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Engineering strategies of Anti-HIV Antibody Therapeutics in Clinical Development

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Structured Abstract

Purpose: Anti-HIV antibody-based therapeutics offer an alternative treatment option to current antiretroviral drugs. This review aims to provide an overview of the Fc- and Fab-engineering strategies that have been developed to optimize broadly neutralizing antibodies and discuss recent findings from preclinical and clinical studies.

Recent Findings: Multispecific antibodies, including bispecific and trispecific antibodies, DART molecules, and BiTEs, as well as Fc-optimized antibodies, have emerged as promising therapeutic candidates for the treatment of HIV. These engineered antibodies engage multiple epitopes on the HIV envelope protein and human receptors, resulting in increased potency and breadth of activity. Additionally, Fc-enhanced antibodies have demonstrated extended half-life and improved effector function.

Summary: The development of Fc and Fab-engineered antibodies for the treatment of HIV continues to show promising progress. These novel therapies have the potential to overcome the limitations of current antiretroviral pharmacologic agents by more effectively suppressing viral load and targeting latent reservoirs in individuals living with HIV. Further studies are needed to fully understand the safety and efficacy of these therapies, but the growing body of evidence supports their potential as a new class of therapeutics for the treatment of HIV.

Keywords

Fc effector function; Fab domains; antibody engineering; HIV; antibody therapy

Introduction

Since the emergence of AIDS in 1981, HIV infections have remained a prevalent and persisting global epidemic. There are still many challenges in the treatment of HIV due to high mutation rates (1), conformational heterogeneity, polymorphisms (2), altered glycosylation patterns (3, 4), and subsequent glycan shielding (5–7). Current therapies include antiretroviral treatment (ART): a non-curative, life-long treatment that suppresses viral replication (8, 9). However, ART is unable to clear the viral reservoir and can often lead to drug-related toxicities over time (10).

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Antibody therapies present a potential alternative treatment option to ART and their potential use in the management of HIV infection has been explored in recent years (11–16). Their appeal as pharmaceutical agents stems from their dual functionality, allowing them to utilize host effector functions with the constant region (Fc domain), while targeting specific viral epitopes with their variable region (Fab). The Fab domain of the broadly neutralizing antibodies specifically targets one of the neutralizing epitope clusters on the viral envelope glycoproteins, such as the V1/V2 region, CD4-binding site (CD4bs), V3 glycan, and MPER, to effectively block viral entry. At the same time, the Fc domain of IgG antibodies interacts with Fcγ receptors (FcγRs) expressed on the surface of effector leukocytes to mediate diverse effector activities. Fc effector functions, including antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis, as well as the induction of protective CD8 T cell responses through modulation of dendritic cell function, play a critical role in the clearance of HIV virions and the cytotoxic elimination of infected cells (17, 18). Unlike ART, through their pleiotropic Fc effector functions, antibodies can effectively target viral reservoirs, thereby providing durable and long-term control of HIV infection (18–24).

Broadly neutralizing antibody (bNAb) therapies provide the building blocks for these antibody-based therapies. On their own they have shown success in the control of HIV infection (25, 26), however, resistant viruses can quickly emerge under bNAb selection, thereby rendering the treatment ineffective (25, 27, 28). To combat these escape mutants, combination therapies consisting of antibody cocktails targeting multiple epitopes of the HIV envelope protein (Env) at once have been explored (29–31) and proven effective with sustained virological suppression in recent clinical studies (32, 33). Although enticing, the combination of multiple antibodies into a single treatment presents challenges in production and clinical application (35). Alternatively, an antibody can be engineered to target multiple epitopes on the Env with greater breadth and potency (36–38). Engineered antibodies come in a variety of different formats including antibodies with multiple distant Fabs, rationally designed Fab regions, backbone substitutions, enhanced Fc receptor engagement, and extended half-life.

In this review, we will explore the variety and application of Fab and Fc engineering in improving anti-HIV antibody therapeutics in clinical development. The goal is to provide a comprehensive understanding of how these strategies can be used to overcome the limitations of current treatments and potentially lead to new therapeutic options for HIV management.

Fab Engineering

Fab engineering aims to increase the potency and breadth of singular antibody treatments to improve the effectiveness of anti-HIV antibodies. These treatments have grown to encompass many types of therapeutic molecules based on the structure of human IgG (Figure 1a), including bispecific antibodies (biAbs), tri-specific antibodies (tsAbs), and other multispecific molecules like Dual Affinity Re-Targeting (DART) proteins and Bispecific T-Cell Engagers (BiTE). The Fab domain can be directly targeted in engineering pursuits to have increased affinity for highly conserved binding sites on the antigen that are necessary for viral fitness (35). Rational engineering of antibodies can increase Env recognition while

also decreasing the likelihood of the emergence of escape mutants (39). While rationally designed Fabs have the potential to increase the efficacy of HIV treatments, already engineered bnAbs can also provide the basis for better targeting of the Env epitopes by pairing different Fabs in a singular antibody.

Bispecific Antibodies

Bispecific antibodies (biAbs) offer an effective approach to increase the potency and breadth of an antibody, while reducing production difficulties. These molecules combine two distinct Fab regions into one antibody, with each region targeting different neutralizing epitopes on the HIV envelope protein (Env) or engaging other human receptors. The correct heterodimerization of the heavy chains in biAbs is achieved by “knob in hole” mutations at the CH3 domain (40). This mutation is performed concurrently with a CH1-CL swap made in the bispecific’s arm with the “hole” mutation (T366S, L368A, Y349C, and Y407V), while the other arm with the “knob” mutation (T366W and S354C) remains unchanged (37, 41). BiNAbs can also be created by adding a full-length bNAb using a flexible linker (42, 43), or by exchanging one of the single-chain fragment variables of a bNAb for that of another bNAb (Figure 1b) (44).

Pairings of two distinct Fab regions in biAbs come primarily in two different forms: both Fabs can bind to target epitopes on the Env (36, 37), or one Fab can bind to the Env while the other binds to a human receptor such as CD4 or CCR5 (41, 45). Preclinical studies have tested and designed many bispecific antibodies in the former capacity, successfully displaying features of both antibody specificities and broader coverage in some cases (36, 46, 47). However, there is some variability in the performance of biAbs compared to their parent antibodies, with either breadth (37) or potency (45) not fully captured in some biAb iterations. Preclinical studies also encompass bispecific antibodies with one Fab targeting an epitope on the Env while the other engages human CD4 receptors, such as Ibalizumab (46, 48–51), or human CCR5 co-receptors, such as PRO140 (52, 53). Ibalizumab (iMab) is an FDA-approved monoclonal antibody treatment for the clinical management of multidrug-resistant HIV-1 infection, making it an attractive candidate for use in combination with HIV envelope targeting Fabs in a bispecific antibody format. 10E3V2.0-iMab has been shown to have exquisite neutralizing activity, reduce virus load substantially, and provide protection if administered before exposure (41), and now 10E8/iMab is in a clinical trial (NCT03875209). Other preclinical iterations of biAbs include iMab-CAP256, which targets the V1/V2 loop with CAP256 (54) and iMab-N6, which targets the CD4 binding site with N6 (45).

Hinge Domain Engineering

Hinge domain engineering provides another way to enhance the therapeutic effects of biNAbs. This involves substituting an IgG1, 2, or 4 backbone for the IgG3 backbone, resulting in increased hinge length. Alternative formats based on the IgG3 hinge include variants (termed IgG3C-) modified to remove many disulfide bonds from the hinge region, increasing both length and flexibility (36) (Figure 1c). Substituting the backbone of IgG1 with the IgG3C- hinge variant has shown improved neutralization potency and in vivo

protective activity against HIV-1 infection due to the longer, more flexible hinge region of IgG3 (36).

Trispecific Antibodies

Trispecific antibodies (tsAbs) are a promising approach to improve the potency and breadth of antibody treatments. Unlike biAbs, tsAbs contain three Fabs and many are currently in development (38, 42, 55). These antibodies are engineered using either the “knob-in-hole” heterodimerization, in which one arm is derived from a normal immunoglobulin (IgG) and the other is a double Fab arm, or by substituting one Fab of a bNAb with another bNAb’s and linking the third Fab in a reverse-order tandem-forming cross-over dual variable Ig (55) (Figure 1d). 10E3 targeting MPER is often included as one of the Fabs while the other two target other Env epitopes in addition to their Fc region. With the increased number of Fabs, the enhanced potency of these antibodies is attributed to simultaneous epitope engagement of the same Env (37, 43). Many tsAbs have shown superior breadth against SHIV in macaques (38, 55), and VRC01/PGDM1400–10E8v4 (SAR441236) is currently tested in a phase I clinical trial (NCT03705169).

Dual Affinity Re-Targeting Molecules and Bispecific T-Cell Engagers

Multispecific molecules, such as DARTs and BiTEs, provide an alternative strategy to increase the efficacy of antibody treatments for HIV. DARTs are typically composed of two chains, where the VL partner of one chain is noncovalently associated with the VH partner of the other chain, forming the antigen-binding domain (56, 57) (Figure 1e). These molecules lack an Fc domain, resulting in a shorter half-life, but their design allows for better tissue penetration and improved engagement of T-cells, increasing the efficiency of T cell activation and directed lysis of infected cells (56). The disulfide bridge stabilizing the DART molecule can also allow for the attachment of an Fc region (58), facilitating the recruitment of effector cells (59) and increasing the half-life (60). MDG014, a bispecific HIVxCD3 DART molecule, engages both the Env and the CD3 receptor on T cells to mediate cell lysis of infected cells. It recently completed a phase 1 safety study in people living with HIV on ART (NCT03570918) with no dose-limiting toxicities or serious adverse events noted. Other DART molecules have shown efficacy in directing T cell-mediated cytolysis of latently HIV-infected cells, clearing viral reservoirs in addition to managing the viral load (57).

BiTEs are another antibody-like approach to HIV treatment that consist of two or three single-chain variable fragments in tandem, targeting human receptors such as anti-CD3 and an epitope on the Env (Figure 1f) (61). BiTEs lack an Fc region and can engage T cells to redirect them to lyse HIV gp120-transfected cells, inhibiting HIV replication in infected PBMCs and macrophages when cocultured with CD8+ T cells (62). However, BiTE therapies containing CD4 targeting domains have not yet advanced to clinical trials for HIV treatment as they have been shown to promote HIV infection of human CD4-CD8+ T cells, possibly due to conformational changes of the gp120 induced by the presence of the CD4 domain. Nevertheless, the BiTE blinatumomab has been clinically successful in

treating non-Hodgkin's lymphoma or B-cell lymphoblastic leukemia (63, 64), and other BiTE candidates are currently undergoing clinical investigation (65, 66).

Fc Engineering

The importance of the Fc region in meditating effector functions in antibody therapies for HIV has been thoroughly established (18–23) making it an attractive target to optimize in HIV treatment therapies. The Fc domain is vital to the engagement of FcγR-expressing effector leukocytes to promote antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) of virions and infected cells as well as modulation of dendritic cell function to elicit protective antiviral CD8 T cell responses (Figure 2) (18, 67, 68).

Extended Half-Life Mutations

While the absence of an Fc domain reduces the half-life of antibodies, Fc engineering offers a promising avenue to increase it (69–72). This is achieved by introducing mutations that enhance the antibody binding affinity to the neonatal Fc receptor (FcRn) (71, 73–75) without impairing antibody function. One such variant is LS (M428L/N434S), which has been shown to increase serum half-life by 2–3 fold in non-human primates and from 15 to 71 days uninfected individuals (71, 74). The LS mutation is being tested in several established bNAb treatments (69, 74, 76), and ongoing clinical trials on 3BNC117-LS (NCT03254277), PGT121.414.LS (NCT04212091), 10–1074LS (NCT03554408), and CAP256V2LS (NCT04408963) are expected to yield promising results. In addition to prolonging the effectiveness of single antibody treatments, Fc-modified antibodies administered through intravenous infusion have been shown to increase the viral challenge required for infection and delay acquisition (71). Improved half-life antibodies offer clear benefits to individuals living with HIV by maintaining therapeutic levels with less frequent administration, making it a promising approach for HIV prevention as well as the long-term control of HIV replication in those diagnosed with HIV.

Enhanced FcγR Engagement

Similar to rational Fab domain engineering, Fc domains can also be rationally engineered to enhance FcγR binding (72, 77). In several preclinical studies, mutations that increase binding to activating FcγR receptors resulted in augmented effector functions and consequently in improved antiviral efficacy in vivo (18, 78–79). Over the past few years, several antibodies targeting tumor and viral antigens have been optimized for enhanced FcγR affinity and many of these Fc-optimized antibodies are currently in clinical testing as well as in clinical use (79). Fc engineering of anti-HIV bNAbs has previously resulted in durable control of HIV-infection in humanized mouse models of HIV infection (18), guiding the clinical development of bNAbs optimized for enhanced FcγR engagement. GS-9722, also known as Elipovimab, is a first-in-class effector-enhanced bNAb treatment that shows improved activating FcγR and FcRn binding. It is derived from PGT121, which targets the V3 glycan motif, and is currently being evaluated for safety and effectiveness in clinical trials.

Conclusion

Fc and Fab engineering are promising approaches to improve the efficacy, breadth, and durability of HIV antibody therapies. Researchers have developed a range of novel antibody therapies that show great promise in preclinical studies, and as clinical trials emerge, their potential to revolutionize the field of HIV treatment becomes clearer. Once individual Fc or Fab alterations are proven safe and effective, combining them in singular antibody treatments may enhance breadth and potency, prolonged half-life, and enhanced Fc effector functions. Ultimately, this could lead to a new generation of HIV antibody therapies with better outcomes and fewer side effects.

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Key Points

- Fab and Fc-engineered anti-HIV antibody therapeutics represent promising candidates for the treatment and long-term management of HIV infection.
- Multispecific antibody therapeutics (biAbs, tsAbs, DART, BiTE) offer increased breadth and potency maximizing the efficacy of HIV treatments.
- Fc optimization increases effector cell engagement and improves antibody half-life.

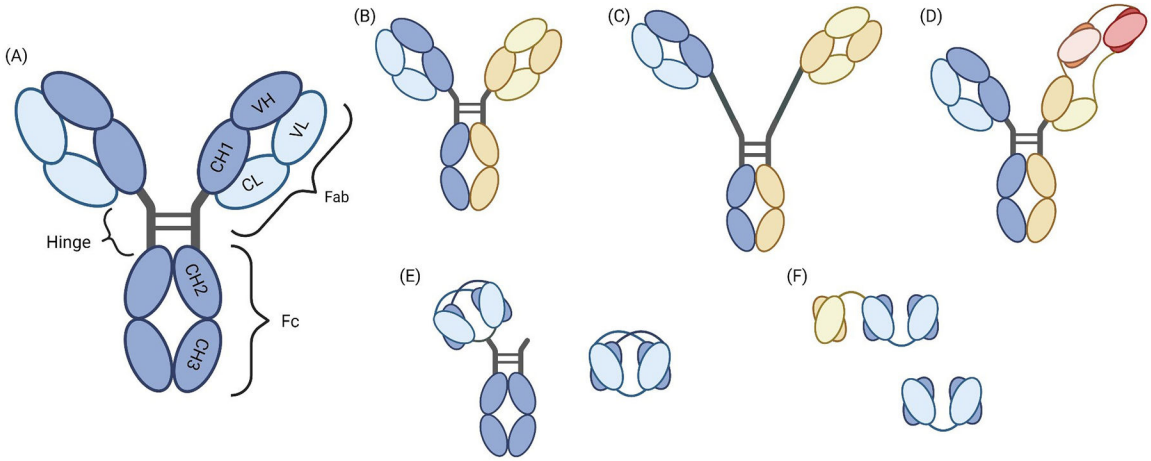

Figure 1. Structures of multispecific, Fab engineered antibodies to improve breadth and potency.

These antibodies demonstrate the diversity of forms and engineering methods that allow for the simultaneous targeting of multiple epitopes on the Env and/or human cellular receptors. Antigen binding is facilitated via the Fab region while effector function engagement is mediated via the Fc as shown in (A). The structures represent (A) non-engineered IgG, (B) bispecific antibody, (C) hinge-domain engineered antibody, (D) trispecific antibody, (E) DART molecule with and without a Fc, and (F) BiTEs.

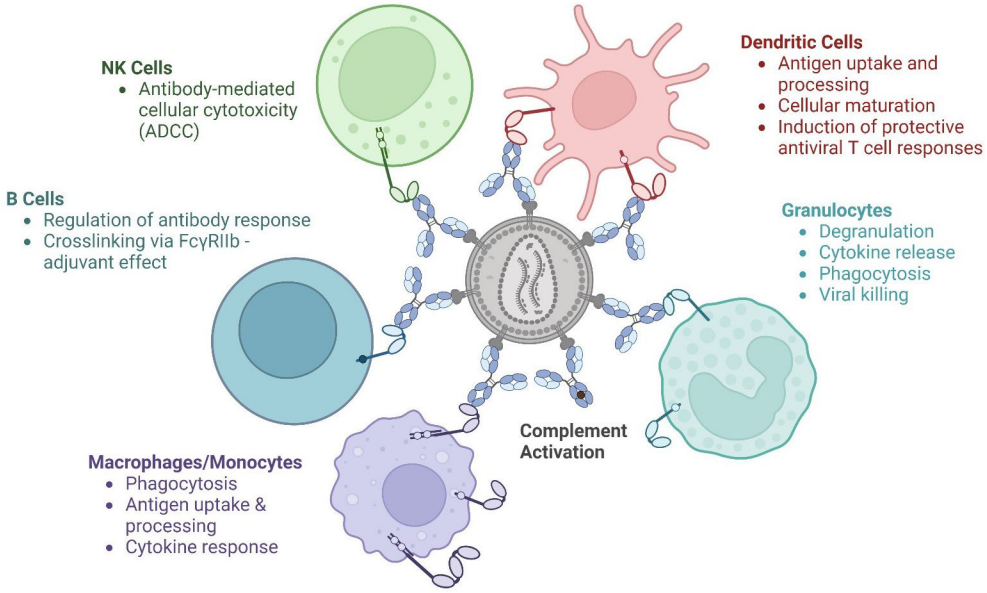

Figure 2. Diversity of effector functions mediated by IgG antibodies.

IgG Fab domain binds to the Env presented on HIV infected cells or virions thereby recruiting effector cell functions through the engagement of FcγRs by the IgG Fc domain. These pleiotropic effector functions are the outcome of the engagement of specific FcγRs on B cells, natural killer (NK) cells, dendritic cells (DC), granulocytes, monocytes, and macrophages.