocktail: once one form of a relatively conserved PTE is included in one component of the cocktail, other common forms of the PTE are favored for inclusion in other components. This criterion also minimizes the inclusion of rare amino acids and rare combinations of neighboring amino acids, which would favor type-specific vaccine responses. (This latter point is important: for example, any given natural Env protein contains, on average, 130 unique 9-mers that are so rare they are not repeated in any other sequence among the many thousands in the database alignments; any vaccine response targeting one of these very rare PTEs would likely be specific to that sequence alone, and therefore useless against real-world HIV exposure.) Mosaics can be full proteins^{38,55} or span shorter protein regions.^{19,21} The inclusion of multiple common epitope variants in mosaic vaccine cocktails has been shown experimentally to stimulate responses that recognize diverse natural forms of the epitope, sometimes beyond just those included in the vaccine cocktail.^{38,56} We typically optimize mosaics for vaccine coverage of 9-mers, the most common length of a CD8+ T-cell epitope,³⁶ and also the length of the core interaction region between CD4+ T-cell epitopes and class II molecules.⁶⁴ Although mosaics are artificially engineered to optimize PTE coverage, they are also locally (over short stretches) natural, and indistinguishable from natural proteins in an alignment. Mosaic

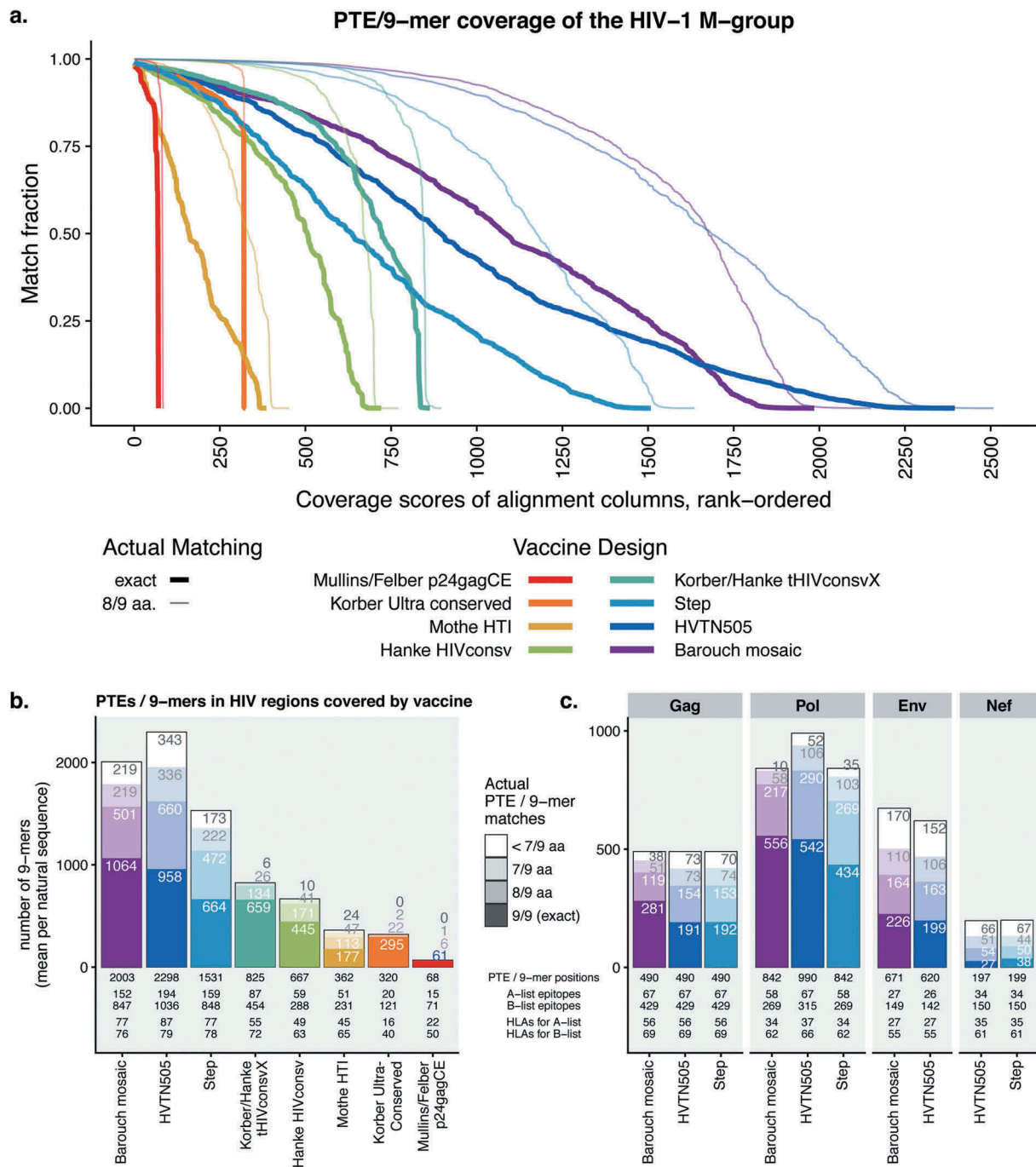


Figure 2. Summarized matching of PTEs/9-mers between natural M group HIV sequences and vaccines. (a). Coverage of 9-mers between the vaccine and natural sequence alignments, ordered by protein location in Figure 1, is reordered by coverage rank (proportion of natural-sequence 9-mers present at each alignment location) for each vaccine, to enable direct comparisons. Area under the curve indicates the total number of 9-mers matched. Solid heavy lines denote exact-match coverage frequencies (9/9 amino acids identical); lighter lines, sum of exact and 1-off matches (9/9 plus 8/9 amino-acids matches) as some T-cell responses may cross-recognize some epitope variants. The right end of each line indicates the total number of included PTEs. The slope of the left-to-right decline indicates the level of inclusion of increasingly poorly covered PTE/9-mers. (b and c). Counts of matched 9-mers (potential T-cell epitopes) in the HIV regions presented by each candidate vaccine, and the number of known epitopes included in the regions. For every sequence in a global M-group HIV-1 alignment, the counts of matching 9-mers in the region(s) presented by each vaccine were computed; the mean per-sequence values are shown (for exact, 8/9, and 7/9 amino-acid matches). The bars are annotated with numerical values (mean counts) for each match category. Several other values relating to the proteomic regions presented in each vaccine are shown in the numeric table below the bars: (i) the total number of HIV-1 PTEs/9-mers in the vaccine regions; (ii) the number of distinct peptide regions that serve as an epitope for one or more HLA class I molecules based on the Los Alamos HIV database "A-list" of best defined CD8+ T-cell epitopes, (iii) the number of distinct peptides that are recognized in the context of one or more HLAs on the B-list, a comprehensive listing of all epitopes available at the Los Alamos HIV Database; (iv) the number of unique HLAs, primarily 4-digit, reported to restrict the A-list epitopes in the vaccine regions; and (v) the number of unique 2-digit HLAs reported for the B-list epitopes in the vaccine regions. (b). Combined values for all regions of each vaccine. Note that the number of known HLAs is greater for the p24GagCE vaccine than the Ultra Conserved, but the total number of PTEs is much greater in the Ultra Conserved. This is because many more epitopes from natural infection have been defined for Gag than Pol; part of the intent of the inclusion of Pol in the Ultra Conserved vaccine was to elicit *de novo* responses by vaccination to otherwise poorly targeted but very highly conserved regions. (c). As panel b., with separate values for individual protein components of the three vaccines that include essentially full-length proteins (Barouch mosaic, HVTN505, and Step 2008). HVTN505 was the only vaccine to include the highly conserved Protease region of Pol (Figure 1), which increases the relative number of conserved epitopes present compared with the Step and Barouch mosaic vaccines.

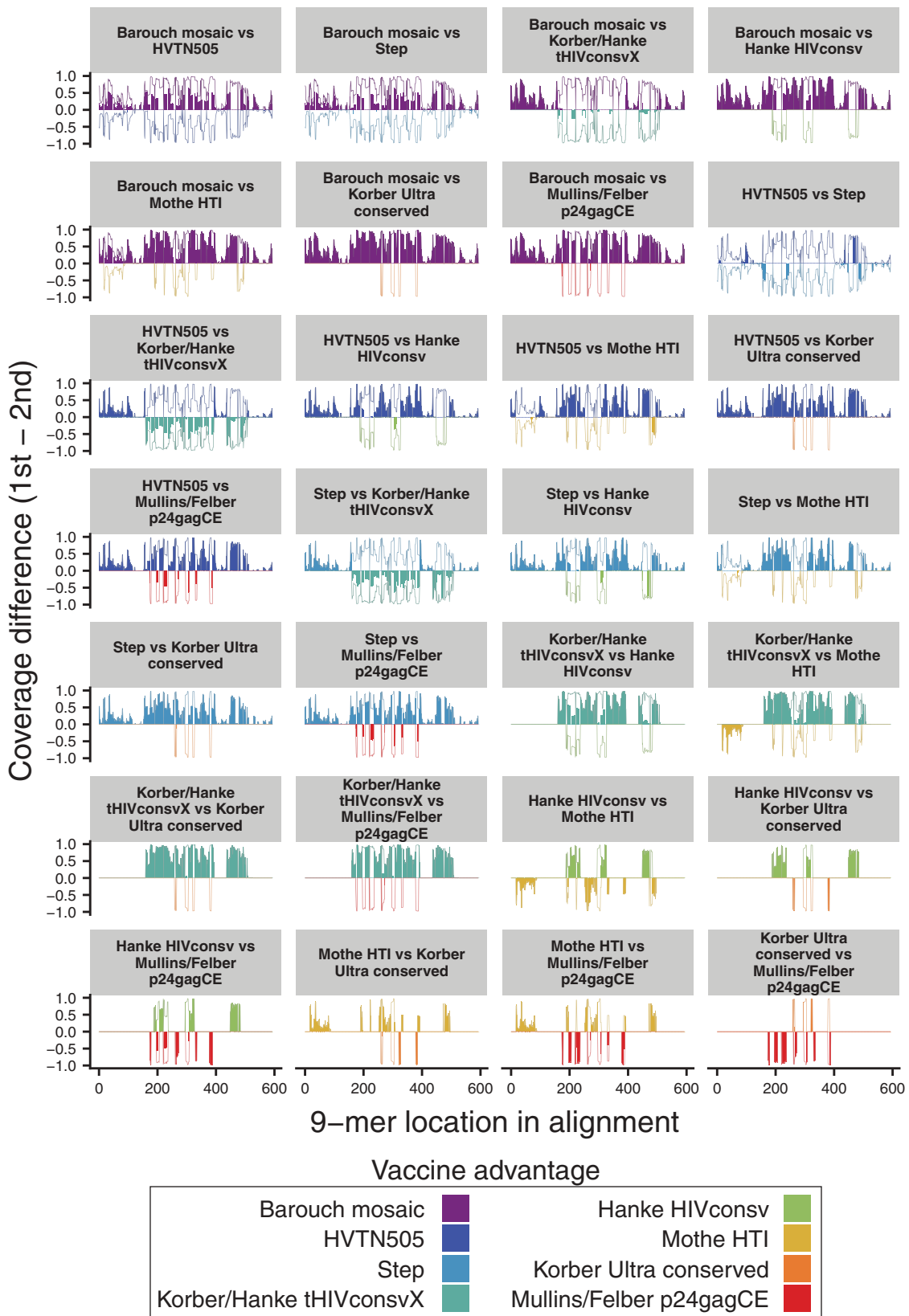


Figure 3. Matching of PTEs/9-mers compared between vaccine candidates (Gag only). Each panel shows a pairwise comparison of two vaccines, one above the x-axis, one below. Coverage by position is shown as thin lines; differences between the two vaccines are shown as solid bars (colored by vaccine as in other figures). Bars and coverage lines *above* the axis relate to the first-named vaccine (i.e. 'x' in 'x vs y'); bars and lines *below* the axis relate to the second-named vaccine ('y' in 'x vs y'). Solid bars mark positions where one vaccine has greater coverage (and the magnitude of the difference); areas enclosed by lines, but not containing solid color, indicate regions where both vaccines present 9-mers and the coverage is essentially identical.

proteins are typically stable and highly immunogenic,^{38,55,67} and Env mosaics are generally well-folded in that they bind neutralizing antibodies with discontinuous epitopes and elicit antibodies that interact with natural strains.⁶⁷

In NHP studies, HIV-1 mosaics induce significantly higher numbers of more cross-reactive CD8+ and CD4+ T-cell responses than do natural protein vaccines.^{38,55} Furthermore, Env mosaic vaccines have elicited both non-neutralizing antibodies and total T-cell vaccine responses that correlate both with protection from infection in SHIV challenge models and better viral control and survival upon breakthrough infection.^{37,58} An HIV-1 mosaic Gag, Pol, and Env vaccine was tested for immunogenicity and protection in an NHP SHIV challenge study testing delivery strategies, and an Ad26 prime/Ad26 plus gp140 boost delivery was both the most immunogenic and conferred the greatest level of protection, with a 94% reduction in per-exposure acquisition risk.³⁷ The impact of the HIV Gag and Pol vaccine responses could not be determined in this NHP model, as the SIV analogs of these proteins in SHIVs are too highly diverged for cross-recognition, so any observed protection in this model would have been Env driven. The principal immune correlates of protection were ELISA (antibody) and total ELISPOT (T-cell) responses. This NHP study was run in parallel with the human “APPROACH” Phase I study. The vaccines were safe, and the Ad26 prime/Ad26 plus gp140 boost delivery was also most immunogenic in humans, and the ELISA and ELISPOT response levels were comparable in macaques and humans.³⁷ Based on these results, the Phase 2b (Imbokodo/HVTN705) clinical trial in Southern Africa was initiated; compared to the natural HIV proteins used in the HVTN505 and STEP trials, these mosaic vaccine antigens have a much greater potential to induce more CD8+ T cell responses with greater cross-reactivity (Figures 1–3). As of this writing (June 2019), the Imbokodo trial is now fully enrolled, a Phase 3 study (Mosaico; HVTN706) is slated to start this summer, and a therapeutic vaccine phase I is also underway (NCT03307915). (Complete listings of vaccine trials underway and completed for evaluating therapeutic vaccines can be found at the Treatment Action Group website (www.treatmentactiongroup.org/cure/trials)).

Conserved-region vaccine approaches include larger stretches of relatively conserved protein regions, while conserved-element approaches incorporate shorter peptides spanning either very highly conserved or beneficial epitopes. The first polypeptide HIV vaccine to present directly concatenated epitopes was immunogenic in mice and macaques,⁶⁸ but elicited very poor responses in humans.⁶⁹ The second included 21 desirable HIV epitopes linked by bioinformatically designed spacers intended to improve epitope processing.⁷⁰ Despite promising immunogenicity in vaccinated HLA-transgenic mice,⁴² in a human Phase I study only 1 of 42 vaccinated adults made a detectable ELISPOT response, to a single non-HIV vaccine epitope.⁷¹ Because of the concerns these studies raised, Hanke and colleagues designed and tested the first HIV conserved-region vaccine, HIVconsv, containing 14 larger protein regions (Figure 1), each region spanning many conserved epitopes presented by many distinct HLAs (Figure 2).¹⁷ HIVconsv is highly immunogenic in humans and induces novel responses to conserved epitopes.^{16,26,72}

A second-generation conserved region vaccine, tHIVconsvX (Figures 1–3) used computational strategies to systematically

define conserved PTEs across the HIV proteome. The regions in tHIVconsvX are substantially more conserved than those in HIVconsv (Figures 1–3) and were restricted to conserved regions known to encompass only epitopes associated with lower viral loads.¹⁸ To minimize responses against unnatural epitopes formed at regional junctions,¹⁶ tHIVconsvX included only 6 instead of 14 regions, and, to prevent junctional-epitope boosting, separate constructs with different fragment orders were used for priming and boosting.¹⁹ tHIVconsvX encoded 895 aa, designed to occupy the maximal insert length readily accommodated by the intended vector (~900 aa), to maximize both the number of PTEs and the number of HLAs presenting peptide regions included in the vaccine (Figure 2); thus, it is longer than HIVconsv’s 806 aa. Since even conserved regions of HIV are somewhat variable, two complementary global mosaics were designed to improve PTE coverage of the selected regions. (Yang and colleagues²¹ used three mosaics to span the conserved regions they favored, instead of just two – the choice weighs vaccine complexity with more complete diversity coverage.) tHIVconsvX was highly immunogenic in mice, and in a Japanese treatment-naïve HIV+ population, natural responses to epitopes within the vaccine were associated with lower viral loads and higher CD4+ T-cell counts.¹⁹ Five specific epitopes were identified in Gag,⁴⁰ and six in Pol,⁴¹ that suppress HIV-1 both *in vivo* and *in vitro* (Figure 1, red annotations); of note, regions containing these potentially advantageous conserved epitopes are usually missing from more tightly focused conserved-region designs (Figure 1). Components of the tHIVconsvX vaccine are currently being tested in a Phase I clinical trial (NCT03844386).

Other CD8+ T-cell vaccine designs involve more precise focusing on local regions that are deemed of particular value, to shift the responses to these regions and avoid so-called decoy epitopes.^{16,18,24} The HTI vaccine includes 16 short regions, joined by poly-Alanine-enriched linkers that span epitopes associated with lower viral loads in natural infection.^{18,25} While both HTI and tHIVconsvX include potentially beneficial epitopes defined by Mothe et al.,¹⁸ they have different emphases in selecting regions for inclusion. The HTI vaccine included all regions enriched for potentially beneficial epitopes, including the quite variable p17 and Vif regions that were excluded from tHIVconsvX because of their diversity (Figures 1 and 3). Because tHIVconsvX includes a bivalent mosaic in a vaccine cocktail, the diversity coverage of the overlapping regions is increased (Figure 3). The HTI vaccine was highly immunogenic when delivered as a DNA.HTI prime with a modified virus Ankara 325 (MVA.HTI) boost both in mice and in Rhesus macaques,²⁵ and it is currently being tested in a phase I clinical trial (NCT03204617).

The p24CE (p24-conserved element) vaccine includes highly conserved regions in p24, again joined by linkers, with two variant peptides for each included region. The vaccine has been described as covering 99% of HIV natural diversity in the regions presented, but we find that on average, 10% of the PTEs spanning these regions in natural HIV-1 strains are not matched by the vaccine (Figure 2). When p24CE is given as a DNA vaccine, CD8+ T cell responses targeting the CEs are enriched, in both mice⁴³ and macaques,²⁴ compared to responses to a full p55Gag DNA vaccine. A p24CE vaccine boosted by p55Gag also shifts the

responses to the conserved regions²⁴ and a phase I trial of this combination is underway (NCT03560258).

Finally, we present coverage for our Ultra Conserved peptide design, which includes short regions of variable length in both Gag and Pol (minimum length 14 aa, to include at least 5 PTEs). Despite meeting the conservation criteria, several short stretches of Env were excluded from our Ultra Conserved design, because of past associations with Env CD8+ T-cell responses and poor viral control,^{18,73,74} as well as for the practical reason that excluding Env would enable sequencing to be restricted to the 5' half of the genome in future clinical studies (Figure 1). This design is currently being evaluated in a dendritic-cell-based vaccination strategy (NCT03758625),^{75,76} which obviates the need to concatenate epitopes into polypeptide strings in a therapeutic setting.^{69,71}

An additional novel alternative approach for CD8 + T-cell vaccine design proposes a selection of regions for inclusion informed by structure-based network analysis, which identifies the regions of the highest importance to HIV-1 protein tertiary and quaternary structure.⁶⁶ CD8+ T cell targeting of these highly networked regions is associated with control of HIV in natural infections.⁶⁶

Conclusions

Multiple well-reasoned and distinctive strategies for CD8 + T-cell-directed vaccine antigen design are currently being pursued. These different designs reflect different weightings of factors that may be important for success: the number of known epitope responses and distinct HLAs covered, diversity coverage, conservation, exclusion of variable decoy epitopes, numbers of unnatural junctions, and overall vaccine complexity. Direct experimental comparisons of antigen design approaches are complicated by different vaccine delivery strategies, different ways of assessing responses, and the fact that the extent of cross-reactivity of a response is seldom assessed; thus, we do not yet know which of these strategies will deliver the best outcomes in the prevention or therapeutic scenarios. Animal studies suggest that *all* of the current candidates offer potential benefits over the vaccines used in the first human clinical trials, Step and HVTN 505, that showed no overall efficacy but did identify cellular immune correlates of vaccine protection. The HVTN 702 trial, now in progress, is exploring whether results from the RV144 trial (a 31% reduction in new infections associated with vaccine-elicited non-neutralizing antibodies, in a Thai cohort), can be translated to a Southern African C-clade epidemic setting, with enhanced protective effects. The HVTN 705 Imbokodo Phase 2b efficacy vaccine trial, also underway and now fully enrolled, will test if non-neutralizing antibody responses and CD8+ T-cell responses against whole protein Gag/Pol/Env mosaics inserts (Figure 3 (b)),^{36,38} delivered using an Ad26/Ad26 gp140 boost strategy optimized in an NHP challenge model,³⁷ can contribute to either protection from infection or post-infection viral control. Other CD8+ T-cell vaccine designs may also be useful in a prevention setting but may be of particular benefit as therapeutic vaccines, by enabling a refocusing of the immune response to conserved or beneficial epitopes.^{24,26} Iterative testing could be used to directly compare the most promising of these alternative design strategies.⁷⁷

In our opinion, the “Holy Grail” of HIV vaccine research is, or should be, a vaccine that works. bNAb induction offers a promising approach toward this goal, albeit with significant challenges. Even if consistent induction of bNAbs with heterologous breadth can ultimately be achieved, the most efficient route to vaccine efficacy may nevertheless be an integrated strategy that combines optimized vaccine induction of bNAbs, non-neutralizing antibodies, and T-cell responses. The main advantage of CD8+ T-cell designs may be their timing. The immunogen strategies described in this review focus on CD8+ T-cell vaccine antigen design, to improve specificity and diversity coverage, but the right means of immunogen delivery will also be needed to induce sufficient frequencies, breadth, functionality, homing, longevity and proliferative capacity of T cells to achieve HIV-1 control.

Methods

Vaccine coverage comparisons used data from HIV-1 M group sequence filtered web alignments from the Los Alamos HIV Database (www.hiv.lanl.gov) circa May 2019. These include one sequence per subject, each alignment representing thousands of HIV infections. They were used for vaccine coverage comparisons, based on analysis tools available through mosaic and epigraph tools at the Los Alamos HIV database.^{36,78,79} Summaries of known CD8+ T cell epitopes within vaccine regions are available through the LANL HIV Immunology Database. The A list presents the most precisely experimentally defined epitopes; the B list is much more comprehensive, but less detailed. A web-based tool for the analyses and plots included here will be made available from the LANL HIV Database at https://hiv.lanl.gov/content/sequence/VACC_COVER/vacc_cover.html.

Disclosure of potential conflicts of interest

We did the computational designs for, and so are on patents for, several vaccines discussed here (BK and WF Imbokodo) (BK, tHIVconsV) (BK, Ultra CE).

Funding

This work was supported by the National Institute of Allergy and Infectious Diseases [UM1-1AI126603].

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