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Engineering Antibody-based molecules for HIV treatment and cure.

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Abstract

Purpose of the review: Immunotherapy strategies alternative to current antiretroviral therapies will need to address viral diversity while increasing the immune system's ability to efficiently target the latent virus reservoir. Antibody-based molecules can be designed based on broadly neutralizing and non-neutralizing antibodies that target free virions and infected cells. These multispecific molecules, either by IgG-like or non-IgG-like in structure, aim to target several independent HIV-1 epitopes and/or engage effector cells to eliminate the replicating virus and infected cells. This detailed review is intended to stimulate discussion on future requirements for novel immunotherapeutic molecules.

Recent findings: Bispecific (bs−) and trispecific antibodies (tsAbs) are engineered as a single molecules to target two or more independent epitopes on the HIV-1 Envelope (Env). These Ab-based molecules have increased avidity for Env, leading to improved neutralization potency and breadth compared to single parental Abs. Furthermore, bs- and tsAbs that engage cellular receptors with one arm of the molecule help concentrate inhibitory molecules to the sites of potential infection and facilitate engagement of immune effector cells and Env-expressing target for their elimination.

Summary: Recently engineered Ab-based molecules of different sizes and structures show promise *in vitro* or *in vivo* and are encouraging candidates for HIV treatment.

Keywords

HIV; bispecific; trispecific; Ab-based molecules; cure

INTRODUCTION

The treatment of HIV-1 infection with antiretroviral therapy (ART) has been effective in controlling virus replication, delaying disease progression, and reducing HIV-1 transmission

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Conflicts of interest

G. F. has patents submitted on HIV antibodies and DART® molecules described in this article. M.T. reports no conflicts of interest.

(1). However, life-long daily administration of ART can cause drug-related toxicities (2). Infusion of broadly neutralizing antibodies (bNAbs) as an alternative therapeutic strategy to ART offers several advantages that include lower toxicity, improved pharmacokinetics (3, 4), Fc-mediated effector functions (5-7) to eliminate infected cells, and diversity of treatment options for patients not responding to ART (8, 9). Infusion of single bNAb or combination of two bNAbs which target independent sites on the HIV-1 Envelope (Env) spike, has mediated suppression of viremia (10-13) and delayed virus rebound during analytical treatment interruption (ATI) (8, 10, 11, 13-17). However, it has also been shown that outgrowth of pre-existent resistant viral variants (11-13, 16, 17) or the development of resistance (15, 16, 18) limits the efficacy of bNAb immunotherapy. Therefore, a combination of bNAbs is necessary for broader coverage of the epidemic and to prevent development of escape mutations following treatment pressure (19). Selected individual and combinations bNAbs currently tested in clinical trials are listed in Table 1.

Bispecific (bs−) and trispecific (ts−) bNAbs are single molecules designed to simultaneously bind two or three distinct antigens, respectively. Engineered bsAbs and tsAbs represent a promising alternative to bNAb combination therapy by pursuing multiple targets on the Env protein. This approach may provide increased breadth to overcome HIV's diversity and to cover natural resistance. Moreover, these Ab-based molecules can overcome complications related to infusion of multiple bNAbs that include costs associated with preclinical testing, manufacturing, and delivery. These molecules and their mechanisms of actions are the scope of the current review.

ENGINEERING MULTI-SPECIFIC AB-BASED MOLECULES FOR HIV CURE

Bispecific-Abs are designed to either recognize two distinct HIV-1 Env epitopes via the single chain variable fragments (scFv) of two independent bNAbs or to engage cellular receptors with one scFv and a single HIV-1 Env epitope with another scFv. The scFvs of these molecules are either joined by a single fragment constant region (Fc) to form a traditional Y-shaped Ab structure or are connected via a linker. Thus, bsAb molecules can be separated into a class of IgG-like molecules and a class of non-IgG-like molecules. When designing Ab-based molecules, the optimal molecules for HIV treatment and cure should combine bNAbs that display adequate coverage of the viral swarms representing the latent HIV-1 reservoir (20) and can be present in sufficient concentrations at the site of viral reactivation in order to be effective. Ab-based molecules that have neutralizing function can bind free virions during acute infection, or post LRA-treatment. This will neutralize the virus and prevent re-infection of other target cells. The summary of recently developed Ab-based molecules is shown in Table 1. These molecules were tested against different panels of HIV- isolates with different assay platforms. Therefore, the neutralization breadth and potency or cell-mediated killing of each Ab-based molecule is reflective of the utilized panel. It should be noted that not every molecule was tested for Fc-mediated functions, which could be relevant for eradication of the reservoir (21). The potency of the molecules discussed here is based on their neutralizing functions unless otherwise stated.

IgG-like bsAbs

CrossMAb technology has allowed combining scFvs with a single Fc chain from two distinct Abs to form a traditional Y-shaped Ab structure. “Knob-in-hole” modification of Fc regions favors the formation of heavy chain heterodimers of desired bsAb (22). Meanwhile, the “crossover” of CL and CH1 sequences in one arm of the Ab favors correct heavy (H) and light (L) chain pairings in both arms (22). This allows for the generation of a typical monoclonal Ab (mAb) in terms of mass and architecture with association of the desired H and L chains (23, 24*, 25, 26**) (Figure 1 A-B). Another approach is to exchange the scFv of one bNAb with the scFv of another bNAb (26**), or fuse it to a full length bNAb via a flexible $(G_4S)_n$ linker in a scFv tandem format (27*, 28**) (Figure 1C). Both of these types of bsAbs demonstrated an increase in neutralization breadth and potency compared to the single parental Abs (Table 1). In addition, they have a functional Fc region that can engage Fcγ-receptors-bearing effectors to mediate lysis of HIV-infected target cells or phagocytosis of virions.

Enhancement in neutralization potency appeared to be more pronounced when combining scFvs targeting HIV-1 Env epitopes with those targeting host-cell receptors CD4 or CCR5 using a CrossMAb approach (27*, 29, 30*) (Figure 1B). iMab is a mAb that binds to domain 2 of human cellular receptor CD4, on the opposite side of gp120 and MHC class II binding, and potently inhibits HIV-1 entry via a noncompetitive mechanism (29, 31). PRO 140 is a mAb targeting cellular receptor CCR5 and prevents it from binding to gp120 (32). The proposed mechanism of enhanced potency relies on anchoring of the bsAbs by binding the cellular receptors, to both effectively concentrate inhibitory molecules at the cell surface and to better engage the Env epitopes during virus–cell interaction (29, 33). One bsAb of this class, 10E8.4/iMab, is in a phase 1 clinical trial (NCT03875209, Table 1).

Another format of bsAbs uses the heavy chain of llama-derived heavy-chain-only antibodies (VHH), which are therefore smaller than typical IgG molecules (34*). The small size of VHH (13-15 kDa) allows them to bind the cavities that are difficult to reach for traditional Abs (150 kDa), and for the CDR3 loop to protrude further within the cavity to reach neutralizing epitopes on the Env. These epitopes are hidden on native HIV-1 Env trimers by conformation or glycosylation, and Abs of large size or with short CDR3 length fail to reach these neutralizing epitopes. Using covalently linked VHH with “knob-in-hole” technology also increases potency due to an increase in avidity (35, 36) (Figure 1D). VHH Abs can also be pared with those binding the CD4bs or the co-receptor binding site. The arms of multispecific VHH Abs can be joined by flexible $(G_4S)_7$ linkers to allow plasticity of the molecule and for the arm to reach their target epitopes. Unlike other bsAbs paired with iMab or PRO 140, VHH bsAbs combined with other llama-isolated Abs targeting these receptors, demonstrated increased breadth but not potency (37). FcR-mediated functions of these bsAbs depend on their structure.

Non-IgG-like bsAbs

Besides targeting CD4 and CCR5 receptors necessary for HIV-1 entry, bispecific molecules that engage other cellular receptors have been designed. Bispecific T cell engagers (BiTEs®) bind to CD3 or CD16 with one arm and to HIV-1 Env with another (38) (Figure 1E). A

similar concept, Dual Affinity Re-Targeting (DART®) molecules demonstrated higher K_D with CD3, improved stability and half-life of the molecule due to the disulfide linking of two arms and, therefore, improved ability to engage target CD4 T cells and effector (CD8) cells (39) (Figure 1F, Table 1). *In vitro* studies showed DART molecules retained the neutralization breadth and potency of the Ab component (40, 41). Importantly, in absence of the Fc-region these molecules mediate lysis of HIV-1-infected cells, measured *in vitro* and *ex vivo*. Using αCD3 and αCD16 arms these molecules recruiting cytotoxic CD8+ T cells or Natural Killer (NK) cells, respectively, to the infected cells expressing the HIV-1 Env, leading to elimination of HIV-infected CD4 T cells (40-43). In addition, these molecules are smaller in size compared to traditional Abs, have better potential to penetrate tissues and a reduced production cost. Currently, enrolment into a phase 1 clinical trial with MGD014 DART, which targets the C1C2 HIV-1 Env epitope with one arm and CD3 with the other, is ongoing (NCT03570918). Nevertheless, the original BiTEs® and DART® molecules had limited *in vivo* pharmacokinetics (bioavailability, solubility, stability, and half-life) compared to traditional Abs (44, 45). To improve half-life, MGD011 DART® intended for B-cell malignancies, was engineered with Fc region (46). Similar approach can potentially be utilized in the design of anti-HIV DART molecules. The BiTE® Blinatumomab, intended for treatment of acute lymphoid leukemia, has been reported to induce immune activation by cytokines (47). This is one of the most serious side effects, although new technologies allow the production of BiTE® molecules with improved pharmacokinetic properties and decreased toxicity (48).

Another format of bsAbs is a tandem single chain variable fragment (scFv1- scFv2). These bsAbs are similar in structure to BiTE® or DART® molecules but target two distinct HIV-1 antigens with each arm (Figure 1G). These bsAbs demonstrated increased neutralization breadth and potency compared to the parental Abs (27*, 28**). However, have poor pharmacokinetics, and do not have an Fc region and thus they lack FcR-mediated function necessary to eliminate infected cells.

Trispecific Abs

In the past three years, several novel tsAbs have been designed (27*, 28**, 38**, 49). A tsAb targeting MPER, V3 glycan and V2 apex 10E8Fab- PGT121fv-PGDM1400fv.V8.4DS, known as SAR441236, protected non-human primates (NHP) against a mucosal challenge with multiple SHIVs, demonstrating superior breadth compared to the parental bNAbs (49**). This tsAb was designed as a PGDM1400 Ab with one Fab was switched to the VRC01 Fab, and the scFv of the other PGDM1400 Fab was linked to the scFv of 10E8.4 in a reverse-order tandem-forming Cross-Over Dual Variable (CODV) Ig (Figure 1H). SAR441236 is currently being tested in a phase 1 clinical trial (NCT03705169). The enhancement in neutralization potency of tsAbs was attributed to improved avidity that allows for simultaneous epitope engagement on the same Env (25, 28**). TsAbs that have an Fc region or αCD3 arm are also able to recruit effector cells and mediate killing of HIV-1-infected cells.

It is important to note that non-IgG-like bsAbs and tsAbs that lack Fc region are cleared from the body by renal cells (50) or undergo FcRn-mediated recycling (51). The unnatural

architecture of many scFv-format Ab-based molecules may also lead to anti-drug antibody responses *in vivo*.

Design challenges: spatial orientation and linker length

Not all HIV-1 Envs expressed on the surface of infected cells are trimers. Therefore, the scFvs that target epitopes based on trimeric assembly of HIV-1 Env will have limited efficacy based on trimeric Env expression (26**). In addition, Env detected on the virion or infected cell surface is sparse and not evenly distributed. Thus, it is possible that two Envs will never be in close enough proximity to be engaged by one bs- or tsAbs molecule at the same time and rather there will be engagement of different epitopes on the same Env (52, 53). Proximity of epitopes is important when engineering bsAbs or tsAbs: the linker between the arms ensures the proper binding of epitopes and the orientations of the VH and VL regions of the scFvs in the bs- or tsAbs can influence the potency of such molecules. The linker length must be sufficiently flexible to allow each arm to bind its epitope but not impair folding and assembly of the rest of the molecule, nor to be cleaved during manufacturing or by the host proteases. Several studies have reported on modifications in the linker between scFvs (49**), as well as with the rest of the molecule (23, 26**, 29, 34*, 54, 55).

Fc-mediated function of Ab-based molecules

In addition to preventing primary infections, bsAbs can prevent cell-to-cell transmission, which likely mediates a significant fraction of viral spread (56). Thus, targeting the cell receptor with one arm could potentially block cell-mediated spreading of infection. Abs can also eliminate infected cells via Fc-mediated functions that include antibody dependent cellular cytotoxicity (ADCC), antibody dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC) and, perhaps, trogocytosis. ADCC activities have been correlated with slow disease progression in HIV-1-infected individuals (57-60). In addition, the Fc region has been shown to enhance protective efficacy *in vivo* (5, 61). Therefore, the Fc region was added to several non-IgG-like molecules to improve overall therapeutic potential of the molecule, such as DART® A32xCD3 MP3 and scFv tandem-Fc 10E8Fab-PGT121fv-PGDM1400fv.V8 (Table 1).

An important factor to consider in the design of Ab-based molecules is modifications in the Fc region to improve Fc-mediated functions of Abs (62). Among those modifications are the triple S298A/E333A/K334A (AAA) (63) and S239D/I332E/A330L (64) amino acid mutations previously reported to augment antibody ability to bind to FcγRIIIa and to enhance ADCC activity. Ramadoss et al. demonstrated fusion of the Fc region of anti-HIV Abs to the scFv of the anti-CD16 Ab, NM3E2, which increased binding to FcγRIIIA on NK cells with subsequent enhancement in killing of infected cells (65). In addition, utilization of an IgG3 Fc backbone instead of IgG1, 2 or 4 allows greater flexibility due to a longer hinge region of IgG3 (23, 66, 67): artificial modification of hinge length has been shown to increase ADCP function of Abs (68). Lastly, glycosylation of the Fc region has also been shown to increase Ab effector functions (69-72). Besides effector function, M428L/N434S (referred to as LS) mutations in Fc improves *in vivo* stability and the half-life of Abs (3).

Targeting tissue reservoirs of HIV

Several studies have shown that B cell follicles, and germinal centers (GCs) in particular, are major sites for HIV-1 reservoir establishment (73). Low levels of HIV-1 replication in lymphatic tissues may also contribute to the persistence of the HIV-1 reservoir (74-76). To overcome this low level expression in the tissues, Latency Reversing Agents (LRAs) have been identified and used to induce proviral transcription in latently infected cells (41, 77) with consequent expression of viral antigens on the cell surface that can be targeted by cytotoxic effector cells. Originally termed the ‘shock and kill’ strategy, this combination of LRAs, ART and virus-induced immune responses has proven to be limited by the ability of the LRAs to induce sufficient virus replication and/or of the cytotoxic effector cells to reach the sites of replications (78, 79). Therefore, the efficacy of Ab-based immunotherapies depends on the recruitment of effector cells in immunologically privileged areas. Besides blood (80) and lymphoid tissues, the HIV-1 reservoir may be found in spleen (81), adipose tissue (82), gut (83), bone marrow (84, 85), CNS (86, 87), lungs (88), kidney (89) and in reproductive organs (90, 91) (Figure 2). ART can penetrate these tissues to various degrees and prevent viral replication but will not eliminate the HIV-1-infected cells (92). Ab-based molecules with effector functions could have a potential to engage tissue resident effector cells (Figure 2) to eliminated infected cells (93-113). In addition to whole virions, shed HIV Env gp120 monomers have been reported to accumulate in lymphoid tissues and other organs during chronic HIV-infection (114). The impact of Abs and Ab-based molecules binding to shed gp120 has not been reported and could be addressed upon completion of ongoing clinical trials with individual or combinations of bNAbs for HIV prevention and treatment. When designing novel Ab-based molecules, the binding to shed gp120 and other “off target” effects should be considered and evaluated in *in vitro* and *in vivo* models.

CONCLUSION

Engineered Ab-based molecules for treatment of HIV have advantages over single Ab or combination of Abs that are mainly related to the ability to target different epitopes using a single molecule which increases breadth and prevents viral escape. These molecules will also provide advantages related to cost effective production and clinical administration, including infusion platforms and dose intervals. Small Ab-based molecules may have improved delivery to the tissues, where reservoir-bearing cells are concentrated. Ab-based molecules will ultimately be required to engage effector cellular subsets at sites of virus replication for improved viral clearance. A critical aspect of the efficacy of engineered antibody-based immunothereapy for cure of HIV-1 will depend on new and improved strategies aimed at reactivating the integrated virus in order to detect the infected cells (115).

It is possible, that one molecule may not have all the necessary features to improve beyond traditional mAbs. Therefore, it will be important to further analyze combinations of IgG-like and non-IgG like Ab-based molecules with complementary functions and different structures to achieve a functional cure.

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Key points

- Abs-based molecules are promising therapeutic candidates for a long-term control and treatment of HIV-1 infection
- bsAbs or tsAbs demonstrate increased antiviral potency compared to single parental Abs
- Engagement of cell-mediated immunity is crucial for elimination of infected cells
- Combination of Ab-based molecules with complementary functions should be considered for HIV therapy and cure

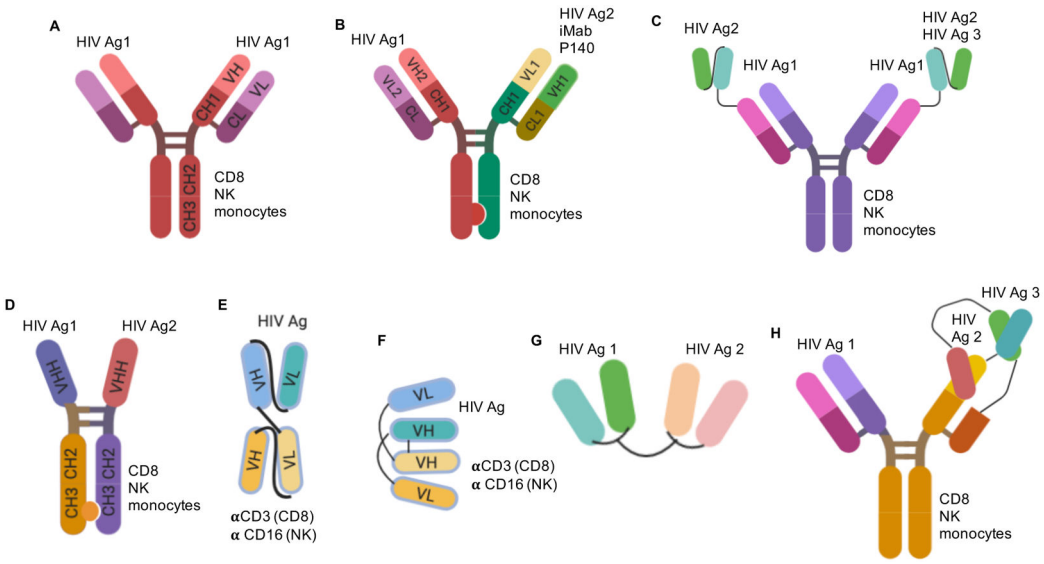

Figure 1. Structures and functions of multi-specific Ab-based molecules.

These constructs represent bsAbs and tsAbs that simultaneously bind with antigen-binding domain to multiple independent HIV-1 antigens (HIV Ags) on virions or HIV-1-infected target cells and/or cellular receptors. Most of the presented constructs (with an exception of **G**) are able to bind to effector cells and induce cell-mediated effector functions. The binding to effector cells is facilitated via Ab Fc region (CD8, NK and/or monocytes) or αCD3 and αCD16 arms to recruit CD8 and NK cells, respectively. (**A**) classical IgG, (**B**) CrossMAb with "knob-in-hole" mutations, (**C**) scFv-(G4S)n-IgG, (**D**) VHH CrossMAb, (**E**) BiTE® (Bi-specific T cell Engager), (**F**) DART® (Dual Affinity Re-Targeting molecule), (**G**) scFv-scFv, (**H**) CODV-Ig. Created with BioRender.com.

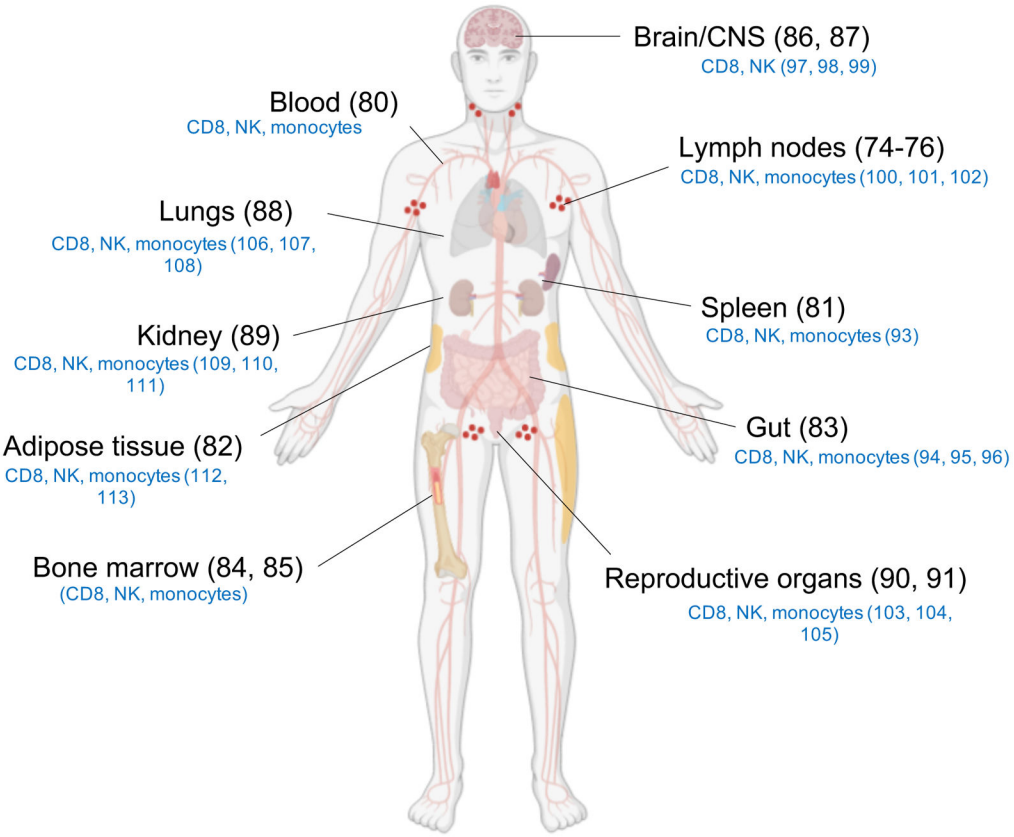

Figure 2. Tissue reservoirs of HIV.

Accumulation of HIV tissue reservoir. Resident effector cells (indicated in blue) that may be utilized in cell-mediated lysis of infected target cells. Created with BioRender.com.

Table 1.

Antibodies and antibody-based molecules in clinical trials and preclinical development.

Type	bNAbs	Epitope	Clinical trial number			Interaction with cellular receptor	Effector cells recruitment	Phase	
mAbs in clinical trial									
mAb	VRC01	CD4bs	NCT02591420			Fc	CD8, NK, monocytes	1, Recruiting	
mAb	VRC07-523LS	CD4bs	NCT03739996			Fc	CD8, NK, monocytes	2, Recruiting	
2 mAbs	VRC01+10-1074	CD4bs+V3 glycan	NCT03831945			Fc	CD8, NK, monocytes	1, Recruiting	
2 mAbs	VRC01+10-1074	CD4bs+V3 glycan	NCT03707977			Fc	CD8, NK, monocytes	1, 2, Recruiting	
2 mAbs	PGT121+VRC07-523LS	V3 glycan+CD4bs	NCT03721510			Fc	CD8, NK, monocytes	1/2a Recruiting	
2 mAbs	3BNC117+10-1074	CD44bs+V3 glycan	NCT03571204			Fc	CD8, NK, monocytes	1, Recruiting	
3 mAbs	PGT121+VRC07-523LS +PGDM1400	V3 glycan+CD4bs+V2 apex	NCT03721510			Fc	CD8, NK, monocytes	1/2a Recruiting	
Type	**bNAbs**	**Epitope**	**# of HIV isolates in panel**	**Neutr/ killing IC_{50}, µg/mL**	**Breadth, %**	**Interaction with cellular receptor**	**Effector cells recruitment**	**Size, kDa**	**Ref.**
IgG-like									
CrossMAb	3BNC117/10-1074	CD4bs/V3 glycan	219	0.11	95.8	Fc	CD8, NK, monocytes	160-180	(23)
	PG16/10-1074	V2 glycan/V3 glycan	219	0.192	87.5	Fc			
	PG16/PGT121	V2 glycan/V3 glycan	219	0.207	91.4	Fc			
	PG16/PGT128	V2 glycan/V3 glycan	219	0.167	88	Fc			
	PGT151/35O22	(gp120-gp41 interface)x2	219	0.22	72.5	Fc			
	3BNC117/PGT135	CD4bs/V3 glycan	219	0.036	93.3	Fc			
	8ANC195/PGT128	gp120-gp41 interface/V3 glycan	219	0.0844	90.5	Fc			
	PGT151/10-1074	gp120-gp41 interface/V3 glycan	219	0.041	85.7	Fc			
CrossMAb	Cap256.VRC26/10-1074 (BICM-1A)	V2 glycan/V3 glycan	15	n.i.	n.i.	Fc	CD8, NK, monocytes	150	(26**)
scFv-Fc	Cap256.VRC26/10-1074 (BISC-1A)	V2 glycan/V3 glycan	15	0.0235	66.7	Fc	CD8, NK, monocytes	150	(26**)
	Cap256.VRC26/PGT121 (BISC-1B)	V2 glycan/V3 glycan	15	0.0246	73.3	Fc			

Type	bNAbs	Epitope	Clinical trial number			Interaction with cellular receptor	Effector cells recruitment	Phase	
	Cap256.VRC26/PGT128 (BISC-1C)	V2 glycan/V3 glycan	15	0.0127	80	Fc			
	PGT145/10-1074 (BISC-2A)	V2 apex/V3 glycan	9	0.051	88.9	Fc			
	PGT145/PGT121 (BISC-2B)	V2 apex/V3 glycan	9	0.162	22.2	Fc			
	PGT145/PGT128 (BISC-2C)	V2 apex/V3 glycan	9	0.022	88.9	Fc			
CrossMAb	VRC01/10E8	CD4bs/MPER	206	0.187	30	Fc	CD8, NK, monocytes	150	(25)
	VRC01/PGT121	CD4bs/V3 glycan	206	0.092	48	Fc			
	VRC01/PG9-16	CD4bs/V2 glycan	206	0.048	56	Fc			
	10E8/PG9-16	MPER/V2 glycan	206	0.123	43	Fc			
scFv-(G4S)n-IgG	VRC01/PGT121	CD4bs/V3 glycan	208	0.38	95.1	Fc	CD8, NK, monocytes		(28**)
CrossMab	iMab-CAP256	**α**CD4/V2 glycan	20	0.00079	100	single Fc chain	reduced, CD8, NK, monocytes	190	(30*)
	10E08-iMab	MPER/ **α**CD4	14	0.0026	100	single Fc chain			
	PG9-iMab	V2 glycan/ **α**CD4	14	0.0079	92.8	single Fc chain			
CrossmAb	10E8/iMab (NCT03875209)	MPER/**α**CD4	118	0.002	100	single Fc chain	reduced, CD8, NK, monocytes	150	(33)
	PGT145/iMab	MPER/**α**CD4	n.i.	n.i.	n.i.	single Fc chain			
	3BNC117/iMab	MPER/**α**CD4	n.i.	n.i.	n.i.	single Fc chain			
	PGT128/iMab	MPER/**α**CD4	n.i.	n.i.	n.i.	single Fc chain			
	PGT151/iMab	MPER/**α**CD4	n.i.	n.i.	n.i.	single Fc chain			
CrossmAb	10E8/P140	MPER/**α**CCR5	118	0.001	99	none	None	150	(33)
	PGT145/P140	MPER/**α**CCR5	n.i.	n.i.	n.i.	none			
	3BNC117/P140	MPER/**α**CCR5	n.i.	n.i.	n.i.	none			
	PGT128/P140	MPER/**α**CCR5	n.i.	n.i.	n.i.	none			
	PGT151/P140	MPER/**α**CCR5	n.i.	n.i.	n.i.	none			
scFv-(G4S)n-IgG	PG9-iMab	V2 glycan/**α**CD4	118	0.004 (<10μg/mL)	100	none	none	204	(29)
	PG9-PRO	V2 glycan/**α**CCR5	118	n.i.		none			
	PG16-iMAbs	V2 glycan/**α**CD4	118	0.0027 (<10μg/mL)	100	none			
VHH CrossMAb	J3/2E7	CD4bs/gp41	8	n.i.	n.i	Fc	CD8, NK, monocytes	80	(34*)

Type	bNAbs	Epitope	Clinical trial number			Interaction with cellular receptor	Effector cells recruitment	Phase	
DART®	A32xCD3 MP3	CD4i	22	n.i.	n.i.	Fc	CD8, NK, monocytes	107	(40**)
non-IgG-like									
DART®	A32xCD3	CD4i	22	160–230 pg/ml	n.i.	**α**CD3	CD8	56	(40**, 42**)
	7B2xCD3	gp41	22	160–230 pg/ml	n.i.	**α**CD3			
	PGT121xCD3	V3 glycan	22	n.i.	n.i.	**α**CD3			
	PGT145xCD3	V2 apex	22	n.i.	n.i.	**α**CD3			
	VRC01xCD3	CD4bs	22	n.i.	n.i.	**α**CD3			
	10E8xCD3	MPER	22	n.i.	n.i.	**α**CD3			
BiTE®	CD4-CD3	CD4	1	0.048	n.i.	αCD3	CD8	54	(38**)
	VRC01-CD3	CD4bs	1	2.86	n.i.	αCD3			
	B12-CD3	CD4bs	1	0.0038	n.i.	**α**CD3			
scFv-Fab	10E8-N6	MPER/CD4bs	109	0.0685	100	none	none	54	(27*)
	m36.4-PRO	gp120 C3/**α**CCR5	117	0.0131	100	none		75	
	PGDM1400-PRO 140	V2 apex/**α**CCR5	117	0.0225	96	none		54	
Bi-scFvs	PGT121-VRC01	V3 glycan/CD4bs	208	0.4	94.7	none	none	54	(28**)
tsAb									
CrossMAb-scFv	VRC01-PGT121-10E8	CD4bs/V3 glycan/ MPER	208	0.071	99.5	Fc	CD8, NK, monocytes	200	(28**)
scFv tandem-Fc	10E8Fab-PGT121fv-PGDM1400fv.V8	MPER/V3 glycan/V2 apex	27	0.1116	89	Fc	CD8, NK, monocytes	150	(27*)
scFv tandem	10E8-PGT121-PGDM1400	MPER/V3 glycan/V2 apex	117	0.0135	99	none	none	100	(27*)
	PRO 140-PGDM1400-PGT121	**α**CCR5/V2 apex/V3 glycan	117	0.026	97	none		100	
	10E8-PGDM1400-PRO	MPER/V2 apex/**α**CCR5	117	0.071	98	none		100	
CODV-IgG	VRC01/PGDM1400-10E8 (NCT03705169)	CD4bs/V2 apex/MPER	208	0.039	98	Fc	CD8, NK, monocytes		(49**)
	N6/PGDM1400-10E8v4	CD4bs/V2 apex/MPER	208	0.02	99.5	Fc			
BiTE®	CD4-17b-CD3	CD4/**α**CCR5	1	0.037		**α**CD3	CD8	80	(38**)

IC50, the half maximal inhibitory concentration. BiTE, bispecific T-cell engager; bNAbs, broadly neutralizing antibodies; Fc, fragment crystallizable region; n.i.-not indicated; NK, natural killer; scFv, single-chain fragment variable; tsAb, trispecific antibody; MPER, membrane-proximal external region.