Strategies for HIV-1 vaccines that induce broadly neutralizing antibodies

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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.

HIV-1 was discovered in 1983 (REF.[1]) and subsequently shown to be the cause of acquired immune deficiency syndrome (AIDS)[2–4]. Effective antiretroviral drug therapy has converted AIDS from a uniformly fatal disease to a chronic disease with a near normal lifespan[5]. Nonetheless, ~1.5 million people each year acquire HIV-1 (REF.[6]). Thus, even with antiretroviral drug therapy 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 owing to the exceptional and increasing genetic diversity of the HIV-1 lentivirus[9,10], its complex mechanisms of immune evasion[11–14] 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 utilized gp120 as antigen for eliciting neutralizing antibodies, whereas more recent trials tested vaccines designed to elicit CD8+ T cell responses and non-neutralizing antibodies[17–19]. 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 trials[21]. However, of two phase IIb/III clinical

trials designed to improve on the RV144 trial — HIV-1 Vaccine Trials Network (HVTN) 702 (NCT02968849)[18] and HVTN 705 (NCT03060629)[15,19] — neither showed significant efficacy[19], suggesting that the RV144 trial may not have been a harbinger of vaccine success. However, an ongoing phase III 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 protection[22]. Thus, for HIV-1, the induction of antibodies that broadly protect against heterologous HIV-1 strains (called broadly neutralizing antibodies (bnAbs)) is a prime goal of HIV-1 vaccine development[23–26].

Importantly, sera from a substantial proportion of untreated individuals with HIV-1 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 response[27–31]. Moreover, in individuals infected with HIV-1 who produce bnAbs, these generally only develop after many months or years owing to infrequent germline B cell priming and the requirement for extensive antibody somatic hypermutation[27–31]. When bnAb activity in serum does develop, it is often mediated by a single clonal lineage (or, more rarely, two or three bnAb lineages) (FIG. 1), and bnAbs constitute a minor component of an individual's overall HIV-1-specific antibody response[32–36]. Recent data suggest that in infants and children, high levels of

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Table 1 | **HIV-1 vaccine efficacy trials completed or in progress**

Trial	Start	End	Vaccine	Location	Result	Refs.
VAX004 (NCT00002441)	1999	January 2000	Bivalent clade B gp120 in alum	United States, Europe	No efficacy	[190–192]
VAX003 (NCT00006327)	March 1999	August 2000	Bivalent CRF_01AE/B gp120 in alum	Thailand	No efficacy	[193–195]
HVTN 502 (Step Study) (NCT00095576)	November 2004	September 2009	Adenovirus type 5 clade B gag/pol/nef	United States	No efficacy; increased infection in vaccinees	[169,196–201]
HVTN 503 (Phambili study) (NCT00413725)	December 2006	July 2015	Adenovirus type 5 clade B gag/pol/nef	South Africa	No efficacy; increased infection in male vaccinees	[170,202–205]
RV144 (NCT00223080)	September 2005	April 2009	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, 206–215]
HVTN 505 (NCT00865566)	May 2009	October 2017	DNAs with clade B gag/pol/nef and DNAs with clade A, B, C Envs; adenovirus type 5 with gag/pol and clade A, B, C Envs	United States	No efficacy	[196,216–222]
HVTN 703/HPTN 081 (NCT02568215)	May 2016	March 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	[111,223,224]
HVTN 704/HPTN085 (NCT02716675)	April 2016	December 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	[111,223,224]
HVTN 702 Uhambo (NCT02968849)	October 2016	September 2021	ALVAC-C with gag/pol/Env; bivalent gp120s in MF59	South Africa	No efficacy	[18,225]
HVTN 705 Imbokodo (NCT03060629)	November 2017	August 2021	Ad26, 4 valent T cell mosaic genes, boost with clade C gp140 Env	Sub-Saharan Africa	No efficacy	[226]
HVTN 706 Mosaico (NCT03964415)	October 2019	Ongoing (est. March 2024)	Ad26, 4 valent T cell mosaic genes, boost with clade C gp140 Env + B cell mosaic gp140 Env	United States, Spain, Central/South America	Ongoing	[227]

Adapted with permission from[227]. HVTN, HIV-1 Vaccine Trials Network; NA, not available.

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 owing to the unusual traits that are required to allow for breadth and neutralization capacity. These include the frequent presence of long heavy chain complementarity determining region 3 (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 analysis[42]. 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 loads[43] and share immunological characteristics such as high levels of circulating CD4+ T follicular helper (T_{FH}) cells, fewer and less potent CD4+ regulatory T (T_{reg}) cells and populations of dysregulated natural killer 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[46] (BOX 1 and FIG. 2). Indeed, numerous 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 antigens[47]) or autoreactivity (antibody reacts with self-antigens[48]) 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 repertoire[49]. The residual polyreactive BCRs augment the breadth and efficacy of normal humoral responses to microbial pathogens[47]. 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 naive B cell precursors with long HCDR3 regions required to bind conserved neutralizing HIV-1 epitopes are relatively rare[50–52]. Thus, a goal of HIV-1 vaccine design is to administer

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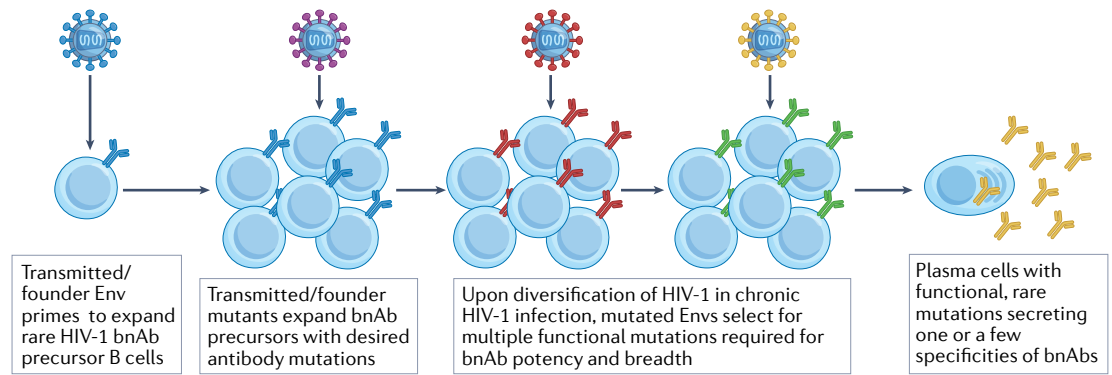
| Transmitted/founder Env primes to expand rare HIV-1 bnAb precursor B cells | Transmitted/founder mutants expand bnAb precursors with desired antibody mutations | Upon diversification of HIV-1 in chronic HIV-1 infection, mutated Envs select for multiple functional mutations required for bnAb potency and breadth | | Plasma cells with functional, rare mutations secreting one or a few specificities of bnAbs |

Fig. 1 | **Model of broadly neutralizing antibody development in humans.** In individuals infected with HIV-1, viral diversification of envelope (Env) sequences was found to be required for broadly neutralizing antibody (bnAb) development[33]. From this work came the concept of transmitted/founder Envs that initiate the infection and B cell lineage design whereby sequential immunogens are chosen from autologous evolved viruses that induced bnAbs, or are structurally designed to have affinity gradients across maturing lineage members and to select for desired mutations to favour bnAb development. bnAb development follows a 'jackpot effect', where each individual with HIV-1 who makes bnAbs has only one or very few bnAb B cell lineages that have made it through a tortuous bnAb maturation pathway requiring multiple rare events stimulated by evolving virus.

immunogens that bind to the BCRs of multiple specificities of B cells that are bnAb precursors[53], with the aim to induce a broader polyclonal B cell response to bnAb Env epitopes than naturally occurs in individuals with HIV-1 infection (FIG. 3). Strategies for bnAb immunogen design are summarized in the legend to FIG. 4.

Durable antibody responses depend on the induction of long-lived plasma cells that persist in bone marrow niches and maintain antibody responses for years or even decades[54]. Scheid et al. have demonstrated that more HIV-1-specific memory B cells are polyreactive than are bone marrow plasma cells with comparable specificity, suggesting that polyreactive bnAb B cells are more likely to develop into memory B cells than long-lived plasma cells[55]. As a large percentage of the HIV-1-targeted antibody response is mediated by polyreactive or autoreactive B cells[40,41,47], the reduced presence of polyreactive bone marrow 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 with protein immunization[58]. Additionally, nucleoside-modified mRNAs in lipid nanoparticles (LNPs) have been shown to induce durable low levels of 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 macaques[62].

HIV-1 infection has been shown to reduce the normal tolerance controls that constrain the development and maturation of polyreactive or autoreactive B cells, thereby creating an immune environment permissive for bnAb development[39,46]. Strategies for HIV-1 vaccine-induced bnAb induction in individuals negative for HIV-1 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 frequencies of which are

needed for bnAb development[39,45], and to minimize the activation of regulatory cells such as CD4+ T_{reg} cells and natural killer 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 centres[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 immunoglobulin 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 germinal centres 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 disfavoured 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 lineage[76]. Thus, a major task of HIV-1 vaccine development is to keep bnAb B cell lineages in germinal centres, or ensure they recirculate back to germinal centres, 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 individuals infected with HIV-1, 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 on HIV-1 bnAbs identified. Moreover, we explain the need for combined T cell and B cell vaccines and discuss the requirements that need to be met for the development of a practical HIV-1 vaccine.

bnAb biology: implications for vaccines

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 or their progeny that are bound by the BCRs of lineage members[80]. Current bnAb vaccine approaches are based on the hypothesis that priming B cells with Env molecules that are capable of binding to and activating naive B cells that express UCAs, followed by sequential Env

immunogens that can select for improbable mutations and guide otherwise disfavoured bnAb B cell lineages to develop into full bnAb capability, can be ultimately successful[25,42,78,81]. An additional requirement for the design of sequential vaccines is the creation of affinity gradients of the Env immunogens 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 maturation[83]. Moreover, vaccine-induced acquisition of fast on-rates is a critical attribute for at least a subset of bnAb B cell lineages to mature[84–89]. Given the complex characteristics that a successful HIV-1 vaccine will require and the physiological factors that disfavour bnAb induction (BOX 1), numerous strategies for sequential immunogen design (B cell lineage immunogen design[25], germline-targeting[25,51,77,78,90], mutation-guided immunogen design[42] and structure-based immunogen design[91]) have been developed (FIG. 4).

With continued exposure to sequential Env immunogens, 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 germinal centres[94,95]. In the case of some bnAbs, the loss of autoreactivity is tantamount with concomitant loss of bnAb activity as autoreactivity can be required for bnAb potency[40,41,47,96].

To guide otherwise disfavoured 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 disfavour 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 with HIV-1 and to the extreme viral diversity of HIV-1 and the relative conservation of SARS-CoV-2 (REFS.[9,97]).

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 FIG. 5) and humanized mouse models (BOX 3).

Types of bnAbs

Numerous 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 (FIG. 3 and TABLE 1). In general, Env

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Box 1 | Roadblocks in the induction of HIV-1 broadly neutralizing antibodies

1. HIV-1 sequence diversity continues to increase.

2. Early in infection, HIV-1 integrates into the host genome and can become 'invisible' to the immune system, thus requiring a successful HIV-1 vaccine to induce sterilizing immunity to prevent sustained HIV-1 infection originating from integrated HIV-1 genomes.

3. Broadly neutralizing antibodies (bnAbs) identified to date have unusual traits such as long heavy chain complementarity determining region 3 (HCDR3s), a high level of improbable mutations and polyreactivity with host or environmental antigens. These are required for bnAb activity.

4. The unusual traits required for bnAb activity can be disfavoured by tolerance mechanisms of the immune system such as deletion or anergy.

5. V3 glycan, V2 glycan and membrane proximal external region (MPER)-targeted bnAbs have long HCDR3 regions. Precursors of B cells capable of producing such bnAbs are usually deleted in the bone marrow owing to autoreactivity. Thus, precursors for such bnAbs may be extremely rare.

6. The HIV-1 envelope (Env) protein, the primary target for bnAbs, is heavily glycosylated and bnAb epitopes are cloaked in both high mannose and complex glycans that are poorly immunogenic.

7. Env has dominant non-neutralizing epitopes that compete with subdominant bnAb epitopes in immune germinal centres for B cells and T follicular helper cells (T_{FH} cells).

8. Owing to lack of HIV-1 transmitted/founder or intermediate virus Envs that are capable of activating B cell lineages that encode unmutated common ancestors (UCAs), Env immunogens must be designed that can bind with high affinity to bnAb B cell lineage UCAs and early intermediates.

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

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

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

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epitopes for vaccine design have been chosen on the basis of the breadth and potency of bnAbs isolated from individuals infected with HIV-1 that bind the respective Env epitopes.

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 with the second type. Examples for CD4 mimic bnAbs are the VRC01 class and the 8ANC131/CH235 class, and an example of an HCDR3-binder bnAb is CH103 (TABLE 1).

CD4 mimic bnAbs. VRC01-class antibodies contain the variable heavy 1–2 (VH1–2) chain and require the use of a five amino acid light chain complementarity determining region 3 (LCDR3)[75,98–101]. 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 antibodies[102]. 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[77,98]. 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 UCA of VRC01 (REF.[77]) has been designed, and binds to most, but not all, putative UCAs of other members of the VRC01 class of bnAbs[75,103]. In VRC01 knock-in mice, eOD-GT8 primes and expands VRC01 precursors, and in combination with sequential boosting immunogens[104] can select for B cells with VRC01 BCRs that have a degree of neutralization breadth[105–107]. eOD-GT8 is immunogenic in macaques, which respond more rapidly to subcutaneous rather than intramuscular injections[108]. In the IAVI G001 human trial, eOD-GT8 expanded B cells bearing BCRs with characteristics of VRC01 precursors[109].

In addition to eOD-GT8, glycan-deleted derivatives of the clade C HIV-1 426c Env have been designed as immunogens that bind to VRC01-class bnAbs[78,90,104,110]. Initially, these designs focused on the strategic removal of glycans, as glycans at Env positions 276, 460 and 463 were found to be major obstacles for binding to VRC01-class precursors[90]. Comparison of eODGT8 and 426c Env immunogens in VRC01 UCA heavy chain knock-in mice showed that the two immunogens engage different VRC01 bnAb precursors[104]. 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[111–113]. Further study showed that for HIV-1 isolates sensitive to VRC01, the level of VRC01 bnAb required for preventing transmission was ~1:200 (REF.[111]). This indicated that a successful HIV-1 vaccine will require high levels of induced bnAbs that are
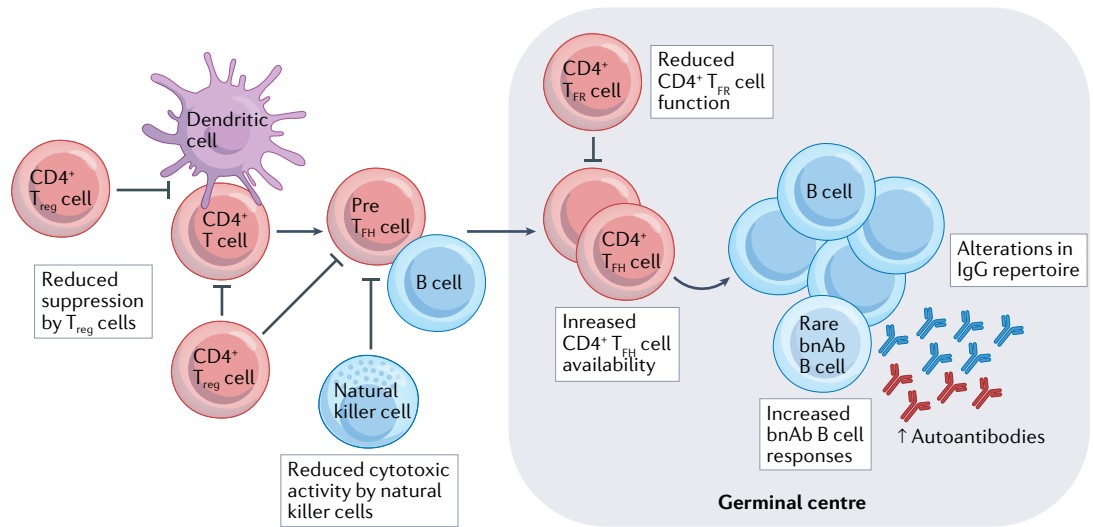

Fig. 2 | **Host immunoregulatory control abnormalities in individuals infected with HIV-1 who make broadly neutralizing antibodies.** In studies of cohorts of individuals positive for HIV-1, those who make broadly neutralizing antibodies (bnAbs) have high levels of circulating CD4+ T follicular helper (T_{FH}) cells, low levels of CD4+ regulatory T (T_{reg}) cells and circulating T follicular regulatory (T_{FR}) cells, high levels of plasma autoantibodies and low levels of functional natural killer cells. Increased availability of CD4+ T_{FH} cells and reductions in the numbers of functional T_{reg} cells and natural killer cells, both of which constrain germinal centre responses to reduce autoantibody production, may enable enhanced B cell somatic hypermutation and repertoire diversification. In addition, individuals infected with HIV-1 who make bnAbs have perturbations in their B cell IgG repertoires such that B cell receptors (BCRs) with longer heavy chain complementarity determining region 3 (HCDR3s) and increased autoreactivity can expand. Thus, HIV-1 infection results in a permissive immunologic environment that favours eventual bnAb development.
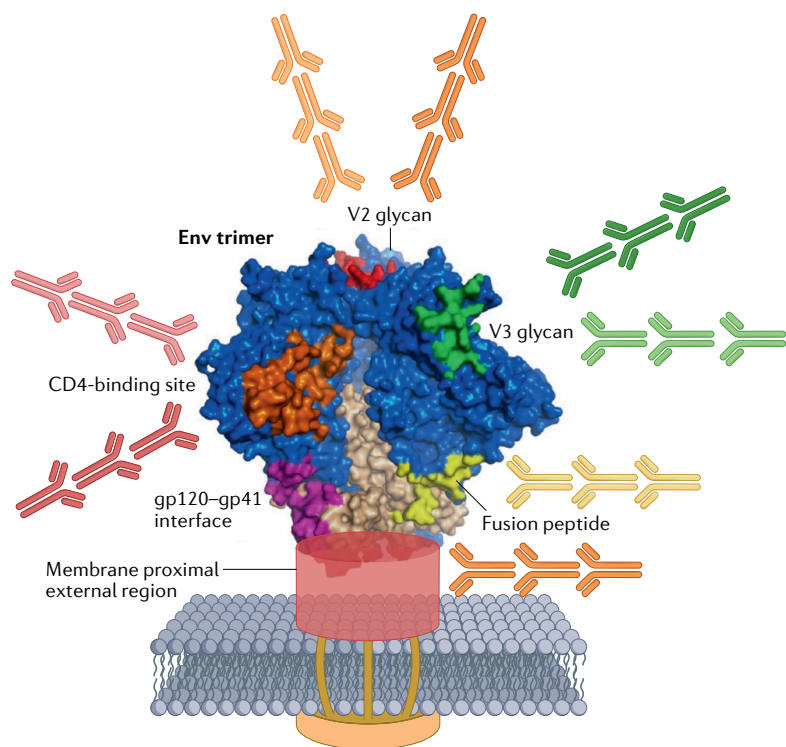
Fig. 3 | **Complexity of a prototype HIV-1 vaccine for the induction of broadly neutralizing antibodies.** HIV-1 envelope (Env) trimer with targets for broadly neutralizing antibodies (bnAbs) shown in colour. A polyclonal multi-B cell lineage response requires development of bnAbs binding to the CD4 binding site and to at least two other epitopes, such as V2 glycan, V3-glycan patch, fusion domain or membrane proximal external region (MPER) sites. Fab-dimerized glycan (FDG) antibodies bind to high mannose residues at multiple sites on Env. Figure derived from structure described by Pancera et al.[230].

maintained at protective levels over long periods of time, and will require bnAb induction to multiple Env epitopes to achieve neutralization breadth[114,115]. Moreover, during the AMP trial, HIV-1 diversification was greater than anticipated[111], 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 1–46 (VH1–46) region but does not require the five amino acid LCDR3 light chain found in VRC01-class antibodies[65,116–119]. 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 (REFS.33,65). 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 cells[32]. A similar mechanism of cooperation between neutralizing lineages was observed in an individual infected with HIV-1 (CH848) who made the DH270 V3-glycan bnAb[70]. In addition, 8ANC131/CH235 class bnAb 1-18 is particularly resistant to HIV-1 escape[117]. Most

HCDR3-binder bnAb lineages that bind the CD4 binding site, such as 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 orthologue for the human VH1–46 region and can make CH235-like antibodies[76]. In addition, Env proteins have been designed that both potently induce 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[76,120]. As 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 disfavouring 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[65,102].

HCDR3-binder CD4 binding site bnAbs. For the induction of HCDR3-binder bnAbs, a transmitted/founder gp120 Env termed CH505 T/F trimer (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 a 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 mice[93], it did not expand CH103 precursors in the HVTN 115 clinical trial[121] (M. Sobieszczyk, W. Williams, B. F. Haynes, J. Kobie, 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].

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 (REFS.122,123). 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 amino acids). There are 6 prototypic V3-glycan bnAb lineages that range in neutralization breadth from 51 to 68% (TABLE 1). Their long HCDR3s are required to reach deep between glycans to bind to the Env polypeptide backbone[123]. 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[51,124,125]. 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 million[51].
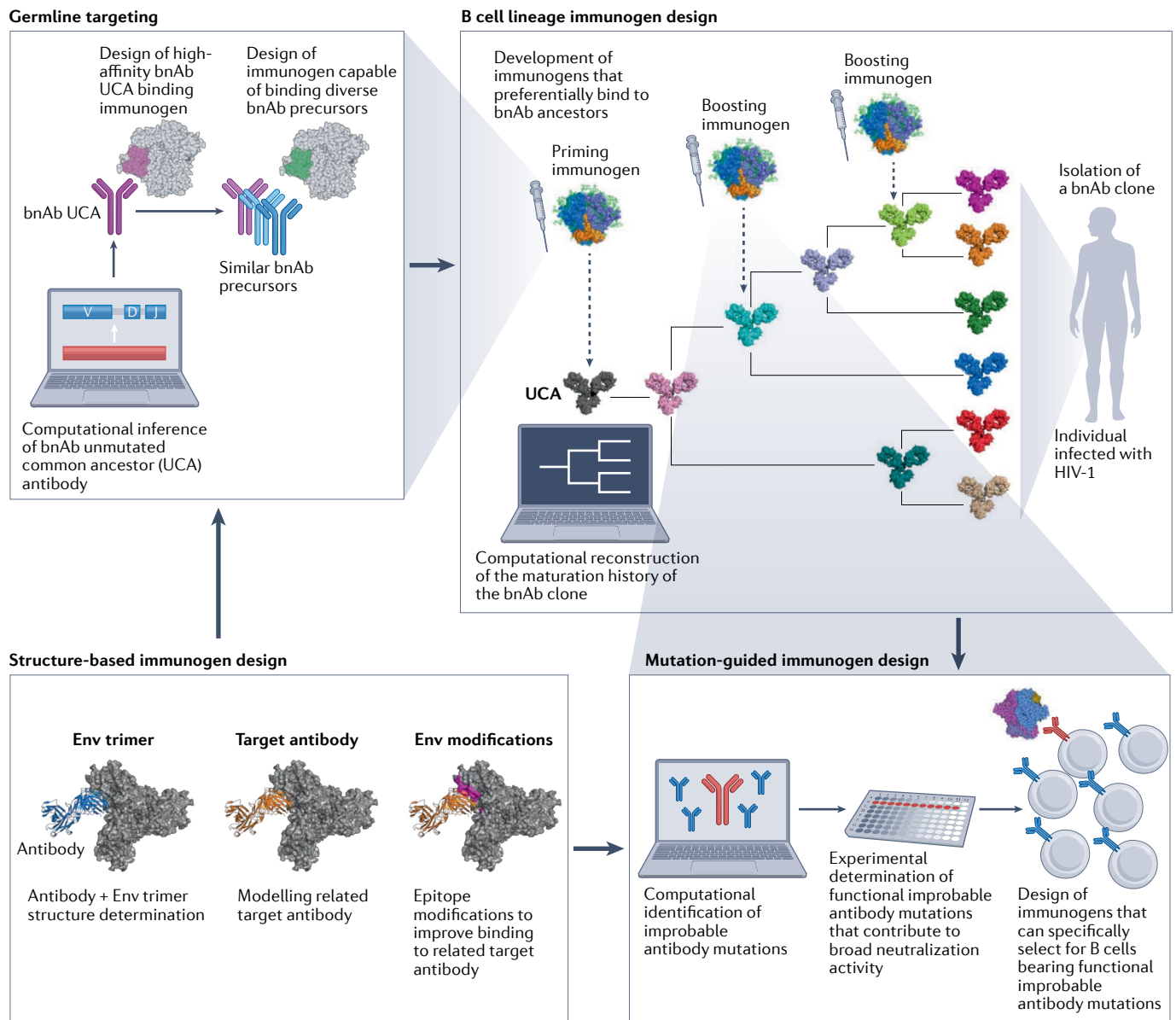
Fig. 4 | Strategies for the design of HIV-1-targeted broadly neutralizing antibody immunogens. B cell lineage immunogen design[25] (upper right) uses insights into HIV-1 virus/broadly neutralizing antibody (bnAb) B cell co-evolution to inform the design of envelope (Env)-based immunogens that can recapitulate generation of similar bnAbs by vaccination. B cell lineage immunogen design starts by isolating a bnAb-producing B cell clone from an individual infected with HIV-1, sequencing its B cell receptors (BCRs) to identify clonal members and, then computationally reconstructing the maturation history of the bnAb. The maturation pathway from the inferred unmutated common ancestor (UCA) antibody (representing the naive bnAb B cell receptor (BCR)) through inferred ancestral intermediates to the bnAb serves as a molecular guide for HIV-1 vaccine design. UCA and inferred antibody intermediates serve as templates for design of immunogens that bind with high affinity. Immunogens can be designed through germline-targeting (upper left), structure-based immunogen design (lower left) and mutation-guided immunogen design (lower right). The goal is for sequentially administered immunogens to provide a selective advantage in the germinal centre to B cells that follow similar desired evolutionary trajectories. Because bnAb UCAs rarely bind with high affinity to unmodified autologous Envs, priming immunogens frequently must be designed with epitope modifications (for example, the shortening of variable loops, or removal of key glycans) in an approach called germline-targeting (upper left). Germine-targeting is based on selection of a transmitted/founder virus or a specifically selected or designed Env immunogen that can bind to a UCA[25,51,77,78,90]. This can be achieved through in vitro selection techniques where UCAs are used to bind and select high-affinity Env antigens from a library of Env variants. These Env ligands are then used to isolate antibodies from the human immunoglobulin repertoire to identify a polyclonal mixture of putative bnAb precursors. A germline-targeting Env antigen can then be re-designed to improve its affinity for many of the isolated putative bnAb precursors using mutation-guided immunogen design and structure-based immunogen design. Mutation-guided immunogen design[42] aims to identify the improbable mutations in bnAbs that are not routinely generated by somatic hypermutation but are critical for broad neutralization. These are then used to inform the design of immunogens that can specifically select for these mutations. Structure-based immunogen design[91] is based on the determination of bnAb–Env complexes that provide atomic-level information that is necessary to computationally model specific bnAbs as templates for immunogen design. These inform the modifications for improving Env immunogen binding to bnAbs or bnAb precursors. Structure-based immunogen design is utilized to inform all stages of the vaccine design strategy.

V3-glycan germline-targeting Envs have been shown to expand these precursors in UCA knock-in mice[51,126,127], while also selecting for improbable mutations required for heterologous breadth[76,121]. Moreover, in mice with a knock-in of a minimally mutated bnAb precursor, sequential immunization with Env immunogens induced antibody affinity maturation[128]. 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 with other V3-glycan bnAbs[129]. 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 challenge[130]. Besides the requirement for long HCDR3 loops, V3-glycan bnAbs have high levels of rare mutations required for acquisition of bnAb breadth[42].

V2 glycan-targeted bnAbs

The V2-glycan epitope includes an N-linked glycan at position 160 and a lysine-rich carboxy-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 strand[131]. The HCDR3 loops of V2-glycan bnAbs are typically even longer (24–36 amino acids) 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 and PCT64, as well as the bnAbs

CAP256.VRC26 and PG16) are characterized by the presence of a YYD tyrosine sulfation motif[131–134].

In one study, antibodies with HCDR3 regions similar to the PG9 bnAb were found by ultra-deep sequencing in only 2 out of 70 individuals naive for HIV-1 (REF.[52]). In UCA knock-in mice, a series of immunogens were used to stimulate V2-glycan bnAb precursors[135], and one study detected V2-glycan bnAb induction by vaccination with a series of engineered Env immunogens in a rhesus macaque[136]. To date, V2-glycan germline-targeting immunogens have yet to be studied in clinical trials, but a chimpanzee V2-glycan UCA-binding Env called MT145KdV5 is in Good Manufacturing Practice (GMP) production for testing in a phase I clinical trial[135].

Although V2-glycan bnAb precursors are rare, one study including a cohort of people of sub-Saharan African descent with primary infection with HIV-1 detected V2-glycan bnAbs in 14% of individuals who had high serum levels of bnAbs, whereas the most common bnAb type was V3-glycan bnAbs, which were observed in 38% of subjects with high levels of bnAbs[137]. V2-glycan bnAbs have also been observed in SHIV-infected macaques[138]. Thus, either HIV-1 or SHIV infection induces a permissive immunologic milieu that facilitates the induction of bnAbs with rare long HCDR3s or the precursor frequency of potential V2-glycan 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 favour the expression of long HCDR3s in those that make bnAbs and the relaxation of immune tolerance mechanisms due to retroviral infection[39,46].

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 individuals infected with HIV-1. 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, have a 24 amino acid HCDR3 and, similar to most other MPER bnAbs, are of the IgG3 isotype[34,139] (TABLE 1). 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, PGZL1 and 4E10 are encoded by the VH1–69 gene segment with the variable light chain VK3–20 (REF.[140]) (TABLE 1). Most MPER bnAbs share the traits of long HCDR3s, have high levels of V_H mutations and bind to a membrane proximal binding epitope that includes binding both the gp41 MPER and viral membranes (TABLE 2). Indeed, 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[96,141]. MPER bnAbs are among the most polyreactive of HIV-1 bnAbs and have been shown to bind, in addition to lipids[40], 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 2F5 (REF.[142]). 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 periphery[143–146]. For 2F5 bnAb knock-in mice, this central tolerance checkpoint appears not to be driven by interaction with lipids, as B cell compartments rescued by receptor editing or anergy lose the capacity to bind kynureninase but retain lipid reactivity[145]. 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 $D \to E$ replacement. In contrast, the SF3B3 proteins of humans and opossums are identical[142]. 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 humans[142]. Thus, in humans, tolerance to kynureninase and SF3B3 likely limits 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 expand[87]. 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 the lack of an improbable proline mutation in the HCDR3 (REF.[87]). 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 an immunogen to determine whether B cell precursors targeting the proximal [683]LDKW[686] epitope of gp41 can be expanded, and bnAb precursors that target this epitope have indeed been identified in clinical trial vaccinees (W. Williams, L. Baden, B. F. Haynes, unpublished observations). However, to date, success has not been achieved in any model system that stimulates the precursors of the more potent distal MPER bnAbs such as 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[40,142]. Moreover, it can prolong the partial thromboplastin time when administered to humans in vivo[147]. In general, however, it is thought that autoreactive HIV-1 bnAbs are not pathogenic. Rather, the polyreactive or autoreactive nature of bnAbs reflects their requirement to have unusual traits such as long HCDR3s for bnAb activity.

Fusion domain-targeted bnAbs

The fusion domain peptide 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 (to enhance immunogen valency and to provide a source of T cell epitopes for CD4^{+} T$_{FH}$ cell induction) has been shown to induce antibodies that neutralize heterologous HIV-1 strains[148–151]. 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 naive B cells, followed by sequential boosts to guide favoured 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[152,153] 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 disfavour 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 $Y \to P$ 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[149,151]. This mutation occurred in the clade of the lineage that
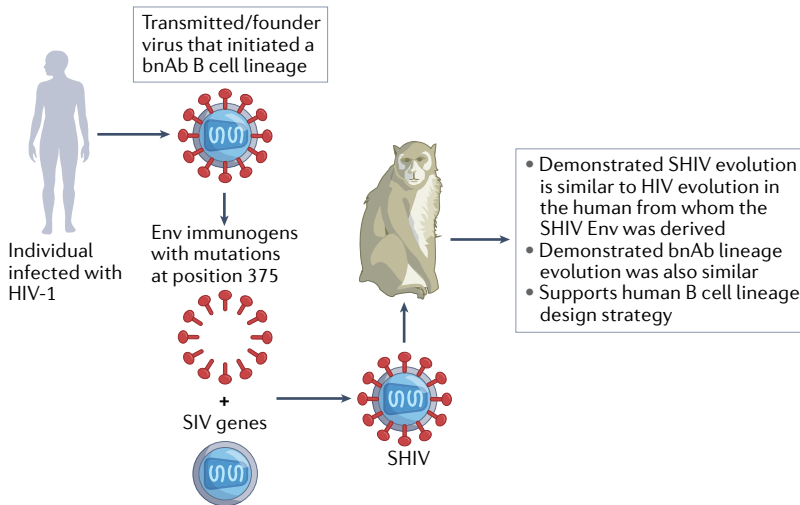
Fig. 5 | **Construction of simian-human immunodeficiency viruses containing clinically relevant primary HIV-1 Env immunogens.** Critical to simian-human immunodeficiency virus (SHIV) design is selection of primary or transmitted/founder HIV-1 envelope (Env) immunogens that exhibit features of clinically relevant viruses. For SHIV construction, a mutation at Env position 375 is made to facilitate macaque CD4^{+} T cell engagement. A HIV transmitted/founder or other Env immunogen is engineered to be expressed on the surface of a simian immunodeficiency virus (SIV) virion to form a chimeric SHIV. In this manner, virtually any HIV-1 Env can be used to produce a SHIV for testing for the ability to induce broadly neutralizing antibodies (bnAbs) in the setting of lentiviral infection.

Figure labels: Transmitted/founder virus that initiated a bnAb B cell lineage · Individual infected with HIV-1 · Env immunogens with mutations at position 375 · + SIV genes · SHIV · Demonstrated SHIV evolution is similar to HIV evolution in the human from whom the SHIV Env was derived · Demonstrated bnAb lineage evolution was also similar · Supports human B cell lineage design strategy

Box 3 | **Humanized mouse models for study of broadly neutralizing antibody B cell development**

The first broadly neutralizing antibody (bnAb) knock-in mouse model studied was the 2F5 V_H knock-in mouse, in which B cells expressed the variable heavy chains of the bnAb 2F5. It demonstrated that B cells expressing the 2F5 B cell receptor (BCR) were mostly deleted at the pre-B cell stage in the bone marrow, and B cells with the 2F5 V_H BCR that made it to the periphery were anergic[144]. Knock-in mice with a mutated 2F5 V_H and knock-in mice that expressed the V_H and V_L regions of the bnAb 4E10 had similar defects[87,110,233]. Interestingly, the first-generation CD4 binding site bnAb 1b12 was found to be autoreactive, but it did not induce bone marrow B cell precursor deletion or anergy in peripheral B cells in knock-in mouse models[234]. By contrast, the CD4 binding site bnAb 3BNC60, also with a VH1-2*02 similar to VRC01 but paired with a different V_L, when this V_H + V_L was knocked into mice, did demonstrate deletion, anergy and receptor editing, suggesting different fates dictated by V_L usage[110].

These initial bnAb knock-in mice were engineered to express the rearranged mature V_H and V_L or the rearranged V_H and V_L sequences of the unmutated common ancestor (UCA) of the respective bnAbs. The expression of pre-rearranged V_H and V_L sequences inhibited the rearrangement and expression of the endogenous mouse immunoglobulin loci. This type of mouse model is useful for testing whether the designed immunogen is capable of activating B cells that express particular bnAb precursors and whether precursor antibodies can mature to functional bnAbs. However, bnAb precursors are present in these mouse models at supra-physiologic frequencies. Immunization under such conditions cannot assess the specificity of immunogens for rare bnAb precursors in complex B cell repertoires[235]. To overcome this limitation, adoptive transfer of B cells that express bnAb precursors to wild type recipient mice can be used to reconstitute B cell compartments with physiological bnAb precursor frequencies[236].

Recently, the groups of Alt, Tian and colleagues have developed HIV-1 bnAb germline-rearranging mice that developmentally generate diverse repertoires of bnAb VRC01-class precursors[106]. This type of mouse model is designed to assess the specificity of an immunogen for bnAb precursors as well as its ability to mature B cell lineages that produce related precursor antibodies towards the production of bnAbs. The latter function is important for immunogen efficacy in the human population, where individuals may have B cell lineages producing various bnAb precursors. In this mouse model, the human variable heavy 1–2 (VH1–2) gene segment recombines with mouse D and J_H gene segments or mouse D segments and human J_H segments to form a diverse range of humanized BCR heavy chains that express a VH1–2 region in association with a multitude of different complementary determining region 3 (CDR3) segments[106]. This model was revolutionary in that it provided for a more diverse repertoire of bnAb-producing B cells, and with a much lower frequency of precursors, resulting in a more physiologic testing environment for potential vaccine immunogens. Indeed, in this model, the eOD-GT8 prime (an immunogen designed to bind to VRC01 germline B cell receptors[77]), coupled with the 426c Env immunogen[104] in a boost regimen, was successful in expanding VRC01 bnAb B cell precursors[106,107]. Mouse models are now being generated that mimic physiological conditions more closely, in which both V_H and V_L rearranging gene segments result in an immense diversity of humanized mouse BCR repertoires. Moreover, new models have been designed to circumvent B cell developmental blocks in the bone marrow by expressing bnAb precursors conditionally in mature B cells[237].

Paratope
The binding region of an antibody for an antigen.

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

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

acquired broad neutralizing activity, but did not occur in other weakly neutralizing clades of the VRC34 lineage[151]. Therefore, the requirement for acquisition of improbable mutations may be one impediment to the development of neutralization breadth by this antibody class[42,151].

Fab-dimerized glycan bnAbs

The glycan-reactive bnAb 2G12 was isolated from an individual infected with HIV-1 in 1996 (REF.[154]) and shown to have a unique V_H domain swap conformation that formed an 'I-shaped' bnAb with an expanded Fab-dimerized paratope capable of Env glycan binding, called Fab-dimerized glycan (FDG) antibodies[155]. 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 macaques[156]. Macaques immunized with a glycosylated V3 peptide and boosted with scaffolded mannose glycans expand FDG precursor antibodies, and FDG bnAbs have also been isolated from SHIV-infected monkeys (bnAbs DH851 and DH1003)[156]. Interestingly, analysis of 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 a source of natural antibody-producing B cells[157,158]. 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 bnAb[156]. 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 to class-switch from IgM to IgG and become bnAbs[156]. Natural glycan-reactive antibodies generally develop in a T cell-independent manner[158]. However, as bnAbs generally develop in germinal centres 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 centres to interact with T_{FH} cells for maturation to neutralization breadth. Studies are underway to test this hypothesis, and novel vaccine delivery regimens are being explored that prolong the germinal centre response and promote CD4+ T_{FH} cell responses[159–162] (BOX 4).

Combined T cell 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} cell responses is required to support vaccine-mediated HIV-1 bnAb induction[39,45]. It was shown that LNP-enclosed mRNA can induce potent T_{FH} cell 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 cells[163–166]. In acute HIV-1 infection, HLA class Ia-restricted T cell responses play an important role in the containment of viraemia and drive a rapid selection for HIV-1 escape mutants[167], and they make a key contribution to sustained control of viral replication in HIV-1 elite controllers[163]. However, they fail to eradicate HIV-1 after viral integration has occurred[167]. 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 set point viral loads after infection in preclinical models[168], clinical trials conducted to date with such vaccines have not succeeded in reducing viraemia[169,170]. Thus, although HLA class Ia-restricted CD8+ T cells clearly have some

Table 2 | **Broadly neutralizing antibodies and their characteristics**

HIV-1 epitope	bnAb	Breadth (%)ᵃ (potency (µg ml⁻¹)ᵇ	V_H/V_L gene	HCDR3 length	LCDR3 length	V_H mutation frequency (%)	V_L mutation frequency (%)	Germline-targeting priming immunogen
CD4 binding site	N49P7	100 (0.10)	VH1–2/VL2–11	19	5	24.5	14.1	NA
	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 (REF.[77])
	VRC01	91 (0.377)	VH1–2/VK3–20	12	5	31.6	17.2	426c.TM4ΔV1-3 (REF.[110]), BG505 SOSIP 4.1 GT1 (REF.[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/VL2–14	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- (REF.[120])
	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 T/F[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 (REF.[126]), RC1-4Fill (REF.[129])
	BG18	61 (0.032)	VH4–4/VL3–25	21	11	21.5	17.6	MD39-11MUTb, N332 GT5 (REF.[51])
	BF520.1	53ᶜ (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 (REF.[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 (REF.[228]), ZM233 (REF.[132]), KER2018 (REF.[133]), BB201.B42 (REF.[133])
	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 (REF.[132]), Q23.17 (REF.[132]), WITO[132], T250 (REF.[131])
	PCT64-35Mᵈ	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	NA
	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	NA
	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	NA
Fab dimer glycan	2G12ᵉ	21 (3.75)	VH3–21/VK1–5	14	9	21.1	11.7	V3-glycopeptide[156]

The following bnAbs met the inclusion criteria but were not included for space considerations: 3BNC55, VRC13, VRC16, VRC18, VRC27. Adapted with permission from REF.[227]. bnAb, broadly neutralizing antibody; MPER, membrane proximal external region; NA, not available; VH1–2, variable heavy 1–2; HCDR3, heavy chain complementarity determining region 3; LCDR3, light chain complementarity determining region 3. ᵃOnly bnAbs that have >45% breadth on a multiclade panel of >50 viruses (from CATNAP database[229]), except where noted. ᵇGeometric mean of detected. ᶜBF520.1 neutralization was only tested on 15 viruses but is included here because it notably was induced in an infant[38]. ᵈPCT64 included because it is only one of two V2 lineages profiled from acute HIV-1 infection to bnAb breadth. ᵉ2G12 does not meet >45% breadth threshold but is included because expected high precursor frequency of natural Fab-dimerized glycan (FDG) antibodies makes this class an attractive target for design.

Box 4 | Env immunogen optimization and delivery regimens

A major advance in the HIV-1 vaccine field was the design and structural definition of a cleaved, stabilized envelope (Env) trimer by addition of the T>C mutation at position 605, A>C mutation at position 501 and I>P mutation at position 559, called the SOSIP.664 envelope gp140 (SOSIP Env trimer)[238–241]. SOSIP Env trimers have been used to select for broadly neutralizing antibodies (bnAbs) targeting the quaternary structure of the Env trimer apex[242]. It is also becoming clear that multimeric, stabilized Env immunogens decorating protein nanoparticles may be more immunogenic than a combination of Env immunogens with small-molecule adjuvants[76,243,244]. This is both because of improved movement of nanoparticles into germinal centres and because of the need to stabilize Env immunogens to prevent the formation of non-neutralizing, diverting epitopes that can take bnAb B cell lineages off-target[245–247]. For example, insufficient glycosylation of Env, resulting in so-called 'glycan holes', can cause this problem[248–252]. The base of the Env trimer also contains diverting epitopes that can impede bnAb development and promote trimer disassembly[253]. Thus, both stabilization and minimization of non-neutralizing, diverting epitopes are important for HIV-1 vaccine immunogen design.

Another aspect to consider in immunogen design is the fact that almost all bnAbs have high levels of mutations, suggesting that B cells either remain in germinal centres for prolonged periods of time or recirculate back into germinal centres to accumulate functional mutations required for bnAb maturation. Therefore, immunization regimens need to prolong germinal centre responses and induce CD4$^+$ T follicular helper cells (T$_{FH}$ cells) to support robust bnAb development in germinal centres[39,45]. In this regard, it was recently demonstrated that modulating the quantity of HIV-1 Env-specific CD4$^+$ T cell help can promote rare B cell responses in mice[160]. In individuals with HIV-1 infection, there is a constant supply of antigenic variants of Env that may, over time, select for rare functional bnAb mutations. This results in bnAb development over 2–5 years in approximately 50% of adults infected with HIV-1[27–31,33,70]. Two studies have found that vaccine strategies that include delivery of antigen either by continuous administration via subcutaneous pumps or by administration of escalating doses of antigen over time result in enhanced antibody responses[159,162]. In macaques, such a regimen sustained germinal centre responses for up to 29 weeks, and with late boosting at 30 weeks, low titres of heterologous HIV-1 neutralizing antibodies were induced[161]. Finally, vaccines based on modified mRNAs in lipid nanoparticles (LNPs) potently stimulate T$_{FH}$ cells[59,64]. After intramuscular immunization, these are rapidly distributed to the liver, spleen and systemic lymph nodes, with prolonged expression of antigen over days[189]. Work has now begun to formulate complex stabilized Env trimer nanoparticle multimers either encoded in mRNAs or delivered as mRNA LNPs[127,188], with some success reported in bnAb unmutated common ancestor (UCA) knock-in mice and in monkeys[188,254]. Thus, development efforts are ongoing to define the optimum platform for delivery of a multicomponent HIV-1 bnAb vaccine.

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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 simian immunodeficiency virus (SIV)-infected cells early in infection in ~55% of animals[164]. The vaccine that achieved this unprecedented protective effect employed a rhesus cytomegalovirus (RhCMV) vector with fortuitous gene deletion that allows it to induce MHC-E-restricted CD8$^+$ T cells[164,165,171,172]. 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 cells[166], and the observation that insertion of orthologous human (H)CMV genes into RhCMV generates a vector that induces

Set-point viral loads
The residual viral loads that result in chronic HIV-1 infection after the peak viral load that occurs during acute HIV-1 infection.

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the same level of protection upon SIV challenge as the original RhCMV vector in rhesus macaques[172] suggests that it may be possible to develop vaccines that induce HLA-E restricted anti-HIV-1 T cells in humans. An 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 with the B cell vaccine alone[173]. Moreover, combination of a T cell immunogen with a B cell immunogen did not compromise the potency of either vaccine in an animal model[174].

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

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 an immunisation strategy where immunogens target naive BCRs, followed by sequential immunogens that select for memory B cells with BCRs with improbable mutations, these become activated, proliferate and continue to acquire functional mutations, resulting in acquisition of bnAb breadth[25,42,51,76,79,81,175,176]. Although the specific design of sequential Env immunogens for a practical vaccine remains to be determined, the desired attributes are now known, and there is a better understanding of the immunobiology of bnAb development and the roadblocks that prevent their induction[11] (BOX 1 and FIG. 1).

The requirements for a multicomponent HIV-1 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$_{FH}$ cell responses in order to stimulate high levels of highly mutated and long-lived bnAbs, and the 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 evolution[177], and such increasing diversity can have an impact on the sensitivity to bnAb neutralization[177,178]. In addition, the complexity of new recombinant forms of HIV-1 and the diversity of clades

and recombinant forms within local geographic populations are increasing[9]. 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 the 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 disfavoured 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 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 occur[81]. In this manner, multiple experimental 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 LNPs or novel vectors. Early after infection, HIV-1 provirus can integrate into the host genome as latent virus without producing viral proteins[179,180], 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[16,181]. Finally, the production of multicomponent protein vaccines will be challenging owing to both the difficulty and time necessary to produce multiple proteins by GMP and the cost of such a vaccine. The modified mRNA and LNP technology that has been so successful for COVID-19 vaccines[182,183] may be more amenable for the production of multicomponent vaccines compared to protein-based vaccines[127,184–189].

The hope is that once a vaccine is designed and shown to be effective, the immune correlates of protection 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

B.F.H. 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 Envs 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 Envs for induction of HIV-1 antibodies.

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