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The potential of engineered antibodies for HIV-1 therapy and cure

Marloes Grobben, Richard AL Stuart and Marit J van Gils

Broadly neutralizing antibodies (bnAbs) are currently under investigation as a therapy for HIV-1 infection and recent clinical trials have shown prolonged viral suppression by bnAbs during antiretroviral treatment interruption. Interestingly, these bnAbs also showed the ability to activate the host immune system to clear HIV-1 infected cells. There are many possibilities to further increase the potential efficacy of bnAbs. Most notably, Fc domain engineering to improve half-life and increase engagement of effector cells will augment two advantages of bnAbs. Moreover, antibody engineering can improve affinity and recognition of conserved epitopes and allows the combination of multiple epitope specificities in a single molecule. These increasingly potent and broad antibodies may prove valuable as alternative HIV-1 therapeutic and possibly in curative approaches.

Address
Department of Medical Microbiology, Amsterdam University Medical Centers, Location AMC, University of Amsterdam, Amsterdam, 1105 AZ, The Netherlands

Corresponding author: van Gils, Marit J (M.J.vangils@amsterdamumc.nl)

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Introduction

HIV-1 is a major global problem due to its substantial mortality and morbidity [1]. The introduction of antiretroviral treatment (ART) was a great step forward in the battle against HIV-1. ART suppresses viral replication and provides patients with a life expectancy close to that of sero-negative individuals [2–4]. While ART is highly effective it is unable to clear the persistent viral reservoir. Therefore, lifelong medication is needed because discontinuation of treatment results in a rapid plasma viral rebound [5–8]. New treatment approaches are needed and the use of broadly neutralizing antibodies (bnAbs) shows potential. BnAbs were shown to protect against HIV-infection [9–12] and their use as a treatment showed potential in the first clinical studies (Table 1). The use of bnAbs as a treatment has some potential advantages not associated with ART. For example, besides their neutralizing capacity, bnAbs can also facilitate clearance of viral reservoir cells by engaging the host immune system via the antibody Fc domain [7,13,14]. Understanding of the role of the Fc domain and its importance in HIV-1 treatment is rapidly progressing and may lead to the development of bnAbs with increased efficacy [15]. In addition, bnAbs have the potential to be administered as a long-acting agent [16••,12]. Here, we will review the recent developments in the use of bnAbs for HIV-1 treatment, options for engineering of bnAbs to improve efficacy and the potential of (engineered) bnAbs for curative approaches.

bnAbs in clinical trials for HIV-1 treatment

A multitude of potent HIV-1 bnAbs have already been tested as an alternative for current HIV-1 treatment. Recent clinical trials using monotherapy of bnAbs 3BNC117, 10-1074 or VRC01 showed a decrease in plasma viremia in the order of 0.8–2.5 Log_{10} copies/mL and this decrease lasted for several weeks in responsive individuals [17,18]. However, in most studies viral rebound was delayed but not prevented. A single bnAb is most likely not suitable for long-term suppression of HIV-1 due to the formation of escape mutants that cause viral rebound [17,19,20]. This is similar to what was historically observed when single antiretroviral compounds were used [21]. The risk of viral escape can be reduced by using a combination therapy with multiple bnAbs. A study testing multiple administrations of the two bnAbs 3BNC117 and 10-1074 in the absence of ART showed that the bnAb therapy was well tolerated and that viral suppression was maintained for a median of 21 weeks in individuals with antibody-sensitive latent reservoirs. In these antibody-sensitive patients, there were no viruses detected that developed resistance to both antibodies [16••,22•]. However, this treatment is only suitable for individuals that carry viruses which are sensitive to both antibodies [16••,22•]. Furthermore, previous preclinical studies in animal models indicated that combinations of three potent bnAbs have high potential for long-term viral suppression [23,24]. Therefore, in the near future, several clinical trials will start that use different combinations of two or even three bnAbs to see if these combinations are more effective (Table 1).

Interestingly, some studies using bnAb therapy showed an improvement in the participants' own intrinsic antibody responses against HIV-1 in addition to the reduction of

Table 1

Overview of bnAb therapy in completed, ongoing and planned clinical trials

bnAb(s)	Most advanced clinical trial status and reference	Findings (completed studies) or previous pre-clinical findings (planned and ongoing studies)
Single bnAb therapy		
F105	Phase 1 Completed (1996) NCT00001105	Safe and non-toxic with a serum half-life of 13 days [103,104].
KD-247	Phase 1 Completed (2012) NCT00917813	Treatment was well tolerated, a significant decrease in HIV-1 RNA was observed, long-term suppression was achieved in one patient [105].
3BNC117	Phase 2 Completed (2017) NCT02446847	Virus was suppressed for a mean of 6.7 weeks (2 infusions) or 9.9 weeks (4 infusions) during ATI, but escape mutants arose [17,106].
VRC01	Phase 2 Completed (2017) NCT02664415	Well tolerated with a serum half-life of 15 days. Suppression of virus for a median of 4 or 5.6 weeks during ATI. 1.1-1.8 $\log_{10}$ copies/mL plasma viremia decrease in (6/8) viremic patients [19,107,18].
10-1074	Phase 1 Completed (2017) NCT02511990	Well tolerated with a serum half-life of 12.8 days in infected individuals. 10-1074-sensitive participants had a mean plasma viremia decline of 1.52 $\log_{10}$ copies/mL [108•].
PGT121	Phase 1 Recruiting NCT02960581	Protects NHP from SHIV infection at low serum concentrations (15 μg/mL) [11].
2G12	Phase 1 Planned NCT02923999	Moderate protection (2/4) and (3/5) in NHP after SHIV vaginal challenge [9,10].
Combination bnAb therapy		
2F5 + 2G12 + 4E10	Phase 2 Completed (2005) NCT00219986	Safe, with serum half-lives of 6.6 (4E10), 3.2 (2F5) and 14.1 days (2G12) but no apparent effect on viremia [109,110].
3BNC117 + 10-1074	Phase 1 Completed (2018) NCT02825797	Well tolerated. Participants that were sensitive to both bnAbs had a mean plasma viremia decline of 2.05 $\log_{10}$ copies/mL that remained reduced for 3 months and no resistance to both antibodies arose [22•,16••].
PGT121 + PGDM1400	Phase 1 Recruiting NCT03205917	Combination therapy in NHP gave 100% (5/5) protection from a SHIV mixture [111].
PGT121 + VRC07-523LS + PGDM1400	Phase 1/2a Recruiting NCT03721510	No information available yet.
VRC01 + 10-1074	Phase 1 Recruiting NCT03831945	No information available yet.
Half-life enhanced bnAb therapy		
VRC01-LS	Phase 1 Completed (2018) NCT02599896	Safe and well tolerated, with a 4-fold extended half-life compared to historical data of VRC01 [68•].
VRC07-523LS	Phase 1 Completed (2018) NCT03015181	Clinical results not available yet; NHP challenge studies showed a plasma EC_{50} of 0.47 μg/mL [112].
VRC07-523LS + 10E8VLS	Phase 1 Suspended NCT03565315	No information available yet.
3BNC117-LS	Phase 1 Recruiting NCT03254277	Protection in NHP for a median of 17 weeks [66•].
N6LS	Phase 1 Recruiting NCT03538626	Potent dose-dependent protection against SHIV challenge in NHP (A Pegu et al., abstract 08.03, HIV Research for Prevention Meeting, Madrid, October 2018).
3BNC117-LS + 10-1074-LS	Phase 1 Recruiting NCT03554408	Protection in NHP for a median of 20 weeks [66•].
Multispecific bnAb therapy		
MGD014 (DART)	Phase 1 Recruiting NCT03570918	No toxicities in NHP and delay in viral rebound after ATI in humanized mice (J Nordstrom et al., abstract in *Journal of Virus Eradication* 2017 3:5) [80].
10E8.4/iMab (bispecific)	Phase 1 Recruiting NCT03875209	Protection and reduction of viral load in humanized mice (CAVD website, URL: https://www.cavd.org/grantees/ Pages/Grantee-Ho4.aspx).
SAR441236 (trispecific)	Phase 1 Planned NCT03705169	100% protection (8/8) in NHP challenged with SHIV [91•].
Effector function enhanced bnAb therapy		
GS-9722	Phase 1 Recruiting No NCT record	bnAb derived from PGT121, engineered for lower immunogenicity, improved PK, enhanced ADCC & ADCP. Tested in preclinical studies. (N Thomsen et al., abstract 356, Conference on Retroviruses and Opportunistic Infections, Seattle, March 2019).

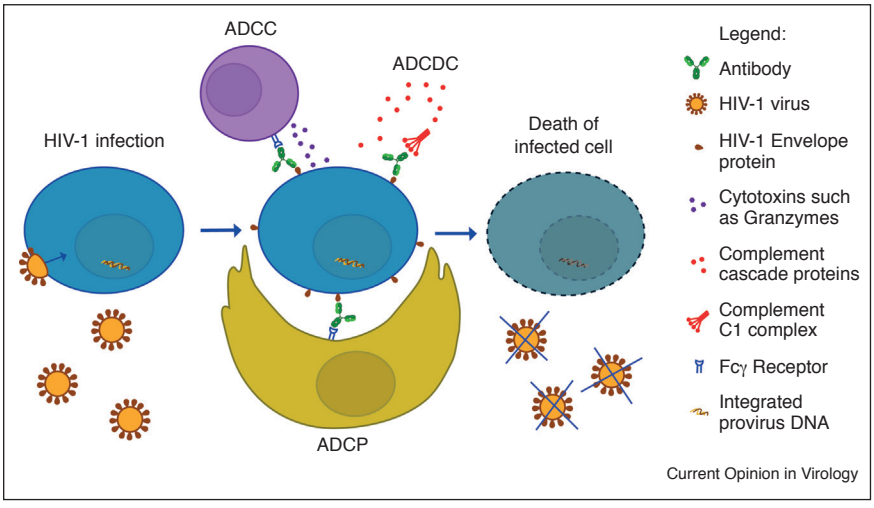
plasma viremia [7,25•]. This activation of the host immune system is most likely caused by the formation of immune complexes of antibody and virus which are taken up by antigen-presenting cells and presented to T and B cells [26,27]. Furthermore, in the study combining 3BNC117 and 10-1074, some individuals with antibody-sensitive viral reservoirs showed a lower frequency of HIV-1 infected cells after 12 weeks. Moreover, in two out of nine individuals, viral loads remained undetectable even when both antibodies could not be detected in serum anymore. This shows that these antibodies might also stimulate the immune system to clear active reservoir cells [16••]. This immune activation gives antibody therapy a potential advantage over other therapies such as ART and may even show the first step towards a functional cure of HIV-1.

The value of antibody effector functions for HIV-1 treatment

By now it is clear that neutralization is not the only factor that determines the efficacy of antibody therapy. Interactions between the antibody Fc domain and Fcγ receptors (FcγRs) were shown to be important for elimination of infected cells and have been associated with protection against disease progression [14,28,29]. Therefore, increased understanding of these antibody Fc domain interactions could contribute to the development of more effective HIV-1 bnAb therapy and possibly a functional cure. The recruitment of immune cells or immune components by the antibody Fc domain after antigen binding triggers antibody effector functions. Recruitment of cytotoxic cells such as NK cells leads to antibody-dependent cellular cytotoxicity (ADCC) via the targeted release of cytotoxins. Recruited phagocytes such as macrophages cause cell death via antibody-dependent cellular phagocytosis (ADCP). Furthermore, clustered Fc domains can trigger antibody-dependent complement-dependent cytotoxicity (ADCDC), a protein cascade that leads to lysis of infected cells [15]. In addition, the combination of antigen binding and effector functions can also lead to clearance of viral particles (Figure 1). Finally, instead of direct cytotoxic responses, transfer of antigen to uninfected cells can also occur via antibody-dependent cellular trogocytosis (ADCT), but there is still little known about this process and its effect on HIV-1 infection [30–32].

In HIV-1 treatment, the effector function ADCC is of particular interest as the increase in magnitude and breadth of the ADCC response has been linked to decreased disease progression [33–37]. This is further exemplified by the fact that even non-neutralizing antibodies capable of mediating ADCC can contribute to the control of HIV-1 infection [38–40]. Moreover, ADCC was correlated with reduced risk of infection in the RV144 vaccine trial [41,42]. In addition, ADCP was demonstrated to play a role in controlling disease progression [43,44] and was recently shown to prevent SHIV infection in preclinical studies [45,46]. Finally, ADCDC has been associated with lower viral loads during acute infection *in vitro* and with the development of bnAbs *in vivo* [47–49]. However, recruitment of complement proteins was also demonstrated to inhibit other effector functions *in vitro* [28,50–53], indicating that further testing is necessary to better understand if this function is desirable or not.

Figure 1



Effector functions induced by antibodies during HIV-1 infection. Active HIV-1 infected cells display the HIV-1 envelope glycoprotein, which is targeted by the antigen binding domain of HIV-1 specific antibodies. The Fc-domain of the antibody can recruit a variety of effector cells and immune components such as NK cells, macrophages, and complement proteins to perform ADCC, ADCP or ADCDC, respectively. All of these processes will eventually lead to death of the HIV-1 infected cell, preventing the production of new virus.

These observations show the importance of antibody mediated effector functions and open up possibilities to add and improve antibody mediated effector functions for a new generation of modified bnAbs with even higher therapeutic potential.

Modulating the Fc domain for enhanced antibody effector functions and half-life

There are currently a number of Fc domain mutations known that increase FcγR binding to improve antibody mediated effector functions such as ADCC and ADCP (as reviewed in [54•]). Effector functions are also dependent on the antibody glycosylation status, as deglycosylation has been demonstrated to abolish FcγR binding [55] and specific Fc-glycan structures were shown to enhance ADCP or ADCC activity [56–59,60•,61,62]. Glyco-engineering could be a good strategy to improve effector functions since glyco-engineered antibodies have already demonstrated their potential in clinical trials for other diseases [63]. Therefore, further advancement in the field of glyco-engineering techniques can prove valuable for HIV-1 bnAb therapy [64,65].

The half-life of an antibody is also important and has been shown to be directly related to efficacy in therapeutic applications [66•,12,67]. Moreover, a longer half-life allows a lower frequency of administration which results in lower treatment costs and a lower burden on the patient [16••]. Multiple studies have demonstrated that the half-life of antibodies can be extended by Fc domain modifications that increase their affinity for the neonatal Fc receptor [66•,68•,69,70]. One highly effective set of modifications is M428L/N434S (referred to as LS). These mutations have been incorporated in multiple bnAbs currently in clinical trials (Table 1) and were shown to highly increase antibody half-life with no effect on antibody function. It is speculated that a single administration consisting of a combination of half-life enhanced bnAbs could potentially suffice for six to twelve months of viral suppression [16••,66•].

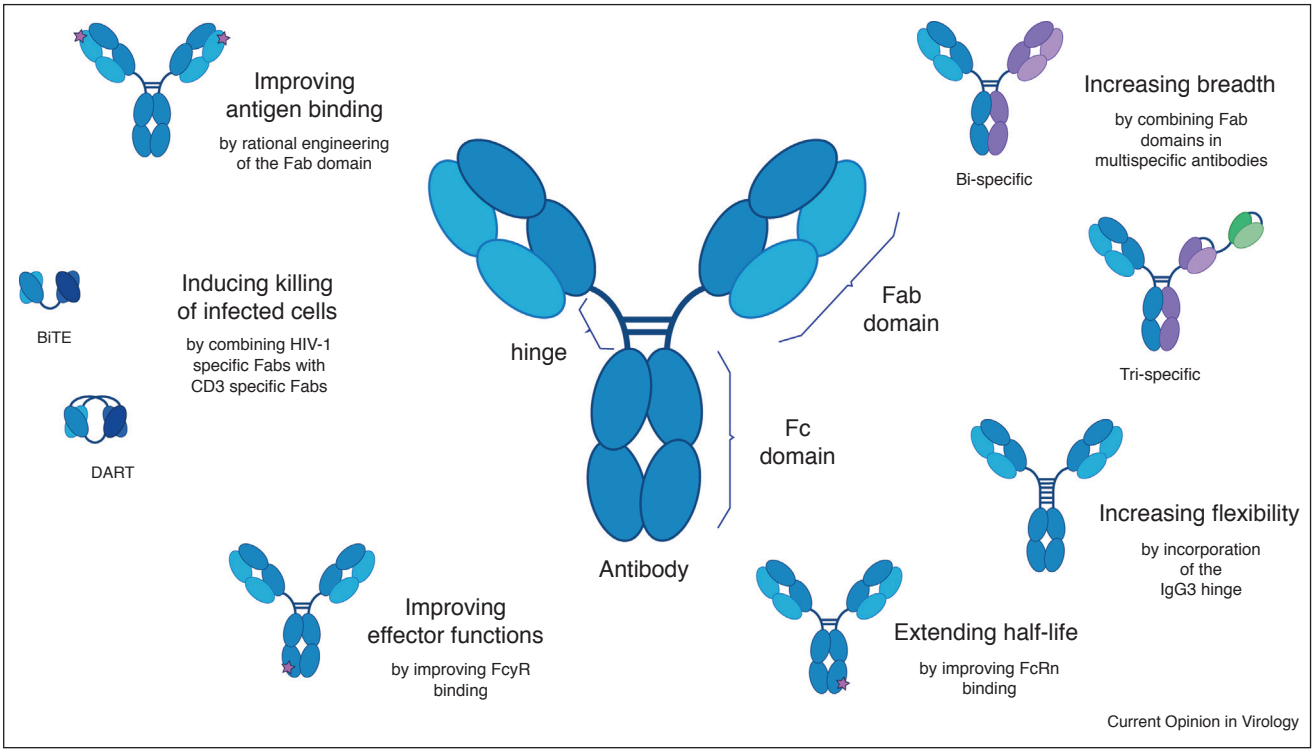
In addition, the different subclasses of IgG can also be used to construct antibodies with more potent and diverse effector functions. Most current IgG therapeutics are of the IgG1 subtype, however the different effector profiles of IgG2, IgG3 and IgG4 are also worth considering for therapeutic use (reviewed by Ref. [71]). IgG3 was shown to have a strong capacity to mediate effector functions [72] and a reduction of IgG3 serum concentration in HIV-1 patients has been linked to faster disease progression [73]. Moreover, the higher flexibility of IgG3 compared to IgG1 could be useful for the dimerization of FcγRs which is needed for efficient effector function [74]. Currently, the therapeutic efficiency of IgG3 is still limited due to its short half-life, but this is a problem that could be solved by antibody engineering as described above [75].

Antibody engineering for improved therapeutic efficacy

Antibody potency and breadth are considered essential for the development of a universal treatment or cure because the ability to potently neutralize many different virus strains is crucial for therapeutic efficacy. The potency and breadth of antibodies can also be improved by antibody engineering (Figure 2). Rational engineering of the antigen binding domain (Fab) can lead to improved affinity for the HIV-1 envelope protein and increases the recognition of conserved sites [77,78,79•,80,81]. Options for viral escape can be limited by targeting conserved epitopes that are needed for viral fitness [76]. Targeting multiple different epitopes in a combination therapy is also a way to further improve neutralization breadth. However, combining multiple antibodies in a single infusion poses difficulties in production and clinical application [77]. These issues may be resolved by combining Fab fragments of multiple bnAbs in a single bispecific antibody. Bispecific antibodies combining bnAbs VRC07/ PG9-16 or 3BNC117/ 10-1074 have a greatly improved neutralization breadth, leading to higher suitability for widespread use [78,74]. In addition, a tri-specific antibody combining bnAbs VRC01, PGT121 and 10E8 showed great promise with near pan-neutralizing breadth and high potency [83•]. Bispecific antibody technology can also be used to combine a HIV-1 specific antibody with a receptor-targeting antibody. For example, the combination of a variety of bnAbs with the human CD4 binding antibody ibalizumab showed very broad and potent neutralization [79•,80–82].

Besides these multispecific antibodies, there are also bispecific molecules in development that combine different antigen binding domains without the Fc domain. This results in a smaller size, better tissue penetration and lower production costs [84]. However, these molecules have a short half-life [85] and cannot mediate effector functions because the Fc domain is absent. In the context of HIV-1, several bispecific molecules have shown interesting results by targeting both virus proteins and host-cell receptors. Bispecific T cell engagers (BiTEs) targeting both HIV-1 envelope and CD3 were shown to stimulate lysis of infected cells, leading to a decreased frequency of HIV-infected CD4 + T cells *in vitro* [86]. In addition, a more compact molecule with higher potency has been developed called Dual Affinity Re-Targeting (DART). DARTs derived from a variety of HIV-1 bnAbs were combined with a CD3 binding arm which induced elimination of HIV-1 infected cells while also retaining their bnAb activity and breadth [80,87]. Despite having no Fc domain, the engineered T cell binding of BiTEs and DARTs allows efficient killing of HIV-1 infected cells by CD8 + T cells, independent of their specificity [80]. These constructs do not activate antibody Fc-mediated effector functions, however with the increasing knowledge on effector functions in HIV-1, other host-targeting

Figure 2



Antibody engineering to improve the efficacy of bnAbs. Different potential ways to engineer antibodies to improve antigen binding, breadth, flexibility, half-life, effector functions or directly induce T cell killing are depicted. Different colors represent different HIV-1 antigen specificities. Dark blue represents CD3 specific Fabs. Light chains are shown in a lighter color than heavy chains. Pink stars represent targeted mutations. Blue curved lines represent linkers.

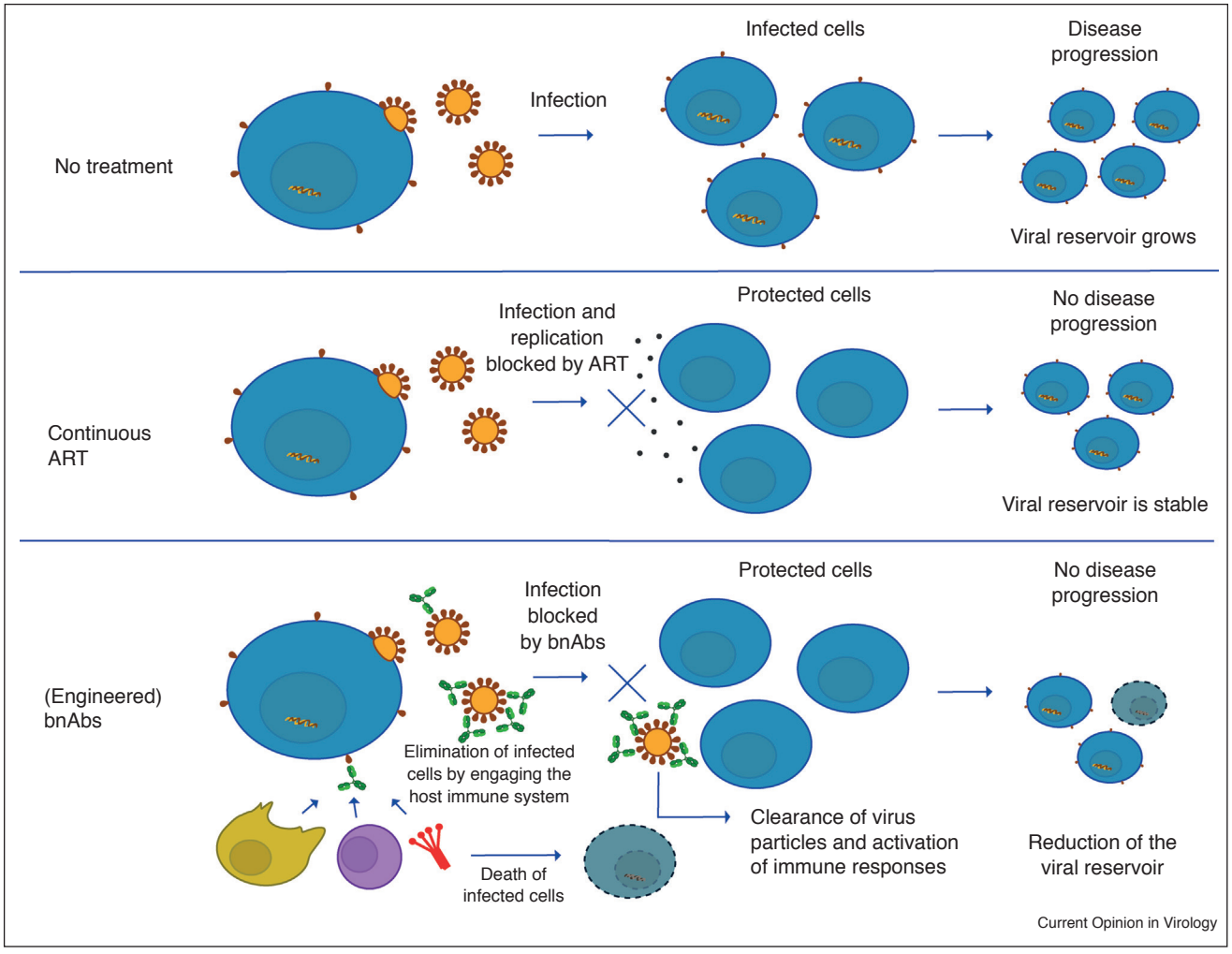
specificities like NK cell or macrophage receptors may also be explored in the future as targets of these small molecules.

The potential of bnAbs for HIV-1 treatment and functional cure

The potential of bnAbs and modified antibodies in the battle against HIV-1 is supported by the substantial amount of research in the field. However, there are several challenges that need to be addressed before bnAbs will be a viable strategy. Allotypic variation of the Fc domain in patients may prevent universal usage of a single allotype antibody treatment due to the risk of an anti-allotype reaction [71]. There is little evidence for this, but development of allotypic bnAbs with lower antigenicity should receive preference in development [88,89,71,90]. In addition, especially when using heavily engineered antibodies, it may be necessary to investigate if there is a higher chance of inducing anti-antibody responses after administration [91•,83•]. Another aspect to consider is the genetic variation in FcγRs of patients, which may prevent universal usage of some effector function enhancing mutations [92,93]. Therefore, it will be important to test the engineered antibodies using preclinical models representing the human FcγR diversity. Finally, antibodies could potentially facilitate antibody-dependent enhancement of HIV-1 and caution has to be taken to circumvent this risk when engineering antibodies [94–97].

A bnAb-based therapy that surmounts these challenges may contribute to a more effective therapy compared to the current treatment standard of life-long ART. Conventional ART is a short-lived therapy requiring daily medication, but longer acting antiretroviral drugs are being developed. Antibody therapy also has a longer half-life, which decreases the frequency of administration and the burden on the patient with lower risk of problems associated with non-adherence to therapy [98]. Antibodies may also be safer in terms of long-term organ toxicities compared to ART [4,99,100]. Most importantly, the ability to engage the host immune system increases the potential effectiveness of the therapy by inducing elimination of HIV-1 infected cells (Figure 3) [7,13]. This valuable property of bnAbs could potentially lead to reduction of the viral reservoir [13], which could be an important step towards the development of a functional cure. However, to achieve a functional cure for HIV-1,

Figure 3



Comparison of untreated HIV-1 infection, treatment with ART and treatment with (engineered) bnAbs. Without treatment, virus replicates and spreads to other cells leading to disease progression. In patients taking (daily) ART medication, virus replication and/or infection is blocked by ART and no disease progression takes place. By administration of bnAbs, infection of new cells is blocked, free virus is cleared and in addition, infected cells may be eliminated by a variety of effector functions activated by the Fc domain of the bnAb. Dark grey dots represent ART, other symbols are explained in the legend of Figure 1.

more research is still needed. Challenges lie mainly in the detection and elimination of all latent reservoirs of HIV-1 infected cells, which are responsible for viral rebound. A potential solution could be the shock-and-kill strategy, which combines compounds that activate latently infected cells with compounds that induce cellular immunity to clear the infection [101,102]. In this strategy, bnAbs could be very suitable candidates to facilitate the killing of the activated infected cells via effector functions.

A viable bnAb strategy for a functional cure will likely contain the following components: multiple target epitopes to prevent viral escape, high potency to allow affordable concentrations of therapeutics, high breadth to ensure efficacy for all patients, a long half-life to increase the effectiveness of the therapy and keep the administration frequency low, activation of effector functions to clear infected cells and optionally the combination with a reservoir-activating component that will ascertain that more HIV-1 infected cells can be cleared.

Conclusion

The recent clinical study using bnAbs for HIV-1 treatment [16••] showed that the potential of bnAbs may go beyond suppression of viral replication alone because bnAbs also have the capacity to reduce the viral reservoir and activate the patient's own immune response. A variety of bnAbs and modified antibodies are being tested in ongoing clinical trials and these are expected to provide

interesting new insights. The discovery of increasingly potent bnAbs and the possibility to engineer bnAbs with optimized potency, breadth, half-life and effector functions, will pave the way towards developing increasingly effective antibody-based therapies for HIV-1. More information on the safety, immunogenicity, options for viral escape and possible side-effects of engineered antibodies is required and may reveal the role of bnAbs in future strategies for treatment and possibly for a functional cure of HIV-1.

Conflict of interest

The authors declare no conflict of interest.

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