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Candidate Antibody-Based Therapeutics Against HIV-1

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Abstract

Antibody-based therapeutics have been successfully used for the treatment of various diseases and as research tools. Several well characterized, broadly neutralizing monoclonal antibodies (bnmAbs) targeting HIV-1 envelope glycoproteins or related host cell surface proteins show sterilizing protection of animals, but they are not effective when used for therapy of an established infection in humans. Recently, a number of novel bnmAbs, engineered antibody domains (eAds), and multifunctional fusion proteins have been reported which exhibit exceptionally potent and broad neutralizing activity against a wide range of HIV-1 isolates from diverse genetic subtypes. eAds could be more effective *in vivo* than conventional full-size antibodies generated by the human immune system. Because of their small size (12~15kD), they can better access sterically restricted epitopes and penetrate densely packed tissue where HIV-1 replicates than the larger full-size antibodies. HIV-1 possesses a number of mechanisms to escape neutralization by full-size antibodies but could be less likely to develop resistance to eAds. Here, we review the *in vitro* and *in vivo* antiviral efficacies of existing HIV-1 bnmAbs, summarize the development of eAds and multispecific fusion proteins as novel types of HIV-1 inhibitors, and discuss possible strategies to generate more potent antibody-based candidate therapeutics against HIV-1, including some that could be used to eradicate the virus.

1. Introduction

More than 60 million people have been infected by HIV-1 and more than 25 million people have died of HIV-1-related diseases. Currently, a variety of treatments are available. A highly active antiretroviral therapy (HAART) or ‘cocktail therapy’, that includes two nucleoside/nucleotide reverse-transcriptase inhibitors (NRTIs/NtRTIs) plus a non-nucleoside reverse-transcriptase inhibitor (NNRTI) or a protease inhibitor (PI) or another NRTI, is a highly efficient method in reducing the number of HIV-1 particles in the bloodstream to undetectable levels and providing clinical benefits.^[1–3] However, HAART can lead to serious adverse effects^[4,5] and the emergence of drug-resistant mutants.^[6,7] Several entry inhibitors were developed, including the HIV-1 entry inhibitor T20 (Enfuvirtide, Fuzeon) and the C-C chemokine receptor type 5 (CCR5) antagonist maraviroc (Selzentry®, or Celsentri® outside the US). Very recently, raltegravir (Isentress®), which represents a new class of integrase inhibitors, was approved by the US FDA for clinical use. Still, primary resistance has already been observed with the use of these new drugs.^[8]

A major problem for these therapeutics is that long-term use can lead to drug-related toxicities. In addition, there is a continuous need for new drugs to cope with potential drug resistance. Monoclonal antibodies (mAbs) are, in general, safer than small molecule drugs and have been successful in the prevention and/or treatment of various diseases including cancers, immune disorders, and infectious diseases. To date, more than 30 mAbs have been approved in the US and EU, and hundreds of mAbs have been evaluated in clinical trials in the past decade.^[9] However, none of the approved mAbs is for the treatment of HIV-1-infected patients. Palivizumab (Synagis®), which targets the respiratory syncytial virus (RSV), is the only mAb approved for clinical use against any infectious disease, and it is for prevention, not for therapy.

One of the difficulties in developing mAbs as HIV-1 therapeutics is the extreme variability of the virus and the rapid emergence of resistant mutants.^[10] This requires that antibodies exhibit sufficient level of breadth and potentially neutralize genetically diverse HIV-1 isolates. Despite the disappointing results from several broadly neutralizing mAbs (bnmAbs) tested in human clinical trials, the fact that passive immunization is protective as prophylaxis in animals and the recent identification of novel, more potent bnmAbs indicate some potential for antibody-based therapeutics against HIV-1.

2. Targets

HIV-1 entry is initiated by binding of its envelope glycoprotein (Env) gp120 to receptor CD4 on the target cell surface. The binding induces extensive conformational changes in gp120, resulting in the formation of the coreceptor-binding site and enabling binding of gp120 to a coreceptor, either CCR5 or C-X-C chemokine receptor type 4 (CXCR4). Substantial structural rearrangements then occur in the Env gp41 which eventually causes virus-cell fusion and injection of the viral genomic DNA into target cells.

2.1 HIV-1 Envelope Glycoproteins (Envs)

The Env is a typical type I viral envelope protein. It is synthesized as a precursor glycoprotein, gp160, which is proteolytically cleaved into a surface subunit, gp120 (for receptor and coreceptor binding), and a transmembrane subunit, gp41 (for virus-cell fusion). The two subunits exist as a trimeric state on the viral spike and interact with each other non-covalently (figure 1).

Gp120 is highly glycosylated and consists of five conserved domains, C1–C5, and five variable loops, V1–V5.^[13,14] The receptor CD4-binding site (CD4bs) on gp120 is a conserved pocket-like structure flanked by some of the loops (figure 2). Although it is recessed to a certain extent from antibody access, broad and potent HIV-1 neutralization by sera from some HIV-1-infected patients is mediated to a significant extent by CD4bs antibodies. Another highly conserved structure on gp120, the coreceptor binding site (CoRbs), is exposed and/or formed after virus attachment to host cells via CD4 binding and, therefore, antibodies targeting the CoRbs are called CD4-induced (CD4i) antibodies (figure 2). This structure is also highly immunogenic. However, because the CoRbs is oriented closely toward the cellular membrane, which leaves a very small space in between, full-size CD4i antibodies generated by the immune system are generally less potent and broad

compared with the CD4bs antibodies. Among the loop structures, V3 (figure 2) is particularly functionally important. First, it recognizes a coreceptor.^[17] Second, it may determine the overall sensitivity of the virus to neutralization through interaction with other elements on the viral spike.^[18,19] It is highly immunogenic and, therefore, represents one of the major targets of neutralizing responses elicited by infection or immunization with HIV-1 Envs.^[20,21] However, the antibodies targeting V3 might exhibit limited breadth of neutralization or may be isolate-specific due to high variability of the loop sequences and structures. Although non-immunogenic, the ‘glycan shield’ on gp120 can, unusually, elicit immune responses in a small number of HIV-1-infected subjects (figure 2), which potentially and broadly neutralize primary and T-cell line-adapted HIV-1 isolates and inhibit syncytium formation in cell lines.^[22,23] It has been found that gp120 in trimeric states adopts some quaternary conformations that are primarily located in the conserved regions of the V2 and V3 loops of gp120.^[24,25] MAbs isolated against these epitopes exhibit a high level of neutralizing activities and breadth comparable to or higher than those of the bnmAbs to monomeric gp120. This finding suggests additional conserved regions on the native Env trimer may exist and remain to be defined. Antibodies to these regions could better affect virus entry, have more potential as therapeutics, and are highly valuable for vaccine immunogen design.

Gp41 (figure 1) is directly involved in viral-cell membrane fusion and, therefore, is an important target for antibody intervention. It is composed of a fusion peptide (FP) in the N terminus followed by the polar region, the N-terminal heptad repeat (NHR), the C-terminal heptad repeat (CHR), the immunodominant region and the membrane proximal external region (MPER).^[26,27] The FP is highly hydrophobic and is hidden before gp120 binds to CD4.^[28] Previous studies showed that an FP variant, in which the highly conserved residues are replaced by their D-enantiomers, could inhibit HIV-1-mediated cell-cell fusion.^[29] Moreover, a synthetic peptide targeting the FP is capable of inhibiting a wide variety of HIV-1 strains.^[30] After exposure of gp41, the NHR and the CHR can interact with each other and form a six-stranded α -helical bundle consisting of an inner triple stranded coiled-coil buttressed by three C-helices, which is the fusion core structure of gp41 and crucial for membrane fusion^[31,32] (figure 1). These two structures are ‘hot regions’ because peptides from the CHR (e.g. C34^[33,34]) could inhibit the viral entry efficiently through interaction with the NHR. Between the CHR and the transmembrane domain is the MPER, a conserved tryptophan-rich region containing about 20 residues (figure 2). Mutational and biophysical analysis has provided compelling evidence to support the involvement of the MPER in HIV-1 fusion.^[35,36] Therefore, antibodies against these regions could have the potential of inhibiting the entry of HIV-1 into host cells.

2.2 Receptor Molecules

In contrast to the Env, host receptor molecules, which are important for viral entry, are conserved. The HIV-1 primary receptor CD4 is a type I integral membrane glycoprotein (58 kDa) that is expressed mainly on the surface of thymocytes, T-helper lymphocytes, and cells of the macrophage/monocyte lineage.^[37–39] CD4 is required for shaping the T-cell repertoire during thymic development, is necessary for appropriate activation of mature T cells,^[40] and helps B cells induce antibody responses.^[41] CD4 consists of an extracellular region of 370

amino acid residues organized in four immunoglobulin-like domains (D1–D4).^[42,43] The crystal structure of D1–D4 (Protein Data Bank [pdb]: 1WIP^[11,12]) was rendered by PyMOL (The PyMOL Molecular Graphics System, Version 1.2, Schrödinger, LLC) (figure 1). The first domain, D1, directly binds to gp120.^[44,45] It has been found that CD4 also contains a binding site for protein disulfide isomerase (PDI) and forms a PDI-CD4-gp120 complex, causing major conformational changes in gp120 to activate the fusogenic potential of HIV-1 Env.^[46–54] Gp120-CD4 interaction induces conformational changes not only in gp120 but also in CD4. Therefore, interference with CD4 conformational changes by antibodies interacting with native CD4 but not with gp120-complexed CD4 represents a novel neutralization principle in addition to directly blocking the gp120-binding surface.

HIV-1 coreceptors (CoRs) [figure 1], CCR5^[55–60] and CXCR4,^[61] are also required for viral entry and some of the HIV-1 isolates can infect coreceptor-expressing cells in a CD4-independent manner, which makes coreceptors an attractive target for drug development.^[62–65] In addition, unlike CD4, CCR5 may not be functionally important for host cells. A minority of people (about 1/1000 Northern Europeans) are born with CCR5 deletion mutation, which endows them with inherent resistance to HIV-1 infection.^[66,67] Therefore, blockade and depletion of the coreceptor may not result in adverse effects such as immunosuppression caused by some of the CD4 antibodies. The receptor and coreceptor are self-antigens and do not elicit antibody responses in humans. However, antibodies against these molecules can be produced by immunization of animals followed by humanization to reduce immunogenicity. Such antibodies can also be selected from libraries where heavy and light chains of naturally occurring human antibodies are randomly assembled.

3. Full-Size Monoclonal Antibodies

3.1 Targeting Envs

The mAb b12 (see table I for some representatives of anti-HIV-1 antibodies and antibody-based proteins) is the first reported representative of the bnmAbs that targets the CD4bs on gp120 as a competitive inhibitor of CD4 binding. It was selected from a phage-displayed antibody library constructed from the bone marrow of an HIV-1-infected donor.^[68,69] B12 efficiently neutralizes a wide range of HIV-1 isolates from different clades *in vitro*^[70,71] and can protect macaques against vaginal challenge with a simian-human immunodeficiency virus (SHIV) that uses CCR5 as a coreceptor.^[72–74] It was also found that b12 could mediate strong antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) of HIV-1-infected cells *in vitro*.^[75] However, ADCC is probably the major effector function for protection against HIV-1 infection *in vivo*.^[76] The four other mAbs: F105,^[77] b13,^[78] m14,^[79] and m18,^[80] which also interact with the CD4bs, have only modest neutralizing activities. Structure analysis reveals that slight differences in recognition result in substantial differences in F105 and b13-bound conformations relative to b12-bound gp120.^[81,82] Modeling and binding experiments showed these conformations are poorly compatible with the viral spike. These findings indicate that the accuracy of CD4bs recognition is crucial for neutralization of HIV-1. About 20 years after the discovery of b12, two mAbs targeting the CD4bs, VRC01 and VRC02, were recently identified by isolation of single mAb-producing B cells against an antigenically resurfaced HIV-1 gp120 specific for

the structurally conserved site of initial CD4 receptor binding.^[83] Notably, VRC01 and VRC02 can neutralize over 90% of circulating HIV-1 isolates with high potency *in vitro*. Although VRC01 partially mimics the binding of CD4 to gp120, a shift from the CD4-defined orientation focuses VRC01 onto the vulnerable site of initial CD4 attachment, allowing it to overcome the glycan and conformational masking that diminishes the neutralization potency of most CD4bs antibodies.^[84] Interestingly, a single substitution in VRC01 heavy-chain complementarity determining region 2 (CDR2) could increase contact with the gp120 bridging sheet and improve breadth and potency, critical properties for potential clinical use.^[85] The characterization of 576 new HIV-1 antibodies from four unrelated individuals showed these new antibodies shared a consensus sequence of 68 immunoglobulin heavy-chain amino acids and arose independently from two related heavy-chain genes.^[86] The crystal structure of one of these antibodies and VRC01 shared conserved contacts to the HIV-1 Env.^[86]

17b and 48d are human mAbs isolated from different HIV-1-infected individuals.^[87] They bind to CD4i epitopes that overlap with the CoRbs.^[88] The crystal structure of gp120 core/two-domain soluble CD4 (sCD4)/antigen-binding fragment (Fab) 17b shows a cavity-laden CD4-gp120 interface and a conserved binding site for the coreceptor, and provides information for conformational changes of gp120 upon CD4 binding, the nature of CD4i epitopes, and possible mechanisms of immune evasion (figure 2).^[15,89] CD4i antibodies 412d and E51 are obtained from an HIV-1-infected individual by selection of the potent enzyme-linked immunosorbent assay (ELISA) response of his serum to the HIV-1 gp120^[90] that functionally emulates CCR5. The tyrosine sulfation on 412d and E51 can contribute to the potency and diversity of the human humoral response.^[91,92] Although the conformations of tyrosine-sulfated regions of CCR5 (α -helix) and 412d (extended-loop) are dramatically different, a critical sulfotyrosine on CCR5 and on 412d induces similar structural rearrangements in gp120.^[93] A sulfated peptide derived from the heavy-chain complementarity determining region 3 (CDR3) of E51 can efficiently bind gp120 in the absence of CD4 and neutralizes HIV-1 more effectively than peptides based on the CCR5 amino terminus.^[94] X5 is a CD4i antibody selected from a phage-displayed library from a seropositive donor with a relatively high broadly neutralizing serum antibody titer, which neutralizes a number of virus isolates as Fab but has more limited and weaker neutralizing activity as IgG1.^[95,96] These results provide proof-of-concept that the receptor-inducible gp120 epitopes elicit potent broadly cross-reactive neutralizing antibody responses in HIV-1-infected patients and could be potentially useful for development of vaccines and inhibitors. They also show that the size of the CD4i antibodies is inversely correlated with their ability to neutralize viruses, suggesting that further decreasing the antibody molecular size may be beneficial.

C β 1, a humanized IgG chimeric mAb^[97] originating from a murine IgG 0.5 β ,^[98] is the first characterized mAb targeting V3 of gp120. In an animal study, it protected chimpanzee from HIV-1 infection during a 52-week observation period, providing direct evidence that anti-V3 loop antibodies can prevent an HIV-1 infection in the absence of other virus-specific immune responses.^[97] After the discovery of C β 1, other anti-V3 antibodies were also found. hNM01 is a humanized monoclonal antibody based on murine mAb mNM01 that neutralizes the virus by the activation of complement and the subsequent disruption of the viral

envelope after binding to V3.^[99,100] The results of a phase I clinical trial were somewhat encouraging but further evaluation with higher doses in larger numbers of patients is required.^[101,102] KD-247,^[103,104] a newer anti-V3 antibody, is currently being evaluated in a phase I clinical trial now.^[105] Although the ability of anti-V3 mAbs to neutralize a significant proportion of HIV-1 isolates from different subtypes (clades) has remained controversial due to its high variability, more anti-V3 mAbs have been identified which display cross-clade neutralizing activity.^[106,107] The evaluation in an animal study reveals that a significant proportion of viruses can be neutralized by the newly identified anti-V3 mAbs such as HGN194.^[108] These results further confirm that the anti-V3 mAbs can prevent infection from different clades, implying that V3 is still a useful target.

2G12, a carbohydrate-targeted bnmAb, was selected from 33 hybridomas producing human mAbs against HIV-1 which were established by cell fusion or EBV transformation.^[23,109–112] Unlike the conventional antibodies, 2G12 adopts a multivalent binding surface by using a unique domain-exchanged structure, in which the heavy-chain variable domains (VHs) of two antibody molecules are swapped. This extraordinary configuration provides two conventional antigen-combining sites and a third potential noncanonical binding site at the novel VH/VH interface for multivalent interactions with a conserved cluster of oligomannose type sugars on the surface of gp120.^[113,114] 2G12 alone and in combination with other antibodies (antibody cocktail) exhibits neutralizing activity in animal experiments^[115–117] and clinical trials.^[118–120] Escape mutant analysis showed that the activity of 2G12 was crucial for the *in vivo* effect of the neutralizing antibody cocktail.^[121] Interestingly, 2G12 could form dimers that are more potent than monomeric 2G12 in neutralization of various strains of HIV-1 *in vitro*.^[122] The 2G12 dimer could elicit ADCC at a lower concentration compared with monomeric 2G12.^[123] Dimeric 2G12, therefore, could be a potent prophylactic reagent against HIV-1 *in vivo* and be used as a part of an antibody cocktail to prevent HIV-1 infection.^[124] Recently, a panel of new bnmAbs have been identified that are almost 10-fold more potent than VRC01, PG9, and PG16 (see below for PG9 and PG16), and 100-fold more potent than the conventional mAbs.^[125] The crystal structure of one of the antibodies, PGT 128, complexed with a fully glycosylated gp120 outer domain at 3.25 Å reveals that the antibody penetrates the glycan shield and recognizes two conserved glycans as well as a short β -strand segment of the V3 loop, accounting for its high binding affinity and broad specificity.^[126] However, their neutralizing breadth is relatively narrower than that of VRC01 when compared at concentrations that produce 50% inhibition (IC₅₀s) of between 1–50 μ g/mL. These data raise a general concern that further increasing antibody potency against certain isolates may result in a substantial decrease in neutralization breadth due to the high variability of the virus. It is therefore likely that an ideal potency and breadth could never be reached by using a single bnmAb.

PG9 and PG16 are two bnmAbs targeting conserved regions of the variable loops of the gp120 subunit preferentially expressed on trimeric Envs. They were selected from a high-throughput neutralization screen of antibody-containing culture supernatants of about 30 000 activated memory B cells from a clade A-infected African donor.^[127] The epitopes defined by PG9 and PG16 are associated with the conserved regions of V1, V2, and V3 loops. They overlap with the CD4bs and possibly with the CoRbs, are sensitive to a loss of the glycan at N332 and an I165A substitution in gp120, but are distinct from that recognized by 2G12.

[128,129] The crystal structure of V1/V2 in complex with PG9 has been solved, which is helpful for identification of conserved features that enable recognition of gp120.^[130] Very recently, plasma bnmAbs specific for epitopes that include carbohydrates and are critically dependent on the glycan at position 332 of Env gp120 were found in CCR5-tropic SHIV-infected macaque.^[131] These quaternary structure-specific antibodies appear to target antigenic variants of the same epitope, with neutralization breadth determined by the prevalence of recognized variants among circulating isolates, which provides important and novel information that will assist in improving the engineering of Env-based immunogens.^[132–134]

As described above, the formation of the fusion core in gp41 is one of the key steps in viral entry. The gp41 NHR structure is relatively conserved and has been successfully targeted by small molecule inhibitors. A mAb targeting the hydrophobic pocket situated in the NHR groove, designated D5, was identified that inhibited the infection of diverse HIV-1 clinical isolates, although relatively weakly.^[135] m44, a mAb presumably targeting the CHR of gp41, can neutralize most of the 22 HIV-1 primary isolates from different clades as tested in assays based on infection of peripheral blood mononuclear cells (PBMCs) by replication-competent viruses.^[136] Recently, a panel of anti-gp41 antibodies were also isolated from memory B cells of HIV-1-infected patients, which targeted different epitopes in gp41 and could neutralize tier-2 viruses.^[137] These findings indicate that the conserved conformational epitopes on gp41 could be useful in the design of vaccine immunogens and as a target for therapeutics.

The identification of three bnmAbs targeting the MPER of gp41 (2F5,^[138] Z13, and 4E10^[139]) has implicated this region as a highly promising vaccine target and has, therefore, spurred interest in its structural characterization.^[140–146] In general, there are two distinct models of the binding patterns and disruption of HIV-1 MPER fusogenic functions by these antibodies. 2F5, like 4E10, induces large conformational changes in the MPER relative to the membrane. The difference is that 4E10 straddles the hinge and extracts residues W672 and F673 while 2F5 lifts up N-terminal residues to the hinge region by exposing L669 and W670 in MPER. In contrast, Z13e1,^[147] a matured version of Z13, does not cause significant change in membrane orientation or conformation, but rather immobilizes the MPER hinge through extensive rigidifying surface contacts.^[144] The difference in binding mechanism also leads to a difference in neutralization activities.^[139] The animal studies showed that a triple combination of 2G12/2F5/4E10 could protect neonatal rhesus macaques from infection and no virus was detected in the plasma, PBMCs, or lymph nodes for more than 1 year.^[148] Therefore, the same combination was also chosen for human clinical trials. In a phase I evaluation, seven individuals showed no drug-related adverse effects.^[120,149] During the phase II clinical trial, chronically and acutely infected individuals were recruited.^[121] These subjects were on antiretroviral treatment (ART) before antibody administration. After administration of these antibodies, the viral rebound was delayed in four of six acutely infected patients whereas only two of eight chronically infected patients showed a profound delay of rebound, suggesting that these bnmAbs could be more effective in early infection. Further evaluation of these three antibodies in a passive immunization trial indicates that the *in vivo* activities are likely due to direct neutralization or Fc receptor-mediated mechanisms, such as phagocytosis and ADCC, or both, but not due to CDC,^[150] which is consistent with

the previous study.^[76] Recently, a new MPER-targeted mAb, m66.6, was reported that binds to essentially the same epitope as 2F5.^[151] Interestingly, both the heavy and light chains of m66.6 are from different germ lines and less mutated than 2F5, suggesting that neutralizing responses against the 2F5/m66.6 epitope can be elicited through different maturation pathways.

3.2 Receptor Molecules

Host receptor molecules are conserved and, therefore, antibodies targeting them could be more efficient than those against the Envs to overcome the high mutation frequency used by HIV-1 to escape from the human immune system surveillance.

A humanized mouse mAb, TNX-355 (ibalizumab), was developed against the D2 domain of human and rhesus CD4. TNX-355 in an IgG4 format inhibited HIV and simian immunodeficiency virus (SIV) replication *in vitro* and was safely administered to rhesus monkeys without depleting CD4⁺ T cells.^[152–156] A phase I human clinical trial with TNX-355 demonstrated that the antibody was well tolerated. A single dose of TNX-355 conferred a significant decrease in viral load and increase in CD4⁺ T cell counts in HIV-1-infected subjects.^[157,158] TNX-355 is currently being developed by TaiMed Biologics, through licensing with Genetech, Inc, and a phase II trial is ongoing.^[159]

The HIV-1 coreceptors, CCR5 or CXCR4, are also attractive targets for development of bnmAbs. A humanized mouse anti-CCR5 mAb, PRO 140, and its murine progenitor, PA14, exhibits potent and broad-spectrum inhibition of primary HIV-1 R5 isolates from different clades but does not affect CCR5 activity.^[160,161] A phase I human clinical trial showed that PRO 140 given intravenously was generally well tolerated and exhibited potent, rapid, prolonged and dose-dependent antiviral activity in the subjects with early-stage HIV-1 infection.^[162,163] Two phase II studies of the safety and pharmacokinetics of PRO 140 given intravenously and subcutaneously performed by Progenics Pharmaceuticals, Inc. are ongoing.^[164,165]

4. Engineered Antibody Domains (eAds)

HIV-1 uses strategies to escape the neutralizing immune responses, one of which is steric occlusion of their highly conserved functionally important structures on Envs. We therefore have hypothesized that antibody fragments of smaller size, especially engineered antibody domains (eAds) [11–15 kDa], could be more effective than full-size antibodies.^[166] Not only is the overall size of eAds much smaller than that of full-size antibodies but also their paratopes are concentrated over a smaller area so that eAds provide the capability of interacting with novel epitopes that are inaccessible to conventional antibodies or antibody fragments with paired light-chain variable domains (VLs) and VHs. eAds could also be more resistant to HIV-1 escape than full-size antibodies because they may bind to the epitopes which are more conserved as required for the maintenance of substantial binding to receptor or coreceptor. Moreover, eAds may have better binding kinetics (e.g. increased association rate) while recognizing and anchoring the physically obstructed epitopes on HIV-1 Envs.

In the following sections, we discuss the progress of the development of two types of eAds against HIV-1 including antibody variable domain-based (V domain-based) and antibody constant domain-based (C domain-based) eAds.

4.1 V Domain-Based eAds

About twenty years ago, an isolated mouse single VH was shown to maintain the binding activity to lysozymes, indicating that such domains are functional.^[167] Later, it was found that a unique kind of antibody composed only of heavy chains is naturally formed in camelids (camels and llamas) [designated HCABs] and cartilaginous fishes (wobbegong and nurse sharks) [designated Ig-NAR],^[168–170] suggesting their variable regions (referred to as V_HHs in camelids and V-NAR in cartilaginous fish) could also recognize antigens as single domain fragments.^[171] Therefore, a direction is the development of binders based on VHs or VLs from full-size antibodies. We term them ‘variable domain-based engineered antibody domains (V domain-based eAds)’.

m36 is the first reported V domain-based eAd based on a human antibody VH (~15 kDa) that targets a sterically restricted CD4i epitope and potently neutralizes genetically diverse HIV-1 isolates *in vitro*.^[166] The structure of m36 was modeled by SWISS-MODEL^[172–174] and rendered by PyMOL (figure 3). In a humanized non-obese diabetic (NOD)/severe combined immunodeficiency (SCID)/ γ_c^{null} mouse model,^[176] m36.4, an affinity-matured version of m36, provided sterilizing protection in four of six animals against intrasplenic challenge with high-titer HIV-1 (>1000 TCID₅₀s [50% median tissue culture infectious dose]) while extensive infection was detected in all four control animals.^[177]

Recently, several V_HHs selected from libraries constructed from immunized camelids by HIV-1 Envs have been characterized as potent HIV-1 inhibitors by targeting the CD4bs or CXCR4.^[178–181] In addition, a novel eAd was identified from a llama immunized with a recombinant form of Nef, an HIV-1 nonstructural protein found in the cytoplasm of infected cells and in association with cellular membranes.^[182] It binds to Nef with a high affinity and can inhibit critical biologic activities of Nef both *in vitro* and *in vivo*. This anti-Nef eAd may represent an efficient tool to elucidate the molecular functions of Nef in the virus life cycle and could help to develop new strategies for the control of AIDS.

4.2 C Domain-Based eAds

The second domain of the IgG, IgA, and IgD heavy chain constant regions, CH₂, is unique in that it exhibits very weak carbohydrate-mediated interchain protein-protein interactions in contrast to the extensive interchain interactions that occur between the other domains. The expression of murine CH₂ in bacteria, which do not support glycosylation, results in a monomeric protein.^[183] It has been hypothesized that isolated CH₂ (from IgG, IgA, and IgD) and CH₃ (equivalent to CH₂ in other antibody isotypes including IgE and IgM) could be used as a promising scaffold for eAd library construction due to their solubility and stability.^[184] Moreover, they could offer additional favorable properties compared with V domain-based scaffolds because they contain binding sites or portions of binding sites for Fc receptors such as neonatal Fc receptor (FcRn) and Fc gamma receptors (Fc γ R), and complement C1q,^[185–190] which extend the half-life and mediate stability and effector

functions *in vivo*. In line with this hypothesis is the finding that the half-life of human CH2 (about 70 hours) in rabbits is much longer than that of human CH3 and Fab (about 15 hours), and that human CH2 might activate the complement cascade.^[191,192] We term the binders based on CH2 scaffold ‘constant domain-based engineered antibody domains (C domain-based eAds)’.

A previous study reported the construction of a large (5×10^{10} members) phage-displayed antibody library by using the human CH2 as a scaffold and mutating all residues in its two loops (BC and FG) to four residues (Y, A, D, or S). The library was panned with a gp120-sCD4 complex, and several binders against HIV-1 were selected.^[193] The highest-affinity binder, m1a1, specifically recognizes a highly conserved CD4i epitope overlapping with that of m36 and can neutralize seven of nine HIV-1 isolates from different clades.^[193] The structure of m1a1 was also modeled by SWISS-MODEL and rendered by PyMOL (figure 3).

However, the native CH2 domain has significantly lower thermal stability compared with other small scaffolds such as the 10th type III domain of human fibronectin (FN3),^[183,194,195] and has a high probability of instability when engineered for binding to antigens and enhanced effector functions. We have recently generated a shortened human CH2 variant, m01s, by introducing an additional disulfide bond between CH2 strands A and G, and removing seven residues from the N-terminus, which showed not only increased stability but also enhanced binding to human FcRn.^[196] A library was constructed by using m01s as a scaffold, from which a binder was identified that bound to HIV-1 MPER and could neutralize a panel of HIV-1 isolates (Gong et al., unpublished data). Because m01s binds to FcRn in a pH-dependent manner,^[196] binders selected from this library might have a longer half-life *in vivo* compared with antibody variable domain-based binders.

Although some success in development of eAds as candidate therapeutics has already been achieved, there are still some issues that need to be addressed before eAds can be suitable for *in vivo* use. The camelid V_HH and shark V-NAR domains are in general soluble and are stable *in vitro*.^[197] However, for *in vivo* administration, humanization (or deimmunization) may be crucial to reduce immunogenicity. Compared with V_HHs and V-NARs, other V_H domains such as human V_H domains may be relatively unstable and tend to aggregate, and their unfolding is normally irreversible because of the lack of V_L partners. Therefore, stabilization is required to make these domains aggregation-resistant and unfolding-reversible.^[198–200] It has been found that V domain-based antibodies had significantly different reaction kinetics compared with the fragments consisting of paired heavy and light chain domains, which likely leads to lower affinity to their targets.^[201] Accordingly, further maturation may be needed to achieve high affinity. In addition, the half-lives of V domain-based eAds in circulation are short and they do not have biological effector functions, as has been described for other antibody fragments, including Fabs and single-chain variable fragments (scFvs). Some methods,^[202] including introduction of serum albumin binding peptide,^[203,204] conjugation of polyethylene glycol (PEG),^[205] and fusion with anti-serum albumin V domain-based eAd,^[206] were developed to extend the half-life, but potential risks might exist. The C domain-based eAds need to be further characterized for their biophysical and biological properties.

5. Increasing Potency

HIV-1 uses multiple strategies to escape from the host immune system surveillance. These include rapid generation of viral variants, high variability of Envs, steric occlusion of conserved neutralizing epitopes on Envs that limits access of bnmAbs, mimicry of human self-antigens by conserved neutralizing epitopes on Envs that results in polyspecificity and autoreactivity of broadly neutralizing antibodies, and encoding of immunogenic conserved non-neutralizing or infection-enhancement epitopes on Envs. Although a number of bnmAbs have been identified, some of which show potent and broad neutralizing activity against HIV-1 *in vitro* and in animal models, we are still far away from the use of these antibodies in the treatment of HIV-infected patients. Improvement of the efficacy of the existing bnmAbs and identification of novel more potent antibodies are still urgent tasks.

5.1 Design of Antigens and Novel Targets

Some (10–25%) HIV-1-infected individuals develop broadly neutralizing sera, from which bnmAbs have been successfully isolated by using recombinantly expressed Envs. However, the structural differences between the isolated Env and the native Env on the viral spike may result in an unpredictable loss of antibodies that preferentially recognize the native Env structures when the isolated Env is used. The design of soluble versions of trimeric Env that display structural and functional properties similar to those observed on intact viruses is highly desirable.^[207] Therefore, efforts have been made to preserve or re-construct functional trimeric Env, and immunization with this resulted in improved elicitation of cross-reactive neutralizing antibodies against primary isolates. These trimeric Env could be useful for selection of novel potent neutralizing antibodies. HIV-1 entry requires extensive conformational changes in both gp120 and gp41, leading to the formation of highly conserved structures. These structures, however, are either sterically hidden or only transiently exposed during entry. Therefore, stabilized fusion intermediates could be valuable antigens for antibody selection. Previous studies have demonstrated efficient isolation of X5 and m36 against the CD4i epitopes on gp120 by using gp120-sCD4 complexes. Immediately prior to fusion, gp41 adopts a prehairpin intermediate state in which the N- (N-trimer) and C-terminal (C-trimer) trimers of gp41 are exposed. Neutralization of HIV-1 by IgGs 2F5 and 4E10 could be potentiated by the addition of a peptide that holds gp41 in this state. Both the N- and C-trimers have been successfully targeted by peptide inhibitors, suggesting that they could also be targeted by antibodies. A recent study opened a new avenue for design of antigens for selection of antibodies against specific epitopes of interest.^[83] HIV-1 Env is a large protein recognized by several types of antibodies including isolate-specific non-neutralizing antibodies, some of which compete with cross-reactive neutralizing antibodies in binding to Envs. Therefore, the use of original Env for selection may not be efficient. Based on this concept, a modified gp120, RSC3, was designed that specifically reacts with antibodies directed against the CD4bs. The use of RSC3 led to the discovery of exceptionally potent and broad antibodies, VRC01 and VRC02.^[83,84] This approach can be extended to select novel bnmAbs against other highly conserved, functionally important epitopes. In addition, further characterization of core epitopes on Env that is conserved and indispensable for viral infectivity could also be helpful for design of antigens.^[208]

Besides the widely used antigens (i.e. Env, receptor and coreceptor) for HIV-1 bnmAb selection, other new interesting targets have been or are being identified with the increase in our knowledge of HIV-1. As mentioned above, inhibition of the activity of PDI on the surface of CD4⁺ cells can prevent the activation of gp41 and HIV-1 entry into target cells. PDI catalyzes the formation of a disulfide bond between the cysteines in gp120 after formation of PDI-CD4-gp120 complex. Therefore, PDI and PDI-CD4-gp120 complex are potentially useful targets for antibodies that could block the enzymatic activity of PDI or its interactions with CD4-gp120 or the subsequent fusion process. Such antibodies have been reported that could potentially inhibit HIV-1 infection.^[47,50,53,209]

5.2 Libraries

Highly diverse antibody libraries have become important sources for selection of antibodies with high affinity and novel properties. Combinatorial strategies provide efficient ways of creating antibody libraries containing a large number of individual clones. These strategies include the reassembly of naturally occurring genes encoding the heavy and light chains from either immune or nonimmune B cell sources, and introduction of synthetic diversity to either the framework regions or the CDRs of the variable domains of antibodies. Phage display and other display technologies (e.g. yeast display and ribosome display) have already been used to construct large antibody libraries (up to 10¹¹ or higher), from which various useful antibodies could be selected through several rounds of biopanning and screening. Compared with the traditional method based on hybridoma, these library methodologies are much more efficient. However, natural antibody repertoires are subject to change; those harvested at a specific time point for library construction cannot cover all theoretical genetic diversity. Moreover, antibodies from such libraries may not represent the native antibodies because the heavy and light chains are randomly combined. Although the preservation of cognate pairing between heavy and light chains may not be critical for therapeutic antibodies, it is relevant to studying antibody maturation pathways. These leave scope for antibody libraries based on the conventional designs to be improved.

The advent of the high-throughput next-generation 454 sequencing technology has allowed direct observation of millions of diverse antibody variants that are generated by the immune systems at different time points and have survived positive, negative, and antigen-driven selection. Recently, large numbers of antibodies from humans have been sequenced and analyzed using this method.^[210–216] Based on a collection of antibody sequences from the literature, large libraries could therefore be designed and directly synthesized through a novel gene synthesis technology that enables not only precise determination of diversity but also positional control of amino acid composition and incorporation frequencies.^[217] The obtained library could reach a diversity beyond that of the donor-derived natural repertoire due to recombination.

The single-cell PCR methodology allows for construction of libraries with original pairs of VH and VL. This methodology is particularly useful in exploration of the natural human antibody responses. Although progress has been made,^[218] some technical difficulties remain to be solved.

5.3 Screening Methods

The selection of a large number of anti-HIV-1 bnmAbs from HIV-1 envelope-binding memory B cells from HIV-1 infected patients which show different types of broad activity exhibited by b12, 2F5, 4E10, or 2G12 indicates the possibility of finding novel bnmAbs by direct B-cell screening.^[219] The successful identification of PG9 and PG16 by functional high-throughput screening of B cells sets a good example for development of new approaches to isolate more potent broadly neutralization antibodies.^[127] This approach is particularly useful to identify broadly neutralizing antibodies that bind poorly to recombinant forms of Env. With this functional screening method, many new bnmAbs have been recently identified which show almost 10-fold higher potency than the previously described VRC01, PG9, and PG16, and are 100-fold more potent than the prototype HIV-1 bnmAbs such as b12.^[220] The discovery of VRC01 and VRC02 by using an antigenically resurfaced HIV-1 gp120, in which the neutralizing surface of CD4bs was preserved while other antigenic regions were eliminated, is another good example for efficient isolation of antibodies targeting a specific surface area of Env.

All existing bnmAbs are isolated from HIV-1-infected individuals, whose sera exhibit broadly neutralizing activity. Due to the limitations of the current screening methods, however, the most potent and broadest bnmAbs in the patients may not be selected. Recent studies show that all HIV-1 bnmAbs are heavily mutated compared with their corresponding germlines and antibodies with high degrees of somatic hypermutation (SHM) generally exhibit higher potency and cross-reactivity than their less mutated intermediates.^[151,215,221,222] Therefore, the large-scale sequencing of potentially a whole antibody repertoire offers another novel approach to select possibly more potent antibodies based on their sequence similarities and levels of SHM compared with the known bnmAbs selected from the same patients via conventional methods.^[216]

5.4 Multifunctional Antibody-Based Fusion Proteins

It has been proposed that a combination of multiple entry inhibitors with the same or different mechanisms of action would increase the antiviral potency and durability to resistance, and provide a broader coverage of the virus. PRO 542 (CD4-IgG2) is a fusion protein comprising human IgG2 in which the variable fragment (Fv) portions of both heavy and light chains have been replaced by the D1 and D2 domains of human CD4, which can protect the human-peripheral blood lymphocyte (PBL)-SCID mouse model^[223] from infection by primary isolates of HIV-1 *in vivo*.^[224,225] It has also been shown that PRO 542 and T-20 are potentially synergistic in blocking virus-cell and cell-cell fusion.^[226] In a clinical trial, both single and multiple doses of PRO 542 were well tolerated.^[227] Statistically significant acute reductions in HIV-1 RNA levels were observed across all study patients including children and adults, and greater antiviral effects were observed in the cohort of patients with more advanced HIV-1 disease.^[228,229] Further evaluation of PRO 542 was performed by Progenics Pharmaceuticals, Inc ().^[230] Fusion of two-domain sCD4 to 17b exhibited very broad and potent activity superior to that of b12, 2F5, 4E10, and 2G12 in neutralization against a large panel of isolates.^[231] Combinations of other CD4i mAbs (e.g. E51 and m36) with sCD4 and antibody Fc also resulted in increased neutralizing activities.^[232,233] The fully functional soluble stable single-domain sCD4 was also generated, which

could be used to decrease the molecular size of large fusion proteins.^[234] A novel, bifunctional HIV-1 entry inhibitor by combining the anti-CD4 mAb 6314 with a fusion inhibitor similar to T-651 (anti-CD4 mAb based bi-functional fusion inhibitor, CD4-BFFI) was described that showed higher antiviral potency compared with the fusion inhibitor T-651 or the anti-CD4 mAb 6314 used independently.^[235,236] Importantly, CD4-BFFI can inhibit all the tested HIV-1 strains among which many strains are only partially inhibited by 6314. This CD4-BFFI also retains antiviral potency against virus strains resistant to two fusion inhibitors, a CCR5 antagonist and an anti-CCR5 mAb. Besides the fusion of antibodies with other non-Ig molecules, the bispecific antibodies through connection of scFvs with IgGs targeting two alternative docking sites of CCR5-tropic HIV strains on CCR5 showed 18- to 57-fold increased antiviral activities compared with the parental antibodies.^[237] In addition, one prototypic tetravalent CCR5 antibody, CCR5-2320, had antiviral activity against virus strains resistant to the single parental antibodies.^[237] These results indicate that bispecific antibodies with tetra-valence produced by physical linkage of two CCR5 antibodies targeting different epitopes on CCR5 have enhanced antiviral potency against wild-type and CCR5 antibody-resistant HIV-1 strains.

In addition to direct fusion of two independent antibodies, some new approaches have been developed to facilitate the assembly of bi-functional antibodies without significantly increasing antibody molecular size. For example, based on the knobs-into-holes technology that enables heterodimerization of the heavy chains, correct association of the light chains and their cognate heavy chains is achieved by exchange of heavy-chain and light-chain domains within Fab of one half of the bifunctional antibody.^[238,239] Through this method, the bispecific anti-HIV-1 antibodies (BiAbs) that can bind bivalently by virtue of one scFv arm that binds to gp120 and a second arm to the gp41 subunit of gp160 were engineered by heterologation, which showed the bivalent could enhance neutralization compared with the parental antibodies.^[240,241]

6. Conclusions and Perspectives

The continued spread of HIV-1 worldwide and, in particular, in sub-Saharan Africa, where an estimated 22 million people are currently living with HIV-1/AIDS, underscores the urgent need for preventative or therapeutic antibodies. However, despite nearly 25 years of intense international research, an efficient vaccine or antibody is not yet available for clinical use. Although antibodies with broadly neutralizing activities can confer sterilizing protection against infection in animal models, some of them have failed for the therapy of HIV-1 infection in humans, and the clinical trials of others are still ongoing. None have entered phase III trials (summary in table I). This is in contrast to the clinical benefits provided by the currently approved therapeutic antibodies, such as those against cancer and immune disorders. There are no such antibodies against HIV-1, most likely because they are not capable of circumventing the escape mechanisms used by the virus, among other reasons. Thus, further studies are needed that could provide valuable insights into the interplay between the virus and the human immune system and how we could provide clinical benefits for HIV-1-infected humans by using antibodies. Finally, antibodies undoubtedly could play a critical role for reaching the ultimate goal – eradication of the virus.

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Fig. 1.

Entry of HIV-1 into CD4⁺ cell. After binding of gp120 to CD4 (pdb: 1WIP,^[11,12] rendered by PyMOL), serial conformational change occurs including: (i) exposure of co-receptor binding site and subsequent binding of gp120 to co-receptor; (ii) exposure of fusion peptide; (iii) formation of fusion core on gp41 through interaction between NHR and CHR; (iv) rearrangement of MEPR region, etc. These events lead to exposure of the new epitope on the Env which could be targeted by broadly neutralizing antibodies. **CHR** = C-terminal heptad repeat; **CoR** = coreceptor; **D1–D4** = domain 1–domain 4; **Env** = envelope glycoprotein; **FP** = fusion peptide; **MPER** = membrane proximal external region; **NHR** = N-terminal heptad repeat.

Fig. 2. Neutralizing epitopes on HIV-1 Env gp160. The structure (pdb: 1G9M^[15,16]) of a gp120 core from the HXBc2 laboratory-adapted isolate in complex with a 2 domain (D1 and D2) fragment of CD4 and Fab 17b targeting CD4i epitope was rendered by PyMOL. The positions of V1/V2 and V3 are also indicated. **CD4bs** = CD4 binding site; **CHR** = C-terminal heptad repeat; **CoRbs (CD4i)** = coreceptor binding site (CD4 induced epitope); **Env** = envelope glycoprotein; **MPER** = membrane proximal external region; **NHR** = N-terminal heptad repeat.

Fig. 3. Models of engineered antibody domains based on V or C domains. m36 was selected from a library based on the heavy chain variable domain (VH) of a human IgG (pdb: 1HZH,^[81,175] a model) while m1a1 was identified from a library based on the second constant domain (CH2) scaffold.

Table I.

Some representatives of antibodies and antibody-like fusion proteins against HIV-1

Epitope	Name	Year	Type	Animal/virus	Clinical trial	<i>In vivo</i> efficacy	Comment
CD4bs	b12	1991	Human IgG1	Macaque/CCR5-tropic SHIV (162P4)	No	4/4 animals were protected from vaginal infection at dose of 25mg/kg through intravenous injection; 9/12 animals were protected from vaginal infection at dose of 5 mg/kg through vaginal administration	First reported bnmAb targeting CD4bs, solved crystal structure
	VRC01 VRC02	2010	Human IgG1	NA	No	NA	Can neutralize over 90% of circulating HIV-1 isolates with high potency <i>in vitro</i> , solved crystal structure of VRC01
	PRO 542 (CD4-IgG2)	1995	IgG-like fusion protein	Human-PBL-SCID mouse/HIV-1	Phase II	Well tolerated at 25 mg/kg in 12 HIV-infected individuals, mediated an 80% response rate and statistically significant $\approx 0.5 \log_{10}$ mean reductions in viral load for 4–6 wk posttreatment in advanced disease (HIV-1 RNA >100 000copies/mL; CD4 lymphocytes <200 cells/mm ³)	Fusion of CD4 D1 and D2 domains to IgG2, longer half-life (3 days) than sCD4 (45 min), does not appear to bind to Fc receptors
CoRbs (CD4i)	17b	1996	Human Fab/IgG1	NA	No	NA	Binds to CD4i epitope that overlap with the CoRbs, solved crystal structure of gp120 core/two-domain soluble CD4/Fab 17b
	412d	2001	Human IgG1	NA	No	NA	A critical sulfotyrosine on CCR5 and on 412d induces similar structural rearrangements in gp120
	X5	2002	Human Fab/IgG1	NA	No	NA	Shows the existence of conserved receptor-inducible gp120 epitopes that can serve as targets
V3	m36	2008	Human IgG1 V domain	Humanized NOD/SCID/ γ_c^{null} mouse/HIV-1	No	1 mg m36.4, an affinity-matured version of m36, provided sterilizing protection of 4/6 animals against intrasplenic challenge with high-titer HIV-1	First reported V domain-based eAd that targets a sterically restricted CD4i epitope and potently neutralizes genetically diverse HIV-1 isolates <i>in vitro</i>
	m1a1	2009	Human IgG1 C domain	No	No	NA	First reported C domain-based eAd that recognizes a highly conserved CD4i epitope overlapping with that of m36 CD4i epitope, modest neutralization activity <i>in vitro</i>
	C β i	1988	Humanized mouse IgG	Chimpanzee/HIV-1 (11b)	No	Protected a chimpanzee at dose of 36 mg/kg from HIV-1 infection during 52-week observation period	First characterized bnmAb targeting V3 of gp120
	hNM01	2004	Humanized mouse IgG1	NA	Phase I	Dose: day 1: 0.2mg/kg, day 15: 1 mg/kg, day 29: 5 mg/kg, and day 43: 5 mg/kg; 3/4 patients had a decrease in their plasma viral load measurements; no significant change in CD4 counts	Neutralizes the virus by the activation of complement and the subsequent disruption of the viral envelope after binding to V3
	KD-247	2006	Humanized mouse IgG1	Cynomolgus/SHIV (C2/1)	Phase I	Passive immunization at a single dose of 45 mg/kg 24 h prior to viral	Efficiently neutralizes CXCR4- and CCR5-tropic primary HIV-1 clade B and clade B' and suppress the <i>ex vivo</i>

Epitope	Name	Year	Type	Animal/virus	Clinical trial	In vivo efficacy	Comment
Quarter-nary structure	HGN194	2010	Human IgG1	Macaque/SHIV (CCR5-tropic, Clade C)	No	challenge completely protected animals from viral challenge; phase I study is ongoing	generation of primary HIV-1 quasi-species in peripheral blood mononuclear cell cultures from HIV-1 - infected individuals
	PG9 PG16	2009	Human IgG1	NA	No	NA	Identified by using appropriate screening methods, neutralizes all Tier-1 and a proportion of Tier-2 pseudoviruses tested, irrespective of clade
	PG9 PG16	2009	Human IgG1	NA	No	NA	Selected from a high-throughput neutralization screen of antibody-containing culture supernatants of about 30 000 activated memory B cells from a clade A-infected African donor, solved crystal structure of V1/V2 in complex with PG9
	PG9 PG16	2009	Human IgG1	NA	No	NA	Selected from a high-throughput neutralization screen of antibody-containing culture supernatants of about 30 000 activated memory B cells from a clade A-infected African donor, solved crystal structure of V1/V2 in complex with PG9
Carbohydrate	PGT128	2011	Human IgG1	NA	No	NA	Cystal structure of complex of Fab PGT 128/fully glycosylated gp120 outer domain reveals that the antibody penetrates the glycan shield and recognizes two conserved glycans as well as a short β -strand segment of the gp120 V3 loop, accounting for its high binding affinity and broad specificity
MPER	2G12	1996	Human IgG1	Macaque/SHIV (89.6PD and vpu +)	Phase II	Administered 1 g 2G12, 1 g 4E10 and 1.3g 2F5 (combination) sequentially in each infusion; 2/8 chronically and 4/6 acutely infected individuals showed evidence of a pronounced delay in viral rebound during	Adopts a multivalent binding surface by using a unique domain-exchanged structure, in which the heavy chain variable domains of two antibody molecules are swapped
	2F5	1993	Human IgG1	Macaque/SHIV (89.6PD and vpu +)	Phase II	See description in 2G12	Spurs interest in its structural characterization: these two bnmAbs induces large conformational changes in the MPER relative to the membrane, phase II clinical trial is ongoing
	4E10	2001	Human IgG1	NA	Phase II	Treatment of arm A: 10 mg/kg, weekly, 10 doses; arm B: a single loading dose of 10 mg/kg on day 1, followed by 5 maintenance doses of 6 mg/kg biweekly and arm C: 25 mg/kg, biweekly, 5 doses resulted in substantial reductions in HIV-1 RNA levels (0.5 to 1.7log ₁₀) in 20 of 22 subjects	Targets the D2 domain of human and rhesus CD4; phase II clinical trial is ongoing
CD4	TNX-355 (ibalizumab, hu5A8)	1992	Humanized IgG4	Macaque/S IV	Phase II		
	CD4-BFFI	2009	IgG-like fusion protein	NA	No	NA	Combines the anti-CD4 mAb 6314 with a fusion inhibitor similar to T-651; <i>in vivo</i> pharmacokinetic studies demonstrate that CD4-BFFI is stable in monkey blood, and a dose of 10 mg/kg maintains serum concentrations greater than 2000-fold over the IC ₅₀ value for 7 days post-

Epitope	Name	Year	Type	Animal/virus	Clinical trial	<i>In vivo</i> efficacy	Comment
CCR5	PRO 140	1999	Humanized IgG4	NA	Phase II	Subcutaneous PRO 140 demonstrated potent and prolonged antiretroviral activity. Mean log ₁₀ reductions in HIV-1 RNA level were 0.23, 0.99, 1.37, and 1.65 for the placebo, 162 mg weekly, 324 mg biweekly, and 324 mg weekly dose groups, respectively	dosing; higher antiviral potency compared with the fusion inhibitor T-651 or the anti-CD4 mAb 6314 Exhibits potent and broad-spectrum inhibition of primary HIV-1 R5 isolates from different clades but does not affect CCR5 activity; phase II clinical trial is ongoing
	CCR5-2320	2011	IgG-like fusion protein	NA	No	NA	Constructed by connection of scFvs with IgGs, in contrast to monospecific CCR5 antibodies; bispecific antibody derivatives block two alternative docking sites of CCR5-tropic HIV strains on the CCR5 coreceptor; consequently, these molecules show significantly increased antiviral activity compared with the parent antibodies

hmmAbs = broadly neutralizing monoclonal antibodies; **CCR5** = C-C chemokine receptor type 5; **CD4bs** = receptor CD4-binding site; **CD4i** = CD4-induced; **CoRbs** = coreceptor binding site; **CXCR4** = C-X-C chemokine receptor type 4; **eAd** = engineered antibody domain; **IC90** = inhibitory concentration at which 90% of bacteria are inhibited; **MPER** = membrane proximal external region; **NA** = not applicable; **NOD** = non-obese diabetic; **PBL** = peripheral blood lymphocyte; **sCD4** = soluble CD4; **scFvs** = single-chain variable fragments; **SCID** = severe combined immunodeficiency; **SHIV** = simian-human immunodeficiency virus; **SIV** = simian immunodeficiency virus.