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Strategies for a multi-stage neutralizing antibody-based HIV vaccine

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A critical property of a prophylactic HIV vaccine is likely to be its ability to elicit broadly neutralizing antibodies (bnAbs). BnAbs typically have multiple unusual features and are generated in a fraction of HIV-infected individuals through complex pathways. Current vaccine design approaches seek to trigger rare B cell precursors and then steer affinity maturation toward bnAbs in a multi-stage multi-component immunization approach. These vaccine design strategies have been facilitated by molecular descriptions of bnAb interactions with stabilized HIV trimers, the use of an array of sophisticated approaches for immunogen design, the development of novel animal models for immunogen evaluation and advanced technologies to interrogate antibody responses. In this review, we will discuss leading HIV bnAb vaccine immunogens, immunization strategies and future improvements.

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Introduction

A prophylactic vaccine that can induce protective antibodies against HIV is paramount for controlling the HIV pandemic, which remains a major global health problem. Although an HIV vaccine remains elusive, remarkable progress has been made over the last decade. This progress includes the isolation of more than 200 broadly neutralizing antibodies (bnAbs) from HIV infected donors, which has revealed critical vaccine targets on the envelope (Env) protein [1–4,5••,6,7]. A second significant discovery was the stabilization and structural characterization of a recombinant soluble Env trimer that mimics native Env present on the viral surface [8–11]. The coupling of these bnAbs and the trimer with structural studies have immensely facilitated structure-guided immunogen design and have made the development of an HIV neutralizing antibody vaccine appear to be an achievable goal.

A relatively small fraction of HIV-infected humans elicit potent bnAb responses, which target the Env spike or trimer and are effective against a wide range of viral isolates [2]. Passive transfer of these bnAbs into non-human primates (NHPs) provides complete protection against mucosal challenge with the virus [2,12]. These proof-of-concept studies suggest that a vaccine that elicits bnAbs of sufficient breadth, potency and concentration may exhibit sterilizing HIV immunity in humans. However, classical vaccine approaches have failed to elicit bnAbs for multiple reasons, including sequence variation in Env, difficulties of accessing bnAb epitopes hidden beneath the canopy of the Env glycan shield and unique genetic requirements that reduce the frequency of precursors encoding bnAbs [1–3,6,13–16]. Recent immunogen design strategies seek to tackle these obstacles by adopting a multi-stage vaccine approach, which involves priming with bnAb precursor targeting molecules to initiate appropriate B cell lineages followed by sequential and/or cocktail immunogen boosts to drive affinity maturation along pathways to bnAbs.

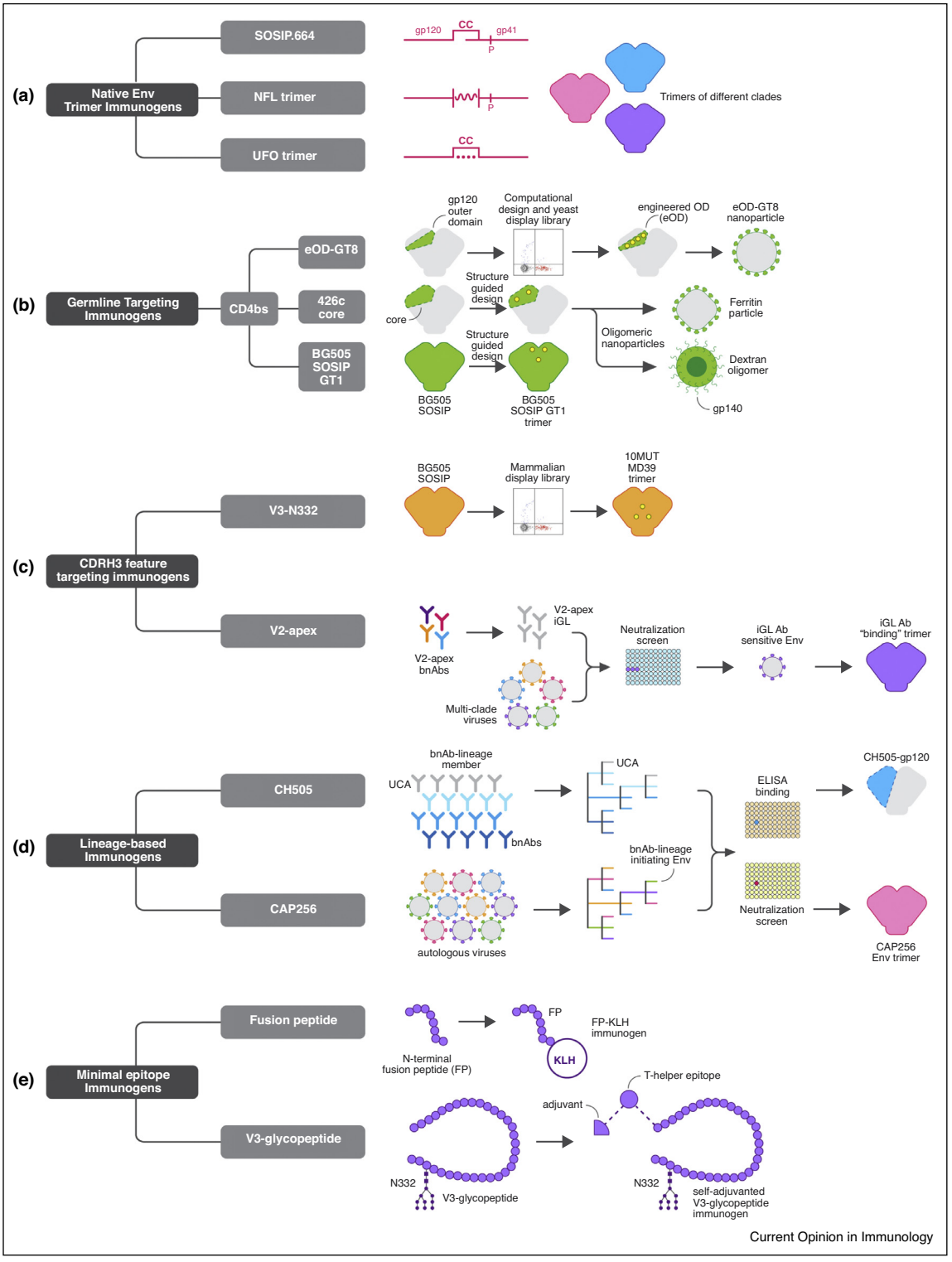
In this review, we will focus on current HIV bnAb immunogen design strategies, recent progress made in the development of animal models to evaluate potential vaccine candidates, advances in the technology to analyze antibody responses, and emerging concepts in understanding B cell developmental pathways that may facilitate HIV vaccine design strategies.

Approaches to design immunogens that can contribute to the elicitation of HIV bnAbs

Native Env trimer immunogens

A major immunogen design effort focuses on the stabilization of native-like soluble trimers (Figure 1a). The

Figure 1



Immunogens that can form components of a neutralizing antibody-based HIV vaccine. **(a)** Schematic showing native envelope trimer immunogens (SOSIP.664, native flexibly-linked (NFL) and uncleaved prefusion optimized (UFO) platforms), which form the basis of both priming and boosting immunization steps are shown. Trimers from multiple clades can be generated. **(b)** CD4 binding site (CD4bs) germline-targeting immunogens, including the engineered outer domain germline-targeting version 8 (eOD-GT8) immunogen (generated by computational approaches and refined through yeast-display and multimerized on nanoparticles), the 426c gp120 core (which incorporates multiple glycan deletions around the CD4bs) and its oligomeric forms on ferritin and dextran and BG505 SOSIP.GT1 (SOSIP.664 modified to have enhanced binding of CD4bs

SOSIP.664 design platform, which links the gp120 and gp41 subunits by a disulfide bond and stabilizes the trimer in the pre-fusion state through an I559P substitution, is the lead strategy in this category [8,17–22]. In contrast, design approaches that incorporate a linker between gp120 and gp41 have also been employed [23–25]. These native-like trimers are particularly advantageous, because they sequester inter-protomer surfaces to occlude immunodominant, non-neutralizing epitopes while presenting broadly neutralizing epitopes, including quaternary sites. Previous immunizations with gp120 or non-native trimers have generally produced tier 1 nAb responses but failed to induce autologous tier 2 nAb titers. Immunizations in rabbits, guinea pigs and NHPs with the SOSIP trimer have produced autologous tier 2 neutralizing responses, indicating the presentation of at least some elements of native Env structure to the humoral immune system [18,20–22]. However, these native trimer immunizations on their own have yielded little in the way of neutralization breadth [18,20–22] and are consistent with the notion discussed above of the need for a multi-immunogen approach to HIV vaccine development.

Studies of natural HIV infection demonstrated that exposure to viral variation within a bnAb epitope drives antibody maturation toward neutralization breadth [1,3,26]. Based on this, one multi-immunogen approach to eliciting a protective nAb response incorporates trimeric immunogens derived from Envs representative of the global viral diversity within specific bnAb epitopes. In this approach, immunizations consisting of a cocktail of multi-clade Env-derived trimers would be administered repeatedly until bnAbs develop. These native trimer immunogen design approaches incorporate stabilizing mutations to increase thermostablity, prevent CD4-induced trimer opening, and improve immunogenicity by masking non-essential immunodominant epitopes [17,19,27,28].

bnAb germline-targeting immunogens

The concept of targeting germline-encoded features of bnAb precursors arose from the observations that inferred germline-reverted precursors (iGLs) of HIV bnAbs lack detectable binding affinity for native Env proteins [29], and that CD4 binding site (CD4bs) bnAbs isolated from multiple donors used a common germline-derived heavy chain immunoglobulin (Ig) gene, VH1-2*02 [16]. Accordingly, Jardine *et al.* employed computational design coupled with a yeast display library platform to generate an outer domain (OD) molecule of Env gp120 with binding affinity for multiple VRC01-class bnAb precursors [15] (Figure 1b). The resulting germline-targeting molecule termed 'eOD-GT6' was displayed on nanoparticles to increase multivalency for more efficient activation of VRC01 precursor B cells *in vivo*. Indeed, a successor to this molecule, eOD-GT8, successfully primed the appropriate precursors in a VRC01 GL knock-in (KI) mouse and enriched for VRC01-class precursors from naïve human B cells of HIV seronegative donors [30,31]. In addition, these eOD-GT8 primed responses, when appropriately boosted, displayed accumulation of somatic hypermutation along the VRC01-class maturation pathway [32•]. However, the responses were not sufficient to broadly neutralize tier 2 viruses dictated by their inability to tolerate the N276 glycan that lies over the CD4 binding site [32•,33]. Trimeric boosting immunogens with staged increasing glycan complexity have been proposed to overcome the N276 hurdle to neutralization [32•].

Although, eOD-GT8 immunogen can effectively prime the VRC01-like B cell precursors in appropriate animal models, its ability to select these germline B cell precursors varied substantially across different model systems [31,33–35]. While eOD-GT8 was most effective in the VRC01 GL KI mouse model, its ability to engage 3BNC60 germline expressing mouse B cell receptors (BCRs) was substantially lower and even weaker still in more competitive polyclonal mouse B cell immune systems that express either germline VH1-2*02 (excluding the CDRH3) or the entire human heavy chain Ig locus KI mouse models [33,35]. For example, in the human Ig transgenic mouse model (Kymab), a single immunization with eOD-GT8 revealed that only 1% of eOD-GT8 specific Ab responses were VRC01-like, suggesting that boosting strategies to reduce the competing B cells will be required to immunofocus the appropriate responses in an Ab recall response [35]. The variable efficiency of eOD-GT8 to engage VRC01-like B cell precursors from different animal models could be attributed to various factors including its diverse affinities for VRC01 GLs and differences in the frequencies of appropriate precursors compared to the off-target B cells. [32•,33]. Based on these animal studies, the eOD-GT8 priming immunogen will be tested in a Phase I clinical trial in 2018 to assess its ability to select VRC01-like precursors in humans.

In addition to eOD-GT8, other germline-targeting immunogens are being developed, including a core gp120 or gp120 oligomers derived from a clade C Env, 426c, which binds VRC01-class germline precursors and primes germline receptor-expressing B cells *in vivo* [34,36], and modified versions of the BG505 SOSIP.664

(Figure 1 Legend Continued) germline-reverted antibodies) are illustrated. **(c)** Immunogens designed to select for antibodies with a long heavy chain complementarity determining region 3 (CDRH3), including the BG505 10MUT MD39 trimer to elicit PGT121-class V3-N332 bnAbs and trimers possessing 'glycan holes' close to the trimer apex to generate V2-apex bnAbs. **(d)** Lineage-based immunogens, derived from virus–antibody co-evolution studies in donors CH505 and CAP256, are in development for the elicitation of CD4bs and V2-apex bnAbs. **(e)** Minimal epitope immunogens, including the N-terminal region of the fusion peptide (FP) fused to keyhole limpet hemocyanin (KLH) and V3-glycopeptides coupled to a T-helper epitope.

trimer, which can bind multiple bnAb germline specificities, including VRC01 (Figure 1b) [37]. Overall, these strategies aim to activate VRC01-like precursor BCRs with a germline-targeting immunogen, followed by boosting with more native-like Env molecules to shepherd Ab responses along favorable bnAb developmental pathways [31,32•,33–35].

CDRH3-feature targeting immunogens

Following the encouraging results of the VRC01 bnAb germline-targeting approach, a cell-surface mammalian display library platform was employed to select mutations within the BG505 SOSIP trimer backbone that would enable binding to iGL precursors of the PGT121-class of V3-N332 glycan-dependent bnAbs [38••] (Figure 1c). Since bnAbs in this class use a long CDRH3 to bind the glycoprotein epitope at the base of the V3 loop and adjacent V1V2 loops, the authors created a glycan 'hole' by removing specific glycans, altering variable loop lengths and substituting several residues within and around the epitope [14,38••]. Immunizations with this precursor-targeting BG505 SOSIP trimer in a KI-mouse model expressing the PGT121 iGL BCR and further sequential boosting with trimer variants that serially restored the native surface around the bnAb epitope followed by final boosting with a variable loop trimer cocktail induced a nAb response with notable breadth [39••]. This five-month immunization scheme sequentially incorporated gradual changes to restore the native bnAb epitope, followed by diversification through variable loop length modifications in the final boosts. The study demonstrated that an HIV Env glycan-dependent nAb response could be triggered by a rationally designed sequential immunization strategy and represented the first example of a vaccine-induced cross-reactive nAb response in any animal model [39••].

Another example of long CDRH3 germline feature targeting is apparent for V2-apex bnAbs, which bind to a glycoprotein epitope at the 3-fold axis of the trimer apex. These bnAbs use an unusually long anionic CDRH3 loop to reach the positively charged lysine-rich patch that forms the core protein epitope. Two independent studies by Andrabi *et al.*, and Gorman *et al.* searched for Env sequences that could interact with iGL versions of the V2-apex bnAbs [40,41] (Figure 1c). Both studies identified viruses that were sensitive to neutralization by V2 apex bnAb iGL antibodies and the corresponding Envs could be adapted as soluble trimer immunogens. Notably, the majority of these Envs possessed uncommon glycan holes around the apex that may provide increased access to the core lysine patch at the V2-apex [22]. Trimer immunization in rabbits induced autologous neutralizing responses that largely targeted basic residues within the V2 region that forms the core bnAb epitope for human V2-apex bnAbs [22]. We have extended these V2-apex germline targeting efforts to chimeric V1V2 HIV trimers and a chimpanzee SIVcpz*Ptt* Env (which shares the V2-apex bnAb epitope with HIV-1)-based trimer designs. Administration of these trimers in a sequential prime/boost immunization strategy in a V2-apex CH01 unmutated common ancestor (UCA)-expressing KI-mouse model induced Ab responses with some cross neutralization targeted to the V2 apex region (Andrabi *et al.*, unpublished data).

The use of naturally existing or artificially created glycan holes on Env trimer immunogens to increase accessibility of immunoquiescent epitopes or to improve binding with the bnAb iGLs has also been extended to the CD4bs bnAb epitope [37,42,43]. However, in increasing accessibility to immunoquiescent, difficult-to-reach bnAb epitopes one is also likely to increase accessibility to otherwise hidden strain-specific neutralizing and perhaps non-neutralizing Ab epitopes that may be quite immunodominant when exposed. Non-desirable antibody responses to these immunodominant epitopes may present considerable competition for relatively rare long CDRH3-bearing responses and hinder their initiation. Exceptionally strong selection may then be required at the boosting stage to favor the rare responses via immunofocusing strategies.

Lineage-based immunogens

Approaches to understand bnAb development in natural infection have focused on identification of the ancestor precursor B cell, the corresponding viruses that initiated these precursor responses, and longitudinal tracing of their co-evolutionary developmental pathways. The ultimate goal is to use this information to design immunogens and strategies to recapitulate elicitation of these responses through vaccination. To date, virus and antibody co-evolution studies of HIV-infected individuals who developed bnAb responses to the CD4bs, V2-apex and V3-N332 bnAb sites have been described in great detail [1,3,6,44] (Figure 1d). These studies described the viral variants responsible for both the elicitation of rare bnAb precursors and the maturation of these responses to neutralization breadth.

The first of these studies described the development of a CD4bs bnAb lineage, CH103, presumably initiated by the founder Env, CH505, that exhibited detectable binding with the UCA Ab [6]. Further, it was demonstrated that a helper B cell lineage, CH235, which itself became a bnAb lineage later, in this donor was critical for CH103 Ab bnAb development [1,4,6]. Unlike the VRC01-class CD4bs bnAbs, whose binding activity is in large part achieved by heavy chain V-gene encoded residues, the CH103 and CH235 CD4bs bnAbs are CDRH3-dominated [45]. The presence of the CD4bs bnAb site on gp120 subunit proteins is demonstrated by the high affinity binding of precursor and broad members of the CH103 and CH235 lineages. Therefore, a series of

gp120 immunogens aimed at mimicking the evolution of the virus in the CH505 donor have been defined [1,46]. This approach includes priming with the founder virus-derived gp120 and boosting with various gp120s that incorporate the Env diversity observed during the development of the bnAb response.

Another strategy based on virus–antibody co-evolution in donor CAP256 is being pursued to elicit V2-apex bnAbs. In this case, the use of trimeric immunogens is required, as the V2-apex bnAb epitope is only present on native pre-fusion trimer and not on Env subunits. This approach will make use of bnAb-initiating Env immunogens to prime these responses followed by a series of sequential immunogens that incorporate diversity at key strand-C residues, as well as variable loop lengths and alternate glycosylations to train B cells to accumulate somatic mutations, including those specific to complex glycans around the V2-apex [3,26,47].

Lineage-based and germline-targeting approaches both begin with the activation of germline-like precursor B cells. Historically, the distinction between the two is largely based around the attention paid to how neutralization breadth is achieved in natural infection. The lineage-based approach has classically tried to design a set of immunogens based on critical points along the natural infection pathway and begins with a single bnAb germline precursor antibody. The germline targeting approach, for example, eOD-GT8 designs an immunogen that binds to multiple bnAb germline precursor antibodies of a given class and then designs subsequent immunogens based on a variety of criteria but largely independent of the course of natural infection.

Minimal epitope immunogens

Env trimers or monomeric gp120 immunogens display a large antigenic surface that can engage diverse BCRs. One strategy for immunofocusing to a very limited set of BCRs is to immunize with minimal Env constructs that incorporate a portion of a bnAb epitope. Constructs meeting this requirement are gp41-fusion peptides and molecules including the base of the V3 loop protein and critical glycans (Figure 1e).

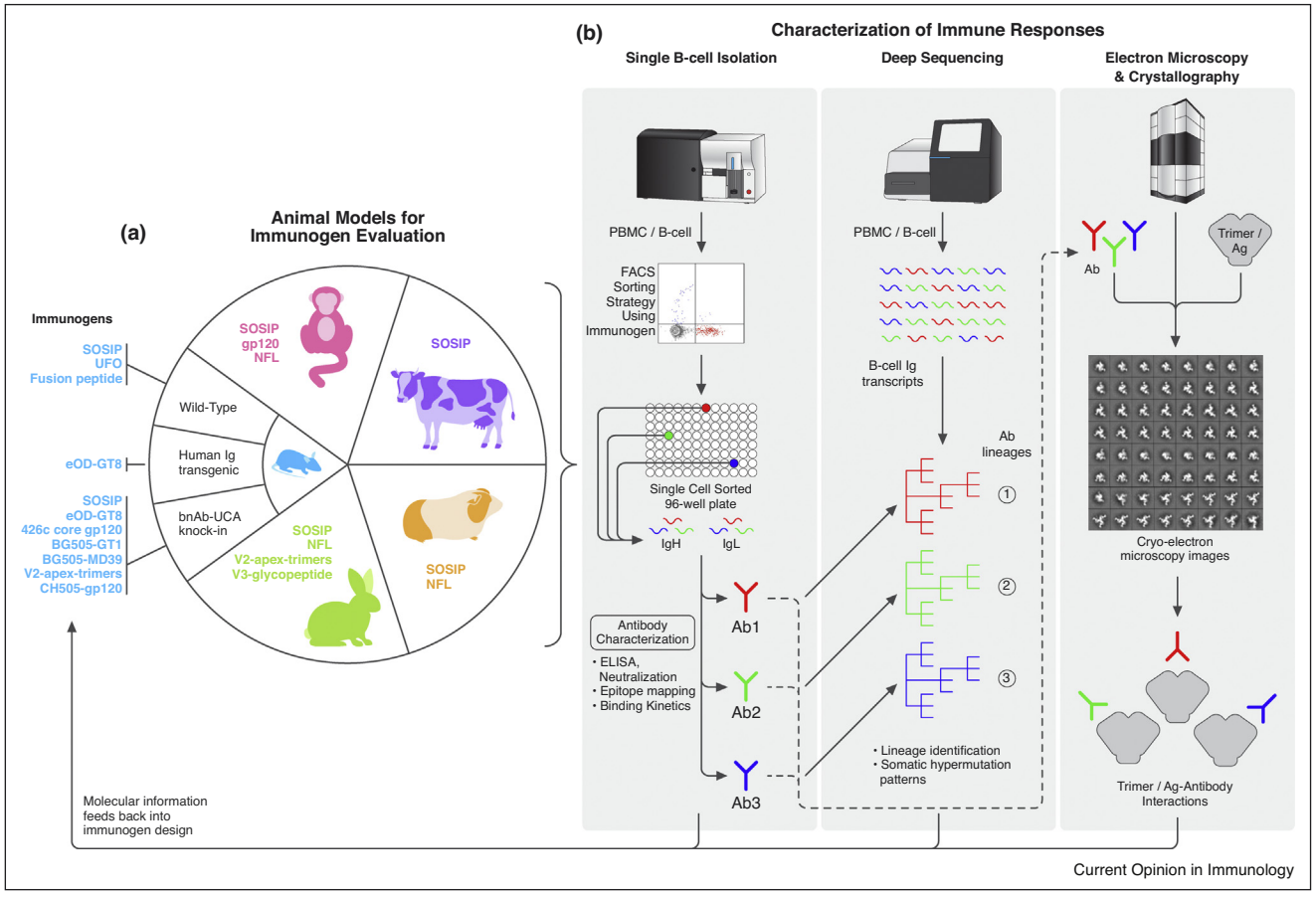
One approach uses an 8-amino acid residue stretch of the N-terminus of a gp41 fusion peptide (FP) that binds to FP-targeting bnAb, VRC34 [5••]. This FP, conjugated to keyhole limpet hemocyanin (KLH) through maleimide-linkage chemistry, was used to immunize mice, resulting in the induction of Abs that cross-neutralized ~20% of a global panel of viruses. (Kai Xu, Peter Kwong, *et al.*, Keystone-2018, unpublished). These initial FP-KLH immunizations mark important progress toward HIV vaccine-induced neutralizing responses in an unbiased B cell repertoire model. However further boosting sequentially with SOSIP trimers to encourage Ab recognition of key glycans surrounding the FP bnAb epitope will be critical to expand the neutralization breadth.

To design V3-glycopeptide based immunogens, another study used a chemoenzymatic method to synthesize and design a cyclic 33-mer gp120-V3 loop glycopeptide that represented a minimal epitope for the V3-glycan-dependent bnAbs, PGT128 and 10-1074 [48]. This V3-glycopeptide was further conjugated with a universal T-helper epitope and a Toll-like receptor 2 lipopeptide ligand (Pam3CSK4) to enhance immunogenicity. Immunizations in rabbits with this synthetic self-adjuvanted V3-glycopeptide elicited glycan-dependent antibody responses that were not neutralizing but displayed broader recognition of HIV-1 gp120s than the response induced by a non-glycosylated V3 peptide version.

Development of animal models for vaccine evaluation

Significant progress has been made over the past few years in the generation of immunoglobulin (Ig) KI mouse models that express the pre-rearranged V(D)J-encoding inferred germline genes of HIV bnAbs precursors to evaluate candidate immunogens (Figure 2a) [31,33,34,39••,49]. Much of this activity has focused on VRC01-bnAb GL KI models, but expansion to other bnAb precursors is in progress [39••,50]. Immunizations in these models have yielded valuable insights, especially with respect to selection and early maturation of B cell precursors by germline-targeting immunogens. These bnAb GL KI mouse models typically can be seen as providing a 'low bar' for immunogen assessment since the precursor frequencies of bnAb gene segments are high. For example, the frequency of CD4bs epitope-specific B cell precursors in the germline HC VRC01 KI-mouse model is approximately 400-fold higher than those predicted for humans (1 in ~1000 compared to 1 in ~400 000 B cells) and this high frequency of precursors may therefore overestimate the immunogen-induced B cell response in humans [30,31]. On the other hand, some KI-transgenic and Ig-transgenic mouse models especially those with more complete human BCR repertoire diversity possess much lower precursor frequencies that may be more reflective of the physiological frequencies in humans and thus provide a 'higher bar' to test these candidate vaccines [33,35]. In addition, precursor B cell adoptive transfers into wild-type animals can provide precursor frequencies that more closely resemble those anticipated in humans and can provide estimates of the affinities of immunogens that may be required to trigger appropriate B cell lineages [51•]. It should also be noted that, while the KI mouse studies have been informative in a number of cases, B cell receptor editing and peripheral anergy have been described for some mice such as those carrying unmutated germline versions of bnAbs 4E10, 2F5 and 3BNC60 bnAbs reducing their value [49].

Figure 2



Animal models for immunogen evaluation and analysis of antibody responses. **(a)** Five animal models (non-human primates, cows, guinea pigs, rabbits and mice) have been used to evaluate the immunogenicity of several current vaccine candidates. Within the mouse model, wild-type, immunoglobulin (Ig) gene knock-in mice (containing the rearranged Ig genes for the inferred unmutated common ancestor (UCA) versions of HIV bnAbs), and mice transgenic for the human Ig locus have been used. The particular immunogen design platforms tested within each animal model system are shown. **(b)** Schematic depicting three critical technologies that have been used to evaluate vaccine-elicited antibody responses. Monoclonal antibodies (mAbs), isolated by antigen-specific single B cell sorting, report directly on responses. Deep sequencing of plasmablast transcripts can be used to report directly on responses; deep sequencing of memory B cell transcripts together with mAbs can report on members of B cell lineages associated with given responses. Electron microscopy and crystallography can be used to identify the epitopes of the vaccine-elicited antibodies. Information derived from these technologies can be fed back to support iterative immunogen design.

Animal models such as wild-type mice, guinea pigs, rabbits and NHPs have been mainly used to evaluate the immunogenicity of native-like trimer immunogens (Figure 2a). These studies have demonstrated induction of autologous tier 2 neutralizing responses by native-like trimers, which often, especially in rabbits, target immunodominant Env glycan holes, that may potentially distract from desired immune responses [18,20,21]. The responses, however, do vary across different model species. Interestingly, repeated immunization in cows with a single immunogen, the BG505 SOSIP trimer, induced rapid bnAb responses [52••]. This contrasted sharply with the theory that sequential immunizations with different antigens will be required to generate bnAbs. However, these cow antibodies, which targeted the CD4bs, possessed ultra-long CDRH3 loops encoded by a VH-DH germline gene combination that is unique to the cow B cell repertoire. This ultra-long CDRH3 alone facilitated access to the CD4bs, which is otherwise sterically shielded from more traditional human bnAbs through glycosylation and oligomerization of the trimer [52••]. Nevertheless, why immunization with a single immunogen produced continuous neutralization broadening rather than a narrower reactivity and higher affinity to the immunizing strain (BG505) is unclear.

Technological innovations to interrogate immune responses

The iterative design of HIV vaccine immunogens has been immensely facilitated by extensive characterization of the Abs isolated from both infected individuals and immunized animals. Technological advances in three

areas have particularly contributed to this progress including, (i) ability to rescue Ig transcripts from single B cells, (ii) deep sequencing to identify bnAb lineages and trace their development through affinity maturation, and, (iii) recent advances in structural biology tools (cryo-electron microscopy and X-ray crystallography) that have revealed critical interaction of Abs and antigen to facilitate structure-guided design strategies (Figure 2b). Historically, conventional approaches investigated vaccine-elicited Ab responses by ELISA or virus neutralization. However, such approaches are inadequate to deal with sequential staged immunization strategies that may only reveal the desired response such as virus neutralization at the final stage. It then becomes crucial to monitor progress at individual stages in the immunization strategy. Single B cell Ab isolation and next generation sequencing (NGS) of B cell expressed Ig transcripts made possible with the emergence of high-throughput sequencing platforms such as Illumina, have enabled the identification of the origin of B cell lineages and trace their development during affinity maturation in natural infection and in vaccination [3,6,32•,35,44,53,54].

Recent advances in understanding the basis of B cell affinity maturation

The HIV Env trimer represents a complex antigenic surface, and the B cell affinity maturation processes to generate HIV bnAbs is intricate [3,6,26,47]. Therefore, understanding the biological basis of germinal center (GC) B cell selection to HIV immunogens at the primary and secondary stages of immune evaluation is important to design robust vaccine immunogens and strategies. Unfortunately, current understanding of antigen driven GC B cell selection comes from rather simple antigens and studies have only recently begun to investigate B cell responses to complex antigens like influenza hemagglutinin and *Bacillus anthracis* protective antigen to observe a higher complexity of B cells responses [55,56••]. Interestingly, these studies revealed that the GC B cell selection driven by complex antigens is more permissive to B cell clonal diversity and a range of BCR affinities than non-complex antigens described in earlier studies. Further studies of the complex antigen driven GC B cell selection as a function of change in antigenic surface under different immunization schemes at primary and secondary stages of immune responses will improve our understanding of how effective Ab responses are generated and facilitate design of better vaccine strategies.

Conclusions

The overall course of HIV vaccine development looks promising. One of the important questions to be addressed for germline-targeting immunogens is what are the affinity and frequency requirements to effectively trigger a rare bnAb precursor BCR. Multivalent display of immunogens [57–59] can activate precursor BCRs more efficiently and strategies that can reduce off-target B cell responses may guide immunofocused responses [60]. Deep mining of heathy human naïve B cell repertoires to determine precursor frequencies for each bnAb specificity will be an important consideration to devise optimal design strategies. Whether immunogen trafficking to secondary lymphoid follicles can be enhanced by immune complexes is an area of growing interest [61]. Also, a deeper understanding of GC B cell selection and Tfh biology for complex antigens at the primary and secondary immunization stages will be critical for developing more effective vaccine strategies. Finally, a multi-stage HIV vaccine will require manufacturing of several HIV vaccine immunogens, setting up of human clinical trials and an extensive pipeline for analysis of the immune responses.

Acknowledgments

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- • of special interest
- •• of outstanding interest

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