

Strategies for HIV-1 vaccines that induce broadly neutralizing antibodies

Barton F. Haynes^{1,2,3*}, Kevin Wiehe^{1,2}, Persephone Borrow⁴, Kevin O. Saunders^{1,5}, Bette Korber⁶, Kshitij Wagh⁶, Andrew J. McMichael⁴, Garnett Kelsoe^{1,3,5}, Beatrice H. Hahn⁷, Frederick Alt⁸, George M. Shaw⁷

¹ Duke Human Vaccine Institute, Duke University School of Medicine, Durham, NC 27707

²Department of Medicine, Duke University School of Medicine, Durham, NC 27710

³Dept of Immunology, Duke University of School of Medicine, Durham, NC 27710

⁴Nuffield Department of Clinical Medicine, University of Oxford, Oxford, OX3 7FZ, UK.

⁵Department of Surgery, Duke University School of Medicine, Durham, NC 27707

⁶T-6: Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, New Mexico, 87545, USA; New Mexico Consortium, Los Alamos, New Mexico, 87545

⁷Departments of Medicine and Microbiology, University of Pennsylvania, Philadelphia, PA, 19104

⁸Program in Cellular and Molecular Medicine, Boston Children's Hospital, Department of Genetics, Harvard Medical School, Howard Hughes Medical Institute, Boston Children's Hospital, Boston, MA 02115

*Correspondence to BFH, barton.haynes@duke.edu

Barton F. Haynes, M.D.
Director, Duke Human Vaccine Institute
Frederic M. Hanes Professor of Medicine
Professor of Immunology and Global Health
2 Genome Court
MSRBII Bldg. Room 4090
DUMC 103020
Duke University Medical Center
Durham, NC 27710
Ph: 919-684-5279
Fx: 919-684-5230
barton.haynes@duke.edu

Abstract

After nearly four decades of research, a safe and effective HIV-1 vaccine remains elusive. There are many reasons why the development of a potent and durable HIV-1 vaccine is challenging, including the extraordinary genetic diversity of HIV-1 and its complex mechanisms of immune evasion. HIV-1 envelope glycoproteins are poorly recognized by the immune system, which means that potent broadly neutralizing antibodies (bnAbs) are only infrequently induced in the setting of HIV-1 infection or through vaccination. Thus, the biology of HIV-1-host interactions necessitates novel strategies for vaccine development to be designed to activate and expand rare bnAb-producing B cell lineages and to select for the acquisition of critical improbable bnAb mutations. Here we discuss strategies for the induction of potent and broad HIV-1 bnAbs and outline the steps that may be necessary for ultimate success.

Introduction

HIV-1 was discovered in 1983¹ and subsequently shown to be the cause of the acquired immune deficiency syndrome (AIDS)²⁻⁴. Effective anti-retroviral drug therapy (ART) has converted AIDS from a uniformly fatal disease to a chronic disease with a near normal lifespan⁵. Nonetheless, ~1.5 million people each year acquire HIV-1⁶. Thus, even with ART as prevention or treatment^{7,8}, there is an urgent need for a safe and effective HIV-1 vaccine.

The development of an effective HIV-1 vaccine is particularly challenging due to the exceptional and increasing genetic diversity of the HIV-1 lentivirus^{9,10}, its complex mechanisms of immune evasion¹¹⁻¹⁴, and the ability of HIV-1 to integrate into host immune cells to become resistant to host immunity and treatment regimens^{15,16}.

The first-generation of vaccines tested in clinical trials were optimized for the induction of CD8⁺ T cells¹⁷, whereas more recent trials tested vaccines designed to induce non-neutralizing antibodies^{18,19}. Out of eight HIV-1 vaccine efficacy trials completed so far, all but one failed (**Table 1**). The only trial that showed a small degree of estimated efficacy in reducing HIV-1 transmission (31.2%) was the RV144 (NCT00223080) trial of the CRFAE_01 canarypox/gp120 vaccine in Thailand^{20,21}. This trial showed that high levels of antibodies binding to the HIV-1 variable loop 2 (V2) and low levels of IgA specific for the envelope (Env) protein correlated with decreased transmission and guided the design of subsequent clinical trials²¹. However, of two phase 2b/3 clinical trials designed to improve on the RV144 (HIV-1 Vaccine Trials Network [HVTN] 702 (NCT02968849)¹⁸ and HVTN 705 (NCT03060629)^{15,19}, neither showed significant efficacy¹⁹, suggesting that the RV144 trial may not have been a harbinger of vaccine success. However, an ongoing Phase 3 trial is further exploring the concept of inducing high titres of non-neutralizing antibodies (HVTN 706, NCT03964415).

Currently, the most successful non-HIV-1 vaccines in clinical use induce neutralizing antibodies as their primary mode of protection²². Thus, for HIV-1, the induction of antibodies that broadly protect against heterologous HIV-1 strains (called *broadly neutralizing antibodies* or bnAbs) is a prime goal of HIV-1 vaccine development²³⁻²⁶.

Importantly, sera from a substantial proportion of untreated HIV-1 individuals will neutralize 50% or more of heterologous viruses, yet only a small subset of these individuals produce bnAbs with the high levels of neutralization breadth and potency that would be necessary for a protective vaccine response²⁷⁻³¹. Moreover, in HIV-1-infected individuals who produce bnAbs, these generally only develop after many months or years due to infrequent germline B cell priming and the requirement for extensive antibody somatic hypermutation²⁷⁻³¹. When bnAb activity in serum does develop, it is often mediated by a single clonal lineage (or more rarely two or three bnAb lineages) (**Figure 1**), and bnAbs constitute a minor component of an individual's overall HIV-1-specific antibody response³²⁻³⁶. Recent data suggest that in infants and children, high levels of bnAbs may develop over a shorter period of time and require fewer mutations than in adults, although the factors governing this remain unclear^{37,38}.

Overall, it is clear that the induction of bnAbs is challenging due to the unusual traits that are required to allow for breadth and neutralization capacity. These include the frequent presence of long heavy chain third complementarity determining regions (HCDR3s)³⁹⁻⁴¹ and extensive somatic hypermutation, including the selection of improbable antibody mutations that are necessary for bnAb activity as defined by structural and functional analysis⁴². The particular challenges for the induction of bnAbs are summarized in **Box 1**.

Individuals who do make potent bnAbs typically have moderate to high viral loads⁴³ and share immunological characteristics such as high levels of circulating CD4⁺ T follicular helper (T_{FH})

cells, fewer and less potent regulatory CD4⁺ T (T_{reg}) cells, and populations of dysregulated natural killer (NK) cells with reduced immunoregulatory activity^{39,44,45}. The ability to make bnAbs in the setting of HIV-1 infection is also associated with a shift in the overall B cell repertoire, with higher frequencies of B cells that produce antibodies with autoantibody-like features (**Figure 2, Box 1**)⁴⁶. Indeed, a number of studies have demonstrated that many bnAb-producing B cell lineages have features associated with polyreactivity (the ability of an antibody to bind to multiple structurally unrelated antigens⁴⁷) or autoreactivity (antibody reacts with self antigens⁴⁸) that are associated with the development of HIV-1 neutralizing antibody breadth^{40,41}. About 40% of early immature B cells in healthy humans express autoreactive and polyreactive B cell receptors (BCRs), but these are usually culled at the early stages of B cell development and only make up about 20% of the mature B-cell repertoire⁴⁹. The residual polyreactive B-cell receptors (BCRs) augment the breadth and efficacy of normal humoral responses to microbial pathogens⁴⁷. That HIV-1 bnAbs are frequently polyreactive or autoreactive can be an obstacle for both the induction and the durability of bnAb responses. Studies of the human antibody repertoire have suggested that bnAb naïve B cell precursors with long HCDR3 regions required to bind conserved neutralizing HIV-1 epitopes are relatively rare⁵⁰⁻⁵². Thus, a goal of HIV-1 vaccine design is to administer immunogens that bind to the BCRs of multiple specificities of B cells that are bnAb precursors⁵³, with the aim to induce a broader polyclonal B cell response to bnAb Env epitopes than naturally occurs in individuals with HIV-1 infection (**Figure 3**).

Strategies for bnAb immunogen design are summarized in the legend to **Figure 4**.

Durable antibody responses depend on the induction of long-lived plasma cells that persist in bone marrow (BM) niches and maintain antibody responses for years or even decades⁵⁴. Scheid et al. have demonstrated that more HIV-1 specific memory B cells are polyreactive than are BM

plasma cells with comparable specificity, suggesting that polyreactive bnAb B cells are more likely to develop into memory B cells than long lived plasma cells⁵⁵. Since a large percentage of the HIV-1 – targeted antibody response is mediated by poly- or autoreactive B cells^{40,41,47}, the reduced presence of polyreactive BM plasma cells may contribute to the inability of current HIV-1 vaccines to induce durable Env-targeted antibody responses^{20,56,57}. Novel vaccine platforms such as integrase-deficient lentiviral vectors have shown improved HIV-1 specific serum antibody durability compared to protein immunization⁵⁸. Additionally, nucleoside-modified mRNAs in lipid nanoparticles (LNPs) have been shown to induce durable low levels plasma ZIKA virus and HIV-1 Env-targeted antibody levels in rhesus macaques,⁵⁹⁻⁶¹ and a TLR7 and TLR8 agonist adjuvant has induced durable HIV-1-specific plasma cell responses in macaques⁶².

HIV-1 infection has been shown to reduce the normal tolerance controls that constrain the development and maturation of poly- or auto-reactive B cells, thereby creating an immune environment permissive for bnAb development^{39,46}. Strategies for HIV-1 vaccine-induced bnAb induction in HIV-1-negative individuals must be able to safely recreate, as much as possible, the permissive immune microenvironment that occurs in HIV-1 infection. One way to achieve this may be by selecting adjuvants that preferentially induce CD4⁺ T_{FH} cell differentiation, high titres of which are needed for bnAb development^{39,45}, and to minimize the activation of regulatory cells such as CD4⁺ T_{reg} cells and NK cells. The nucleoside-modified mRNA – LNP vaccine platform may be advantageous in this regard as it has been shown to elicit potent CD4⁺ T_{FH} cell responses to vaccine immunogens^{59,63,64}.

Another major difficulty in inducing bnAb development is the large number of mutations that are required for bnAb maturation in germinal centers (GCs)⁴². Mutations that are likely to be

introduced by the activity of activation induced cytidine deaminase (AID) arise frequently, a phenomenon termed *intrinsic mutability* of Ig genes^{42,65-69}. However, bnAbs are highly enriched in mutations that are rare during affinity maturation. The acquisition of such ‘improbable’ mutations can be guided by BCR ligands that specifically promote the expansion of B cells that have acquired such mutations^{42,70,71}. Thus, the acquisition of improbable mutations in GCs can be rate-limiting for bnAb development, as the selection of improbable mutations requires specific Env epitopes to be present at the time the rare mutation arises.

Exploring the natural pathways of bnAb generation has provided key insights into the requirements for the induction of protective neutralizing antibody responses^{33,65,70-75}. The HIV-1 vaccine field is essentially having to learn how to “engineer” the adaptive immune system to generate bnAb B cell lineages that are highly disfavored in the setting of vaccination^{51,76}. Success in the induction of multiple probable and improbable mutations in a bnAb B cell lineage in humanized mouse models has been achieved, but the critical mutations that are required generally occur sporadically and independently, reflecting unlinked mutation rather than focused accumulation of combinations of mutations in a single B cell lineage⁷⁶. Thus, a major task of HIV-1 vaccine development is to keep bnAb B cell lineages in GCs, or ensure they recirculate back to GCs, where they may accumulate the improbable mutations required for bnAb activity. Moreover, a successful bnAb-inducing vaccine will need to reduce the time needed for bnAb affinity maturation by providing optimal Env epitopes that are required to select for improbable bnAb mutations. In antibody-virus co-evolution studies in HIV-1-infected individuals, it was found that bnAb development requires viral diversification, suggesting the need for sequential immunizations with diverse Env epitopes that are capable of selecting for key mutations^{32-34,65,70,72,73,75,77,78}. In this Review, we discuss current strategies for HIV-1 bnAb vaccine

development, reflect on progress made to date in induction of bnAb lineages in animals and humans, and describe the different classes of HIV-1 bnAbs identified to date. Moreover, we explain the need for combined T and B cell vaccines and discuss the requirements that need to be met for the development of a practical HIV-1 vaccine.

[H1] BnAb biology: vaccine implications

Detailed studies of HIV-1-neutralizing antibody co-evolution have been carried out to elucidate the “arms race” between the evolving virus and B-cell lineages that mature to produce bnAbs^{32-34,65,70,72,73,75}. These studies have identified clonal “trees” of bnAb B cells, their inferred bnAb unmutated common ancestors (UCAs)⁷⁹, and the transmitted/founder viruses [G] or their progeny that are bound by the BCRs of lineage members⁸⁰. Current bnAb vaccine approaches are based on the hypothesis that priming B cells with Env molecules that are capable of binding to and activating naïve B cells that express UCAs, followed by sequential Env immunogens that can select for improbable mutations and guide otherwise disfavored bnAb B cell lineages to develop into full bnAb capability, can be ultimately successful^{42,81}. Additional requirements for the design of sequential vaccines are the creation of affinity gradients of the Env immunogen across stages of bnAb B cell lineage development^{25,76,82}, in order to create an “affinity pull” across the lineage such that a ceiling of affinity is not reached until the B cell lineage achieves full maturation⁸³. Moreover, vaccine-induced acquisition of fast on-rates [G] is a critical attribute for at least a subset of bnAb B cell lineages to mature⁸⁴⁻⁸⁹. Given the complex characteristics that a successful HIV-1 vaccine will require and the physiological factors that disfavor bnAb induction (**Box 1**), a number of strategies for sequential immunogen design (B cell lineage

immunogen design²⁵, germline targeting^{25,51,77,78,90}, mutation-guided immunogen design⁴², and structure-based immunogen design⁹¹) have been developed (**Figure 4**).

With continued exposure to sequential Envs, bnAb B cell lineages can either remain on the evolutionary track to bnAb maturation or go off track by the accumulation of mutations that disable Env reactivity^{70,92,93}. In particular, the autoreactivity of some bnAb B cells raises the possibility that bnAb B-cell maturation may be subjected to the process of “antibody redemption”, whereby autoreactive B-cell lineages that have escaped central and peripheral tolerance checkpoints are rendered less or non-autoreactive during their somatic evolution in GCs^{94,95}. In the case of some bnAbs, the loss of autoreactivity is tantamount with concomitant loss of bnAb activity since autoreactivity can be required for bnAb potency^{40,41,47,96}.

To guide otherwise disfavored bnAb B cell lineages to affinity mature to acquire full neutralization capacity, sequential protein Env immunogens should be formulated with adjuvants that promote T_{FH} cell responses and disfavor T_{reg} cell induction in order to overcome peripheral tolerance mechanisms such as B-cell anergy. The engineering required to design immunogens that induce bnAb-precursor-producing B-cell lineages to affinity mature in the face of multiple developmental roadblocks is unprecedented in vaccinology and requires an in depth understanding of B cell development and differentiation in the setting of vaccination. This contrasts with the development of COVID-19 vaccines in less than a year, owing both to the dramatically different host-virus interactions of SARS-CoV-2 compared to HIV-1, and to the extreme viral diversity of HIV-1 and the relative conservation of SARS-CoV-2^{97,98}.

Animal models are critical for the preclinical work towards the development of an HIV-1 vaccine that induces bnAbs. The two most useful models for validating immunogen design are

simian-human immunodeficiency viruses (SHIVs) for infection of rhesus macaques (**Box 2**) and humanized mouse models (**Box 3**).

[H1] Types of bnAbs

A number of bnAbs have been identified in individuals with HIV-1, and have undergone in-depth characterization. These bind to several different conserved epitopes on the HIV-1 Env (**Figure 3, Table 1**). In general, Env epitopes for vaccine design have been chosen on the basis of the breadth and potency of bnAbs isolated from HIV-1 infected individuals that bind the respective Env epitopes.

[H2] CD4 binding site-targeted bnAbs

There are two types of bnAbs that bind the CD4 binding site of Env; CD4 mimic bnAbs and HCDR3-binder CD4 binding site bnAbs (HCDR3-binder bnAbs). These are prime targets of HIV-1 vaccine development efforts. BnAbs of the first type mimic the CD4 contact residues for the CD4 binding site of Env via their HCDR2 regions and rely less on HCDR3 contacts with Env compared to the second type. Examples for CD4 mimic bnAbs are the VRC01-class and the 8ANC131/CH235-class, and an example of a HCDR3-binder bnAb is CH103. (**Table 1**)

[H3] CD4 mimic bnAbs

VRC01-class antibodies contain the variable heavy 1-2 (VH1-2) heavy chain and require the use of a five amino acid light chain complementary determining region 3 (LCDR3)^{75,99-102}. However, one VH1-2 bnAb in this class (IOMA) has a normal length LCDR3 and fewer mutations and insertions or deletions than other VRC01-class antibodies¹⁰³. VRC01 bnAbs are among the most potent and broad of all bnAbs isolated to date and their precursors are more common than other bnAb precursors^{77,99}. However, a major roadblock for VRC01-class bnAb induction is the

requirement for many improbable mutations, as well as antibody insertions and deletions, in order to acquire full breadth^{42,86}. The VRC01-class is represented by 11 prototypic B cell lineages, which range from 80% neutralization breadth of heterologous primary HIV-1 strains (VRC-PG20) to 100% breadth (bnAb N49P7) (**Table 1**).

A germline targeting immunogen, termed eOD-GT8, with picomolar affinity for the inferred unmutated common ancestor (UCA) of VRC01⁷⁷ has been designed, and binds to most but not all putative UCAs of other members of the VRC01-class of bnAbs^{75,104}. In VRC01 knock-in mice, eOD-GT8 primes and expands VRC01 precursors, and in combination with sequential boosting immunogens¹⁰⁵, can select for B cells with VRC01 BCRs that have a degree of neutralization breadth¹⁰⁶⁻¹⁰⁸. eOD-GT8 is immunogenic in macaques, which respond more rapidly to subcutaneous rather than intramuscular injections¹⁰⁹. In the IAVI G001 human trial, eOD-GT8 expanded B cells bearing BCRs with characteristics of VRC01 precursors¹¹⁰.

In addition to eOD-GT8, glycan-deleted derivatives of the clade C HIV-1 envelope 426c Env have been designed as immunogens that bind to VRC01-class bnAbs^{78,90,105,111}. Initially, these designs focused on the strategic removal of glycans, since glycans at Env positions 276, 460, and 463 were found to be major obstacles for binding to VRC01-class precursors⁹⁰. Comparison of eODGT8 and 426c Env immunogens in VRC01 UCA heavy chain knock-in mice showed that the two immunogens engage different VRC01 bnAb precursors¹⁰⁵. However, the Antibody Mediated Protection (AMP) trials in South Africa, which studied the protective efficacy of passively administered VRC01 bnAb, showed no overall protection from HIV-1 infection by VRC01 bnAb administration¹¹²⁻¹¹⁴. Further study showed that for HIV-1 isolates sensitive to VRC01, the level of VRC01 bnAb required for preventing transmission was ~1:200¹¹². This indicated that a successful HIV-1 vaccine will require high levels of induced bnAbs that are

maintained at protective levels over long periods of time, and will require bnAb induction to multiple Env epitopes to achieve neutralization breadth^{115,116}. Moreover, during the AMP trial, HIV-1 diversification was greater than anticipated¹¹², and the continuing evolution of HIV-1 will need to be considered.

The second type of CD4-mimic bnAbs, the 8ANC131/CH235-class, exclusively uses the variable heavy chain region 1-46 (VH1-46) but does not require the 5 aa LCDR3 light chain found in VRC01-class antibodies^{33,65,117-120}. The 8ANC131/CH235-class includes the bnAbs CH235, 8ANC131, 1-18 and 1B2530, and their neutralization breadth ranges from 97% neutralization for the bnAb 1-18 to 71% for the bnAb 8ANC131 (**Table 1**). An individual (CH505) in the CHAVI001 acute HIV-1 infection cohort made both the CH235 bnAb as well as a member of the second type of CD4 binding site bnAb, the HCDR3-binder CH103. In this individual, the bnAb response required cooperation between the two neutralizing antibody lineages, whereby an early autologous neutralizing antibody of the CH235 lineage selected for viral escape mutants that were sensitive to the CH103 bnAbs produced by affinity matured B cells³². A similar mechanism of cooperation between neutralizing lineages was observed in an HIV-1 infected individual (CH848) who made the DH270 V3-glycan bnAb⁷⁰. In addition, VRC01-class bnAb 1-18 is particularly resistant to HIV-1 escape¹¹⁸. Most HCDR3 binder- bnAb lineages that bind the CD4 binding site, like the CH103 bnAb lineage, do not have the same level of neutralization breadth as the CD4 mimicking bnAb lineages (for example, the CH103 bnAb lineage achieves only 67% neutralization breadth)³³.

Rhesus macaques have an ortholog for the human VH1-46 region and can make CH235-like antibodies⁷⁶. In addition, Env proteins have been designed that potently induce both the expansion of CH235 precursors and select for a subset of improbable functional mutations that

are necessary for acquiring heterologous neutralizing breadth in both UCA knock-in mice and in rhesus macaques^{76,121}. Like observed for VRC01-class bnAbs, the frequency of 8ANC131/CH235-class bnAb precursors does not appear to be limiting, but the very high frequency of required improbable mutations appears to be the predominant factor disfavoring induction of this bnAb class. Unlike VRC01-class bnAbs, most CH235 bnAbs do not require insertions or deletions (rare events in B cell maturation) for acquisition of bnAb breadth^{65,103}.

[H3] HCDR3-binder bnAbs

For the induction of HCDR3-binder bnAbs, a transmitted/founder gp120 Env, termed CH505 T/F trimer (it was derived from individual CH505 from the CHAVI001 acute HIV-1 infection cohort) with relatively low affinity for binding to the CH103 UCA was designed and tested as priming immunogen in the HVTN115 trial (NCT03220724)³³. Although the CH505 T/F gp120 monomer had expanded CH103 UCA B cells in UCA knock-in mice⁹³, it did not expand CH103 precursors in the HVTN 115 clinical trial¹²² (Sobieszczyk, M, Williams W, Haynes BF, Kobie, J unpublished results). As a follow-up to test the effect of affinity in mediating bnAb precursor expansion, a near-native stabilized CH505 T/F trimer with higher affinity for the CH103 UCA is now being tested in humans in the HVTN 300 clinical trial (NCT04915768). An additional factor limiting induction of HCDR3-binder or other CD4 binding site bnAbs may be the acquisition of autoreactivity at various stages in the bnAb B cell lineages^{11,33}.

[H2] V3-glycan-targeted bnAbs.

A second major bnAb epitope of Env, the V3-glycan patch, is located at the base of its V3 loop region at the 'GDIR' sequence (amino acids 324-327), located between two N-linked glycans at positions 301 and 332^{123,124}. Unlike CD4 mimic bnAbs that utilize VH1 heavy chains and have

normal length HCDR3s, bnAbs targeted at V3-glycans are encoded by multiple V_H gene segments and have long HCDR3s (18-24 aa). There are 6 prototypic V3-glycan bnAb lineages that range in neutralization breadth from 51-68% (**Table 1**). Their long HCDR3s are required to reach deep between glycans to bind to the Env polypeptide backbone¹²⁴. However, for vaccine development, the requirement for a long HCDR3 region means that V3-glycan precursors are very rare due to the deletion of B cells with BCRs with long HCDR3s by immune tolerance mechanisms^{51,125,126}. For example, V3-glycan precursors of the BG18 bnAb B cell lineage have been found to be present at a frequency of only 1 in 53 million⁵¹. V3-glycan germline targeting Envs have been shown to expand these precursors in UCA knock-in mice^{51,127,128}, while also expanding precursors that have acquired the improbable mutations required for expanding heterologous breadth^{76,122}. Moreover, in mice with a knock-in of a minimally mutated bnAb precursor, sequential immunization with Env immunogens induced antibody affinity maturation¹²⁹. Immunization of macaques with the Env immunogen RC1, which has mutations in its V1 region and glycan deletions that result in increased accessibility of the V3 glycan site, elicited V3 glycan-targeted antibodies that were dependent on the glycan at amino acid position 332 of Env, but structural analysis revealed differences in antibody orientation compared to other V3-glycan bnAbs¹³⁰. Boosting of macaques with sequential heterologous Env immunogens induced low levels of heterologous neutralizing antibodies targeting the V3-glycan patch, but also promoted the development of antibodies that bound off-target and offered minimal protection following SHIV challenge¹³¹. Besides the requirement for long HCDR3 regions, V3 glycan bnAbs have high levels of rare mutations required for acquisition of bnAb breadth⁴².

[H2] *V2-glycan-targeted bnAbs.*

The V2-glycan epitope includes an N-linked glycan at position 160 and a lysine-rich C-terminal strand (around positions 168-171). There are five prototypic V2-glycan bnAb B cell lineages termed PG9, PGDM1400, VRC26.25, CH01 and PCT64 (**Table 1**). Many V2-glycan-targeted bnAbs utilize their long negatively charged HCDR3 loops and sulfated tyrosines to penetrate the Env glycan shield and interact with the positively charged C-terminal strand¹³². The HCDR3 loops of V2-glycan bnAbs are typically even longer (24-36 aa) than those found in V3-glycan bnAbs (**Table 1**). This requirement makes precursors of V2-glycan bnAbs exceedingly rare. The long HCDR3s of many V2-glycan antibodies (including the prototypic bnAbs PG9, CH01, PCT64, as well as the non-prototypic bnAbs CAP256.VRC26 and PG16) are characterized by the presence of a YYD tyrosine sulfation motif¹³²⁻¹³⁵.

In one study, antibodies with HCDR3 regions similar to the PG9 bnAb were found by ultra-deep sequencing in only 2 out of 70 HIV-1 naive individuals⁵². In UCA knock-in mice, a series of immunogens was used to stimulate V2-glycan bnAb precursors¹³⁶, and one study detected V2-glycan bnAb induction by vaccination with a series of engineered Env immunogens in rhesus macaques¹³⁷. To date, V2 glycan germline targeting immunogens have yet to be studied in clinical trials, however a chimpanzee V2 glycan UCA-binding Env called MT145KdV5 is in GMP production for testing in a Phase 1 clinical trial¹³⁶.

Although V2-glycan bnAb precursors are rare, a study of a Sub-Saharan HIV-1 primary infection cohort detected V2-glycan bnAbs in 14% of HIV-1 infected individuals who had high serum levels of bnAbs, whereas the most common bnAb type was V3-glycan bnAbs, which were observed in 38% of bnAb-high subjects¹³⁸. V2-glycan bnAbs have also been observed in SHIV-infected macaques¹³⁹. Thus, HIV-1 or SHIV infection induces either a permissive immunologic milieu that facilitates the induction of bnAbs with rare long HCDR3s, or the

precursor frequency of potential V2 bnAb BCRs in humans and macaques is greater than previously estimated. Precisely why retroviral infection leads to bnAb induction in only a subset of humans and macaques is not completely understood. The reasons are likely multifactorial, including shifts in the B cell repertoire that favor the expression of long HCDR3s and the relaxation of immune tolerance mechanisms due to retroviral infection^{39,46}.

[H2] *BnAbs targeting the membrane proximal external region of gp41*

Two different regions of the membrane proximal external region (MPER) of gp41 (the transmembrane domain of Env), are also targets of bnAbs and have both been found to be immunogenic in HIV-1 infected individuals. bnAbs targeting these regions are represented by six prototypic B cell lineages (**Table 1**). bnAbs targeted at the distal MPER, such as 10E8 and DH511.2-K3, are the most potent members of this class and both of these bnAbs use the same VH3-15 gene segment, and have 24 aa HCDR3, and like most other MPER bnAbs, are of the IgG3 isotype (**Table 1**)^{34,140}. Notably, the bnAbs 10E8 and DH511.2-K3 are also among the broadest (~99%) HIV-1 neutralizing antibodies isolated to date, making them attractive vaccine design targets. Other distal MPER bnAbs such as VRC42.1, CH12, PGZL1 and 4E10 are encoded by the VH1-69 gene segment with the variable light chain VK3-20¹⁴¹ (**Table 1**). Most MPER bnAbs share the traits of long HCDR3s, high levels of V_H mutations, and bind to a membrane proximal binding epitope that includes binding both the gp41 MPER and to viral membranes (**Table 3**). Indeed, all MPER bnAbs must also bind lipids in order to neutralize HIV-1, as this is thought to tether the bnAb to the virus lipid bilayer to be available for binding of the MPER after epitope exposure during receptor-mediated Env activation [**G**]^{96,142}. MPER bnAbs are among the most polyreactive of HIV-1 bnAbs and have been shown to bind, in addition to

lipids⁴⁰, host proteins including the splicing factor SF3B3 (4E10 bnAb) and kynureninase. Kynureninase carries a dimerization motif (ELDKWA) that is identical to an epitope in the proximal MPER bound by the bnAb 2F5¹⁴³. Mice engineered to express mature MPER bnAbs or UCAs of MPER bnAbs characteristically exhibit deletion or receptor editing of bnAb precursors in the bone marrow and anergy of residual bnAb precursor B cell lineages in the periphery¹⁴⁴⁻¹⁴⁷. For 2F5 bnAb knock-in mice, this central tolerance checkpoint appears not to be driven by interaction with lipids, since B cell compartments rescued by receptor editing or anergy lose the capacity to bind kynureninase but retain lipid-reactivity¹⁴⁶. Interestingly, in opossums, a point mutation in the kynureninase gene results in a dimerization motif (ELEKWA) that differs from that in humans by the single D→E replacement. In contrast, the SF3B3 proteins of humans and opossums are identical¹⁴³. When opossums were immunized with gp41 MPER immunogens, they responded robustly to the ELDKWA epitope, presumably in the absence of tolerance imposed by the endogenous ELEKWA motif, but had little or no response to the SF3B3 protein they share with humans¹⁴³. Thus, in humans, tolerance to kynureninase and SF3B3 likely limit the response to both the proximal and distal MPER bnAb epitopes.

Nonetheless, immunization of monkeys and UCA knock-in mice with an MPER-peptide liposome containing the proximal and distal MPER bnAb epitopes induced precursors of 2F5-like antibodies to expand⁸⁷. In monkeys, a 2F5-like bnAb B cell lineage expanded and underwent affinity maturation but was limited in its capacity to undergo full bnAb maturation by lack of an improbable proline mutation in the HCDR3⁸⁷. Thus, in mice and monkeys, it was shown that antibodies binding the proximal MPER epitope can be induced with germline targeting immunogens.

The human immunization trial HVTN133 (NCT03934541) tested an MPER peptide-liposome as immunogen to determine whether B cell precursors targeting the proximal ⁶⁸³LDKW⁶⁸⁶ epitope of gp41 can be expanded, and bnAb precursors that target this epitope have indeed been identified in clinical trial vaccinees (Williams, W, Baden, L, Haynes, BF, unpublished results). However, to date, success has not been achieved in any model system that stimulates the precursors of the more potent distal MPER bnAbs like 10E8 or DH511-K3. This may be due, in part, to the close proximity of the distal MPER epitope to the viral membrane and to the extraordinary autoreactivity of some of the distal MPER antibodies. For example, 4E10 cross-reacts with SF3B3, has strong reactivity to lipids such as cardiolipin and has lupus anticoagulant activity^{40,143}. Moreover, it can prolong the partial thromboplastin time [G] when administered to humans *in vivo*¹⁴⁸. In general, however, it is thought that autoreactive HIV-1 bnAbs are not pathogenic. Rather, the poly- or autoreactive nature of bnAbs reflects their requirement to have unusual traits such as long HCDR3s for bnAb activity.

[H2] *Fusion domain-targeted bnAbs.*

The fusion domain peptide [G] is exposed on the surface of the HIV-1 Env trimer, and bnAbs targeting this epitope, such as the VRC34 lineage, have been identified. Vaccination of mice or monkeys with fusion domain peptides conjugated to carrier proteins [G] (to enhance immunogen valency and to provide a source of T cell epitopes for CD4⁺ T_{FH} induction) has been shown to induce antibodies that neutralize heterologous HIV-1 strains¹⁴⁹⁻¹⁵². Whereas the general strategy for vaccine induction of most HIV-1 bnAbs has been to employ some form of B cell lineage design by targeting UCAs or germline naïve B cells, followed by sequential boosts to guide favored bnAb B cell lineages, a different strategy is pursued for fusion domain-targeted bnAbs. Here, vaccination with a fusion domain linear peptide sequence is followed by

immunofocusing^{153,154} [G] with fusion domain peptides. Although fusion domain-targeted bnAbs are not as potent or broad as other types of HIV-1 bnAbs, fusion domain-targeted antibodies could potentially be an important component of a vaccine-induced polyclonal and multi-epitope neutralizing antibody response to Env. There are currently no known immune mechanisms that disfavor the formation of fusion domain-targeted antibodies, although to date, potent fusion domain bnAbs have been difficult to induce. An analysis of the fusion peptide-targeted bnAb lineage VRC34 revealed an accumulation of improbable mutations during affinity maturation. In particular, a Y→P mutation at position 33 was a key functional improbable mutation that occurred during affinity maturation and regulated the interaction between VRC34 intermediate antibodies and the fusion peptide^{150,152}. This mutation occurred in the clade of the lineage that acquired broad neutralizing activity, but did not occur in other weakly neutralizing clades of the VRC34 lineage¹⁵². Therefore, the requirement for acquisition of improbable mutations may be one impediment to the development of neutralization breadth by this antibody class^{42,152}.

[H2] *Fab dimerized glycan bnAbs.*

The glycan reactive bnAb 2G12 was isolated from an HIV-1-infected individual in 1996¹⁵⁵ and shown to have a unique V_H domain swap conformation that formed an “I-shaped” bnAb with an expanded fab-dimerized paratope [G] capable of Env glycan binding, called Fab dimerised glycan (FDG)¹⁵⁶. Recently, Williams et al. isolated two new FDG-targeted bnAbs with the characteristic “I-shape”, but without the V_H domain swap found in the bnAb 2G12, from SHIV-infected macaques¹⁵⁷. Macaques immunized with a glycosylated V3-peptide and boosted with scaffolded mannose glycans [G] expand FDG precursor antibodies, and FDG bnAbs have also been isolated from SHIV-infected monkeys (bnAbs DH851 and DH1003)¹⁵⁷. Interestingly, human FDG precursor antibodies that were isolated with high mannose-containing ‘hooks’

demonstrated that they were predominantly of the IgM isotype and were present in the CD27⁺, IgM⁺, IgD⁻ marginal zone B cell pool, which is thought to be the source of natural antibody-producing B cells^{158,159}. In contrast, in the context of SHIV infection, FDG precursor antibodies that have bnAb activity are of the IgG isotype, whereas FDG precursor antibodies of the IgM isotype have no heterologous neutralizing activity. FDG bnAbs can target multiple glycan sites on HIV-1 Env and are therefore a promising component of a polyclonal bnAb response, despite the current lack of neutralization breadth of any particular FDG bnAb¹⁵⁷. Thus, one strategy to elicit FDG antibodies (and perhaps other glycan-reactive HIV-1 bnAbs) with vaccines is to stimulate the pool of glycan-reactive natural antibodies [G] to class-switch from IgM to IgG and become bnAbs¹⁵⁷. Natural glycan-reactive antibodies generally develop in a T cell-independent manner¹⁵⁹. However, since bnAbs generally develop in germinal centers with help provided by CD4⁺ T_{FH} cells, these data suggest that a successful HIV-1 vaccine strategy for some types of bnAbs will be to recruit the precursors of extrafollicular natural glycan- antibody-producing B cells into germinal centers to interact with T_{FH} cells for maturation to neutralization breadth. Studies are underway to test this hypothesis, and different vaccine delivery regimens are being explored that prolong the GC response and promote CD4⁺ T_{FH} responses¹⁶⁰⁻¹⁶³ [G] (**Box 4**).

[H1] Combined T and B Cell Vaccines

T cell responses can contribute to HIV-1 vaccine efficacy in two ways. First, the induction of strong CD4⁺ T_{FH} responses is required to support vaccine-mediated HIV-1 bnAb induction^{39,45}. It was shown that lipid nanoparticle (LNP) enclosed mRNA can induce potent T_{FH} responses. Moreover, ionizable LNPs can be used as an adjuvant in combination with protein vaccines to promote CD4⁺ T cell help^{59,64}. Second, T cells can act as direct effectors of vaccine-mediated

protection by mechanisms including cytolysis of HIV-1-infected cells by CD8⁺ T cells¹⁶⁴⁻¹⁶⁷. In acute HIV-1 infection, HLA class Ia-restricted T cell responses play an important role in the containment of viremia and drive a rapid selection for HIV-1 escape mutants¹⁶⁸, and they make a key contribution to sustained control of viral replication in HIV-1 elite controllers¹⁶⁴. However, they fail to eradicate HIV-1 after viral integration has occurred¹⁶⁸. Vaccines that are primarily designed to induce HLA class Ia-restricted CD8⁺ T cell responses have failed to prevent HIV-1 transmission to date. Although limited success has been achieved with CD8⁺ T cell-inducing vaccines in reducing setpoint viral loads [G] after infection in preclinical models¹⁶⁹, clinical trials conducted to date with such vaccines have not succeeded in reducing viremia^{170,171}. Thus, although HLA class Ia-restricted CD8⁺ T cells clearly have some degree of anti-HIV-1 activity in animal models, they have not yet been shown to protect against infection in the setting of vaccination in humans, nor are they able to clear infection in the setting of acute HIV-1 infection.

However, experiments in rhesus macaques have shown that a vaccine that induces HIV-1-reactive CD8⁺ T cells restricted by the MHC class Ib molecule MHC-E shows some protection from infection, where it eradicated SIV infected cells early in infection in ~55% of animals¹⁶⁵. The vaccine that achieved this unprecedented protective effect employed a rhesus cytomegalovirus (RhCMV) vector with a fortuitous gene deletion that allows it to induce MHC-E-restricted CD8⁺ T cells^{165,166,172,173}. Although this vaccine did not prevent initial infection, it did eliminate virus-infected cells before a durable latent pool of infected CD4⁺ T cells was established, at least in ~55% of the animals. In vitro experiments have shown that it is possible to prime human HIV-1 targeted HLA-E-restricted CD8⁺ T cells¹⁶⁷, and the observation that insertion of orthologous human (H)CMV genes into RhCMV generates a vector that induces the same level of protection upon SIV challenge as the original RhCMV vector in rhesus

macaques¹⁷³ suggest that it may be possible to develop vaccines that induce HLA-E restricted anti-HIV-1 T cells in humans. A HCMV-vectored HIV-1 vaccine is currently in development (NCT0472587).

Notably, experiments in macaques have shown that the combination of a potent T cell vaccine, which stimulates classical HLA-Ia restricted CD8⁺ T cells, with a B cell vaccine that induces autologous neutralizing antibodies, resulted in protection from infection at lower bnAb titres compared to the B cell vaccine alone¹⁷⁴. Moreover, combination of a T cell immunogen with a B cell immunogen did not compromise the potency of either vaccine in an animal model¹⁷⁵.

Therefore, a combination of a next-generation T cell vaccine with a bnAb-inducing vaccine may be more effective than either alone. Moreover, a combination of a T cell vaccine that can induce anti-HIV-1 HLA-E-restricted responses with CD4⁺ T_{FH}- inducing and bnAb-inducing vaccine constructs, may hold promise.

[H1] Outlook

Much progress has been made in B cell lineage immunogen design, germline targeting, immunofocusing and structure-based immunogen design. Overall, the field is using a strategy of targeting immunogens to naïve BCRs followed by sequential immunogens that select memory B cells with BCRs with improbable mutations to be activated to proliferate and continue to acquire functional mutations that result in acquisition of bnAb breadth^{25,42,51,76,79,81,176,177}. Although the specific design of sequential envelopes for a practical vaccine remains to be determined, the desired attributes are known, and there is now a better understanding of the immunobiology of bnAb development and the roadblocks that prevent their induction¹¹ (**Figure 1** and **Box 1**).

The requirements for a multicomponent vaccine that is capable of inducing bnAb lineages are daunting. Sequential immunogens need to keep bnAb maturation on track and avoid the selection of antibody mutations that interfere with binding to the Env bnAb epitope. If a single Env protein is to be used as a priming immunogen, it must bind to many diverse bnAb UCAs. Similarly, studies are underway to determine whether many steps of a sequential vaccine can be administered simultaneously to simplify vaccination regimens. As noted above, bnAb-inducing vaccines also need to elicit potent CD4⁺ T_{FH} responses in order to stimulate high levels of highly mutated and long-lived bnAbs, and a final version of a practical HIV-1 vaccine will likely also need a component that can induce broadly cross-reactive CD8⁺ T cell responses to kill HIV-1 infected CD4⁺ T cells. A key attribute of a successful HIV-1 vaccine will be to prevent infection by the majority of circulating HIV-1 strains. HIV-1 has continued to diversify in terms of within-clade evolution¹⁷⁸, and such increasing diversity can have an impact on their sensitivity to bnAb neutralization^{178,179}. In addition, the complexity of new recombinant forms of HIV-1 and the diversity of clades and recombinant forms within local geographic populations are increasing⁹⁸. The panels of HIV-1 pseudoviruses currently in use to evaluate vaccine induced neutralizing antibody responses are based on older HIV-1 strains, and are in the process of being updated to better represent contemporary diversity of HIV-1 as well as physiologically relevant transmitted founder viruses.

Despite the utility of the bnAb UCA knock-in mouse model and SHIV challenges in rhesus macaques, studies of complex HIV-1 vaccines in humans are necessary to learn the precise rules for shaping the B cell response to disfavored Env bnAb epitopes and for determining the response of the outbred human B cell repertoire to experimental vaccine components and full

practical vaccines. To accomplish this evaluation of bnAb vaccines *in vivo*, the HIV Vaccine Trials Network (HVTN) has established the HVTN Experimental Medicine Trials protocol for testing immunogens in small groups of human volunteers so that rapid vaccine design iteration can occur⁸⁰. In this manner, multiple vaccines can be tested in the context of the human immune system.

A protective HIV-1 vaccine will likely be the most complex vaccine ever designed, employing novel vaccine platform technologies such as modified mRNAs in lipid nanoparticles or novel vectors. Given that early after infection, HIV-1 provirus can integrate into the host genome as latent virus without producing viral proteins^{180,181} becoming, in effect, invisible to the immune system. For this reason, an effective HIV-1 bnAb vaccine that aims to prevent transmission with sterilizing immunity will need to be essentially 100% effective for both blood and mucosal exposure, an extraordinary bar that no vaccine has yet achieved^{16,182}. Finally, the production of multicomponent protein vaccines will be challenging both due to the difficulty and time necessary to produce multiple proteins by good manufacturing practice and from the cost of such a vaccine. The modified mRNA and LNP technology that has been so successful for COVID-19 vaccines^{183,184} may be more amenable for production of multicomponent vaccines^{128,185-190}.

The hope is that once a vaccine is designed and shown to be effective, the correlates of protection [G] of a successful bnAb vaccine will be identified and the complex nature of the immunogen can be simplified and made practical for administration in countries around the globe.

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Author contributions

BFH researched and wrote the first draft of the paper. All authors contributed to the content and edited the manuscript.

Competing Interests

K.O.S. and B.F.H. are inventors on International Patent Application PCT/US2018/020788 submitted by Duke University, that covers the composition and use of CH848 HIV-1 envelopes for induction of HIV-1 antibodies. K.O.S., K.W., and B.F.H. are inventors on International Patent Application PCT/US2018/03477 submitted by Duke University, that covers the composition and use of CH505 HIV-1 envelopes for induction of HIV-1 antibodies.

Peer review information

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Table 1. HIV-1 vaccine efficacy trials completed or in progress.

Trial	Start	End	Vaccine	Location	Result	References
VAX004 (NCT00002441)	1999	Jan 2000	Bivalent Clade B gp120 in Alum	U.S., Europe	No Efficacy	191-193
VAX003 (NCT00006327)	Mar 1999	Aug 2000	Bivalent CRF_01AE/B gp120 in Alum	Thailand	No Efficacy	194-196
HVTN 502 (Step) (NCT00095576)	Nov 2004	Sep 2009	Adenovirus type 5 Clade B gag/pol/nef	U.S.	No Efficacy; increased infection in vaccinees	170,197-202
HVTN 503 (Phambili) (NCT00413725)	Dec 2006	Jul 2015	Adenovirus type 5 Clade B gag/pol/nef	South Africa	No Efficacy; increased infection in male vaccinees	171,203-206
RV144 (NCT00223080)	Sep 2005	Apr 2019	ALVAC with gag/pro/Env; Bivalent CRF_01AE/B gp120 in Alum	Thailand	Estimated 31.2% vaccine efficacy at 42 months; 12-month efficacy, 60%	20,21,207-216
HVTN 505 (NCT00865566)	May 2009	Oct 2017	DNAs with Clade B gag/pol/nef and DNAs with Clade A, B, C Envs Adenovirus type 5 with gag/pol and Clades A,B,C Envs	U.S.	No Efficacy	197,217-223
HVTN 703/ HPTN 081 (NCT02568215)	May 2016	Mar 2021	Antibody Mediated Protection (AMP) Trial of VRC01 neutralizing antibody infusion IV	Sub-Saharan Africa	No overall Efficacy; protection from only highly sensitive HIV-1 strains	112,224,225
HVTN 704/ HPTN085 (NCT02716675)	Apr 2016	Dec 2020	Antibody Mediated Protection (AMP) Trial of VRC01 neutralizing antibody infusion IV	North America, South America, Switzerland	No overall Efficacy; protection from only highly sensitive HIV-1 strains	112,224,225
HVTN 702 Uhambo (NCT02968849)	Oct 2016	Sep 2021	ALVAC-C with gag/pol/Env; Bivalent gp120s in MF59	South Africa	No Efficacy	18,226
HVTN 705 Imbokodo (NCT03060629)	Nov 2017	August 2021	Ad26, 4 valent T cell mosaic genes, boost with clade C gp140 Env	Subsaharan Africa	No Efficacy	227
HVTN 706 Mosaico (NCT03964415)	Oct 2019	Ongoing (est. Mar 2024)	Ad26, 4 valent T cell mosaic genes, boost with clade C gp140 Env + B cell mosaic gp140 Env	US, Spain, Central/South America	Ongoing	N/A

1 **Table 2. Broadly neutralizing antibodies and their characteristics**

HIV-1 Epitope	BnAb	Breadth ^a (Potency ^b)	V _H /V _L Gene	CDRH3 Length	CDRL3 Length	V _H Mut Freq	V _L Mut Freq	Germline Targeting Priming Immunogen
CD4 Binding Site	N49P7	100% (0.10)	VH1-2 /VL2-11	19	5	24.5%	14.1%	
	N6	99% (0.062)	VH1-2 /VK1-33	13	5	30.2%	22.4%	
	12A12	93% (0.221)	VH1-2 /VK1-33	13	5	21.9%	15.5%	eOD-GT8 ⁷⁷
	VRC01	91% (0.377)	VH1-2 /VK3-20	12	5	31.6%	17.2%	426c.TM4ΔV1-3 ¹¹¹ , BG505 SOSIP 4.1 GT1 ⁵³ , eOD-GT8
	3BNC117	89% (0.116)	VH1-2/ VK1-33	10	5	23.7%	14.8%	eOD-GT8
	VRC-CH31	84% (0.321)	VH1-2 /VK1-33	13	5	20.2%	15.2%	426c.TM4ΔV1-3, eOD-GT8
	PCIN63.71I	84% (0.46)	VH1-2 /VK1-6	13	5	14.6%	12.5%	
	VRC-PG04	81% (0.317)	VH1-2 /VK3-40	14	5	28.6%	15.2%	eOD-GT8
	VRC-PG20	80% (0.226)	VH1-2 /VL-2-14	13	5	24.0%	14.8%	426c.TM4ΔV1-3, eOD-GT8
	IOMA	49% (2.33)	VH1-2 /VL2-23	15	8	25.4%	23.3%	
	1-18	97% (0.048)	VH1-46 /VK3-20	16	9	26.4%	20.2%	
	CH235.12	89% (0.70)	VH1-46 /VK3-15	13	8	25.0%	14.8%	CH505.M5.G458Y.GNTI-121
	1B2530	72% (3.62)	VH1-46 /VL1-47	16	11	27.8%	15.7%	
	8ANC131	71% (1.78)	VH1-46 /VK3-20	16	9	25.7%	17.2%	
	CH103	67% (2.28)	VH4-59 /VL3-1	13	10	16.9%	11.1%	CH505 TF ³³
V3 Glycan	PGT128	68% (0.064)	VH4-39 /VL2-8	19	10	19.1%	7.0%	
	PGT121	66% (0.072)	VH4-59 /VL3-21	24	12	19.6%	16.5%	MD39-11MUTb ¹²⁷ , RC1-4Fill ¹³⁰
	BG18	61% (0.032)	VH4-4 /VL3-25	21	11	21.5%	17.6%	MD39-11MUTb, N332 GT5 ⁵¹
	BF520.1	53%* (7.31)	VH1-2 /VK3-15	18	11	6.6%	5.3%	
	PGDM12	54% (0.14)	VH3-11 /VK2-24	19	9	19.1%	14.3%	
	DH270.6	51% (0.21)	VH1-2 /VL2-23	18	10	12.8%	6.7%	CH848 SOSIP 10.17 DT ⁷⁶
	PCDN76-33A	46% (0.50)	VH4-34 /VK3-20	20	8	13.0%	11.6%	
V2 Apex	PG9	87% (0.154)	VH3-33 /VL2-14	28	10	12.6%	6.3%	BG505 SOSIP 4.1 GT1, BG505 SOSIP 4.1 GT1.1 ²²⁸ , ZM233 ¹³³ , KER2018 ¹³⁴ , BB201.B42 ¹³⁴
	PGDM1400	83% (0.02)	VH1-8 /VK2-28	32	9	26.4%	11.8%	
	VRC26.25	70% (0.004)	VH3-30 /VL1-51	36	12	12.2%	8.6%	
	CH01	54% (1.38)	VH3-20 /VK3-20	24	9	16.7%	11.2%	BG505 SOSIP 4.1 GT1, BG505 SOSIP 4.1 GT1.1, ZM233, CM244 ¹³³ , Q23.17 ¹³³ , WITO ¹³³ , T250 ¹³²
	PCT64-35M [^]	35% (0.41)	VH3-15 /VK3-20	23	8	11.2%	4.9%	

MPER	10E8	98% (0.356)	VH3-15 /VL3-19	20	12	21.4%	13.4%	
	DH511.2	98% (0.943)	VH3-15 /VK1-39	21	11	19.8%	14.0%	
	4E10	98% (1.81)	VH1-69 /VK3-20	18	9	6.9%	4.1%	
	VRC42.1	96% (4.09)	VH1-69 /VK3-20	15	9	10.8%	5.6%	
	VRC43.1	63% (1.34)	VH4-4 /VL7-43	19	9	11.1%	8.5%	
	PGZL1	84% (6.06)	VH1-69 /VK3-20	15	9	20.9%	11.8%	
	2F5	58% (2.83)	VH2-5 /VK1-13	22	9	13.1%	11.0%	MPER-Liposome ⁸⁷
Fusion Peptide	PGT151	73% (0.04)	VH3-30 /VK2D-20	26	9	20.8%	11.5%	
	VRC34	47% (0.32)	VH1-2 /VK1-9	13	9	14.9%	8.7%	
Silent Face	SF12	62% (0.20)	VH4-59 /VK3-20	21	6	16.3%	13.9%	
Fab Dimer Glycan	2G12**	21% (3.75)	VH3-21 /VK1-5	14	9	21.1%	11.7%	

^aOnly bnAbs that have >45% breadth on a multiclade panel of >50 viruses (from CATNAP database²²⁹), except where noted

^bug/ml, geometric mean of detected

*BF520.1 neutralization was only tested on 15 viruses but is included here because it notably was induced in an infant³⁸

**2G12 does not meet >45% breadth threshold but is included because expected high precursor frequency of natural FDG antibodies makes this class an attractive target for design

[^]PCT64 included because it is only one of 2 V2 lineages profiled from acute HIV-1 infection to bnAb breadth.

Note: The following bnAbs met the inclusion criteria but were not included for space considerations:

3BNC55, VRC13, VRC16, VRC18, VRC27

2

3 Legends to Figures

4 **Figure 1. Model of broadly neutralizing antibody development in humans.** In HIV-1 infected

5 individuals, viral diversification of Env sequences was found to be required for bnAb

6 development³³. From this work came the concept of the transmitted/founder Env that initiated the

7 infection and B cell lineage design whereby sequential immunogens are chosen from autologous

8 evolved viruses that induced bnAbs, or are structurally designed to have affinity gradients across

9 maturing lineage members and to select for desired mutations to favor bnAb development. BnAb

10 development follows a “jackpot effect”, where each individual with HIV-1 who makes bnAbs
11 has only one or very few bnAb B cell lineages that have made it through a tortuous bnAb
12 maturation pathway requiring multiple rare events stimulated by evolving virus.

13

14 **Figure 2. Host immunoregulatory control abnormalities in individuals infected with HIV-1**
15 **who make broadly neutralizing antibodies.** In studies of cohorts of HIV-1 positive individuals,
16 it was found that those who make bnAbs have high levels of circulating CD4⁺ T follicular helper
17 (T_{FH}) cells, low levels of CD4⁺ regulatory T (T_{reg}) cells and circulating T follicular regulatory
18 (T_{FR}) cells, high levels of plasma autoantibodies and low levels of functional NK cells. Increased
19 availability of CD4⁺ T_{FH} cells and reductions in the numbers of functional T_{reg} and NK cells, both
20 of which constrain germinal center responses to reduce autoantibody production, may enable
21 enhanced B cell somatic hypermutation and repertoire diversification. In addition, HIV-1
22 infected individuals who make bnAbs have perturbations in their B cell Immunoglobulin G (IgG)
23 repertoires such that BCRs with longer HCDR3s and increased autoreactivity can expand. Thus,
24 HIV-1 infection results in a permissive immunologic environment that favors eventual bnAb
25 development.

26

27 **Figure 3. The complexity of a prototype HIV-1 vaccine for the induction of broadly**
28 **neutralizing antibodies.** An HIV-1 Env trimer is shown with targets for bnAbs shown in colour.
29 A polyclonal multi-B cell lineage response requires the development of bnAbs binding to the
30 CD4 binding and to at least two other epitopes, such as the V1V2 glycan, the V3 glycan patch,
31 fusion domain, or the membrane proximal external region sites. Fab Dimerized Glycan abs bind
32 to high mannose residues at multiple sites on Env.

33

34 **Figure 4. Strategies for the design of HIV-1-targeted broadly neutralizing antibody**
35 **immunogens.**

36 B cell lineage immunogen design²⁵ (**upper right**) uses insights into HIV-1 virus/bnAb B cell co-
37 evolution to inform the design of Env-based immunogens that can recapitulate the generation of
38 similar bnAbs by vaccination. B cell lineage immunogen design starts by isolating a bnAb-
39 producing B cell clone from an HIV-1 infected individual, sequencing its BCRs to identify
40 clonal members, and then computationally reconstructing the maturation history of the bnAb.
41 The maturation pathway from the inferred unmutated common ancestor (UCA) antibody
42 (representing the naïve bnAb B cell receptor) through inferred ancestral intermediates to the
43 bnAb serves as a molecular guide for HIV-1 vaccine design. The UCA and inferred antibody
44 intermediates serve as templates for the design of immunogens that bind with high affinity.
45 Immunogens can be designed through germline targeting (upper left), structure-based
46 immunogen design (lower left) and mutation-guided immunogen design (lower right). The goal
47 is for sequentially administered immunogens to provide a selective advantage in the germinal
48 center to B cells that follow similar desired evolutionary trajectories.

49 Because bnAb UCAs rarely bind with high affinity to unmodified autologous Envs, priming
50 immunogens must be designed with epitope modifications (for example, the shortening of
51 variable loops, or removal of key glycans) in an approach called Germline Targeting (**upper left**
52 **panel**). Germline targeting is based on the selection of a transmitted/founder virus or a
53 specifically selected or designed Env immunogen that can bind to a UCA^{25,51,77,78,90}. This can be
54 achieved through *in vitro* selection techniques where UCAs are used to bind and select high
55 affinity Env antigens from a library of Env variants. These Env ligands are then used to isolate

56 antibodies from the human immunoglobulin repertoire to identify a polyclonal mixture of
57 putative bnAb precursors. A germline-targeting Env antigen can then be re-designed to improve
58 its affinity for many of the isolated putative bnAb precursors using mutation-guided immunogen
59 design and structure-based immunogen design.

60 Mutation-guided immunogen design⁴² aims to identify the improbable mutations in bnAbs that
61 are not routinely generated by somatic hypermutation but are critical for broad neutralization.
62 These are then used to inform the design immunogens that can specifically select for these
63 mutations.

64 Structure-based immunogen design⁹¹ is based on the determination of bnAb-Env complexes that
65 provide the atomic-level information that is necessary to computationally model specific bnAbs
66 as templates for immunogen design. These inform the modifications for improving Env
67 immunogen binding to bnAbs or bnAb precursors. Structure-based immunogen design is utilized
68 to inform all stages of the vaccine design strategy.

69 **Figure 5. Construction of Simian-Human Immunodeficiency Viruses (SHIVs) containing**
70 **clinically-relevant primary HIV-1 Envs.** Critical to SHIV design is selection of primary or
71 transmitted/founder (TF) HIV-1 Envs that exhibit features of clinically-relevant viruses and
72 introduction of a mutation at envelope position 375 that facilitates macaque CD4 engagement.
73 Mutations are made at Env aa 375 to facilitate interaction with macaque T cell CD4. The HIV
74 transmitted/founder or other Env is engineered to be expressed on the surface of a SIV virion to
75 form a chimeric SHIV. In this manner, virtually any HIV-1 Env can be used to produce a SHIV
76 for testing for the ability to induce bnAbs in the setting of lentiviral infection.

77

78

79 **Boxes**

80 **Box 1.**

81 **Roadblocks in the induction of HIV-1 broadly neutralizing antibodies.**

- 82 1. HIV-1 sequence diversity continues to increase.
- 83 2. Early in infection, HIV-1 integrates into the host genome and can become ‘invisible’ to
84 the immune system, thus requiring a successful HIV-1 vaccine to induce sterilizing
85 immunity to prevent sustained HIV-1 infection originating from integrated HIV-1
86 genomes.
- 87 3. bnAbs identified to date have unusual traits such as long heavy chain complementary
88 region 3s (HCDR3s), a high level of improbable mutations and polyreactivity with host
89 or environmental antigens. These are required for bnAb activity.
- 90 4. The unusual traits required for bnAb activity can be disfavored by tolerance mechanisms
91 of the immune system such as deletion or anergy.
- 92 5. V3-glycan, V2-glycan and MPER-targeted bnAbs have long HCDR3 regions. Precursors
93 of B cells capable of producing such bnAbs are usually deleted in the bone marrow due to
94 autoreactivity. Thus, precursors for such bnAbs may be extremely rare.
- 95 6. The HIV-1 envelope (Env) protein, the primary target for bnAbs, is heavily glycosylated
96 and bnAb epitopes are cloaked in both high mannose and complex glycans that are poorly
97 immunogenic.
- 98 7. Env has dominant non-neutralizing epitopes that compete with subdominant bnAb
99 epitopes in immune germinal centers for B cells and T follicular helper cells.
- 100 8. Due to lack of HIV-1 transmitted/founder or intermediate virus Envs that are capable of
101 activating B cell lineages that encode unmutated common ancestors (UCAs), Envs must

102 be designed that can bind with high affinity to bnAb B cell lineage UCAs and early
103 intermediates.

104 9. For a vaccine to be protective, multiple specificities of bnAb precursors need to be
105 induced simultaneously.

106 10. The bar for protective neutralization breadth, potency and durability is very high, as
107 determined in the Antibody Mediated Protection (AMP) trial.

108 11. The vaccine will need to protect against both blood and mucosal HIV-1 transmission.

109

110

111 **Box 2:**

112 **Construction of Simian-Human Immunodeficiency Viruses (SHIVs) containing clinically-**
113 **relevant primary HIV-1 Envs.**

114 Shaw and colleagues have taken a unique approach to SHIV design by mutating the CD4 binding
115 site of primary or transmitted/founder HIV-1 Envs⁸⁰ to make them more infectious in rhesus
116 macaques^{139,230,231} (**Figure 5**). Remarkably, when SHIVs were constructed with Envs derived
117 from transmitted/founder viruses from individuals who made bnAbs, and used to infect monkeys,
118 the Env evolution in monkeys was frequently quite similar to Env evolution in the HIV-1
119 infected human from whom the transmitted/founder Envs were derived¹³⁹. Moreover, among 150
120 SHIV-infected macaques, ~15% of animals developed bnAbs after 3-24 months of infection.

121 These bnAbs targeted canonical bnAb epitopes on the HIV-1 trimer, including the V2 glycan, V3
122 glycan patch, CD4 binding site and fusion peptide¹³⁹. One rhesus bnAb (RHA1) was strikingly
123 similar in structure and paratope-epitope interactions to the human V2 bnAbs PCT64 and
124 PGT145¹³⁹. SHIV-induced rhesus macaque bnAbs provide a powerful experimental model of

125 bnAb development in that V2 apex, V3 glycan, CD4 binding site and fusion peptide neutralizing
126 epitopes bear striking resemblances to those of human bnAbs in structure, immunogenetics and
127 antibody sequences needed for epitope recognition. Thus, the SHIV-infected rhesus macaque
128 provides a powerful model for evaluating common pathways of bnAb development. Most
129 recently, Fab-dimerized glycan (FDG) bnAbs (DH851, DH1003) that bind to the Env glycan
130 shield were isolated from SHIV-infected macaques, extending the value of the SHIV model¹⁵⁷.
131 Finally, a considerable advantage of the SHIV infection model is that bnAb lineages can be
132 followed from before infection, throughout the affinity maturation process, culminating with
133 appearance of bnAbs. Identification of viral Envs that guide bnAb maturation can inform novel
134 immunogen designs.

135

136 **Box 3 Envelope immunogen optimization and delivery regimens**

137 A major advance in the HIV-1 vaccine field was the design and structural definition of a
138 cleaved, stabilized envelope (Env) trimer by addition of the T>C mutation at position 605, A>C
139 mutation at position 501 and I>P mutation at position 559, called the SOSIP.664 envelope gp140
140 (SOSIP Env trimer)²³²⁻²³⁵. SOSIP Env trimers have allowed to select for bnAbs targeting the
141 quaternary structure of the Env trimer apex²³⁶. It is also becoming clear that multimeric,
142 stabilized Envs decorating protein nanoparticles may be more immunogenic than a combination
143 of Env immunogens with small molecule adjuvants^{76,237,238}. This is both because of improved
144 movement of nanoparticles into germinal centers and because of the need to stabilize Envs to
145 prevent the formation of non-neutralizing, diverting epitopes that can take bnAb B cell lineages
146 off-target²³⁹⁻²⁴¹. For example, insufficient glycosylation of Env, resulting in so called “glycan
147 holes” , can cause this problem²⁴²⁻²⁴⁶. The base of the Env trimer also contains diverting epitopes

148 that can impede bnAb development and promote trimer disassembly²⁴⁷. Thus, both stabilization
149 and minimization of non-neutralizing, diverting epitopes are important for HIV-1 vaccine
150 immunogen design.

151 Another aspect for immunogen design is the fact that almost all bnAbs have high levels of
152 mutations, suggesting that B cells either remain in germinal centers for prolonged periods of time
153 or recirculate back into germinal centers to accumulate functional mutations required for bnAb
154 maturation. Therefore, immunizations regimens need to prolong GC responses and induce CD4⁺
155 T_{FH} cells to support robust bnAb development in GCs^{39,45}. In this regard, it was recently
156 demonstrated that modulating the quantity of HIV-1 Env-specific CD4⁺ T cell help can promote
157 rare B cell responses in mice¹⁶¹. In individuals with HIV-1 infection, there is a constant supply of
158 antigenic variants of Env that may, over time, select for rare functional bnAb mutations. This
159 results in bnAb development over 2-5 years in in ca 50% of HIV-1 infected adults^{27-31,33,70}. Two
160 studies have found that vaccine strategies that include delivery of antigen by either continuous
161 administration via subcutaneous pumps or by administration of escalating doses of antigen over
162 time results in enhanced antibody responses^{160,163}. In macaques, such a regimen sustained
163 germinal center responses for up to 29 weeks, and with late boosting at 30 weeks, low titres of
164 heterologous HIV-1 neutralizing antibodies were induced¹⁶². Finally, vaccines based on modified
165 mRNAs in LNPs potently stimulate T_{FH} cells^{59,64}. After intramuscular immunization, these are
166 rapidly distributed to the liver, spleen and systemic lymph nodes, with prolonged expression of
167 antigen over days¹⁹⁰. Work has now begun to formulate complex stabilized Env trimer
168 nanoparticle multimers encoded either in mRNAs and delivered as mRNA-LNPs^{128,189}, with
169 some success reported in bnAb UCA knock-in mice and in monkeys^{189,248}. Thus, development

170 efforts are ongoing to define the optimum platform for delivery of a multicomponent HIV-1
171 bnAb vaccine.

172

173 **Box 4**

174 **Humanized mouse models for study of bnAb B cell development**

175 The first bnAb knock-in mouse model studied was the 2F5 V_H knock-in mouse, in which B cells
176 expressed the variable heavy chains of the bnAb 2F5. It demonstrated that B cells expressing the
177 2F5 BCR were mostly deleted at the pre-B cell stage in the bone marrow, and B cells with the
178 2F5 V_H BCR that made it to the periphery were anergic¹⁴⁵. Knock-in mice with a mutated 2F5
179 V_H and knock-in mice that expressed the V_H and V_L regions of the bnAb 4E10 had similar
180 defects^{87,111,249}. Interestingly, the first generation CD4 binding site bnAb 1b12 was found to be
181 autoreactive, but it did not induce bone marrow B cell precursor deletion or anergy in peripheral
182 B cells in knock-in mouse models²⁵⁰. In contrast, the CD4 binding site bnAb 3BNC60, also with
183 a V_H1-2*02 similar to VRC01 but paired with a different V_L, when this V_H + V_L was knocked
184 into mice, they did demonstrate deletion, anergy and receptor editing, suggesting different fates
185 dictated by V_L usage¹¹¹.

186 These initial bnAb-knock-in mice were engineered to express the rearranged mature V_H and V_L
187 or the rearranged V_H and V_L sequences of the unmutated common ancestor (UCA) of the
188 respective bnAbs. The expression of pre-rearranged V_H and V_L sequences inhibited the
189 rearrangement and expression of the endogenous mouse immunoglobulin loci. This type of
190 mouse model is useful for testing whether the designed immunogen is capable of activating B
191 cells that express particular bnAb precursors and whether precursor antibodies can mature to

192 functional bnAbs. However, bnAb precursors are present at supra-physiologic frequencies.
193 Immunization under such conditions cannot assess the specificity of immunogens for rare bnAb
194 precursors in complex B cell repertoires²⁵¹. To overcome this limitation, adoptive transfer of B
195 cells that express bnAb precursors to wild-type recipient mice can be used to reconstitute B cell
196 compartments with physiological bnAb precursor frequencies²⁵².

197 Recently, the groups of Alt, Tian and colleagues have developed HIV-1 bnAb germline
198 rearranging mice that developmentally generate diverse repertoires of bnAb VRC01-class
199 precursors¹⁰⁷. This type of mouse model is designed to assess the specificity of an immunogen
200 for bnAb precursors as well as its ability to mature B cell lineages that produce related precursor
201 antibodies toward the production of bnAbs. The latter function is important for immunogen
202 efficacy in the human population, where individuals may have B cell lineages producing a
203 variety of bnAb precursors. In this mouse model, the human VH1-2 gene segment recombines
204 with mouse D and J_H gene segments or mouse D segments and human J_H segments to form a
205 diverse range of humanized BCR heavy chains that express a VH1-2 region in association with a
206 multitude of different complementary determining region 3 (CDR3) segments¹⁰⁷. This model was
207 revolutionary in that it provided for a more diverse repertoire of bnAb-producing B cells, and
208 with a much lower frequency of precursors, resulting in a more physiologic testing environment
209 for potential vaccine immunogens. Indeed, in this model, the eOD-GT8 prime (an immunogen
210 designed to bind to VRC01 germline B cell receptors⁷⁷), coupled with the 426c Env
211 immunogen¹⁰⁵ in a boost regimen, was successful in expanding VRC01 bnAb B cell
212 precursors^{107,108}. Mouse models are now being generated that mimic physiological conditions
213 more closely, in which both V_H and V_L rearranging gene segments result in an immense diversity
214 of humanized mouse B cell receptor repertoires. Moreover, new models have been designed to

215 circumvent B cell developmental blocks in the bone marrow by expressing bnAb precursors
216 conditionally in mature B cells²⁵³.

217

218 **Glossary**

219 Carrier proteins- molecules added to small non-immunogenic molecules such as peptides to
220 make them more immunogenic. Examples of carrier proteins are Keyhole limpet hemocyanin
221 and tetanus toxoid.

222 CD4 T follicular helper (T_{FH}) responses- CD4 helper cell responses in the germinal center that
223 promote somatic hypermutation and affinity maturation of B cells.

224 HIV-1 fusion domain peptide- short peptide at the N-terminus of gp41 that inserts into the host
225 cell membrane after receptor-mediated Env activation and effects virus and host cell fusion. The
226 fusion peptide is a target for HIV-1 bnAbs.

227 Immune correlates of protection- the immune responses induced by a vaccine that are
228 responsible for vaccine efficacy.

229 Immunofocusing- the design of immunogens to produce antibodies with a defined specificity.

230 Natural antibodies- antibodies produced by B1 or marginal zone B cells that are present before
231 antigen stimulation and act early in host defense prior to the adaptive, secondary B cell response.

232 Paratope- the binding region of an antibody for an antigen.

233 On rate- the rate constant (K_{on} or K_a) of association of an antibody to the envelope.

234 Partial Thromboplasin time (PTT)- test that measures the time it takes for a blood sample to clot.
235 The PTT is prolonged in the presence of anti-cardiolipin antibodies.

236 Receptor mediated Env activation- the events that transpire subsequent to CD4 binding the HIV-
237 1 envelope (Env).

238 Scaffolded mannose glycans- the design of HIV-1 Env peptides with high mannose glycans
239 displayed to mimic bnAb epitopes on Env.

240 Set point viral load- the residual viral load that results in chronic HIV-1 infection after the peak
241 viral load that occurs during acute HIV-1 infection.

242 Transmitted/founder HIV-1 strains- one or a few HIV-1 strains that establish transmission after
243 HIV-1 mucosal exposure.

244