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**Authors**

Sievers, Stuart A  
Scharf, Louise  
West, Anthony P  
et al.

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# Antibody engineering for increased potency, breadth and half-life

Stuart A. Sievers<sup>a</sup>, Louise Scharf<sup>a</sup>, Anthony P. West Jr<sup>a</sup>, and Pamela J. Bjorkman<sup>a,b</sup>

## Purpose of review

This review highlights recent developments in HIV-1 antibody engineering and discusses the effects of increased polyreactivity on serum half-lives of engineered antibodies.

## Recent findings

Recent studies have uncovered a wealth of information about the relationship between the sequences and efficacies of anti-HIV-1 antibodies through a combination of bioinformatics, structural characterization and *in vivo* studies. This knowledge has stimulated efforts to enhance antibody breadth and potency for therapeutic use. Although some engineered antibodies have shown increased polyreactivity and short half-lives, promising efforts are circumventing these problems.

## Summary

Antibodies are desirable as therapeutics due to their ability to recognize targets with both specificity and high affinity. Furthermore, the ability of antibodies to stimulate Fc-mediated effector functions can increase their utility. Thus, mAbs have become central to strategies for the treatment of various diseases. Using both targeted and library-based approaches, antibodies can be engineered to improve their therapeutic properties. This article will discuss recent antibody engineering efforts to improve the breadth and potency of anti-HIV-1 antibodies. The polyreactivity of engineered HIV-1 bNAbs and the effect on serum half-life will be explored along with strategies to overcome problems introduced by engineering antibodies. Finally, advances in creating bispecific anti-HIV-1 reagents are discussed.

## Keywords

antibody engineering, bispecific reagents, breadth, HIV-1, polyreactivity, potency

## INTRODUCTION

HIV-1 effectively evades antibody-mediated responses due to rapid mutation of gp120 and gp41, the two glycoproteins that comprise the envelope (Env) spike (Fig. 1) [1<sup>a</sup>,2<sup>a</sup>,4<sup>a</sup>], and structural features that enable Env to hide conserved epitopes, including a shield of host-derived carbohydrates [5], conformational masking [6], steric occlusion [7–10] and flexible variable loops [8,11]. The low number and density of envelope spikes on HIV-1 virions may also contribute to the ability of HIV-1 to evade antibodies by preventing most IgGs from binding simultaneously with both Fabs [12,13<sup>a</sup>]. These features usually result in production of strain-specific antibodies. However, recent advances in single-cell cloning techniques have led to the identification of extremely potent, broadly neutralizing HIV-1 IgG antibodies (bNAbs) from infected donors [14–16]. When delivered passively in animal models of HIV-1 infection, anti-HIV-1 bNAbs can prevent [17–20] or suppress infection [21–24], prompting efforts to improve their potency and/or breadth in order to increase their

efficacy as therapeutics. Characterization of bNAbs through structural and bioinformatic approaches has identified epitopes on HIV-1 Env, including the gp120 V1V2 and V3 loops, the CD4-binding site (CD4bs), the gp41 membrane proximal external region (MPER) and sites bridging gp120 and gp41 (Fig. 1) [25–30].

<sup>a</sup>Division of Biology and Biological Engineering, California Institute of Technology, 1200 East California Boulevard, Pasadena, CA 91125, USA and <sup>b</sup>Howard Hughes Medical Institute, Chevy Chase, MD 20815-6789, USA

Correspondence to Pamela J. Bjorkman, Division of Biology and Biological Engineering, California Institute of Technology, 1200 E. California Blvd., MC 114-96, Pasadena, CA 91125, USA. Tel: +1 626 395 8351; e-mail: bjorkman@caltech.edu

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**KEY POINTS**

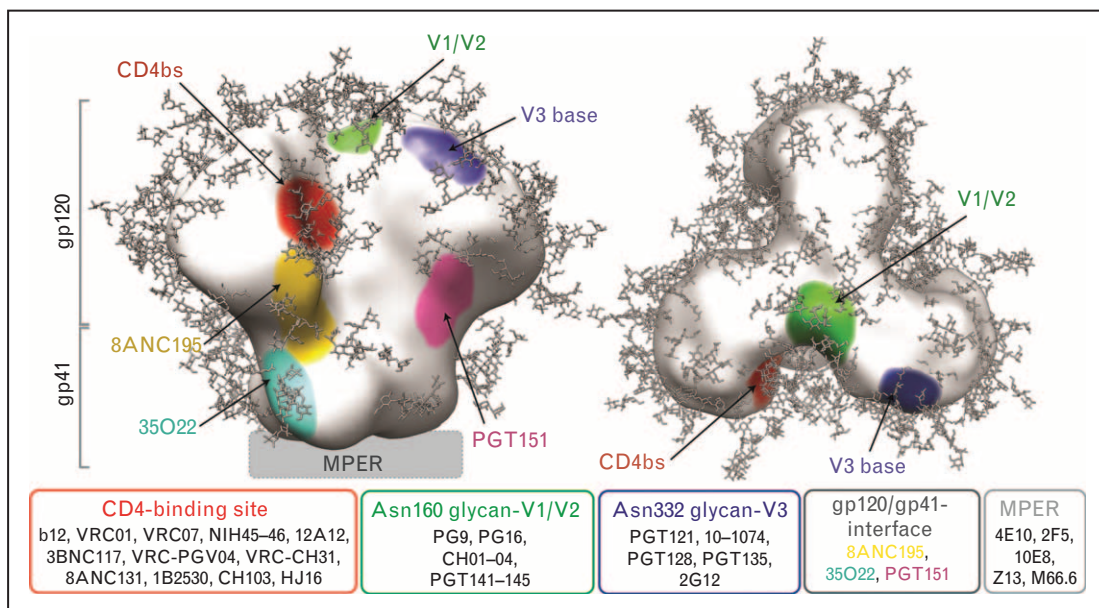
- Library-based and structure-design-based strategies for engineering anti-HIV-1 antibodies can increase both breadth and potency, even of potent donor-derived antibodies.
- Antibody engineering may introduce polyreactivity, which correlates with short serum half-life.
- Antibody engineering efforts can overcome problems of short half-life and other poor pharmacokinetic properties.
- Bispecific and chemically-conjugated antibody reagents provide an alternative strategy to target multiple HIV-1 epitopes to control viral escape and engineer avidity.

This review will discuss engineering efforts to create more potent anti-HIV-1 antibodies, using both traditional IgG and modified architectures. We also consider how engineering efforts can result in polyreactivity, the ability of an antibody to bind to more than one antigen, which can lead to auto-reactivity and/or decreased *in vivo* half-life.

**ENGINEERING HIV-1 ANTIBODIES**

Techniques to improve IgG affinities [31<sup>■</sup>,32], broaden their specificity to related antigens [33–35] and

improve their expression and solubility [32] include computational techniques [36–39] and directed mutagenesis to introduce diversity coupled with selection techniques, for example phage, yeast, mRNA and ribosome display [32]. Early library-based engineering efforts to improve HIV-1 antibodies involved b12, one of the first HIV-1 bNABs. The affinity of b12 was enhanced by nearly 400-fold by selecting for gp120 binding from libraries of phage-displayed mutants in complementarity-determining regions (CDRs) [40]. These studies demonstrated that increasing affinity through in-vitro evolution could also increase breadth [41]. The engineering of the HIV-1 m9 antibody used a modified approach termed sequential antigen planning to improve both affinity and breadth: a single-chain variable fragment (scFv) library of a CD4-induced (CD4i) antibody was screened against sequentially changing antigens, ultimately identifying m9 [42]. Although antibodies recently isolated from donors are more promising therapeutically than earlier bNABs [43,44<sup>■</sup>], library-based methods may be important to improve the new generation of antibodies, as they can introduce beneficial changes that might not be anticipated from inspection of antibody–antigen complex structures. However, the large number of HIV-1 strains, including the diversity of the viral swarm within a single infected individual, makes it difficult to select for antibodies that maintain breadth across viral



**FIGURE 1.** Location of bNAb epitopes on HIV-1 Env trimer. The approximate location of epitopes (shown only once per trimer) are highlighted on a surface representation of Env derived from electron microscopy, structure EMD-5782 [1<sup>■</sup>]: CD4-binding site Ab epitope (red), V1/V2 loop/Asn160 Ab epitope (green), V3 loop/Asn332 Ab epitope (blue), 8ANC195 epitope (yellow), 35O22 epitope (cyan), PGT151 epitope (pink), MPER epitope (gray). N-linked glycans shown as grey sticks were added to all potential N-linked glycosylation sites present in the coordinates for BG505 SOSIP Env (PDB 4NCO) [2<sup>■</sup>] using Glyprot [3]. Representative bNABs targeting each epitope are listed.

strains while increasing binding to one or a few Env specificities.

Of relevance to prospects for improving anti-HIV-1 antibodies is the fact that bNAbs isolated from HIV-1 infected individuals, especially the most potent, recently discovered bNAbs, often show high levels of somatic hypermutation [43,44<sup>■</sup>,45<sup>■</sup>]. Somatic mutations in HIV-1 bNAbs can include insertions/deletions in framework regions (FWRs) and the more variable CDRs. Taken together, the V<sub>H</sub> domain alone can include 40–100 nucleotide mutations [14,45<sup>■</sup>,46–51]. Many of the FWR mutations, even those not directly contacting antigen, appear to be important for bNAb function, as reverting mutated noninteracting FWR residues to germline residues resulted in reduced neutralization potency and breadth [45<sup>■</sup>]. Efforts to identify a minimal set of FWR mutations required for bNAbs VRC01 and 10E8 showed that it was possible to revert up to 78% of VRC01 and up to 89% of 10E8 FWR mutations to germline residues while maintaining much of the original potency and breadth [52<sup>■</sup>]. Interestingly, reverting 50% of the light chain FWR mutations to germline improved the potency of VRC01 [52<sup>■</sup>]. Thus, although FWR mutations can contribute antigen contacts in addition to stabilizing CDR conformations and allowing conformational flexibility [45<sup>■</sup>], not all FWR mutations are required for bNAb potency and breadth, and some may even be deleterious.

Some of the most potent antibodies against HIV-1 that are the focus of engineering efforts are VRC01-class antibodies, which target the CD4bs using a VH1-2\*02 derived heavy chain to mimic CD4 recognition of gp120 [8,15,16,46,53,54<sup>■</sup>]. A structure-based approach was taken to improve NIH45-46 [46], a more potent clonal variant of VRC01 [15]. Structural and mutagenesis studies demonstrated that a four-residue insertion within the NIH45-46 CDRH3 loop accounted for its increased potency [55]. Although VRC01-like bNAbs effectively mimic CD4 [48], both VRC01 and NIH45-46 fail to fill a hydrophobic pocket within gp120 to mimic the burying of a hydrophobic CD4 residue, Phe43<sub>CD4</sub>. Mutants were created by substituting the Phe43<sub>CD4</sub>-equivalent residue in NIH45-46, Gly54<sub>NIH45-46</sub>, and improved potencies were found for Trp, Phe, Tyr and His substitutions [55]. The most promising mutant, NIH45-46<sup>G54W</sup>, showed an overall 10-fold increase in neutralization potency and neutralized some NIH45-46 resistant strains [55]. Residue 54 is a Trp in some VRC01-related bNAbs that are less potent than VRC01 or NIH45-46, for example VRC03 [48], demonstrating that a Gly-to-Trp substitution at position 54 is a possible somatic mutation in this bNAb lineage, but that maximal increased potency from

this substitution requires other features of a VRC01-like bNAb, most likely the CDRH3 loop insertion.

A follow-up study [56<sup>■</sup>] used structure-based design to reduce available routes of HIV-1 escape from antibody pressure. Bioinformatic analysis identified gp120 sequence correlates for resistance to VRC01-class antibodies by noting that variations at highly conserved gp120 residues 279<sub>gp120</sub>–280<sub>gp120</sub> and 458<sub>gp120</sub>–459<sub>gp120</sub> (the 'NNGG' motif, named for the Asn and Gly residues at these positions) lead to resistance [53]; these predictions were experimentally verified *in vitro* [53] and in HIV-1 infected humanized mice [22]. To counteract the effects of gp120 escape mutations, rational design was used to create two new mutants: 45–46m2 (S28Y<sub>LC</sub>), which introduces contacts with an Asn276<sub>gp120</sub>-linked glycan, as seen in VRC01/gp120 complexes, but not NIH45–46/gp120 complexes [48,55], and 45–46m7 (W47V<sub>HC</sub>), which removes steric clashes with gp120 substitutions in the GG motif [56<sup>■</sup>]. 45–46m2 neutralized nearly 96% of viruses, an improvement over other CD4bs bNAbs, which typically neutralize nearly 90% of strains. *In vivo* experiments demonstrated that viral escapes were no longer found in the GG motif when HIV-1 infected animals were treated with 45–46m2/45–46m7, and a resulting A281T<sub>gp120</sub> escape variant had reduced viral fitness [56<sup>■</sup>]. These studies demonstrate that even highly somatically mutated bNAbs are not necessarily optimal as isolated from donors, and that even if it is impossible to completely prevent HIV-1 escape from bNAbs, it might be possible to drive the evolution of viruses with reduced fitness that could be more easily combated by host defense mechanisms.

A similar effort to improve a bNAb is represented by development of a more potent variant of a VRC01-class antibody that was isolated from the VRC01/NIH45–46 donor [54<sup>■</sup>]. First, 454 pyrosequencing of B cell antibody gene transcripts allowed identification of a heavy chain clonal variant that was closely related to NIH45–46 and contained the same four-residue CDRH3 insert. When combined with the VRC01 light chain, the new bNAb, VRC07, was about two-fold more potent than VRC01. Although not antibody engineering *per se*, this result illustrates that deeper searching of bNAb donor repertoires can identify more potent clonal relatives; other examples include variants of PGT121 [47,57,58], PGT141 [59] and 8ANC195 [26]. Next, all 20 amino acids were evaluated at VRC07 heavy chain position 54. Similar to results for NIH45–46 Gly54<sub>HC</sub> mutants [55], substitution of VRC07 Gly54 to larger residues, including Trp, Tyr, Phe and His, increased neutralization potency and breadth, but substitutions for large aromatic residues resulted in polyreactive recognition of non-HIV-1 antigens



[54<sup>\*\*\*</sup>]. A Gly54His substitution showed the least increase in polyreactivity while still increasing potency. The antibody light chain was also shortened to address observations that the V5 loop of gp120 interferes with the binding of VRC01-like antibodies [60]. On the basis of results from a screen of light chain truncations and modifications, a two-residue N-terminal truncation as well as a Val3Ser<sub>LC</sub> mutation was selected [54<sup>\*\*\*</sup>]. For *in vivo* experiments, mutations in the Fc region to improve plasma half-life through enhanced binding to FcRn (M428L/N434S, the 'LS' mutant [61]) were introduced. Additional framework mutation reversions and solubility-enhancing mutations were investigated, but the antibody selected after *in vivo* half-life determinations, VRC07-523-LS, did not contain these mutations. VRC07-523 and VRC07-523-LS were five- to eight-fold more potent than VRC01 and neutralized 96% of tested HIV-1 strains *in vitro*. Viral challenge experiments in macaques verified that the increased *in vitro* potency correlated with improved protection from infection *in vivo* [54<sup>\*\*\*</sup>]. Taken together, the NIH45-46 and VRC07 examples illustrate the potential to improve the activity of VRC01-class bNAbs by filling the hydrophobic Phe43<sub>CD4</sub> pocket on gp120.

In a different approach, features of clonally related antibodies with similar specificities can sometimes be combined into one antibody to increase breadth and/or potency. For example, a chimeric version of glycan-dependent bNAbs 10-1074 and PGT121 [14,57] was constructed by grafting five PGT121 heavy chain residues identified as contacting a complex-type N-linked glycan into the 10-1074 heavy chain [57]. The chimera (10-1074<sub>GM</sub>) exhibited improved potency and neutralized some viral strains resistant to wildtype 10-1074. In the case of PG9 and PG16, members of a family of bNAbs targeting the V1V2 loop and glycans at positions Asn160<sub>gp120</sub> and Asn176<sub>gp120</sub> [14], three PG16<sub>LC</sub> residues that contact a complex or hybrid-type N-glycan at Asn176<sub>gp120</sub> were grafted onto LC<sub>PG9</sub> to create PG9-PG16-RSH [62]. The resulting chimeric antibody had improved potency compared with PG9 [62]. In both examples, grafting residues contacting complex-type N-glycans onto a family member that favors a high-mannose N-glycan at the same Env position seemed to allow the antibody to accommodate different types of glycoforms. In another example, the neutralization potency of the MPER antibody 4E10 was improved nearly two-fold by engineering its CDRH3 to mimic lipid-binding properties observed for 10E8 [63].

### ENGINEERING ANTIBODIES CAN AFFECT POLYREACTIVITY AND IN VIVO HALF-LIFE

Antibodies raised against foreign antigens such as HIV-1 Env can be polyreactive or autoreactive, that

is they can bind to more than one antigen (polyreactivity) and/or to self proteins (autoreactivity) [64–66]. Although B cells with autoreactive and polyreactive B cell receptors are largely removed as they pass through tolerance checkpoints [67,68], a fraction of mature B cells remain polyreactive or autoreactive in healthy individuals [69]. Polyreactive antibodies produced by these cells may be generally useful for fighting bacterial infection and enhancing the phagocytosis of apoptotic cells [70,71]. Indeed, some bacterial and viral infections are associated with higher serum levels of autoreactive and polyreactive antibodies [65].

The presence of autoreactive/polyreactive antibodies has been associated with HIV-1 infection for more than 25 years [72–74]. Polyreactivity was first characterized in detail for the 4E10 and 2F5 MPER bNAbs [75]. These bNAbs were described as interacting with cardiolipin, a negatively charged mitochondrial phospholipid [75–80]. Further studies confirmed that binding of 2F5 and 4E10 to HIV-1 Env is enhanced by the presence of the host-derived viral membrane, which would include negatively charged phospholipids [81]. Recent studies have identified human proteins, kynureninase, type 1 inositol trisphosphate receptor and splicing factor 3b subunit 3 as potential self-antigens recognized by 2F5 and 4E10 [82,83]. Another MPER antibody, 10E8, does not exhibit polyreactivity in standard assays [84], yet binds tightly to a self-antigen, FAM84A (family with sequence similarity 84, member A) [85<sup>\*\*\*</sup>]. In a comprehensive study, a majority of more than 200 anti-HIV-1 Env antibodies isolated from HIV-1 infected donors exhibited binding to both self and nonself antigens, with anti-gp41 antibodies showing the most polyreactivity, particularly against lipid antigens [86,87]. In general, broadly neutralizing anti-HIV-1 antibodies exhibit a higher frequency of polyreactivity than nonneutralizing antibodies [85<sup>\*\*\*</sup>].

Polyreactivity may not necessarily be a problem for anti-HIV-1 antibodies, especially as it is a common property of donor-derived bNAbs; however, a correlation between increased polyreactivity and an increased rate of antibody clearance *in vivo* has been observed for IgGs [88]. IgG half-lives are normally extended beyond other serum proteins due to interactions with FcRn, the receptor that protects serum IgG from a default degradative pathway in vascular endothelial cells [89,90]. However, mutations known to increase the half-life of nonpolyreactive IgGs through enhanced interactions with FcRn, such as the LS mutation [61] that was introduced into VRC01 and VRC07–523 [54<sup>\*\*\*</sup>,91], do not generally improve the half-lives of polyreactive IgGs. This is likely because increased FcRn function cannot

compensate for IgG loss due to off-target binding, which absorbs polyreactive IgGs and leads to their rapid clearance [88].

In antibody engineering efforts, it has been observed that improving the breadth and potency of antibodies can lead to increased polyreactivity and/or reduced solubility and increased aggregation propensity [54<sup>\*\*\*</sup>,92–94]; in studies described above, many of the Gly54<sub>HC</sub> substitutions into NIH45–46 and VRC07 derivatives resulted in increased polyreactivity and decreased *in vivo* half-lives [54<sup>\*\*\*</sup>,94]. In addition, the more potent version of 10–1074, 10–1074<sub>GM</sub>, exhibited increased polyreactivity compared with its PGT121 and 10–1074 parents (H. Mouquet, L. Scharf, P.J. Bjorkman and M.C. Nussenzweig, unpublished data).

Strategies being used to improve properties such as poor solubility and aggregation profiles that could lead to polyreactivity include switching the IgG isotype, inserting N-linked glycosylation sites, and making targeted mutations to reduce surface-exposed hydrophobic residues [92–97]. For example, in the engineering of VRC07-523, variants such as VRC07-G54W not only showed increased breadth and potency, but also polyreactivity and poor pharmacokinetics [54<sup>\*\*\*</sup>]. Similar characteristics were observed for NIH45–46<sup>G54W</sup> [94]. In both cases, a histidine substitution (G54H) reduced polyreactivity [54<sup>\*\*\*</sup>,94]. Similarly, several mutations intended to eliminate clashes with the gp120 V5 loop resulted in VRC07 variants with increased polyreactivity, but mutations were identified that yielded a variant exhibiting a favorable pharmacokinetic profile [54<sup>\*\*\*</sup>]. In another study, a structure-based approach guided by cataloguing variations in solubility in clonal relatives of 10E8 was used to improve the solubility of 10E8 nearly 10-fold while maintaining comparable potency, an important step towards making this antibody useful clinically [98].

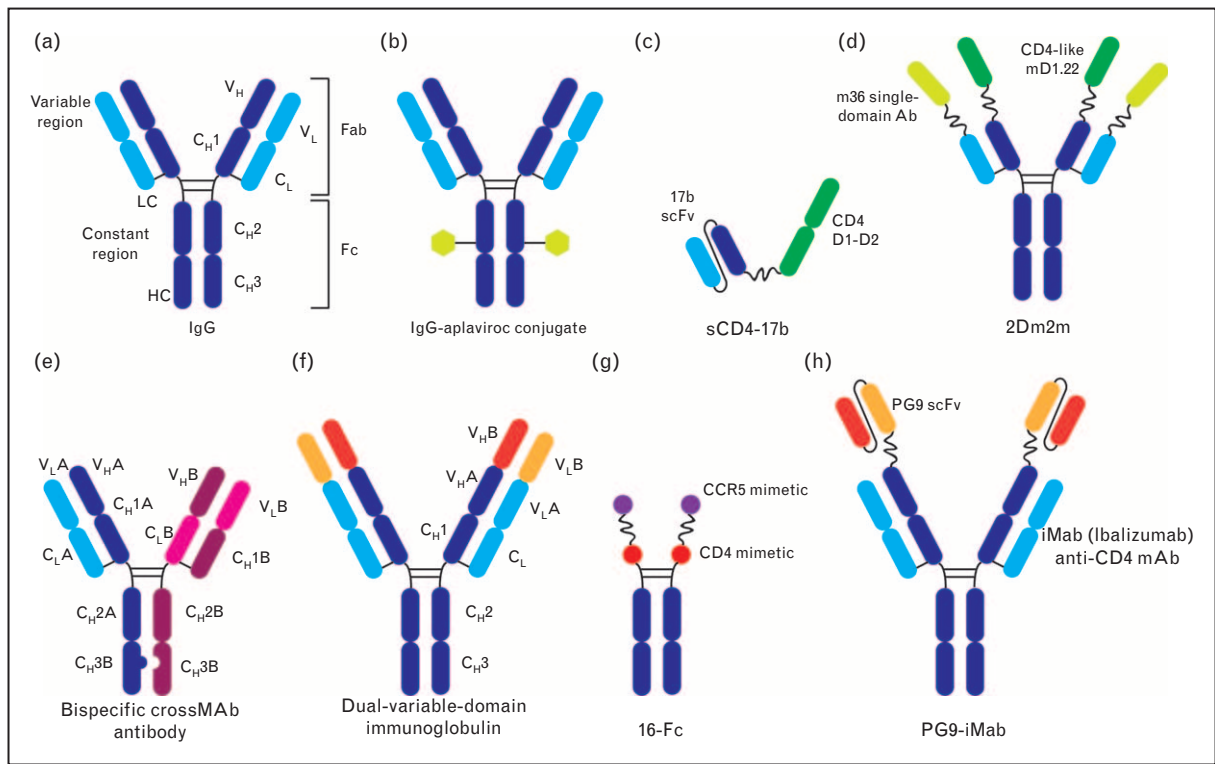
## BISPECIFIC REAGENTS

In addition to traditional IgG architectures (Fig. 2a), other scaffolds have been used to achieve bispecific recognition. Small molecules can be conjugated to IgGs to create bifunctional reagents (Fig. 2b); for example conjugating cholesterol to the light chains of the anti-MPER bNAbs 2F5 and D5 enhanced their potencies through cholesterol-mediated interactions with the viral membrane [99]. In another study, a small-molecule inhibitor of viral entry, aplaviroc, was conjugated to the Fc to enhance neutralization potency [100].

An early example of a bispecific anti-HIV-1 reagent fused the gp120-binding domains of CD4

to an scFv version of a CD4i antibody. CD4i antibodies recognize the conserved coreceptor-binding site on gp120 after it is exposed as a result of CD4 binding [101]. As IgGs, CD4i antibodies exhibit limited neutralization potencies because of steric constraints when gp120 is bound to CD4 on the target cell [7]. The broadly neutralizing reagent sCD4-17b was created by fusing CD4 to the variable regions of a CD4i antibody, 17b, thereby solving the steric problem because the epitope was exposed by CD4 binding when the virion was not bound to the target cell (Fig. 2c) [101]. The CD4-17b reagent was recently expressed in a chimeric antigen receptor (CAR) format on T cells to target and kill HIV-1-infected cells [102<sup>\*</sup>]. Later versions of CD4-CD4i reagents used an IgG architecture by N-terminally fusing CD4 to the Fabs of CD4i antibodies [103]. Despite containing only two CD4 moieties, the IgG-based CD4-CD4i reagents showed increased potencies compared with CD4-IgG2 (PRO542), a tetravalent CD4-Fc fusion protein [104]. A more recent reagent of this class combined a CD4 variant with m36, a CD4i single variable domain selected against different HIV-1 envelope proteins (Fig. 2d) [105<sup>\*</sup>,106].

Bispecific IgGs containing variable regions from two different IgGs have been produced using the crossMab platform in which heavy chain heterodimerization occurs using a ‘knob-into-hole’ Fc architecture and V<sub>H</sub> and V<sub>L</sub> domains are swapped in one half of the reagent to ensure proper heavy chain–light chain pairing [107] (Fig. 2e). Other architectures, such as dual variable domain constructs, attach a second Ig variable domain N-terminal to an IgG (Fig. 2f). A promising reagent of this class was created from bNAbs against gp120 and gp41 [108]. Another potent chimeric reagent was constructed as an Fc fusion with CD4- and coreceptor-mimetic peptides attached to the Fc, thus targeting both the receptor and coreceptor sites on HIV-1 Env (Fig. 2g) [109<sup>\*</sup>]. Bispecific reagents generally function by binding of one or the other Env-targeting component at a time. Simultaneous binding of both components of bispecific reagents to single Env trimers was recently shown to result in synergistic *in vitro* neutralization improvements of two to three orders of magnitude [13<sup>\*</sup>], suggesting a promising strategy for achieving potency, breadth and resistance to viral mutations. Synergistic neutralization resulting in extremely high *in vitro* potencies was also observed for reagents combining anti-Env and antihost specificities (see Ho and Markowitz, this issue). For example, attaching the scFv of PG9 or PG16 to the N-terminus of ibalizumab (anti-CD4) yielded synergistic reagents, PG9-iMab and PG16-iMab, of exceptional potency that neutralized 100%



**FIGURE 2.** Architectures of bispecific anti-HIV-1 reagents. (a) Domain nomenclature for IgG. (b) Conjugate of small molecule antiretroviral aplaviroc with an IgG. (c) Fusion of CD4 D1-D2 with CD4i scFv 17b. (d) Reagent 2Dm2m constructed from CD4-mimetic mD1.22 and m36 single domain antibody. (e) The CrossMab technology allows for specific heavy chain heterodimerization using ‘knob-into-hole’ mutations to combine heavy chains from two IgGs, A and B. Domain exchange of C<sub>H1</sub> with C<sub>L</sub> from the second IgG, labeled C<sub>H1B</sub> and C<sub>L</sub>B, ensure unique light chain pairing. (f) Dual-variable domain IgG that contains a second set of V<sub>H</sub>/V<sub>L</sub> domains. The two variable domains from a second antibody, labeled V<sub>H</sub>B and V<sub>L</sub>B, are added N-terminally to the variable domains of the first IgG, V<sub>H</sub>A and V<sub>L</sub>A. (g) 16-Fc, CD4 and CCR5 mimetic peptides are attached to an Fc. (h) PG9/PG16-iMab, containing scFvs attached to anti-CD4 antibody ibalizumab. C<sub>H1</sub>–3, the heavy chain constant domains; C<sub>L</sub>, light chain constant domain; V<sub>H</sub>, variable heavy chain domain; V<sub>L</sub>, variable light chain domain.

of a 118-virus panel, with the majority of IC<sub>50</sub>s less than 0.01 μg/ml (Fig. 2h) [110<sup>11</sup>]. Another broad and potent bispecific reagent, iMabm36, joined m36 with ibalizumab [111]. This reagent neutralized 96% of a 118-member multiclade pseudovirus panel at a concentration of 10 μg/ml.

**CONCLUSION**

Although an effective HIV-1 vaccine remains an elusive goal, newly discovered potent HIV-1 bNAbs and advances in engineering antibodies offer possibilities for improved passive delivery strategies to prevent or treat HIV-1. More potent bNAbs could be used therapeutically at a lower concentration and thus reduce cost and/or production time, increase the number of patients being treated and lower the potential for immunogenicity or other side effects related to bNAb administration. There also remains a need to create reagents to combat viral mutation and the natural sequence diversity of HIV-1, which

may be possible by identifying common pathways of viral escape and then using structure-based design to engineer resistance to these pathways. Library-based strategies designed to enhance affinity can also lead to increased breadth and block escape. Bispecific reagents and chemically conjugated reagents offer other ways to target multiple sites to prevent viral escape strategies and combat HIV-1 diversity. Although increased polyreactivity associated with some improved bNAbs may not necessarily be dangerous, polyreactivity correlates with shortened plasma half-life and represents an indicator that a reagent needs to be optimized to improve its biophysical and pharmacokinetic properties. Recent successes in creating bispecific, conjugate and engineered antibody reagents with minimal polyreactivity may be important therapeutically, particularly if gene therapy strategies, such as vector-mediated gene transfer (see Balazs and Baltimore, this issue) [112], are used to deliver antibodies and antibody-like reagents.



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## Conflicts of interest

A.P. West and P.J. Bjorkman have patent applications pending on improving CD4-binding site antibodies. The remaining authors have no conflicts of interest.

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- of special interest
- of outstanding interest

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