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Original research

Bispecific antibody-derived molecules to target persistent HIV infection

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A B S T R A C T

HIV infection persists despite durable and potent antiviral therapy. To target persistent HIV infection, one major strategy aims to induce HIV provirus expression using latency reversing agents and then eliminate these reservoir cells via immune responses enhanced by treatment with antibody-derived bispecific molecules. The specificities of anti-HIV-1 envelope monoclonal antibodies have been incorporated into bispecific molecules that can recognize infected cells and recruit cytotoxic immune cells to eliminate them. This concept seeks to engineer a unique and potent effector response based on the opportunity to target conserved viral epitopes on infected cells, and recruit broad populations of immune effector cells that are not limited by major histocompatibility complex restrictions or other programmed specificity constraints. This article provides a review of bispecific DART® molecules and other dual-specificity antibody-based molecules that function by co-engaging CD3-expressing T cells or CD16A-expressing NK cells with HIV-1-infected cells.

1. Introduction

Antiretroviral therapy (ART) has drastically reduced the morbidity and mortality of HIV infection, but generally must be continued indefinitely, as following treatment interruption humoral and cellular components of the immune system generally fail to prevent viral rebound and disease progression. Several strategies to facilitate the clearance of infection that persists despite ART, or to allow control of viral rebound following ART interruption, are under development and discussed in the accompanying reviews. To target these latently-infected cells, one major strategy aims to induce HIV provirus expression using latency reversing agents (LRAs) in order to enhance their elimination by immune effector cells. In this review we will discuss how anti-HIV-1 envelope (Env) monoclonal antibodies (mAbs) have been leveraged to develop bispecific molecules that can recognize infected cells and recruit cytotoxic immune cells to eliminate them. This concept seeks to engineer a unique and potent effector response based on the opportunity to target conserved viral epitopes on infected cells, and recruit broad populations of immune effector cells that are not limited by major histocompatibility complex (MHC) restrictions or other programmed specificity constraints.

The opportunity to leverage mAbs to control or clear HIV infection arises from the observation that both polyclonal and monoclonal Abs can control virus replication (reviewed in Ref. 1). Polyclonal Ab responses which can mediate Fc-dependent functions for engaging immune effector cells have been reported to control virus replication[2–4]; similarly, broadly neutralizing monoclonal Abs (bnmAbs) have also been able to control virus replication together with reducing the size of the pool of latently infected cells in animal models and recent human studies.[5–9] In addition to controlling viremia, the passive infusion of bnmAbs could also prevent virus replication in the weeks and years following their last infusion. In preclinical studies conducted in rhesus monkeys infected by chimeric simian/human immunodeficiency virus (SHIV), the infusion of bnmAbs early after infection resulted in control of viremia that could last for years and was associated with emergence of CD8 T cell responses[10,11] that could contribute to suppression of virus replication. Similar long-term effects on control of HIV-1 replication were observed up to 20 weeks following infusion of bnmAbs in cART-suppressed PLWH upon treatment interruption with concomitant reduction in the size of the latent reservoir.[12] Although the mechanism for this long-lasting control in PLWH is still unclear, the preclinical and clinical studies suggest that passive bnmAbs may positively impact the endogenous immune responses resulting in spontaneous control of virus replications. However, individual bnmAbs have thus far been found to be incompletely effective in preventing infection due to the extensive diversity of HIV-1 isolates circulating within people living with HIV-1 (PLWH) that are the source of viral transmission[13]; and furthermore, they are also incompletely effective at controlling viremia within

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individuals living with HIV due to the selection of resistant variants during mAb therapy.[14,15] The presence of pre-exisiting HIV-1 isolates that are less susceptible to the neutralizing potency of individual bnmAbs has also been reported.[15,16] These findings have prompted current efforts to establish combinations of bnmAbs with optimal neutralizing breadth against HIV-1 species[16–18] and enhanced Fc-mediated functions.[19]

Over the years, we have come to appreciate the significant contributions that cytotoxic cellular subsets of the immune system can make to curb HIV-1 infection. Early studies indicated the relevant association of the appearance of HIV-specific $CD8^+$ T cell responses with decline in the plasma virus load (reviewed in Ref. 20); these initial observations were further expanded by identifying their epitope specificities and characterizing the immune pressure exerted against the replicating virus that contributed to the appearance of escape mutants.[21–26] In PLWH expressing selected human leucocyte antigen (HLA) class I alleles, these antiviral responses were found to be associated with durable control of viremia without progression to immunodeficiency in the absence of antiretroviral treatment, e.g., long-term non-progressor status with low but detectable viremia[27] or elite controller status with undetectable viremia.[28] Further, investigation of the role of $CD8^+$ T cells in rhesus monkeys infected with simian immunodeficiency virus (SIV) or SHIV revealed that $CD8^+$ cell depletion resulted in loss of control of virus replication, further supporting the anti-viral function of $CD8^+$ T cells.[29] In addition, natural killer (NK) cells also contribute to the control of virus replication during acute infection.[30,31] It should be noted that NK cells are the effector cells of choice recruited by Abs capable of mediating antibody-dependent cellular cytotoxicity (ADCC) to confer protection or control of virus replication in pre-clinical vaccine studies.[32]

However, efforts thus far to engage $CD8^+$ T-cell effectors to clear persistent HIV infection by inducing $CD8^+$ T-cell responses with an HIV therapeutic vaccine, or by administering $CD8^+$ T cells themselves, have so far had limited success.[33,34] Similarly, administering bnmAbs to target persistently infected cells and eliminate them via Fc-mediated mechanisms has thus far resulted in minimal effects.[35] The high affinity of binding, the wide breadth of reactivity, and the potent neutralizing activity of bnAbs are features that stimulated the design of bispecific antibody-derived molecules capable of recognizing HIV-1-infected cells and engaging T or NK cells to form signaling synapses that lead to clearance of the infected cells. The importance of recruiting these effector cell subsets is strongly supported by the ability of immune effector cells to counteract natural virus infection, illustrating their ability to converge on sites of active virus replication. In this article, we will provide a review of bispecific DART® molecules and other dual-specificity antibody-based formats that function by co-engaging immune effector cells with HIV-1-infected cells, and thus are promising immunotherapeutic agents for use against persistent HIV-1 infection.

2. CD3-engaging DART molecules

Anti-HIV-1 Env x anti-CD3 bispecific molecules are designed to redirect T cells against HIV-1-infected Env-expressing cells. One specificity targets the HIV-1 envelope protein, the only viral-encoded protein expressed on the surface of HIV-1-infected cells. The other specificity targets human CD3ε. CD3 is an invariant complex of proteins (consisting of one γ chain, one δ chain, two ε chains and two ζ chains) that associate with the T cell receptor (TCR) to form the TCR complex, which is responsible for signal transduction and activation of cytotoxic and helper T cells. CD3 is expressed almost exclusively by T cells and is present on all stages of T cell development.

Bispecific DART proteins are constructed in a diabody format in which each antigen binding domain (Fv) is formed by the noncovalent association of a VL partner on one chain with a VH partner on the second chain, with the overall structure stabilized by a covalent carboxyl terminal disulfide bridge (Fig. 1).[36,37] A crystal structure of a DART molecule shows that the disulfide-stabilized diabody assembles into a compact spherical structure that differs considerably from previously published diabody structures which are more extended, flexible and have the antigen binding sites facing opposing ends of the molecule.[38] The two antigen binding sites of the DART molecule, in contrast, are separated from each other by approximately 30 Å and are facing away from each other at an angle of ~90°.[39] The orientation and short distance between the antigen binding domains of the DART protein appears to allow for more efficient $CD3^+$ T cell-target cell synapse formation and contribute to an increase in potency of T-cell directed lysis compared to other bispecific antibody formats, such as the BiTE format.[36]

The initial strategy to leverage mAbs to design first generation bispecific DART molecules was based on anti-HIV-1 envelope (Env) mAbs that were non-neutralizing, able to mediate potent ADCC, and directed against highly conserved epitopes. The choice of a non-neutralizing mAb as the anti-HIV-1 arm of the DART molecule was intended to focus it on its critical target (viral antigen on the surface of the infected cell), and to minimize its capture by free virions, which could reduce availability or

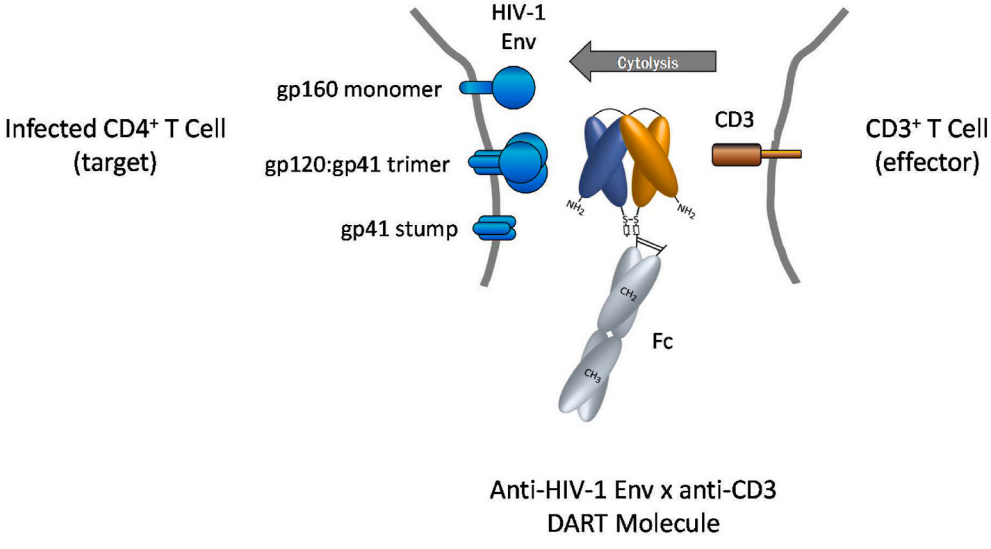

Fig. 1. DART molecule structure and mechanism for redirected $CD3^+$ T cell-mediated cytolysis of HIV-1-infected Env-expressing cells. The anti-CD3 arm (orange) binds to CD3 (brown) at the surface of $CD3^+$ T cells (effector cell), and the anti-HIV-1 env arm (dark blue) binds to HIV-1 Env at the surface of HIV-1 infected $CD4^+$ T cells (target cell). Cell surface HIV-1 Env glycoprotein (light blue) may be in the form of functional mature trimers or nonfunctional variant forms such as gp160 monomers or gp41 stumps. DART molecule-mediated co-engagement of target and effector cells results in activation of $CD3^+$ T cell-mediated cytolytic responses against Env-expressing target cells. The human IgG1 Fc domain (grey), which is inactivated for FcγR binding but retains FcRn binding, is included to confer an extended circulating half-life in vivo. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

possibly facilitate undesired viral entry into $CD3^+$ T cells. The A32 mAb-based DART molecule recognizes a CD4-inducible (CD4i) epitope (epitope cluster A located in the C1 and C2 regions of the gp120 subunit of HIV-1 Env). The 7B2 mAb-based DART molecule recognizes an epitope in the cluster I (or loop) region of the gp41 subunit, a more membrane-proximal epitope that allows recognition of gp41 'stumps', which form upon dissociation of gp120 subunits. Both epitope sequences are highly conserved across all HIV-1 subtypes, which provides strong evidence that they are obligatory for virus function.[40] Although the expression of Nef and Vpu viral gene products can downregulate the expression of CD4 and limit the accessibility of CD4i epitopes,[41] it has been shown that these epitopes can be targeted during virus entry and at the time of virus budding.[42,43] The presence of nonfunctional variant forms of Env on the cell surface, such as gp160 monomers or gp41 stumps (Fig. 1;[44]), also could facilitate recognition by the A32 and 7B2 specificities.

An initial in vitro study by Sung and collaborators[37] demonstrated the ability of the A32 x anti-CD3 and 7B2 x anti-CD3 DART molecules to bind cells infected with different HIV-1 infectious molecular clones expressing subtype AE, B, and C envelopes. These DART molecules were also able to bind to $CD8^+CD3^+$ T cells. Importantly, T-cell activation and cytolytic activity was completely dependent on the DART molecule-mediated coengagement of the HIV-infected target cells with the $CD3^+CD8^+$ T-cell effector cells.[37] A key finding was the ability of the DART molecules to increase the elimination of latently infected cells obtained from ART-treated PLWH by autologous $CD8^+$ cytotoxic T lymphocytes (CTL) upon reactivation of virus replication using PHA stimulation or vorinostat treatment.[37] Thus, $CD8^+$ T cells from PLWH, despite having a potentially exhausted phenotype, were effectively redirected by the DART molecules. Indeed, subsequent studies have demonstrated that $CD8^+$ T cells from healthy people or from PLWH were comparable in their ability to mediate DART molecule activity in vitro (unpublished results). In all experiments, the DART molecules were active at picomolar concentrations, consistent with their suitability for clinical utilization. These findings strongly supported the hypothesis that these bispecific molecules could contribute to strategies to eradicate the latent reservoir.

In a follow up study, Sloan and collaborators evaluated anti-HIV-1 Env x anti-CD3 DART molecules with anti-HIV-1 Env specificities based on bnAbs.[45] In addition to confirming the potency of the A32 and 7B2 mAb-based DART molecules, their data demonstrated that DART molecules based on PGT121 (specific for variable region 3 of gp120) or PGT145 (specific for variable region 2 of gp120) were similarly potent as the A32 and 7B2-based DART molecules in redirecting $CD8^+$ T cells to lyse autologous resting infected $CD4^+$ T cells. Conversely, DART molecules based on VRC01 (specific for CD4 binding site of gp120) or 10E8 (specific for the membrane-proximal external region (MPER) of gp41) were inefficient in their ability to redirect $CD8^+$ T cells to kill infected $CD4^+$ T cells. These findings reflected earlier observations that among the bnmAbs, those recognizing the apex domains of the HIV-1 envelope were among those with the highest ADCC potency.[46,47] Similar to Sung and collaborators,[37] Sloan et al. evaluated the potency of DART molecules to target both resting and reactivated infected $CD4^+$ T cells obtained from eight ART-suppressed PLWH. The authors observed that the release of viral antigen detected after 14 days of culture of unstimulated PBMCs in the presence of DART molecules decreased in every culture with a range from 17% to 96% compared to an inactive control DART molecule. Moreover, the combination of DART molecules based on the PGT121 and 7B2 mAbs always provided the greatest combined effect. An additional key finding was the demonstration that DART molecules based on A32 non-neutralizing mAb or PGT121 bnmAb did not induce cell-to-cell virus spread in resting or activated $CD4^+$ T-cell cultures. This study also reported the evaluation of DART molecules linked to an effector-deficient Fc domain (for the purpose of enhancing the molecule's circulating half-life), demonstrating that both Fc-less and Fc-bearing DART molecules had comparable potency in redirecting $CD3^+$ T cells to lyse HIV-1 infected target cells. Comparison of pharmacokinetics of basic and Fc-bearing DART molecules in human FcRn transgenic mice revealed that the Fc domain conferred an increase in circulating half-life from a few hours to 70 h.

3. Preclinical and clinical studies with MGD014 and MGD020

MGD014 (A32 x anti-CD3) and MGD020 (7B2 x anti-CD3) are Fc-bearing DART molecules that are intended to be used in combination to maximize breadth of reactivity. The combination of the two DART molecules consistently and potently redirects $CD8^+$ T cells to kill $CD4^+$ T cells infected by diverse HIV-1 isolates in vitro (unpublished data). In vivo antiviral activity is being evaluated in HIV-1-infected humanized BLT mice on ART. Because the anti-HIV-1 specificities of MGD014 and MGD020 are non-neutralizing, these studies are designed to directly demonstrate the ability of these DART molecules to clear HIV-1-infected cells in vivo, and to assess the potential for the MGD020+MGD014 combination to mediate enhanced clearance activity in vivo.

No unexpected reactivities were observed when MGD014 and MGD020 were evaluated in good laboratory practice (GLP) human tissue cross-reactivity studies or when they were screened against a comprehensive array of human membrane and secreted proteins (unpublished data). Thus, the risk of off-target effects with these DART molecules is extremely low. A GLP toxicology study with MGD014 was conducted in cynomolgus monkeys.[48] While the anti-HIV-1 Env arm lacks a target in these animals, the anti-CD3 arm cross-reacts with cynomolgus monkey CD3 with nearly equivalent affinity. MGD014 was administered by IV infusion at 0.1, 1 or 10 mg/kg weekly for 6 weeks. The infusions, which resulted in mean C_{max} serum concentrations of 2, 19 or 180 μg/mL, were well tolerated with no adverse events. Dose-dependent binding of MGD014 to circulating monkey $CD4^+$ and $CD8^+$ T cells was observed, but these interactions did not lead to induction of activation markers on circulating T cells or increases in serum cytokines. Thus, monovalent binding of the DART molecule to CD3 did not trigger T-cell activation even when CD3 occupancy was near maximal. This is consistent with the dependency of T-cell activation by DART molecules on the co-engagement of antigen-expressing target cells and CD3-expressing T cells.

Similar results were observed when MGD014 or MGD020 were administered by IV infusion at a dose of 1 mg/kg weekly for 6 weeks to SHIV-infected rhesus monkeys maintained on ART.[49] MGD014 or MGD020 exhibited binding to circulating monkey $CD4^+$ or $CD8^+$ T cells without causing increases in T-cell activation or serum cytokines. However, given alone, the MGD014 or MGD020 treatments did not lead to decreases in persistent virus infection parameters, such as cell-associated viral RNA or time to rebound in viremia following ART interruption. The absence of noticeable indications of clearance activity in these animals is most likely due to the low frequency of Env-expressing target cells when SHIV-infected animals are maintained on long-term ART, particularly in this model system wherein the viral reservoir of persistent infection is of low frequency. These data highlight the need to combine DART molecules with latency reversing agents in future in vivo animal model systems, and in pilot human studies.

The strategy of utilizing of anti-HIV-1 Env x anti-CD3 DART molecules in combination with an LRA to reduce the latent reservoir was recently studied in the rhesus monkeys.[50] The study assessed the ability of a cocktail of 3 DART molecules (based on the A32, 7B2 and PGT145 mAbs) combined with AZD5582, an inhibitor of apoptosis (IAP) antagonist that activates the noncanonical NF-κB pathway, and acts as an LRA. Animals were infected with SHIV C·CH505.375.dCT and then fully suppressed with ART (tenofovir/emtricitabine/dolutegravir). After 42 weeks of ART, 8 SHIV-infected monkeys received DART molecules for 10 weeks at weekly intervals, in concert with weekly receipt of AZD5582. In contrast to the prior study in SIV-infected rhesus monkeys,[51] SHIV-infected animals rarely developed low-level viremia following the administration of AZD5582 in combination with the DART molecule

cocktail.[50] The impact of the treatment on the pool of latently infected cells was evaluated by measuring the levels of SHIV DNA, but did not reveal differences before and after treatment as well as between treatment groups.

In vitro studies indicated that the HIV-1 envelope used to generate the SHIV was sensitive to the activity of the DART molecules, and C_{max} plasma concentrations of the DART molecules achieved in the rhesus monkeys greatly exceeded the 50% effective concentration (EC_{50}) for redirected killing activity in vitro. However, all the animals develop antidrug antibody (ADA) with a subsequent rapid decline in serum concentration following 3 to 6 infusions. This suggested that the assessment of DART molecule activity in the SHIV/rhesus monkey model was hampered by DART molecule immunogenicity, and/or by the relatively low level of plasma viremia induced by SHIV C·CH505.375. dCT prior to ART initiation, resulting in limited seeding of the latent reservoir.

Given this result, Tuyshime and collaborators performed a careful evaluation of the ability of the DART molecules to engage the $CD8^+$ T cell subsets obtained from the rhesus monkeys utilized in the study and to eliminate SHIV-infected cells.[52] The investigators utilized a novel approach that relied on generating SHIV-infected $CD4^+$ T cells as target cells, and then in an autologous effector approach, using resting $CD8^+$ T cells from the same animal as effectors. The study revealed that the DART molecules were indeed capable of recruiting rhesus monkey effector cells to eliminate the infected cells as measured by direct killing, and by the ability to reduce the number of cells that can propagate the infection to uninfected cells.

The problem of eliminating the latent reservoir is not limited to the adult population but also involves the pediatric population. In 2019, WHO estimated that 1.3 million women and girls living with HIV become pregnant each year and, in absence of intervention, the rate of transmission during pregnancy, labor, and breast feeding may vary between 15% and 45%.[53] This has accounted for a consistent worldwide incidence of perinatal HIV transmission (MTCT) that remains above 150,000 infected newborns/year. The negative impact that the current COVID-19 pandemic may have on interventions to prevent HIV infection, with a consequent increase in transmission rate is of continuing concern.[54] In any event, the number of children living with HIV in 2020 due to perinatal transmission was an estimated 150,000, unchanged from 2019, reflecting a failure to achieve the WHO target of limiting MTCT to 20,000 children. To explore the possibility that DART molecules could be utilized early after birth to eliminate HIV-infected cells and, ultimately, impact persistent infection, Pollara and collaborators evaluated the potency of A32 x anti-CD3 and 7B2 x anti-CD3 DART molecules in the newborn population by analyzing the killing capacity of cytotoxic T cells isolated from the cord blood.[55] Their study revealed that DART molecules were indeed capable of redirecting $CD3^+$ cytotoxic T cells to kill HIV-infected $CD4^+$ T cells. However, cellular subsets acquired from cord blood were less potent than those isolated from the adult peripheral blood, indicating a possible limitation of this strategy.

A first-in-human phase 1 safety study of MGD014 in PLWH has been completed.[56] The study evaluated 21 participants in Part 1 (single dose escalation phase with MGD014 doses of 0.1–300 μg/kg in half-log increments) and 3 participants in Part 2 (MGD014 at 300 μg/kg administered at 2-week intervals for a total of 3 doses). There was no dose-limiting toxicity or serious adverse event. At the higher doses, MGD014 bound to the majority of circulating $CD4^+$ and $CD8^+$ T cells without inducing T-cell activation markers or increases in serum cytokines. MGD014 half-life was ~12 days and trough serum concentrations exceeded EC_{90} for redirected $CD8^+$ T cell-mediated killing of HIV-infected $CD4^+$ T cells in vitro by $\geq$ 20-fold, which should favor distribution of effective concentrations of MGD014 into tissues potentially harboring latent infected cells.[57] Low titer ADA was detected at the end of the study in 2 participants who received 1 dose in Part 1 and 3 doses in Part 2; however, there was no noticeable effect on PK. In summary, MGD014 was well-tolerated in PLWH on ART and exhibited a linear PK profile. A first-in-human phase 1 safety study of MGD020 in PLWH on ART will begin in 2022. This study will also evaluate the safety and PK of the MGD014 + MGD020 combination. The results from these studies will support future clinical studies in which the DART molecules will be combined with LRAs.

4. Other bispecific molecules designed to mediate clearance of HIV infection

Bispecific molecules in a variety of formats have been designed and tested for their ability to redirect T cells to lyse HIV-1-infected cells or, in one example, to lyse host cells known to contribute to HIV-1 infection. The molecular formats and specificities of these molecules are summarized in Table 1.

The first example of a bispecific molecule designed to redirect T cells against HIV-1-infected cells consisted of extracellular domains 1 and 2 of human CD4 linked to an anti-human CD3ε single-chain Fv (scFv).[58] The CD4 domains target the CD4 binding site of HIV-1 Env. The CD4 x anti-CD3 bispecific molecule, termed 'Janusin' after Janus, the Roman

Table 1
Bispecific molecules designed to mediate clearance of HIV infection.

Format[a]	Specificities[b]	Antiviral Target	Refs
Bispecific CD3 Engagers			
DART (diabody-Fc) [c]	A32 x anti-CD3 (MGD014)	gp120 C1–C2	34, 42
	7B2 x anti-CD3 (MGD020)	gp41 cluster I	
	PGT121 x anti-CD3	gp120 V3	
	PGT145 x anti-CD3	gp120 V2	
	10E8 x anti-CD3	gp41 MPER	
	VRC01 x anti-CD3	gp120 CD4bs	
BiTE (scFv-scFv)	B12 x anti-CD3	gp120 C2	57
	VRC01 x anti-CD3	gp120 CD4bs	
Fab-scFv	VRC07 x anti-CD3	gp120 CD4bs	56
ImmTAV (soluble TCR-scFv)	SL9 TCR (HLA-A*02:01) x anti-CD3	Gag p17	58
Fab arm exchanged rhesus IgG1 [d]	Anti-CCR5 x anti-CD3	rhesus CCR5	59
ECD-scFv	CD4 ECD_{1+2} x anti-CD3	gp120 CD4bs	55
ECD-scFv	CD4 ECD_{1+2} x anti-CD3	gp120 CD4bs	57
ECD-scFv-scFv	CD4 ECD_{1+2} x 17b x anti-CD3	gp120 CD4bs gp120 CoRBS	
Bispecific CD16 Engagers			
DART (diabody-Fc) [c]	A32 x anti-CD16	gp120 C1–C2	62
	7B2 x anti-CD16	gp41 cluster I	
IgG1-scFv [d]	QA255.006 x anti-CD16	gp41 heptad repeats & fusion region	61
	QA255.072 x anti-CD16	gp41 cluster I	
BiKE (Fab-V_HH)	VRC01 x anti-CD16	gp120 CD4bs	64
BiKE (ECD-VH)	CD4 ECD_1 x anti-CD16A	gp120 CD4bs	63

Abbreviations: BiKE, bispecific killer engager; BiTE, bispecific T cell engager; C1–C2, constant regions 1 and 2; CD4bs, CD4 binding site; CoRBS, coreceptor binding site; ECD, extracellular domain; Env, HIV-1 envelope; Fab, fragment antibody-binding; FcRn, neonatal Fc receptor; HLA, human leucocyte antigen; ImmTAV, immune-mobilizing monoclonal T-cell receptors against viruses; MPER, membrane proximal external region; scFv, single chain Fv; TCR, T-cell receptor; V2, variable region 2; V3, variable region 3; VH, human single variable heavy domain; V_HH, llama-derived single variable heavy domain.

[a] The bispecific molecules are bivalent (monovalent for each specificity) except for IgG1-scFv molecules, which are tetravalent (bivalent for each specificity). The trispecific ECD-scFv-scFv molecule is trivalent (monovalent for each).

[b] A32, 7B2, B12, 17b, QA255.006 and QA255.072 are non-neutralizing *anti*-Env specificities. PGT121, PGT145, 10E8, VRC01 and VRC07 are neutralizing *anti*-Env specificities.

[c] Disulfide-stabilized diabody linked to an IgG1 Fc engineered to be deficient in effector function but retain FcRn binding to prolong circulating half-life.

[d] IgG1 component has a fully functional Fc region.

god of duality who is often pictured with 2 faces, was shown to redirect cytotoxic T cells to lyse HIV-infected Jurkat cells in vitro at ng/mL concentrations.

A bispecific anti-HIV-1 Env x anti-CD3 molecule consisting of the fragment antibody-binding (Fab) region of VRC07 linked to an anti-human CD3 scFv (Fab-scFv) was described by Pegu et al.[59] This VRC07 x anti-CD3 molecule potently redirected $CD8^+$ T cells to mediate the lysis of HIV-infected cell lines expressing Env at elevated or low levels. An unusual property was its ability to increase the level of Env expression on $CD4^+$ T cells when incubated with PBMCs from PLWH on ART, suggesting its ability to directly activate HIV-1 expression in persistently infected, resting $CD4^+$ T cells in the absence of a latency reversing agent or other inducer. A variant version of the molecule (anti-human CD3 arm replaced by an anti-rhesus CD3 arm) was administered to SHIV-infected rhesus monkeys on ART. Shortly after infusion, $CD3^+$ lymphopenia accompanied by substantial increases in serum levels of TNF-α, MIP-1β and IL-10 was observed. These findings demonstrate that low dose administration of the VRC07 x anti-rhesus CD3 molecule resulted in the stimulation of circulating monkey T cells, which differs from that observed when MGD014 or MGD020 were administered to cynomolgus monkeys or SHIV-infected rhesus monkeys on ART.[48,49,56] Because of the low level of Env-expressing cells in SHIV-infected monkeys maintained on ART, the observed T-cell stimulation seems likely to have resulted from direct activation by the anti-rhesus CD3 arm itself (in monomeric or possibly aggregated forms), rather than to co-engagement of Env-expressing and $CD3^+$ T cells by both arms of the molecule.

Anti-HIV-1 Env x anti-CD3 molecules in bispecific T-cell engaging (BiTE) format consisting of tandem scFvs (scFv-scFv) were constructed and evaluated in vitro.[60] The bispecific molecules were B12 x anti-CD3, VRC01 x anti-CD3 and CD4 x anti-CD3 and a trispecific molecule was CD4 x 17b x anti-CD3, where the CD4 component consisted of extracellular domains 1 and 2. These BiTE-based molecules potently redirected T cells to lyse gp120-transfected cell lines and inhibited HIV-1 replication when infected human PBMCs or macrophages were cocultured with $CD8^+$ T cells. The most potent BiTE constructs were the ones containing CD4 domains, but these molecules also promoted HIV infection of human $CD4^-CD8^+$ T cells. This property, which was more prominent for the CD4 x anti-CD3 molecule than for the CD4 x 17b x anti-CD3 molecule, is attributed to the CD4 domain binding to Env of viral particles which trigger conformational changes that enable coreceptor binding and infection of cells lacking CD4 expression. The risk of promoting HIV-1 infection might be an obstacle in the clinical development of such CD4 domain-containing molecules.

Immune-mobilizing monoclonal T-cell receptors against viruses (ImmTAVs) are bispecific molecules consisting of a soluble affinity-enhanced T-cell receptor (TCR) fused to a humanized anti-human CD3 single chain variable fragment (soluble TCR-scFv). The soluble TCR arm recognizes a specific HIV-1 antigen (immunodominant Gag p17 epitope, SL9) presented by HLA-A*02:01 on the surface of infected $CD4^+$ T cells and the other arm binds to $CD3^+$ T cells. ImmTAVs specific for the SL9 Gag epitope potently redirected $CD8^+$ T cells to lyse HIV-1-infected $CD4^+$ T cells.[61] Importantly, the SL9 Gag epitope-specific ImmTAVs redirected $CD8^+$ T cells to lyse PHA-reactivated resting $CD4^+$ T cells obtained from PLWH on ART who were HLA-A*0201-positive. An advantage of ImmTAVs is that they are not dependent on targeting cell surface HIV-1 Env but can target intracellular HIV-1 targets that are processed to peptides and presented on the cell surface by HLA molecules. Some of these HIV-1 peptides may become accessible on the infected cell surface earlier than Env. A disadvantage of ImmTAVs, however, is that their activity is HLA-restricted and thus applicable to a subset of PLWH. ImmTAVs are discussed in more detail in another article in this compilation.

A bispecific antibody with anti-rhesus CCR5 and anti-rhesus CD3 specificities and rhesus IgG1 constant regions was constructed by a Fab arm exchange method.[62] This molecule is monovalent for each specificity. When administered to rhesus monkeys, CCR5+ cells were depleted from blood and colon biopsy samples, which remained depleted in blood for at least 1 week, but returned to pretreatment levels in colon biopsies within 1 week. Administration of the anti-CCR5 x anti-CD3 molecule also caused rapid $CD3^+$ lymphopenia which was restored to pretreatment levels by 1 week. Transient lymphopenia is a common clinical feature of CD3-engaging bispecific antibodies that target tumor antigens, which is due to T-cell activation and cytokine production resulting from co-engagement of $CD3^+$ T cells with antigen-expressing target cells and is prominent when antigen-expressing target cell frequencies are elevated.[63] It is also important to note that the anti-CCR5 x anti-CD3 molecule used here may have an intact Fc region (the authors did not specify that it was disabled for effector functions). The presence of an intact Fc region in a CD3-engaging bispecific molecule could contribute to off-target toxicity by causing Fc-gamma receptor clustering and potentially activating T cells in an antigen-independent manner.[64] By targeting CCR5, the activity of this bispecific molecule is not dependent on the induction of Env expression on latent-infected cells and will mostly target uninfected cells. It will be interesting to see if depletion of CCR5+ cells from SIV or SHIV-infected animals that are maintained on ART will lead to substantial decreases in the size of the latent reservoir, without durable depletion of the uninfected CCR5+ cell pool.

5. Bispecific CD16A-engaging molecules

Bispecific molecules were also designed to engage natural killer (NK) cell through the Fcγ-receptor IIIa (CD16A). An advantage of targeting NK cells is that they are relatively refractory to HIV-1 infection, though it has been reported that $CD56^{high}$ NK cells expressing CD4, CCR5 and CXCR4 could be infected by HIV-1.[65]

7B2 x anti-CD16A DART molecules were tested for ability to redirect adult PBMCs or cord blood mononuclear cells (CBMCs) to lyse HIV-1-infected $CD4^+$ T cells.[66] The subset of cytotoxic $CD56^{dim}CD16^+$ NK cells was the dominant population of NK cells in both cord blood and adult PBMC and treatment with IL-15 increased the activation (upregulated HLA-DR and CD69) of NK cells in adult PBMC and cord blood. However, NK cells in cord blood are less mature, less lymphoid homing, less active and produce less granzyme B compared to NK cells in adult PBMCs. The 7B2 x anti-CD16A molecule had modest ability to redirect resting cord blood NK cells to mediate the lysis of HIV-1-infected $CD4^+$ T cells, but treatment with IL-15 improved killing activity to a level that was comparable to that observed with NK cells from adult PBMCs. These data demonstrate that NK cells in cord blood have potential to be effective mediators of DART molecule-redirected killing after priming with IL-15.

Bispecific anti-gp41 x anti-CD16A antibodies were constructed in an IgG1 x scFv tetravalent format (bivalent for each specificity) in which the Fc region of the IgG1 component was intact and thus also capable of interacting with NK cells as well as other immune effector cells.[67] Two bispecific molecules were evaluated. One had an anti-gp41 specificity similar to that of 7B2 and the other recognized a discontinuous gp41 epitope that included parts of the C- and N-heptad repeat and fusion regions. Both molecules mediated similar levels of lysis of HIV-1-infected $CD4^+$ T cells in the presence of IL-2 stimulated NK cells in vitro.

A bispecific killer cell engager (BiKE) consisting of CD4 extracellular domain 1 fused to an anti-human CD16A VH antibody domain (ECD-VH) was evaluated in vitro.[68] The molecule potently redirected NK cells to lyse cell lines expressing HIV-1 Env and redirected PBMCs to mediate the killing of HIV-1-infected cells, including ones infected by a primary HIV-1 isolate. The small size of the BiKE molecule (MW ~28 kDa) may favor penetration into lymphoid and other tissues where latent infected cells reside, but exposure would be limited by rapid clearance of the molecule.

The Fab portion of VRC01 was linked to a llama-derived anti-CD16

nanobody, which is composed of a single variable heavy (VHH) domain 69. This Fab-VHH molecule demonstrated high affinity binding to both targets and potently activated and redirected NK cells to lyse HIV-1 Env-expressing target cells. Plans are to construct a trispecific killer cell engager (TriKE) that includes an IL-15 domain so that the molecule could potentially activate infected $CD4^+$ T cells in addition to enhancing NK cell killing activity.

6. Conclusion

Bispecific antibody-derived molecules can eradicate hematologic malignancies by retargeting immune effectors to transformed cells expressing a selected antigen. Bispecific DART molecules (or other bispecific formats) may be capable of the same feat in the setting of HIV infection. These reagents are capable of recognizing infected cells expressing HIV envelope glycoproteins on their surface, and recruiting and redirecting cytotoxic T or NK cells immune effector cells to form synapses that lead to cytolysis of the infected cells. Bispecific CD3-engaging molecules recruit $CD3^+$ T cells in an MHC-independent manner, without regard for T-cell specificity. Similarly, bispecific CD16-engaging molecules can recruit a broad population of $CD16^+$ NK cells.

Bispecific molecules may therefore overcome key obstacles to the clearance of HIV infection, during which MHC downregulation in infected cells and exhaustion of HIV-specific T cells render the anti-HIV immune response dysfunctional. When combined with the breadth of activity of broadly binding or neutralizing antibodies, bispecific $CD3^-$or CD16-engaging molecules could prove to be powerful tools against latent HIV infection.

The accessibility of viral antigen targets on persistently, latently HIV-infected cells is incompletely understood, and remains a critical distinction in the use of the bispecific molecule approach to HIV infection. The level and durability of antigen expression required to allow bispecific molecule-directed cell killing is not yet known. Similarly, while several LRAs have been clearly shown to induce cell-associated HIV RNA expression clinical studies, it is still unclear whether current LRA approaches induce sufficient antigen levels in vivo in a sufficient proportion of a latently infected population to allow clinically relevant reservoir clearance (reviewed in Ref. 70).

Ultimately, these questions can only be resolved by clinical studies in PLWH. As DART molecules and other approaches are now advancing into clinical testing, there is hope that HIV-specific $CD3^-$or CD16-engaging molecules, or other immune cell-engaging entities, could be ideal modalities to enhance immune-mediated clearance of persistent HIV infection and allow durable control of infection in the absence of ART, or even completely eradicate chronic HIV infection.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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