ANNUAL REVIEWS

Annual Review of Immunology

Vaccines and Broadly Neutralizing Antibodies for HIV-1 Prevention

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Annu. Rev. Immunol. 2020. 38:673–703

The *Annual Review of Immunology* is online at immunol.annualreviews.org

https://doi.org/10.1146/annurev-immunol-080219-023629

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Keywords

vaccine, broadly neutralizing antibody, HIV-1, viral diversity, prevention, bNAb

Abstract

Development of improved approaches for HIV-1 prevention will likely be required for a durable end to the global AIDS pandemic. Recent advances in preclinical studies and early phase clinical trials offer renewed promise for immunologic strategies for blocking acquisition of HIV-1 infection. Clinical trials are currently underway to evaluate the efficacy of two vaccine candidates and a broadly neutralizing antibody (bNAb) to prevent HIV-1 infection in humans. However, the vast diversity of HIV-1 is a major challenge for both active and passive immunization. Here we review current immunologic strategies for HIV-1 prevention, with a focus on current and next-generation vaccines and bNAbs.

INTRODUCTION

The HIV-1 epidemic continues to threaten global health and economic development, with 1.8 million new HIV-1 infections in 2017 alone (1–3). Behavioral and biomedical interventions have reduced HIV-1 incidence since the height of the global epidemic, but the impact of these interventions has largely plateaued in recent years. For example, global HIV-1 incidence decreased only 5% between 2016 and 2017, despite nearly $10 billion spent on HIV-1 prevention efforts over this period (1–3).

Until recently, HIV-1 prevention efforts have focused primarily on behavioral interventions, male circumcision, and antiretroviral therapy (ART) for preventing mother-to-child transmission and postexposure prophylaxis (4–12). Randomized controlled trials have now established that transmission does not occur between serodiscordant couples if the HIV-1-infected partner has an undetectable viral load ("U=U"), and that uninfected individuals are far less likely to acquire HIV-1 if they take ART, which is termed preexposure prophylaxis (PrEP) (13–28). In particular, studies have demonstrated the efficacy of tenofovir disoproxil fumarate and emtricitabine (TDF-FTC)-based PrEP regimens in reducing HIV-1 transmission in men who have sex with men, transgender women, heterosexual men, and injection drug users (20–28). As a result, PrEP has become a major component of HIV-1 prevention efforts, and current clinical development efforts are focused on long-acting injectable antiretroviral drugs, implantable devices, and vaginal rings.

There are a number of limitations to treatment-as-prevention and PrEP strategies. Most importantly, access and compliance are major challenges for widespread implementation of PrEP. For example, women in sub-Saharan Africa at risk for HIV-1 infection may have suboptimal access to medical care and may face stigma for possession of antiretroviral pills. Long-term use of TDF-FTC can also be associated with renal toxicity and osteoporosis, and patients need to be screened for coinfection with hepatitis B and C before receiving TDF-FTC due to antiviral drug interactions (22, 29–31). Alternative PrEP agents, such as long-acting injectable integrase inhibitors, will likely improve compliance challenges but may lead to additional toxicity concerns during pregnancy (32). There is also a risk that PrEP might be started in patients who are already acutely infected with HIV-1, leading to a suboptimal regimen that leads to drug resistance.

Given the limitations of current biomedical options for HIV-1 prevention, there is a need for the development of new HIV-1 prevention methods. Recent data have generated renewed enthusiasm for immunologic approaches, including active immunization with vaccines and passive immunization with broadly neutralizing antibodies (bNAbs). Historically, vaccines have been the most effective intervention for controlling global pandemics. Five large clinical efficacy trials are currently underway to evaluate both active and passive immunization strategies for HIV-1 (**Table 1**). These trials are testing the clade C canarypox ALVAC (Env/Gag/Pro)/gp120

Table 1 Current efficacy trials of vaccines and broadly neutralizing antibodies for HIV-1 prevention

Trial	Phase	Product type	Product description	Clinical trial
HVTN 702 Uhambo	2b/3	Vaccine	Canarypox vector expressing clade C Env/Gag/Pro + bivalent clade C gp120 with MF59 in sub-Saharan Africa	NCT02968849
HVTN 703 AMP	2b	Antibody	VRC01 in sub-Saharan Africa	NCT02568215
HVTN 704 AMP	2b	Antibody	VRC01 in Americas and Europe	NCT02716675
HVTN 705 HPX 2008 Imbokodo	2b	Vaccine	Trivalent Ad26 vector expressing mosaic Env/Gag/Pol + clade C gp140 with alum in sub-Saharan Africa	NCT03060629
HVTN 706 HPX 3002 Mosaico	3	Vaccine	Tetravalent Ad26 vector expressing mosaic Env/Gag/Pol + bivalent mosaic/clade C gp140 with alum in Americas and Europe	NCT03964415

vaccine (HVTN 702), the global mosaic Ad26 (Env/Gag/Pol)/gp140 vaccine (HVTN 705/706; HPX 2008/3002), and the bNAb VRC01 (HVTN 703/704). In this article, we review active and passive immunization strategies for HIV-1 prevention and discuss the challenge of global HIV-1 diversity for these efforts.

GLOBAL HIV-1 SEQUENCE DIVERSITY

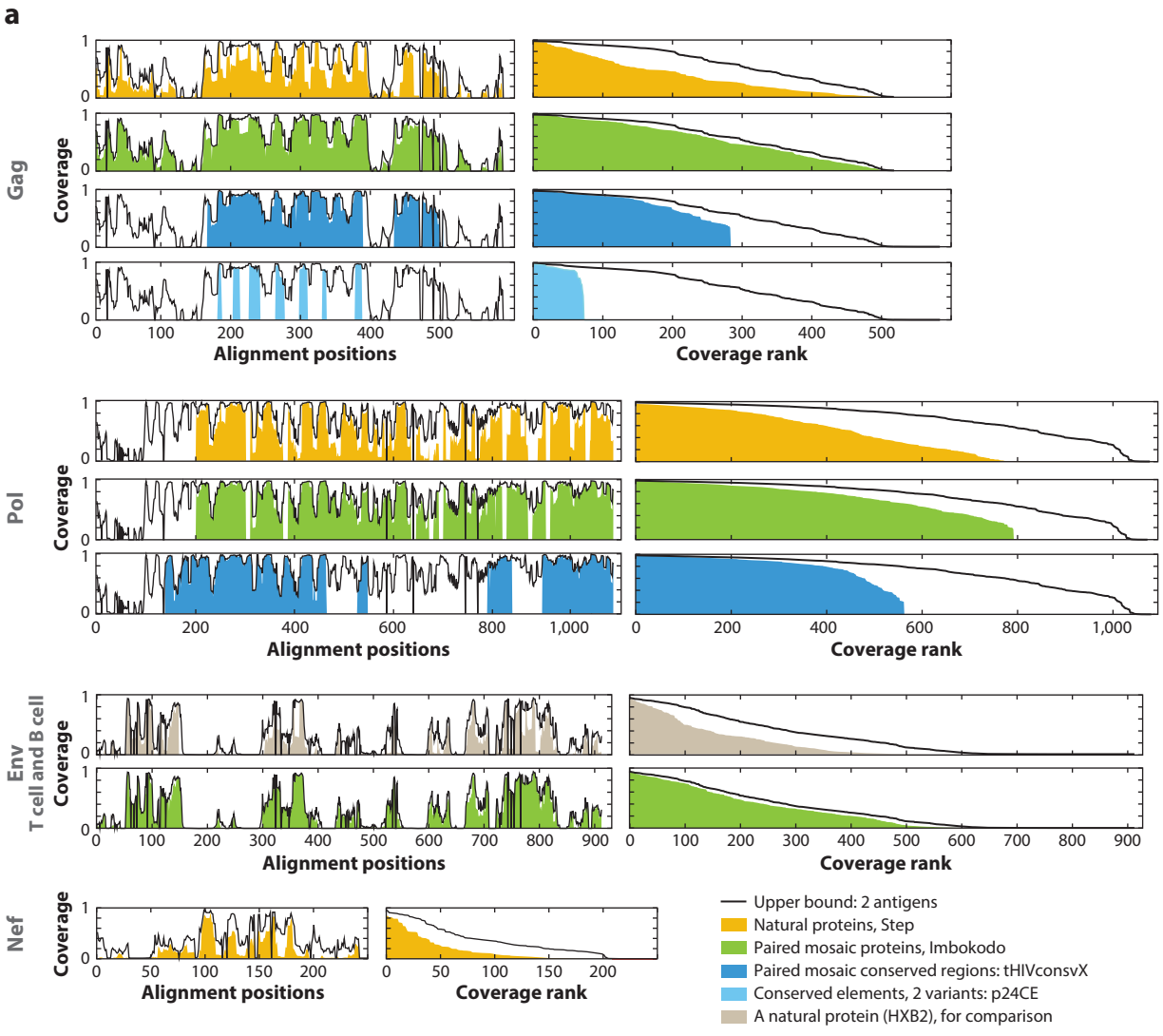
The primary target for HIV-1-specific antibody responses is the surface envelope glycoprotein (Env), which exhibits profound sequence diversity. All HIV-1 proteins are under immune pressure during chronic infection and are highly variable, but the diversity of Env is greater than Gag and Pol, which are good targets for T cell responses (**Figure 1*a***). The diversity of HIV-1 results from rapid virus replication, the mutation-prone reverse transcriptase, the propensity for recombination and insertion and deletion events (indels), and the selection of escape variants from immune selection pressure. During the early expansion of the HIV-1 epidemic in Africa, major clades were established, and based on their phylogenetic relationships, a nomenclature to describe these clades (A–K) was defined (33). These major clades persist, and some clades dominate regionally (e.g., C in southern Africa, B in the United States), while others are very rare; the geographic distribution of these clades in various regions of the world is shown in **Figure 1*b***.

In a comparison of HIV-1 Env protein sequences from clades A–D in the HIV-1 sequence compendium alignment, the within-clade median difference between Env amino acid sequences was 22% (quartiles 20–24%, range 15–29%), while the between-clade median difference was 27% (quartiles 26–29%, range 22–44%). HIV-1 continues to diversify within clades, and on the time scale of decades, the within-clade cross-reactive neutralization potency of sera from natural infection has diminished (34, 35), suggesting that the challenge of HIV-1 diversity for immunologic responses is worsening over time. HIV-1 evolution is further complicated by recombination. Some interclade recombinants [called circulating recombinant forms (CRFs) (33)] have expanded into major epidemic lineages in their own right (36), such as CRF01 (an A/E recombinant), which is dominant in Southeast Asia, and CRF02 (an A/G recombinant), which is dominant in West Africa (**Figure 1*b***). Some less common intersubtype recombinants are only involved in local transmission chains, and so although they are identified as CRFs, they are of more limited epidemiological importance. There are also many recombinants that have unique breakpoints, particularly in geographic regions where multiple clades cocirculate, e.g., A and D clades in Uganda (37).

Intersubtype variability also means that different subtypes and CRFs can have very distinctive neutralization profiles, which is an important consideration for both active immunization with vaccines and passive delivery of bNAbs, and thus heterologous breadth does not necessarily imply global applicability (35, 38). For example, CRF01 strains, which are common in Southeast Asia, are highly resistant to bNAbs targeting Env variable region 3 (V3), whereas clade B strains, which are common in North America and Europe, often have reduced sensitivity to bNAbs that target variable region 2 (V2) (38).

HIV-1 SEQUENCE DIVERSIFICATION DURING INFECTION

HIV-1 evolution within infected individuals begins soon after infection. The sequence diversity that arises results from continuing cycles of immune response and escape (39–44), and thus it is directly relevant to immunologic strategies to prevent HIV-1 infection. Structurally, this diversity is manifested as both variable amino acids and glycans on the HIV-1 Env trimer (**Figure 2*a*,*b***). Such variable positions are found in virtually all bNAb target epitopes (**Figure 2*a***); e.g., bNAbs targeting the CD4 receptor binding site (CD4bs) contact positions in the highly variable V5 loop.



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

HIV-1 global diversity and vaccine antigen design. (*a*) Coverage of linear HIV-1 diversity by four vaccine antigen designs: natural proteins (*gold*), paired mosaic proteins (*green*), paired mosaic and conserved region peptides (*dark blue*), and conserved region peptides (*light blue*). Sequence database alignments for Gag, Pol, Env, and Nef were used to explore the extent of coverage of linear epitope global diversity that is achieved by these representative vaccine antigens. Peptide fragments that are nine amino acids in length (9-mers) were included as a proxy for linear T cell epitopes. If a vaccine response to an epitope has a perfect match for that epitope in a circulating variant, it is very likely that the vaccine will cross-react with that variant. If it is mismatched, the vaccine likely either will not react at all or will react with reduced potency. The graphs on the left span each protein, starting with the 9-mer beginning at position 1 (positions 1–9), shifting to the 9-mer starting at position 2 (positions 2–10), and so on. The black line tracks the maximum coverage that can be achieved by a bivalent (two-antigen) vaccine for a given 9-mer, and represents the sum of the two most common variants of each 9-mer sequence in each column of 9-mers. Given that overlapping 9-mers often have different preferred amino acids in a given position, the upper bound cannot be completely achieved in an assembled protein. The height of the colored region at any position shows the extent of the 9-mer population coverage that is actually achieved by each vaccine. The graphs on the right show the same information reordered, such that potential T cell epitopes (PTEs) with the highest-to-lowest coverage are shown left to right in descending order. Here we illustrate four T cell antigen design concepts: (*i*) Natural proteins, for example the antigens used in the Step trial. The Step vaccine spanning Gag, Pol, and Nef is shown in gold (74). This vaccine did not confer overall protection, and although the majority of people developed immunologic responses, only a small number of responses per person were elicited by this vaccine, and PTE diversity coverage by the vaccine was low, so that responses had limited cross-reactivity for incoming viruses. (*ii*) A computationally designed complementary mosaic pair of proteins that optimizes PTE coverage. The vaccine antigens were used in the nonhuman primate studies that led to the Imbokodo clinical efficacy trial (63, 66) and are shown in green. Note that the green area nearly approaches the black line (the reason it does not do this in Pol on the right is that not all of Pol was included in the vaccine; the coverage is excellent in the parts of Pol that were included, as shown on the left). (*iii*) A conserved region vaccine that includes moderately large fragments of relatively conserved regions spanned by paired mosaics (tHIVconsvX, in *blue*) (95). (*iv*) A conserved element vaccine (p24CE) that is focused on very highly conserved short fragments of HIV-1 located within p24 (92, 99). (*b*) Geographical distribution of clades and circulating recombinant forms (CRFs) worldwide (*left*), in Asia (*center*), and in Africa (*right*). Distribution of subtypes is shown as pie charts with each clade and CRF represented by a different color, shown in the legend. The Geography Search Interface on the Los Alamos National Laboratory HIV Sequence Database was used to generate these figures (June 2019) (**https://www.hiv.lanl.gov/components/sequence/HIV/geo/geo.comp**).

Thus, we have previously argued that bNAbs do not derive their breadth from primarily recognizing conserved epitopes; rather, bNAbs have been selected in vivo to tolerate much of the natural diversity, likely resulting from antibody-Env coevolution (40, 45–47) (**Figure 2*b***). Recombination is also rampant in chronic infections (48) and during viral rebound upon ART interruption (49, 50).

Another aspect of Env variability is the immense length and sequence variation of the hypervariable sections of the variable loops V1, V2, V4, and V5 (**Figure 2*c***). Indels are extremely common in these regions; thus, they cannot be readily aligned, even in chronic infections that were initiated by single founding viruses. Therefore we have advocated for alignment-free characteristics of hypervariable regions, such as their length, net charge, and number of glycans, when considering the impact of these regions on antibody sensitivity (38). For example, M-group Envs show a median V1 hypervariable loop length of 22 amino acids, though the loop length can actually range from 6 to 58 amino acids. Such hypervariable regions can interact with several bNAbs (e.g., hypervariable V1 with V3 glycan bNAbs, hypervariable V2 with V2 apex bNAbs, and hypervariable V5 against CD4bs bNAbs), and they can have a profound impact on bNAb sensitivity (38).

VACCINES THAT GENERATE FUNCTIONAL NONNEUTRALIZING ANTIBODIES

Historically, vaccines have been critical tools for ending viral epidemics (51). The scientific challenges in the development of a prophylactic HIV-1 vaccine, however, are unprecedented, including the vast diversity of global HIV-1 and the difficulties in inducing broadly reactive neutralizing antibodies by vaccination. Accumulating data from clinical and preclinical studies suggest that functional nonneutralizing antibodies (nNAbs) may provide partial protection

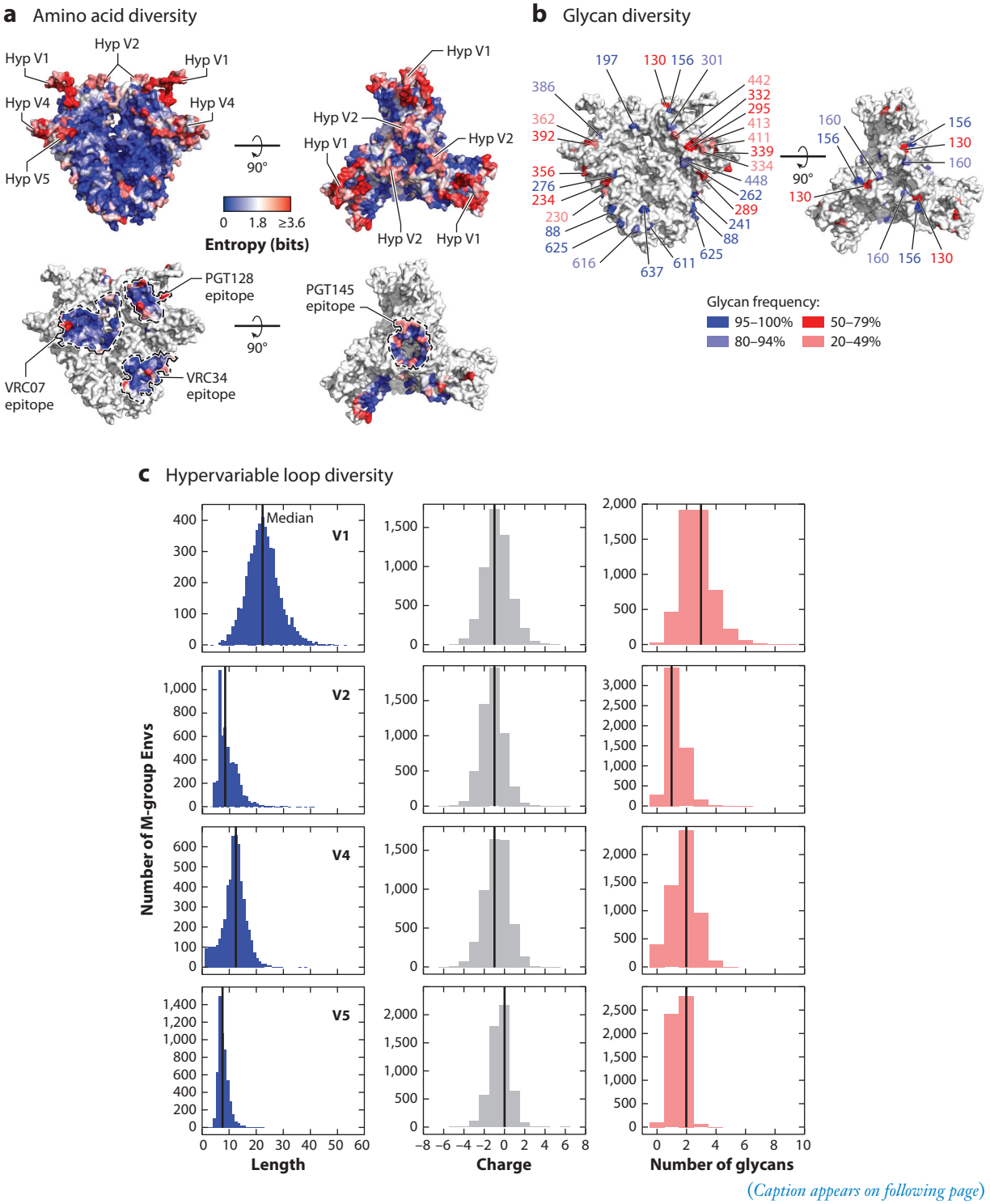


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Figure 2 (*Figure appears on preceding page*)

HIV-1 Env diversity. (*a*) Amino acid diversity. The images on top show the global amino acid diversity per site mapped onto a trimeric Env crystal structure (PDB: 5FYJ from Reference 171). Each site on the trimer is color-coded according to the diversity as measured by sequence entropy (172); dark blue indicates highly conserved sites, while red indicates highly variable sites. The regions of high diversity are typically the hypervariable (hyp) V1, V2, V4, and V5 loops, indicated on the structure. The images on the left show the side view of the Env trimer with the apex on the top and the viral membrane on the bottom, and the images on the right show the view looking down the trimer apex. The images on the bottom highlight the sequence diversity for epitopes of representative bNAbs from four bNAb classes of interest: CD4 binding site (VRC07), V2-apex (PGT145), V3 glycan (PGT128), and fusion peptide (VRC34). Epitope sites are defined as those within 8.5 Å of bNAbs calculated using the following published structures: PGT128 (PDB: 5C7K) (173), PGT145 (PDB: 5V8L) (174), VRC07 (PDB: 4OLW) (133), and VRC34 (PDB: 5I8H) (125). (*b*) Glycan diversity. Mapping of variable and conserved glycan sites on the trimer structure (same views as in panel *a*). Glycan sites are color-coded according to frequency in M-group. The hypervariable loops carry glycans; however, given their high sequence and length variability, an alignment and thus numbering of sites in such regions are not meaningful. Thus, glycan sites in the hypervariable loops are ignored. (*c*) Hypervariable loop diversity. Variation in hypervariable loop characteristics are shown for V1, V2, V4, and V5, from top to bottom. (V3 is relatively conserved and does not have a hypervariable region.) For each hypervariable loop, the left graph shows length variation, the center shows charge, and the right shows the number of glycans. Variability for each hypervariable loop characteristic is shown as a histogram with the characteristic on the horizontal axis and the number of M-group Envs with a particular value of characteristic on the vertical axis. All analyses in this figure were performed using the 2017 Filtered Web Env reference alignment from the Los Alamos National Laboratory HIV Database (a total of 5,398 Env sequences, one per individual and spanning all global subtypes and circulating recombinant forms; **https://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html**).

against HIV-1 infection via alternative mechanisms, such as antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD) (52–54). The RV144 trial of a canarypox vector prime (ALVAC-Env/Gag/Pro), gp120 protein boost in Thailand provided the first clinical evidence of efficacy for any HIV-1 vaccine (55–60). This study demonstrated 31% efficacy, and protection correlated with V1/V2-specific IgG1 and IgG3 responses, ADCC activity, and decreased IgA responses (57). nNAb responses have also been shown to correlate with partial protection in nonhuman primate studies (61–64). Using a comprehensive antibody-profiling approach known as systems serology, we showed that reduced risk of infection correlated with polyfunctional antibody responses, e.g., ADCP, ADCC, and ADCD (61, 65). We also observed that an adenovirus serotype 26 (Ad26)-based vector prime, gp140 protein boost afforded 66% protection against acquisition of infection following simian-human immunodeficiency virus (SHIV)-SF162P3 challenges in nonhuman primates, and both nNAb responses and T cell responses were immune correlates of protection (66).

Based on clinical data with the ALVAC/gp120 vaccine and preclinical data with the Ad26/gp140 vaccine, two parallel vaccine development programs have led to clinical efficacy trials. RV144 involved ALVAC vectors expressing clade B and CRF01_AE antigens boosted with alum-adjuvanted bivalent clade B/E gp120 (ALVAC + gp120 B/E). The current goal of this program is to confirm and extend the RV144 findings using clade C antigens in South Africa, a more potent adjuvant (MF59), and an additional boost immunization. The phase 1/2 study of ALVAC + gp120 C/C showed that it was safe and immunogenic; it elicited binding antibodies in 100% of vaccine recipients, although V1V2-specific responses were lower than those observed in RV144 (67). These responses supported moving this vaccine into a phase 2b/3 efficacy trial of 5,200 men and women in South Africa in the Uhambo study (HVTN 702; **Table 1**). Unfortunately, the results of this study, announced on February 3, 2020, showed no efficacy in terms of prevention of HIV-1 infection (67a).

The second HIV-1 vaccine program currently in clinical efficacy trials involves an Ad26 vector expressing bioinformatically optimized HIV-1 mosaic Env/Gag/Pol antigens and boosted with alum-adjuvanted gp140. A prototype Ad26 vector expressing a single clade A HIV-1 Env immunogen (Ad26.ENVA.01) was shown to be safe, well-tolerated, and immunogenic (68–70). Multivalent Ad26 vectors expressing mosaic HIV-1 Env/Gag/Pol immunogens, Ad26.Mos.HIV,

were manufactured with the goal of enhancing cellular immune breadth and functional antibodies against diverse global viruses (63, 71, 72). In a phase 1/2a clinical trial, we explored the safety and immunogenicity of various regimens involving multivalent Ad26.Mos.HIV vector priming with Ad26.Mos.HIV, MVA.Mos.HIV, and/or Env gp140 boosting in 393 subjects in Rwanda, South Africa, Thailand, Uganda, and the United States (66). Humoral and cellular immune responses in humans proved comparable to those that afforded partial protection in nonhuman primates, and the mosaic Ad26-Env/Gag/Pol prime, Ad26 plus high-dose gp140 boost vaccine was advanced into a phase 2b clinical efficacy trial in 2,600 women in five countries in sub-Saharan Africa called Imbokodo (HVTN 705, HPX 2008) (**Table 1**). Preliminary efficacy results are expected in 2021. A phase 3 trial called Mosaico (HVTN 706, HPX 3002) is currently being launched with a similar vaccine involving the mosaic Ad26-Env/Gag/Pol with bivalent mosaic/clade C gp140 in 3,800 men and transgender individuals who have sex with men/transgender individuals in the Americas and Europe (**Table 1**).

Another vaccine efficacy trial that is being planned involves combining PrEP with active vaccination. This PrEPVac study aims to test the combined efficacy of both modalities of HIV-1 prevention, with PrEP provided for the first six months, when immune responses are being induced by the vaccine. Multiple other vaccine approaches that induce nNAb responses are also being explored in preclinical studies and early phase clinical trials, which are beyond the scope of this review (see **http://www.avac.org/pxrd** for a database of ongoing HIV-1 vaccine trials).

VACCINES THAT GENERATE T CELL RESPONSES

Vaccine approaches aimed at inducing broad and potent T cell responses are also being developed. Two vaccine trials that had no efficacy in terms of preventing HIV-1 infection still showed that T cell responses exerted immune selection pressure in follow-up analyses. The Step trial tested whether T cell responses elicited by an Ad5 vector expressing natural B-clade Gag, Pol, and Nef could protect against HIV-1 infection or impact viral control following infection. The Step study did not show decreased infection risk (73, 74); instead, there was an increase in HIV-1 acquisition among uncircumcised and/or Ad5-seropositive vaccinated study participants (75). Nevertheless, the vaccine led to immunologic pressure at transmission, as viruses from infected vaccinees were genetically further from the vaccine antigens than viruses from placebo recipients, most strikingly within Gag (76, 77). Furthermore, there was an inverse correlation between plasma viremia and the number of vaccine-induced $CD8^+$ T cell responses (78). These clinical data are consistent with nonhuman primate SIV challenge studies that have shown that vaccine-elicited $CD8^+$ T cell responses, particularly those targeting Gag, correlated with improved viral control and survival following infection (63, 79–85).

A second trial, HVTN 505, evaluated a DNA prime, Ad5 boost delivery and included HIV-1 B-clade Gag, Pol, and Nef antigens, as well as three diverse gp145 Envs (86). Again, no overall reduction in HIV-1 acquisition or in viral load upon infection was observed (87). However, a subset analysis identified an association between reduced infection risk and $CD8^+$ T cell vaccine responses (88), as well as an association between reduced infections and IgG responses among those with low T cell responses (89). The associations between vaccine-elicited $CD8^+$ T cell responses and reduced rates of infection (88, 89) or reductions in viral load (76) raise the possibility of a vaccine benefit, but given the lack of overall protection in both the Step and HVTN 505 human trials, this will require further study. Moreover, T cell responses as well as antibody responses correlated with protection against SHIV challenges in nonhuman primates (66).

Several approaches to antigen design have been undertaken to improve vaccine-elicited T cell responses relative to the natural protein vaccines used in the Step and HVTN 505 trials

(**Figure 1*a***). The first vaccine approach is used in the Imbokodo trial and includes essentially complete protein antigens, as did Step and HVTN 505, but instead of natural proteins, complementary pairs of HIV-1 mosaic immunogens were included. Mosaic proteins are computationally designed to provide improved immunologic coverage of global circulating viruses. Two complementary mosaics for Env/Gag/Pol were designed (**Figure 1*a*** displays the predicted coverage by paired mosaic proteins in green) (71). Mosaics not only optimize epitope diversity coverage but also minimize epitope redundancy and rare epitopes that would favor type-specific vaccine responses. In nonhuman primates, mosaic antigens elicited significantly higher T cell breadth and depth than did natural sequence antigens (72, 90, 91). Mosaic Env proteins also elicited nNAb responses that were associated with protection against SHIV challenge in nonhuman primates (63, 66). The second vaccine approach focuses on conserved regions with greater cross-reactive potential, excluding the most variable regions while still retaining substantial regions of the HIV-1 proteins. They include a large number of potential epitopes, reflecting a broad spectrum of HLA presenting molecules (92–97). Some conserved region immunogens are also enriched for epitopes that have been shown to be associated with low viral loads in natural infection (94, 95, 98). Other conserved region vaccines use complementary mosaic designs, as even relatively conserved regions of HIV-1 are still quite variable, and complementary mosaic protein designs can be used to maximize epitope coverage in such vaccines (95, 96). **Figure 1*a*** displays the predicted coverage by a complementary mosaic-conserved region concept in dark blue. The third vaccine approach includes only very short regions that span highly conserved sections of HIV, for example, the p24 conserved element vaccine, p24CE (92, 99). **Figure 1*a*** displays the predicted coverage by a very short conserved region concept in light blue. A proposed alternative to the conserved element approach is to use similarly short regions that are highly networked at the protein level, and so are likely to be functionally critical (100). Both strategies may be advantageous as therapeutic vaccines, by directing immune responses to epitopes that would exact a high fitness cost if mutated (92, 101–103).

A novel approach in the generation of a T cell–based vaccine involves the induction of nonclassical MHC-E restricted $CD8^+$ T cell responses by a modified cytomegalovirus (CMV) vector (104–106). These responses tap into a novel immunological pathway, which has been shown to control and clear newly established SIV infections in approximately half of vaccinated nonhuman primates (106, 107). MHC-E approaches, which require a modified CMV vector for delivery, could also include the HIV-1 antigen design approaches described above, for example using full-length or conserved protein designs to provide better cross-reactive coverage of MHC-E epitope responses.

VACCINES THAT GENERATE bNAbs

A major unsolved problem in the HIV-1 vaccine field is the development of immunogens capable of inducing bNAbs. Such antibodies have proven extremely difficult to elicit by vaccination, and despite large research efforts, no such immunogens yet exist. Challenges include the high degree of Env sequence diversity as well as the extensive glycan shield that protects the Env trimer surface from antibody attack (108). Monoclonal antibodies have been identified in HIV-1-infected humans with exceptional potency and breadth, but typically these bNAbs emerge only after years of chronic HIV-1 infection and after multiple rounds of antibody-Env coevolution (45). These bNAbs often have unusual features, such as long third heavy-chain complementary-determining regions (CDRH3s) and short third light-chain complementary-determining regions (CDRL3s), which are able to penetrate and negotiate the glycan shield, respectively (109). The maturation of bNAbs also typically requires substantial somatic hypermutation, which partially explains the long time for development of bNAbs during chronic HIV-1 infection.

Soluble trimers that mimic the native Env spike have recently been developed. BG505 SOSIP.664 is a soluble protein that links Env gp120 and gp41 subunits with a disulfide bond and is stabilized with an I559P substitution in the prefusion state (108, 110–113). The BG505 SOSIP.664 gp140 vaccine has been shown to elicit potent autologous neutralizing antibody (NAb) responses to BG505 in animal models but with very limited heterologous NAb breadth (111–115). These autologous BG505-specific NAbs target an immunodominant hole in the glycan shield of this virus (109, 112), although other epitopes are also targeted (111). Some differences between SOSIP trimers and virion-expressed Env spikes have also been described (116), and approaches that utilize structural biology to design improved SOSIP immunogens are being actively pursued by multiple laboratories.

It is likely that multiple immunogens will be required to induce bNAbs (41, 117, 118). Some of the strategies that are being pursued are illustrated in **Figure 3**. Studies of B cell ontogeny leading to the development of bNAbs in HIV-1-infected humans have led to the concept of sequential immunization to recapitulate antigenic exposure in chronic HIV-1 infection by a sequence of immunogens. This approach involves priming with an immunogen that can stimulate the desired germline antibody genes, followed by boosting with a series of intermediate constructs to coax B cell development along the specific pathways that lead to bNAbs (43, 119). Stimulation of appropriate antibody germline B cells may be required for vaccine induction of some bNAbs, since such germline B cells have the rare CDR configurations that are likely prerequisites for the development of bNAbs. However, such germline B cells typically show no cross-reactivity with natural Envs, and thus germline-targeting immunogens may be required. For example, eOD-GT8 is a nanoparticle-based engineered immunogen that was designed to stimulate precursor B cells for the VRC01 class of bNAbs, which target the CD4 binding site (120–122), and is currently being explored in a phase 1 clinical trial. Sequential immunogens have also been designed that aim to induce V3-specific bNAbs (see **Figure 2** for Env structure) (123, 124). However, none of these immunogens or combinations of immunogens has yet elicited bNAbs in nonhuman primates or humans.

Epitope-targeted immunogen design strategies are an alternative approach for inducing bNAbs. Examples of this approach include fusion peptide (FP)- and V3-glycopeptide-based immunogens, which present minimal epitope fragments as a means to avoid distracting off-target responses targeting other regions of the full Env spike (125–128). Another approach is the use of signature-based epitope targeted (SET) immunogens. We recently reported the design and testing of V2-SET immunogens that aim to increase induction of V2-specific NAbs using neutralization signatures from large virus panels to optimize exposure and diversity of the V2 epitope on the Env surface (38). Both the FP-targeted and V2-SET vaccines have induced NAb responses with moderate breadth in small animals, and also in nonhuman primates for FP-based immunogens (38, 125, 127).

PASSIVE IMMUNIZATION WITH bNAbs FOR HIV-1 PREVENTION

While passive transfer of monoclonal antibodies for HIV-1 prevention is not a novel concept, there is now renewed interest in this concept as a result of the discovery of multiple bNAbs with higher neutralization breadth and potency. The Antibody Mediated Prevention (AMP) trials are two phase 2b clinical studies (HVTN 703/704) (**Table 1**) that are assessing the protective efficacy of the CD4bs-specific bNAb VRC01 against HIV-1 acquisition in humans (129). These studies are evaluating VRC01 in heterosexual women in sub-Saharan Africa and in men and transgender persons who have sex with men in the Americas and in Europe. Enrollment in these trials is complete and results are anticipated in 2020.

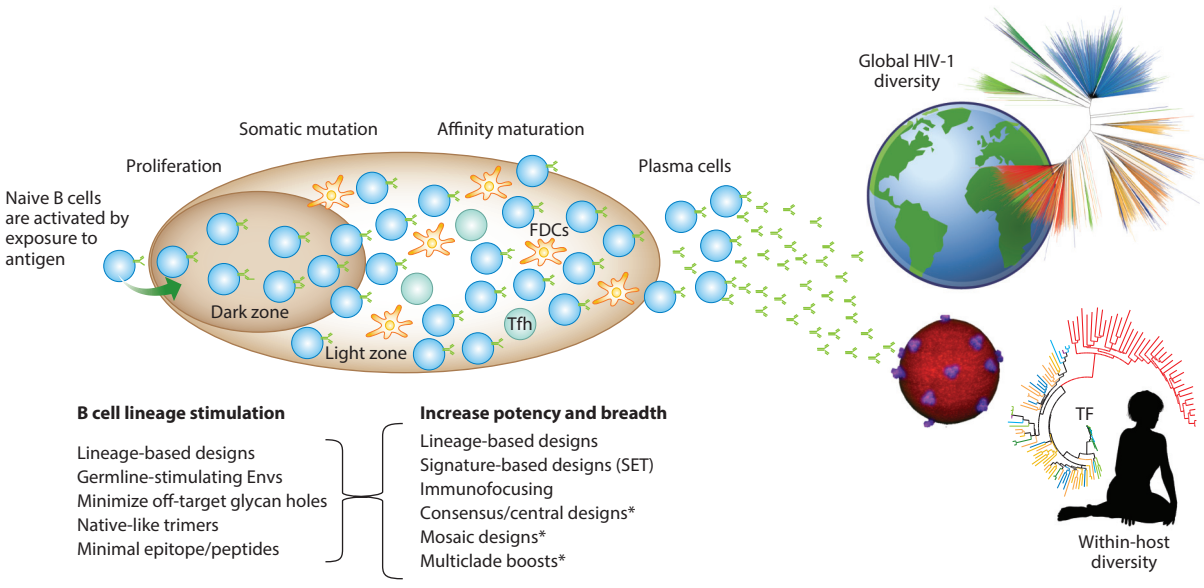


Figure 3

Strategies for designing vaccine antigens to stimulate bNAbs. (*Left*) bNAb induction starts by activation of an appropriate B cell, which then enters the germinal center and begins to proliferate. Germline B cells with the capacity to initiate the bNAb responses in different individuals can be highly similar and have shared characteristics (186, 188). Some vaccine approaches to stimulate them are listed on the left. Lineage-based strategies sample antibody lineages during natural HIV-1 infections as they develop into potent bNAbs, enabling the reconstruction of the unmutated common ancestor (UCA). Natural Envs can sometimes be identified that can bind to the UCA (40, 41, 118), or can be modified based on structural information or bNAb sensitivity signatures to improve binding to the UCA and improve germline stimulation (121, 123, 175, 176, 186, 188). Reducing exposure of off-target immunodominant epitopes that detract from desirable bNAb responses can be aided by minimizing rare holes in HIV-1's glycan shield (109, 112, 177). Designing more native-like Env trimers may be helpful both for triggering initial responses and for guiding affinity maturation toward bNAbs tailored to recognize the natural form of the Env (108, 110, 113). Using minimal peptides that bind well to the UCA of appropriate lineages can also trigger an initial B cell response (127, 193). Further vaccination can then be used to try to enhance the breadth and potency of responses during B cell maturation. (*Center*) Once a B cell lineage begins to proliferate in the germinal center, the antibody it carries undergoes rapid somatic mutation. This is followed by selection for antibodies with greater affinity for their target antigen, and then further rounds of mutation and selection. Follicular dendritic cells (FDCs) and T follicular helper (Tfh) cells critically support the selective processes essential for B cell microevolution. To generate bNAbs, a vaccine must be engineered to steer selection for affinity toward enhanced recognition of HIV-1 diversity. Lineage-based designs can also be used at this stage to try to mimic an evolutionary trajectory that leads to neutralization breadth (40, 41, 186) and to design immunogens that select for critical but difficult to achieve antibody mutations (178). Signature-based epitope targeting (SET) vaccines are designed to incorporate features of Envs that are correlated with bNAb sensitivity levels, and they include relevant and common natural variants in a vaccine cocktail (38). Immunofocusing is being used to direct an initial antibody response exclusively to a targeted epitope of interest (179). Immunogens that present the most common form of epitopes (consensus) (180–182), or computationally optimized combinations of the most common forms of epitopes (mosaics) (66, 71, 72), or that represent common circulating HIV-1 clades (111) are being used to try to select for antibodies with greater cross-reactive potential; such strategies may also enhance the breadth and potency of both bNAbs and beneficial nonneutralizing approaches. (*Right*) For a vaccine to elicit protective B cell responses, ultimately the antibodies must be able to contend with HIV-1 diversity. Global HIV-1 diversity is indicated by a phylogenetic tree based on the M group Env sequences in the HIV-1 database (**https://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html**); different geographic regions of sampling are indicated by different colors in the branches of the global phylogenetic tree. Furthermore, a vaccine response will need to effectively neutralize the array of diverse forms represented in a single host, so it is not readily bypassed by resistant variants upon exposure. This is illustrated by a phylogenetic tree of Env sequences from a single representative individual (48), sampled over two years and colored by time point. The transmitted/founder (TF) viral sequence is in the center of the tree; the first time point is in green, and the two-year time point in red. The image of the Earth is adapted from Wikipedia.

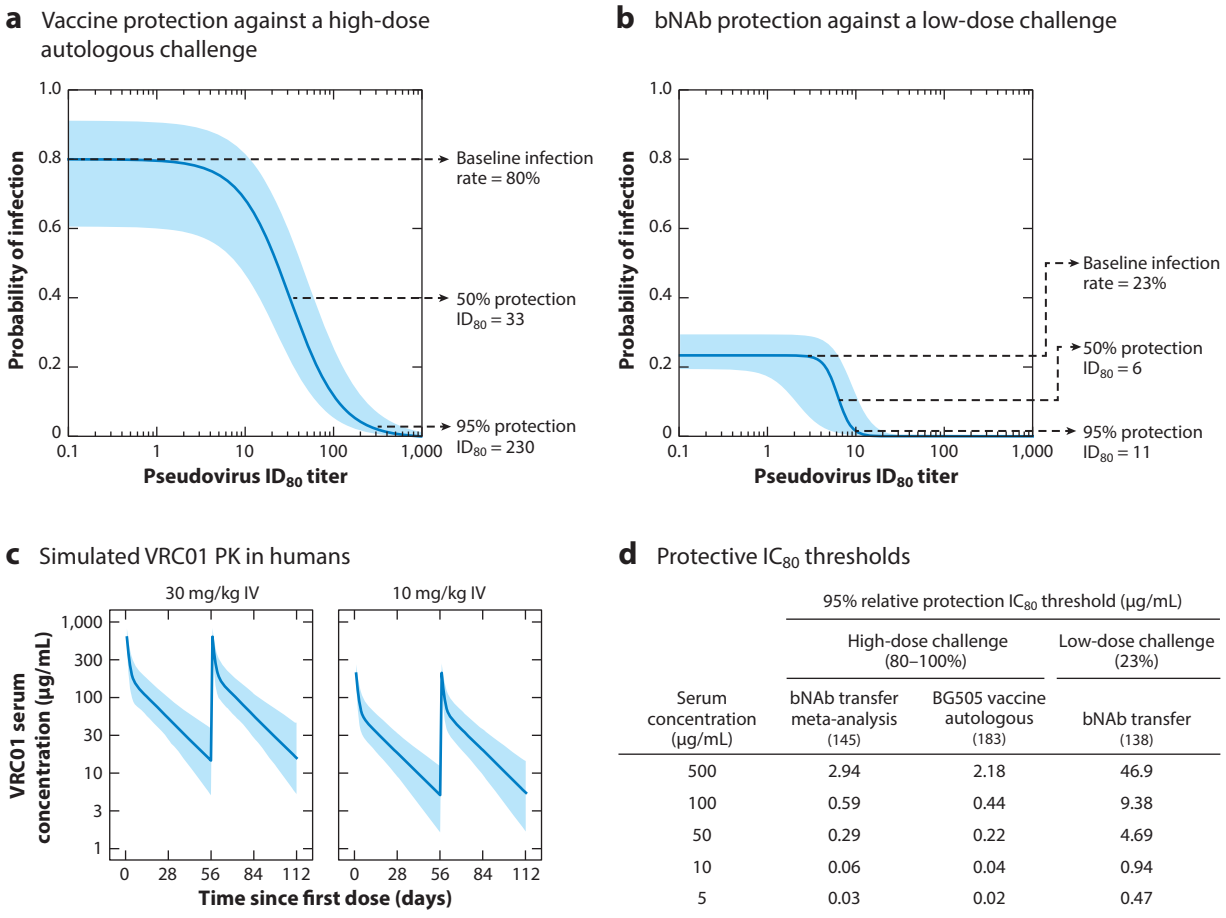
Studies of bNAbs for HIV-1 prevention have two main goals. First, these studies will help define the neutralization titers required for protection, which will inform the development of next-generation vaccines that aim at inducing such responses. Second, passive immunization with bNAbs may also be an HIV-1 prevention strategy in its own right. In particular, long-acting bNAbs may provide a viable alternative to antiretroviral drugs as PrEP. It is also possible that bNAbs will have fewer adverse effects than certain antiretroviral drugs (particularly during pregnancy), and efforts to extend the half-life of bNAbs could lead to infrequent administration. Antibodies have been engineered for enhanced breadth and potency (130–133), and incorporation of "LS" mutations (M428L and N434S in the constant regions of bNAb heavy chains) that improve the affinity between antibody Fc and the neonatal Fc receptor (FcRn) has been shown to extend the in vivo half-life (134).

A critical goal of the AMP trials is to define the degree of neutralization activity that is required to protect against HIV-1 infection in humans, which may result in defining NAb titers as a correlate of protection. Multiple studies have shown the ability of passively transferred bNAbs to protect against SHIV challenge in nonhuman primates (131, 135–142). Serum neutralization titers required for protection typically range from ID_{50} (inhibitory dilution for 50% neutralization) titers of 30 to 1,000, with most studies finding ID_{50} titers of approximately 100 to be protective (142); these ID_{50} values correspond to approximate ID_{80} (inhibitory dilution for 80% neutralization) titers of 7.5, 250, and 25, respectively (143, 144). Recently, a meta-analysis of studies of bNAb passive transfer experiments against high-dose SHIV challenges in nonhuman primates demonstrated a significant correlation between serum neutralization titer and protection, with ID_{50} titers of 91 and 685 required for 50% and 95% protection, or ID_{80} titers of 1.7 and 170 for 50% and 95% protection, respectively (145).

We have also reanalyzed data from two preclinical studies involving two different biological settings: a vaccine study that used medium/high-dose SHIV challenges and a passive bNAb infusion study that used low-dose SHIV challenges. The first study involved an adjuvanted BG505 SOSIP.664 gp140 protein vaccine against repeated medium/high-dose SHIV-BG505 challenges (146). The baseline rate of infection in this study was 80% per challenge in control animals (**Figure 4*a***), which is lower than the 100% per challenge infection rate in the meta-analysis of high-dose SHIV challenge studies (145). Nevertheless, the ID_{80} titer requirements of 33 and 230 for 50% and 95% protection, respectively, were similar (**Figure 4*a***).

The second study involved a passive bNAb transfer experiment with repeated low-dose SHIV-AD8-EO challenges (138). In this study, a single intravenous infusion of 20 mg/kg of various bNAbs was administered, and animals were challenged weekly with low doses of virus that resulted in a 23% per challenge infection rate in controls. It took a median of 3 (range 2–6) challenges to infect the control animals, and thus this low-dose model may be more representative of human HIV-1 exposures than high-dose challenge models. Of note, HIV-1 acquisition risk in humans has been estimated to be approximately 138 per 10,000 exposures for receptive anal intercourse, and 8 per 10,000 for receptive penile-vaginal exposure (147), markedly lower than even the low-dose challenge model in nonhuman primates. We applied a modified logistic regression model to the serum neutralizing activity at the time of each challenge, and whether or not the challenge resulted in infection, similar to the above protective models (148) (**Figure 4*b***; see **Supplemental Methods**). This low-dose challenge model predicted that ID_{80} titers of 6 and 11 were required for 50% and 95% protection, respectively, substantially lower than the ID_{80} titers required for protection against high-dose SHIV challenge. The high-dose challenge contains more infectious virions than the low-dose challenge, and thus a higher fraction of the inoculum presumably needs to be neutralized to achieve complete protection. Other factors may also contribute to these differences, including different characteristics of the various SHIVs used, differences between monoclonal

Supplemental Material >



Serum concentration (µg/mL)	95% relative protection IC_{80} threshold (µg/mL)		
	High-dose challenge (80–100%)		Low-dose challenge (23%)
	bNAb transfer meta-analysis (145)	BG505 vaccine autologous (183)	bNAb transfer (138)
500	2.94	2.18	46.9
100	0.59	0.44	9.38
50	0.29	0.22	4.69
10	0.06	0.04	0.94
5	0.03	0.02	0.47

Figure 4

Potency of neutralization required for protection against SHIV challenges in nonhuman primates. (*a*,*b*) Modeling of protection in SHIV challenge studies as a function of serum neutralization ID_{80} titers using two models of neutralization-based protection. Panel *a* shows protection conferred by BG505 SOSIP vaccine–induced neutralization against an autologous high-dose challenge, as modeled in Pauthner et al. (183). Panel *b* shows the protection conferred by passively transferred bNAbs against a repeated low-dose SHIV challenge in unvaccinated macaques, modeled by Wagh et al. (157) using data from Gautam et al. (138). The light blue shaded areas are approximate 95% confidence intervals for each model. See **Supplemental Methods** for details of calculations. (*c*) Simulated VRC01 pharmacokinetic (PK) profiles in the two dosing groups matching those in the Antibody Mediated Prevention (AMP) trials. Adapted from Huang et al. (149) with permission. (*d*) Predicted protective IC_{80} thresholds for incoming challenge viruses to achieve 95% or higher relative protection are shown based on the two protection models above (*a*,*b*), as well as the protection model for bNAb passive transfer against a high-dose SHIV challenge from the meta-analysis by Pegu et al. (145). The numbers in parentheses indicate the baseline probability of infection per challenge for each model.

Supplemental Material >

and polyclonal antibodies, and experimental specifics such as the use of exogenous human bNAbs in the passive transfer studies compared with vaccine-elicited rhesus NAbs in the active vaccine study.

It remains to be determined which of these preclinical models will best predict protection against HIV-1 infection in humans. They may underestimate the impact of a bNAb, because the baseline rate of infection, by experimental necessity, is much higher in nonhuman primates. On the other hand, these models may overestimate the impact of a bNAb, because nonhuman primate

studies typically utilize a single SHIV challenge strain, whereas HIV-1 exposure in humans exhibits far greater diversity at both the individual and population levels.

In the AMP trials, VRC01 is infused every 8 weeks, and the decay of serum bNAb concentrations was modeled as a biphasic exponential decline (149). For example, an infusion of VRC01 at 30 mg/kg leads to peak serum antibody concentration of ~600 μg/mL, which then declines rapidly over 3 days to ~223 μg/mL (**Figure 4*c***). This is followed by a slower second phase of decline with a half-life of ~14 days, such that serum antibody concentrations are ~12 μg/mL at the end of each 8-week cycle. In predictive models, this ~50-fold decline in bNAb concentration would be expected to result in decreased levels of protection (**Figure 4*d***). Using the least stringent of the protective preclinical models (i.e., the low-dose challenge where an ID_{80} of 10.66 is sufficient for 95% relative protection; **Figure 4*b***), a serum VRC01 concentration of 500 μg/mL would be predicted to protect against incoming challenge viruses with IC_{80} < 46.9 μg/mL (500/10.66), which corresponds to 78% of global circulating viruses. However, toward the end of the infusion cycle, a serum VRC01 concentration of 10 μg/mL would only be predicted to protect against viruses with IC_{80} < 0.94 μg/mL (10/10.66), which corresponds to only 34% of global viruses.

The decline in plasma bNAb concentrations highlights the importance of quantifying the decay in protective efficacy with serum neutralizing activity. Quantification of development of bNAb resistance can also be obtained from phase 1 clinical studies of the therapeutic use of bNAbs in HIV-1-infected individuals who are viremic or undergoing analytical treatment interruptions (150–154). In these studies, most individuals exhibited viral rebound with resistant virus despite high serum bNAb concentrations. For VRC01 and 3BNC117, rebound viruses showed a median increase of 3- to 12-fold in IC_{80} titers, respectively (**Figure 5**). For 10–1074, all rebound viruses showed complete neutralization resistance. Looking at the viral escape pathways, the CD4bs-specific bNAbs appear to select for varied resistance mutations that typically lead to less potent, but not complete loss of, neutralization (150–154). In contrast, resistance to the V3 glycan bNAb 10–1074 showed two common escape pathways: the loss of the glycan at N332 and a mutation at position 325, both of which confer complete 10–1074 resistance (153).

CHALLENGES FOR bNAb PASSIVE PREVENTION: CLADE-SPECIFIC NEUTRALIZATION RESISTANCE

A major challenge facing the development of bNAbs for passive HIV-1 prevention is the prevalence of viral clades that are resistant to any one particular antibody (38, 148). A striking example is the lack of activity of V3 glycan–dependent bNAbs, such as 10–1074 and PGT121, against CRF01 viruses, the major circulating lineage in Southeast Asia. The reason for this is that a critical Env *N*-linked glycosylation site N332 in V3 is lost in this clade (38). Similarly, V2 glycan–dependent antibodies, such as PGDM1400 and CAP256, have less breadth and potency for clade B viruses than clade C viruses (38, 155). There is also an apparent reduced potency of all bNAbs against clade D viruses, although the number of subtype D viruses available for testing is limited (148). Because most geographical regions are enriched for specific clades (**Figure 1*b***), the subtype-specific resistance profiles of bNAbs should be considered in decisions to test bNAbs in particular regions.

A solution for mitigating these issues is to use combinations of bNAbs or alternatively multispecific bNAbs. We showed preclinical proof-of-concept of the benefit of bNAb combinations against a mixed SHIV challenge in nonhuman primates (140). These data are consistent with in vitro studies that have shown that bNAb combinations have significantly improved potency and breadth compared to individual bNAbs (148, 156, 157).

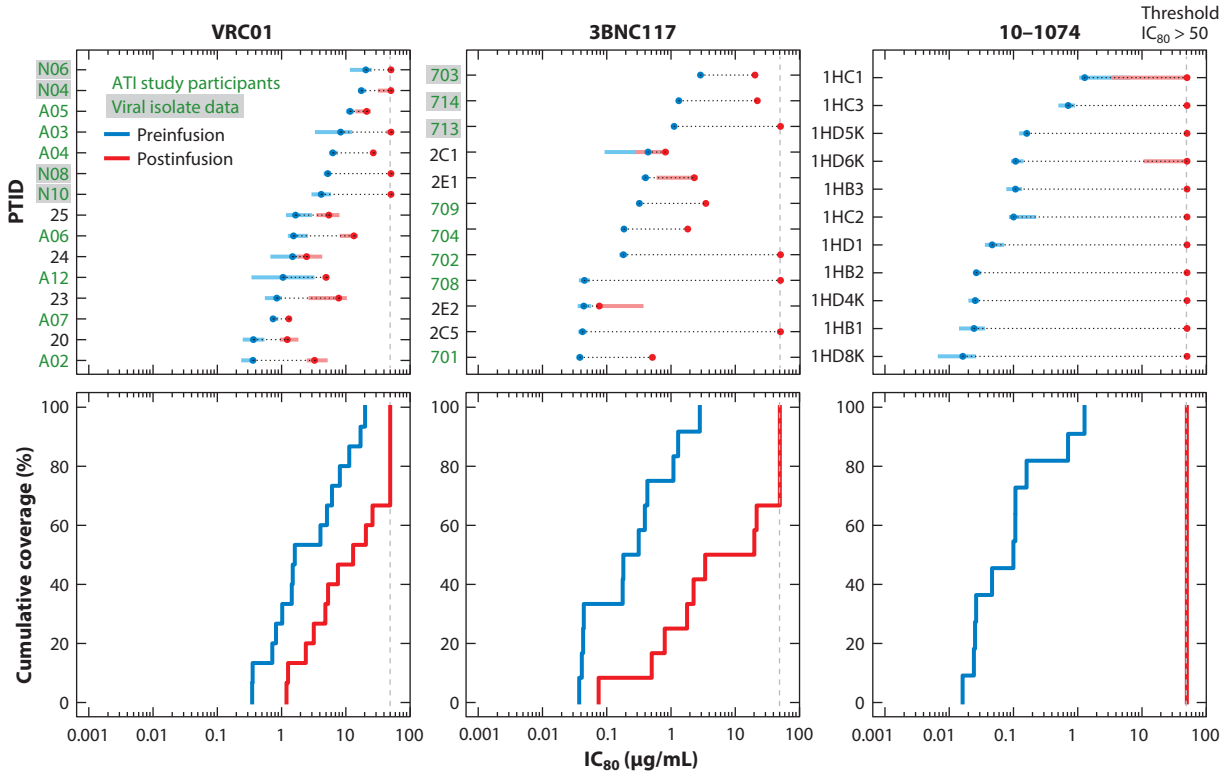


Figure 5

Levels of bNAb resistance that developed in published clinical studies of passively transferred bNAbs [VRC01 (150, 151), 3BNC117 (152, 154), and 10–1074 (153)]. Only data from participants with documented viral escape from bNAbs in the primary publications were used. The top panels show the pre- and postinfusion sensitivity (*x*-axis) of viruses isolated from each participant (*y*-axis). Blue dots indicate median IC_{80} titers preinfusion, and light blue bars the interquartile range. Red dots indicate median IC_{80} titers postinfusion, and pink bars the interquartile range. The bottom panels show the cumulative distributions of median preinfusion titers (*blue curve*) and postinfusion titers (*red curve*), analogous to the breadth-potency plots of in vitro neutralization efficacy of bNAbs against pseudovirus panels. Some studies analyzed bNAb passive transfer in viremic individuals, while others were analytic treatment interruption (ATI) studies. Participant designations from ATI studies are in green. Pseudovirus neutralization data were usually available and were chosen to facilitate comparison with typical in vitro neutralization panel data. However, for a few participants only viral isolate neutralization data were reported, and these are indicated by highlighted participant identifiers (PTIDs). Of note, viral isolates typically show more resistant neutralization profiles relative to matched pseudoviruses (184).

CHALLENGES FOR bNAb PASSIVE PREVENTION: WITHIN-HOST MINORITY RESISTANT VARIANTS

For a bNAb to block HIV-1 infection, two conditions are intuitively critical: (*a*) sufficient bNAb titers at the appropriate anatomic location and (*b*) sensitivity of the challenge virus to the bNAb. With this simple model, the predicted efficacy of an individual bNAb would be a function of how likely it is that an incoming challenge virus will be sufficiently sensitive to the bNAb. However, it is likely that an individual will not be exposed to a single virus sequence, but rather to a swarm of highly diverse viruses with a spectrum of neutralization sensitivities, even if there are only a limited number of founder viruses that actually establish productive infection. Thus, it is possible that the presence of a bNAb may drive transmission events toward acquisition of more resistant

viral variants. Population-level molecular epidemiology (**Figure 1*b***) and neutralization of panels of defined pseudoviruses do not address this complexity. Additionally, it is likely that bNAbs do not work exclusively by blocking virions at the mucosal portal of entry, since we showed that PGT121-mediated protection against SHIV-SF162P3 challenge in nonhuman primates included systemic clearance of distal foci of virus in tissues for up to seven days following challenge (158). Thus, it is theoretically possible that the efficacy of bNAb-based protection may be reduced by minor resistant variants within a challenge swarm, which could potentially be selected with bNAb pressure.

Examples of how within-host diversity can impact bNAb sensitivity are shown in **Figure 6**. Neutralization data for viruses from three chronically infected viremic individuals (153) show that for each individual, there were one to two virus variants that were resistant to at least one bNAb, despite other virus variants being sensitive (**Figure 6*a***). Thus, bNAb combinations that neutralize within-host virus variants with at least two active bNAbs may be necessary to avoid escape. An analysis of within-host sequences from six chronically infected individuals similarly shows that each individual harbored frequent minor variants that were resistant to bNAbs from each class, even if the bulk population was sensitive (**Figure 6*b***). Such resistance signatures can be single amino acids or glycans located in key antibody epitope sites (**Figure 6*b***), or variable loop characteristics that are significantly associated with bNAb sensitivity levels (**Figure 6*b*,*c***).

The problem of within-host diversity is driven by the fact that chronically HIV-1-infected individuals can develop NAbs that target similar epitopes as the bNAbs considered for passive transfer and vaccines. bNAb epitopes are in regions of vulnerability and thus are relatively frequently targeted in natural infection (142, 159). Thus, bNAb-resistant viral variants naturally arise during in vivo escape either from autologous NAbs targeting the bNAb epitope, even if the host's antibody lineage does not acquire breadth, or as part of the intricate process of antibody-virus coevolution that gives rise to neutralization breadth. The latter process involves multiple rounds of viral escape followed by antibody evolution to recognize escaped viruses, and ultimately, most within-host viruses escape matured bNAbs in each infection regardless of their neutralization breadth and potency for heterologous viruses (40, 43, 160). Since bNAbs targeting similar epitopes show similar neutralization profiles and resistance mutations (38), chronically infected individuals who develop bNAbs targeting a particular epitope will often harbor viruses that are relatively resistant to clinically used bNAbs of a similar epitope class. For example, individuals who are known to have developed V2 apex, CD4bs, or V3 glycan bNAbs also typically show resistance mutations in the corresponding bNAb signature sites. Studies of cross-sectional cohorts have established that ~50% of individuals develop ~50% serum breadth against panels of heterologous strains (35, 161). Among such individuals, 12–14% develop V2 apex, 21–36% develop V3 glycan, and 5–26% develop CD4bs bNAbs, depending on the cohort (162, 163).

Extensive characterization of within-host diversity of bNAb sensitivity is available for only a handful of individuals. We therefore cannot assess the coverage of bNAbs against the swarm of viral variants that are present in most chronically HIV-1-infected individuals, and thus the predicted coverage provided by bNAbs based on pseudovirus neutralization assays may be an overestimate. Timing of infection may also be important, as transmissions are most common during acute infection when the within-host viruses are more homogeneous (164–167). Nevertheless, data to date raise substantial concern that within-host minor resistant variants may reduce the projected efficacy of bNAbs in preventing HIV-1 infection. The use of combinations of three or more bNAbs can partially alleviate this concern. If most of the within-host viruses are sensitive to at least two bNAbs in the combination, then selection for minority variants that are simultaneously resistant

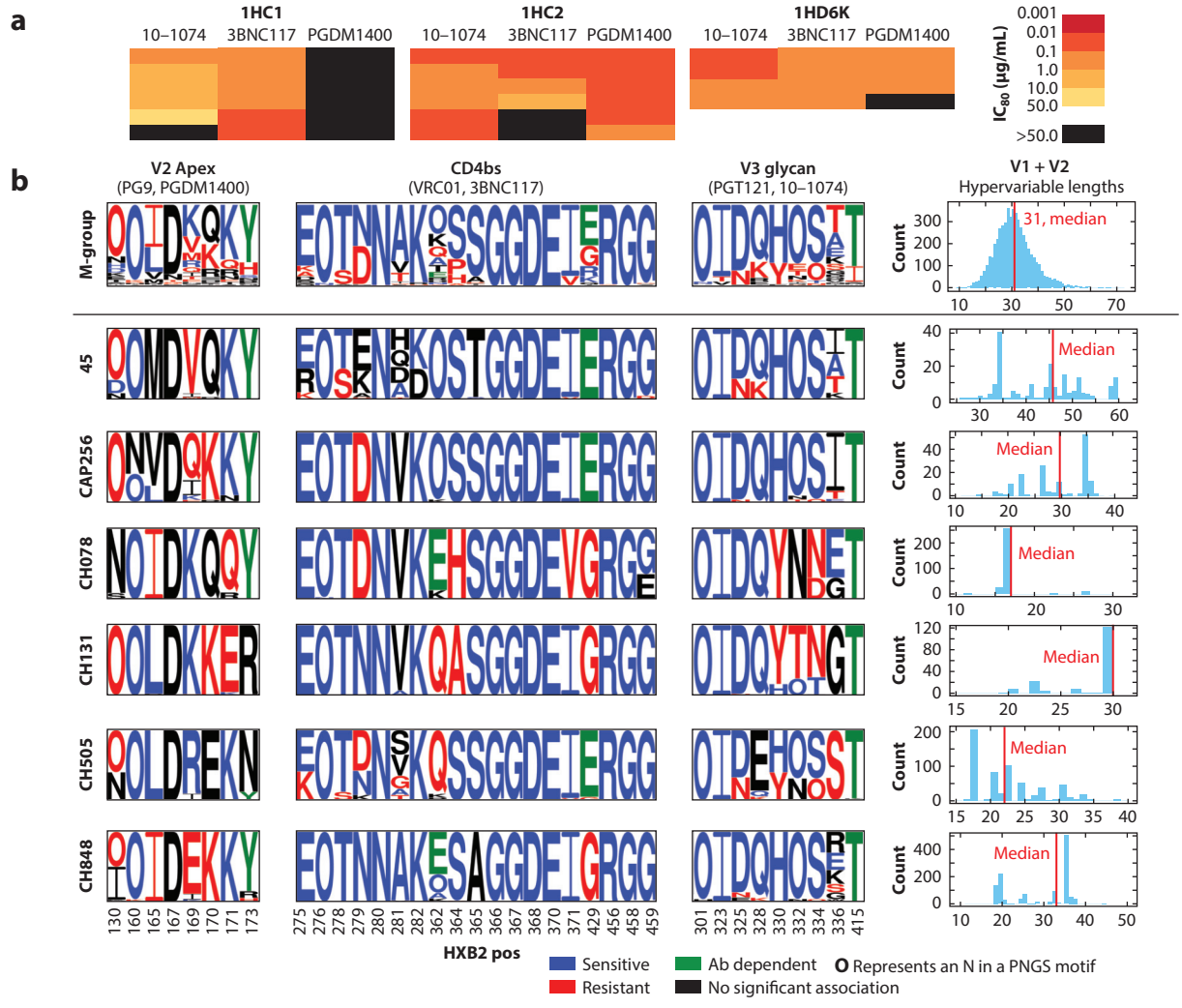


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Figure 6 (*Figure appears on preceding page*)

Natural within-host diversity leads to heterogeneous bNAb profiles. (*a*) Neutralization IC_{80} titers for preinfusion viral variants in Caskey et al. (153). Data from 3 of 11 participants (1HC1, 1HC2, 1HD6K) were selected for display here as examples that illustrate resistance variants comingling with majority sensitive viruses for representative bNAbs in the clinical pipeline (V2 apex, PGDM1400; CD4bs, 3BNC117; and V3, 10–1074). For each participant, viral isolates were obtained from preinfusion samples and tested against three bNAbs. These data are shown as heatmaps where viruses are represented as rows and bNAbs are columns, and each cell shows IC_{80} titers using the color-coding in the legend. Black cells indicate no detected response, with $IC_{80} > 50$ μg/mL. (*b*) HIV-1 bNAb signature amino acids frequencies in the global population and within HIV-1-infected individuals. Amino acids that are significantly associated with bNAb sensitivity (*blue*) or resistance (*red*) across bNAb classes for V2 apex, CD4bs, and V3 glycan bNAbs are illustrated by LOGO plots indicating the frequency of the amino acids by their relative height in relevant positions. LOGOs were made for the M-group HIV-1 Env viruses in the Los Alamos National Laboratory HIV database (5,420 viruses including just 1 sequence per infected individual from **https://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html**, *top* LOGO). To illustrate how subsets of these signature resistance mutations are commonly sampled in natural infections, LOGOs were made representing distinct sequences from six HIV-1 infections. Subjects were selected simply on the basis of being sampled over a period of several years, and having extensive Env sequences available. The sites displayed here are the subset of significant sensitivity or resistance signatures defined in Bricault et al. (38) that are either structurally defined as antibody contact residues or shown to be relevant for neutralization by mutational analysis or emergence of resistance [3BNC117 and VRC01 for CD4bs bNAbs (152, 154, 185–187), PG9 and PGDM1400 for V2 apex bNAbs (188–191), and PGT121 and 10–1074 for V3 glycan bNAbs (192–196)]. Hypervariable region characteristics are highly associated with bNAb sensitivity, and patterns are often shared across antibodies within a class (38). Hypervariable regions rapidly evolve during the course of natural infections by insertions and deletions (indels); generally significant evolution has occurred in these regions within the first year of infection. To illustrate in detail how such characteristics vary, we provide an example of the V1 + V2 hypervariable length variation found at the population level in the M-group alignment, as well as the variation within the 6 individuals included here, to the right of the LOGOs. The V1 + V2 hypervariable regions are bounded by HXB2 positions 132–152 (V1h) + 185–190 (V2h); outside of these regions most viruses are readily aligned, but within these regions alignments are chaotic due to length variation and minimal retention of shared motifs. Long V1+ V2 hypervariable regions are highly associated with bNAb resistance for many bNAbs, including three major classes of antibodies: CD4bs, V2 apex, and V3 glycan. The association is often stronger when considering V1h + V2h combined than when considering V1h or V2h separately. (*c*) Examples illustrating the relationship between variable loop characteristics and bNAb sensitivity, and the extent of the diversity sampled during natural infections. The relationship between pseudovirus bNAb sensitivity, represented here as an average IC_{80} score for a given bNAb/pseudovirus combination using all available M group viral data obtained from the Los Alamos National Laboratory HIV immunology database (**http://www.hiv.lanl.gov/components/sequence/HIV/neutralization/index.html**), and loop characteristics are displayed in the three scatterplots. The plot on the left shows the correlation between net charge of the V2 loop (HXB2 positions 159–197), which contains a hypervariable stretch (HXB2 positions 185–190), with PGDM1400 IC_{80} titers; viruses with more positive V2 loops are generally much more sensitive to V2 apex–binding antibodies (38). (This is true for both the hypervariable region within the V2 loop and the entire V2 loop; the data for the full V2 loop are shown here.) The plot in the center shows how the length of the V5 loop is correlated with 3BNC117 sensitivity. The V5 loop (HXB2 positions 459–470) contains a hypervariable region (V5h, HXB2 460–465). The length of this region as well as the number of PNGS sites encompassed in the region are strongly inversely correlated with CD4bs antibody activity (38). The V5 hypervariable region is embedded in the CD4bs contact surface (**Figure 2*a***), and of note, slight shifts in length can change the relative position of glycans in the V5 loops and impact developing antibody sensitivity (197). To develop breadth, CD4bs antibodies have to be selected to tolerate the natural diversity in the V5 region. On the right is shown an example of an association between 10–1074 and V1h + V2h length. As mentioned above, V3 glycan, V2 apex, and CD4bs (38) bNAbs are all generally more potent against viruses with shorter V1h + V2h lengths. In all three plots, only the positive viruses are shown, as variable region characteristics tend to modulate levels of bNAb sensitivity, but not to completely block activity. Above each of the three scatter plots, using the same *x*-axis, is a display that shows the median and range of loop characteristics of interest in the six subjects used to explore within-subject diversity. Within these subjects, there is always variation in the characteristics sampled, and in some cases nearly the full range of global diversity of these loop characteristics can be recapitulated in a single infection.

to all the bNAbs is less likely during within-host evolution. However, if most viruses are sensitive to only one bNAb in the combination, then there is a chance that minority variants will be completely resistant to the combination.

HOW MANY bNAbs ARE REQUIRED FOR HIV-1 PREVENTION?

A combination of bNAbs, or a multi-specific bNAb, will certainly be required for HIV-1 treatment and cure strategies (150, 152, 153, 168, 169). For the reasons discussed above, a combination

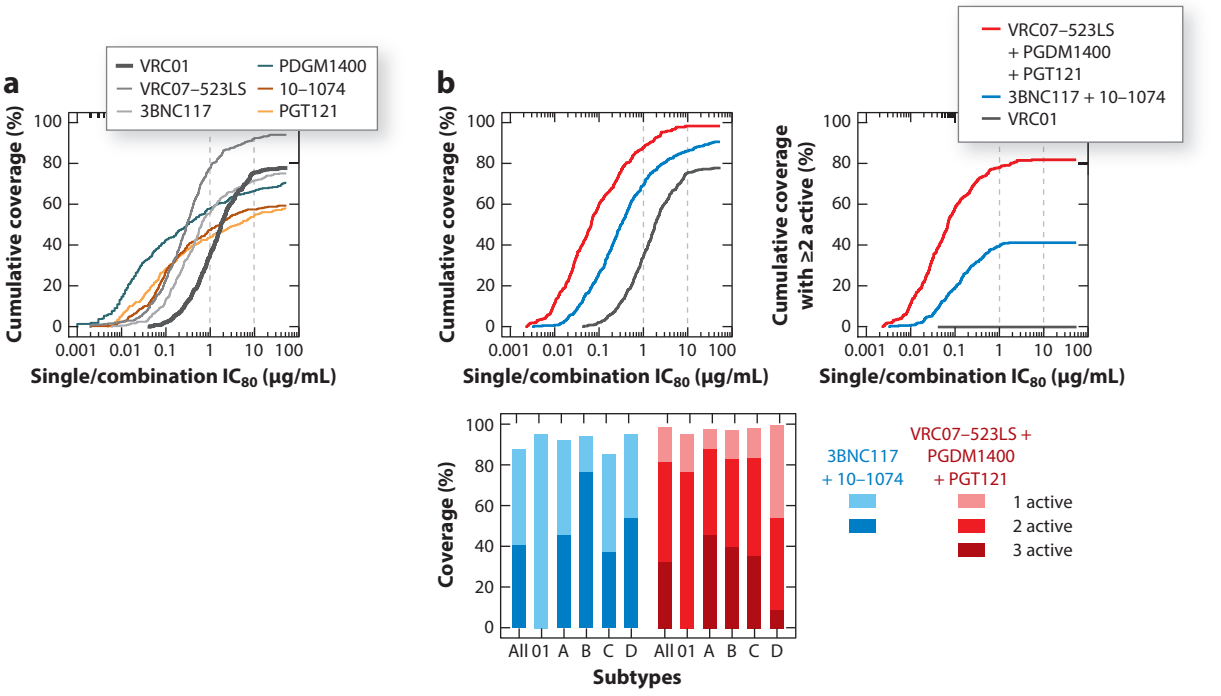
of bNAbs will also likely be required for robust HIV-1 prevention, e.g., to prevent breakthroughs of resistant viral variants transmitted from a diverse viral swarm that is the hallmark of chronic HIV-1 infection (170) (**Figure 6**). A cocktail of bNAbs that target different epitopes may therefore be required for both prevention and treatment strategies. Here we argue that a combination of at least three bNAbs will likely be needed for robust protection against HIV-1 acquisition in diverse populations. We compared the neutralization coverage of monotherapy with VRC01, the dual bNAb combination 3BNC117 + 10–1074, and the triple bNAb combination VRC07–523LS + PGT121 + PGDM1400 (**Figure 7**). These regimens are all currently being explored in clinical trials. The dual bNAb combination provides significantly higher breadth and potency compared to VRC01 monotherapy, and this was further improved by the triple bNAb combination (**Figure 7*a*,*b***). For example, the geometric mean IC_{80} is 2.64 μg/mL, 0.48 μg/mL, and 0.09 μg/mL for VRC01 monotherapy, the dual bNAb combination, and the triple bNAb combination, respectively. Similarly, the breadth of neutralization at $IC_{80} < 10$ μg/mL for viruses with at least one antibody active increases from 76% to 88% to 99%, respectively. Moreover, and perhaps most notably, if it proves necessary to have two or more bNAbs simultaneously active for coverage of within-host diversity as argued above, then the triple bNAb combination still has 82% coverage, whereas the dual bNAb combination has only 41% coverage ($P = 2.2 \times 10^{-31}$, Fisher exact test) (**Figure 7*b***).

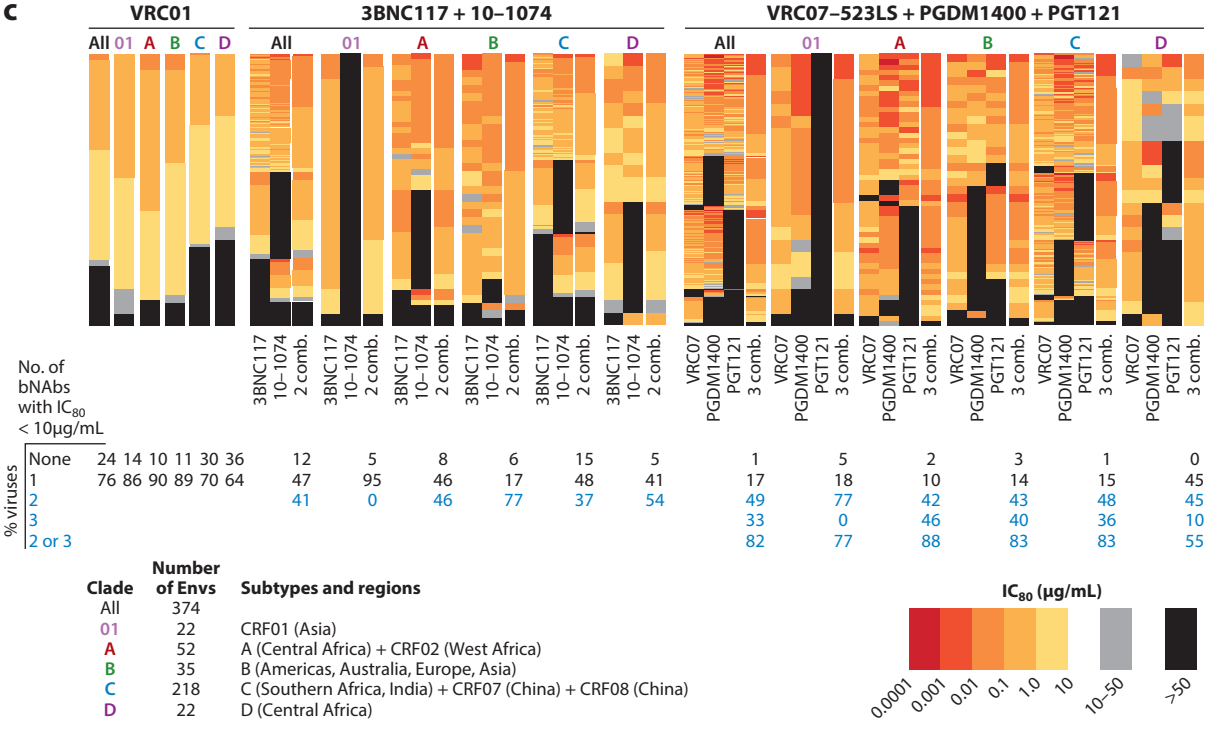
To further highlight the improved performance of the triple bNAb combination, we focused on each of the major HIV-1 subtypes (**Figure 7*c***). These patterns result in dramatic differences in coverage by the triple compared with the dual bNAb combination. For CRF01 viruses, no viruses are covered by both bNAbs in the dual bNAb combination, because CRF01 viruses are completely resistant to 10–1074. For clade C viruses, only 37% of viruses would be covered by both viruses in the dual bNAb combination. On the other hand, both 3BNC117 and 10–1074 have good coverage of clade B viruses, with 77% of subtype B viruses covered by both bNAbs, suggesting that the combination of 3BNC117 and 10–1074 would likely perform better against clade B viruses than against clade C or CRF01 viruses. In contrast, the triple bNAb combination has high coverage with at least two bNAbs in the cocktail for all subtypes (77–88%) except for subtype D (55%). This high coverage reflects the fact that the V2-specific bNAb PGDM1400 and the V3-specific bNAb PGT121 have complementary neutralization patterns (140), and the CD4bs-specific bNAb VRC07–523LS itself has exceptional breadth.

A parallel strategy to building bNAb cocktails is to generate bi- or trispecific antibodies. One such example is the 10E8.4-iMab bispecific antibody that targets the membrane proximal external regions (MPERs) of the virus and host CD4 (130). Another example is the VRC01–PGDM1400–10E8.4 trispecific antibody that simultaneously targets three epitopes on the virus (131). Such multispecific antibodies offer the possibility of extremely robust breadth with a single product and are currently in early phase clinical trials.

CONCLUSIONS

We are at a crossroads in HIV-1 prevention research. There are currently five large phase 2b and phase 3 clinical efficacy trials that will provide substantial data on the clinical efficacy of two vaccine candidates and a bNAb in preventing HIV-1 acquisition in humans. Meanwhile, next-generation vaccines aimed at inducing bNAbs and passive immunization approaches that involve combinations of bNAbs targeting multiple epitopes are being developed. Active and passive immunization efforts will need to address the important challenges of within-host, population-level, and global HIV-1 diversity to achieve optimal efficacy.





(*Caption appears on following page*)

Figure 7 (*Figure appears on preceding page*)

Intersubtype variability of bNAb potency and combination bNAb neutralization profiles. (*a*) Breadth-potency curves for single bNAbs. Each curve shows the cumulative coverage of viruses (*y*-axis) with IC_{80} less than or equal to a given value shown on the *x*-axis. The full neutralization dataset ($N = 374$, all major subtypes and circulating recombinant forms included) was used. The neutralization data for individual bNAbs were extracted from CATNAP using all viruses that had IC_{50} and IC_{80} titers reported for all bNAbs analyzed (**https://www.hiv.lanl.gov/components/sequence/HIV/neutralization/index.html**). (*b*) Neutralization breadth and potency of bNAb combinations. Left graph shows the breadth-potency curves for the dual combination of 3BNC117 and 10–1074, the triple combination of VRC07–523LS, PGT121, and PGDM1400 and for comparison the single bNAb VRC01, used in the ongoing Antibody Mediated Prevention (AMP) trials to explore its potential to prevent HIV-1 infection (129). Right graph shows the breadth-potency curves modified to consider as covered only those viruses that are simultaneously neutralized by two or more bNAbs in the combination at individual bNAb $IC_{80} < 10$ μg/mL threshold. This threshold is based on the bNAb passive transfer model of protection (**Figure 4*b***) and an average serum bNAb concentration of ~100 ug/mL. Bottom graph shows the coverage by the dual and triple combinations for each major subtype analyzed. The percentage of viruses neutralized by one, two, or three bNAbs in combination are shown by the colors indicated in the legend to the right. The clade definition, number of viruses, and geographical regions are shown in the bottom left of panel *c*. IC_{80} titers for bNAb combinations were predicted using the webtool CombiNAber (157) on the individual bNAb titers (see **Supplemental Methods** for details; **https://www.hiv.lanl.gov/content/sequence/COMBINABER/combinaber.html**). Each bNAb is assumed to be at equal concentration in the combination and the combination IC_{80} titers reported are the sum of concentrations of all bNAbs (e.g., if 3BNC117 + 10–1074 IC_{80} is 1 μg/mL, then each bNAb is present at 0.5 μg/mL). (*c*) Subtype-specific distributions of IC_{80} titers for bNAbs and combinations. IC_{80} titers are shown as heatmaps for VRC01 (*left*); 3BNC117, 10–1074, and this dual combination (*center*); and VRC07–523LS, PGDM1400, PGT121, and this triple combination (*right*). In each heatmap, viruses are represented as rows and bNAbs/combinations as columns. Each cell shows IC_{80} titer for each bNAb or combination for each virus and is color-coded according to the legend in the bottom right. Cells colored gray indicate IC_{80} between 10 μg/mL and 50 μg/mL, a range of weak neutralization that may not be adequate to provide a beneficial effect, and those colored black indicate undetectable IC_{80} responses that were above the highest concentration tested (50 μg/mL). Within each grouping of bNAbs/combinations, separate heatmaps are shown for each major subtype. Circulating recombinant forms (CRFs) that are major epidemic lineages and that are similar to a single subtype in Env are grouped with the corresponding subtype: CRF02, an important lineage in West Africa, is subtype A in Env, so it is grouped with subtype A; and similarly, CRF07 and CRF08, important lineages in China, are mostly subtype C in Env and so are grouped with subtype C. The numbers of viruses in each group are shown in the bottom left. The numbers below the heatmaps indicate the percentage of viruses in each subtype that were simultaneously neutralized by one, two, three, and two to three bNAbs in the combination, using the single bNAb $IC_{80} < 10$ μg/mL threshold. As having at least two antibodies active may be important for success, percentages of viruses with two or more bNAbs active are highlighted in blue.

Supplemental Material >

DISCLOSURE STATEMENT

D.H.B. and B.K. are co-inventors on HIV-1 vaccine patents that have been licensed.

ACKNOWLEDGMENTS

The authors acknowledge funding support from the Bill and Melinda Gates Foundation (OPP1032144, OPP1107669, OPP1169339); the National Institutes of Health (AI068614, AI114381, AI124377, AI126603, AI128751, AI129797, OD024917); the Ragon Institute of MGH, MIT, and Harvard; the Henry M. Jackson Foundation; and the American Foundation for AIDS Research. The authors further acknowledge collaborations with David Montefiori and Michael Seaman, in which several of the presented ideas were first analyzed.

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