Minireview

Discoveries and developments of CXCR4-targeted HIV-1 entry inhibitors

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Impact statement

This minireview summarized the current progress in the identification of CXCR4-targeted HIV-1-entry inhibitors based on discovery/developmental approaches. It also provided a discussion of the inhibitor structural features, antiviral activities, and pharmacological properties. Unlike other reviews on anti-HIV-1 drug development, which have generally emphasized inhibitors that target intracellular viral replication and host genomic integration, this review focused on the drug discovery approaches taken to develop viral-entry inhibitors aimed at disturbing the initial step of viral interaction with uninfected host cells and preventing the subsequent viral replication/genomic integration. This review amalgamated recently published and important work on bivalent CXCR4-targeted anti-HIV-1-entry candidates/conjugates, discussed the research challenges faced in developing drugs to prevent and eradicate HIV-1 infection, and provided a perspective on strategies that can lead to future drug discoveries. The findings and strategies summarized in this review will be of interest to investigators throughout the microbiological, pharmaceutical, and translational research communities.

Abstract

The chemokine receptor CXCR4 is required for the entry of human immunodeficiency virus type 1 (HIV-1) into target cells and its expression correlates with more profound pathogenicity, rapid progression to acquired immunodeficiency syndrome (AIDS), and greater AIDS-related mortality. There is still no cure for AIDS and no method for preventing or eradicating HIV-1 infection. HIV-1 entry begins with the interaction of the viral envelope glycoprotein gp120 and the primary receptor CD4, and subsequently with the coreceptors, CCR5 or CXCR4, on the host cells. Blocking the interaction of HIV-1 and its coreceptors is therefore a promising strategy for developing new HIV-1 entry inhibitors. This approach has a dual benefit, as it prevents HIV-1 infection and progression while also targeting the reservoirs of HIV-1 infected, coreceptor positive macrophages and memory T cells. To date, multiple classes of CXCR4-targeted anti-HIV-1 inhibitors have been discovered and are now at different preclinical and clinical stages. In this review, we highlight the studies of CXCR4-targeted small-molecule and peptide HIV-1 entry inhibitors discovered during the last two decades and provide a reference for further potential HIV-1 exploration in the future.

Keywords: CXCR4, gp120, V3 loop, HIV-1 entry inhibitor, X4-tropic

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Introduction

An acquired immunodeficiency syndrome (AIDS) is one of the major diseases with a large public health impact. It is caused by the human immunodeficiency virus 1 (HIV-1) and represents the final stage of HIV-1 infection.[1] One of the hallmarks of HIV-1 infection is the selective destruction of $CD4^+$ T cells.[2] Currently, six main classes of antiretroviral drugs are used to treat HIV-1 infections. These include nucleoside/nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, integrase inhibitors, protease inhibitors, booster drugs, and entry inhibitors.[3] Generally, combinations of double or triple drugs are used to ensure a powerful attack on HIV-1.[4,5] The most difficult issues that restrict the eradication of

HIV-1 infection are rapid integration of a DNA copy of the viral genome into the host cell chromosome, the high mutation rate and tropism changes, and the long-term storage of HIV-1 infected cells in the pool of resting memory T cells ($CD4^+$, $CCR5^+$, $CXCR4^+$).[6–11]

The viral entry process is one of the most promising targets for the development of new anti-HIV-1 drugs that can disturb the interaction of virus with uninfected cells and prevent the subsequent initiation of virus replication cycle for the long-term treatment of patients with AIDS.[12,13] Two types of HIV are recognized, HIV-1, which is responsible for the majority of HIV infections worldwide, and HIV type 2 (HIV-2), which causes the infection endemic in western Africa.[14,15] Here, our review mainly focuses on the worldwide infection caused by HIV-1.

In order to enter a host cell, HIV-1 must attach to the host cell surface and interact with two separate receptors on the cell's surface: the CD4 receptor and a co-receptor, either CCR5 or CXCR4. CD4 is the primary receptor of the HIV-1 envelope glycoprotein 120 (gp120) and is critical for HIV-1 entry into host cells.[16,17] HIV-1 infection is initiated by the binding of gp120 to CD4,[18] which induces a series of conformational changes in gp120 that consequently expose its third variable (V3) loop for specific recognition of the coreceptor, either CCR5 (R5- or M-tropic virus) or CXCR4 (X4- or T-tropic virus).[19–22] The amino acid sequence of the V3 variable domain of gp120 is considered to be the primary structure that determines which coreceptor is utilized (i.e., the tropism of the virus). Indeed, coreceptor binding is the second obligatory event in HIV-1 entry and can also specifically contribute to establishment of the tropism of HIV-1 infection.[23,24] Following the interaction of viral gp120 with its coreceptor, the gp120-gp41 complex undergoes a dramatic conformational change that leads to the formation of a trimeric hairpin structure of gp41, enabling the viral envelope to fuse with the host cell membrane. The result is the release of the viral capsid into the cytoplasm of the target host cell.[25–27] The R5-tropic viruses are the principal circulating strain in most patients with early HIV-1 infection. These are non-syncytium-inducing viruses that target CCR5 and replicate best in macrophage-monocytes and they have less virulent clinical course. By contrast, the X4-tropic viruses are syncytium-inducing virus that target CXCR4 and preferentially replicate in T lymphocytes.[28–30] The X4-tropic virus infection is correlated with a more pathogenic and rapid progression to AIDS and greater AIDS-related mortality caused by progressive and quantitative declines in $CD4^+$ T cells.

CXCR4 is a seven transmembrane helical protein that belongs to the superfamily of G-protein-coupled receptors[31,32] and responds to its physiological (e.g., the C-X-C chemokine stromal-derived factor alpha [SDF-1α], also named CXCL12, and ubiquitin[33,34]) and viral (e.g., vMIP-II, gp120, and gp41) ligands. CXCR4 plays an important role in HIV-1 entry as a coreceptor. The binding pocket of CXCR4 is larger, more open, and located closer to the extracellular surface, and it includes acidic Asp^{187}, Glu^{288}, and Asp^{97}, which are important for SDF-1α binding. The co-crystal structure of CXCR4 and viral macrophage inflammatory protein-II (vMIP-II) has provided further important evidence that residues in the vMIP-II N-terminus and N loop interact with the CXCR4 TM pocket, CRS1, CRS1.5, and CRS2. In CRS2, the chemokine N-terminus forms by hydrogen bonds with CXCR4 residues D97, D262, and E288.[35] Further deciphering of the structure-function details regarding the dynamic changes involved in CXCR4 interactions with its natural and synthetic ligands will surely generate new opportunities for drug discovery efforts that target specific functional residues of this receptor.

Of all the steps in the HIV-1 infection pathway, the viral entry process is still one of the most promising targets for the development of new anti-HIV-1 drugs. These new drugs will aid in initial X4-tropic HIV-1 infection while also retarding the progression of HIV-1 infection to AIDS. This progression is often associated with a switch from R5-tropic to X4-tropic or R5/X4 dual tropic variants.[12,13] The high rate of mutagenesis of gp120 has meant that drug resistance is one of the key problems to consider during the development of anti-HIV-1 therapeutic medicines[36] and must be considered when searching for new potential drug leads targeting gp120. When drug resistance occurs, and especially cross-resistance, the existing drugs that share the same mechanism of action will be rendered ineffective. Thus, the discovery of new HIV-1 inhibitors remains a hot topic for HIV-1 therapy. In this review, we mainly focus on the discoveries and developments of CXCR4-targeted anti-HIV-1 inhibitors.

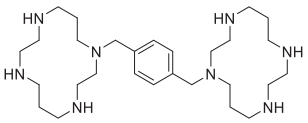
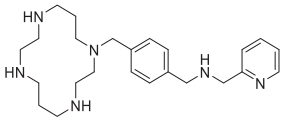
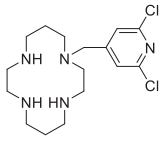
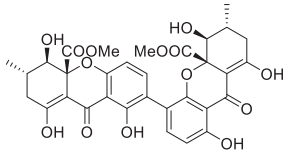
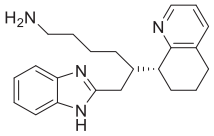
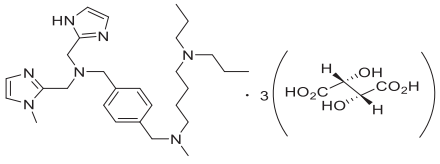
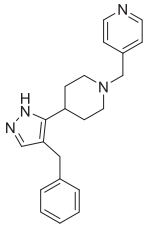
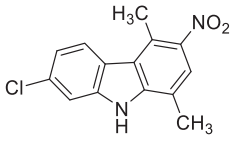
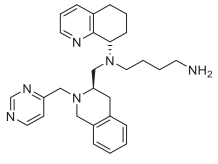
CXCR4-targeted small-molecule HIV-1 entry inhibitors

The effectiveness of coreceptor-based therapeutic approaches for inhibiting HIV-1 infection is supported by observation that individuals with CCR5 delta 32 mutations are healthy and highly resistant to HIV-1 infection, by the successful achievement of long-term remission in HIV-1 patients receiving CCR5 delta mutated stem cell transplantation, and by the potent anti-R5-tropic activity of CCR5-targeted maraviroc in clinical patients.[37] The well-known CXCR4-targeted anti-HIV-1 chemical entities, such as AMD3100, AMD3465, AMD070, and KRH3955 have been described in previous reviews.[38–41] In recent years, some promising anti-HIV-1 entry small-molecule inhibitors that target CXCR4 have been reported (Table 1).[38,41–48]

Dual-targeted anti-HIV-1 entry inhibitors

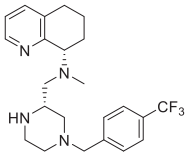
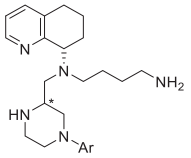
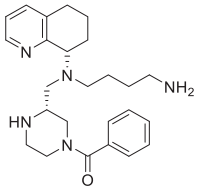
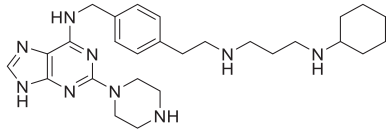
One representative of the next generation of dual anti-HIV-1 entry entities is compound 1, a pyrazolo-piperidine. Like AMD3541 (the first reported dual antagonist of CXCR4 and CCR5), compound 1 is also a dual antagonist of CXCR4 and CCR5.[49] It not only inhibits the non-nucleoside reverse transcriptase, but it also blocks both X4-tropic ($IC_{50} = 36\,\mu M$) and R5-tropic ($IC_{50} = 52\,\mu M$) viral entries at low micromolar levels.[42] A modeling study was carried out and demonstrated that the protonated piperidine ring of compound 1 could undergo an electrostatic interaction with Asp262 and the pyrazole ring; the 4-pyridyl ring could form hydrogen bonds with Gln200,

Table 1. Representatives of CXCR4-targeted small-molecule compounds that inhibit HIV-1 entry into cells.

Name of inhibitor	Chemical structure	Anti-HIV-1 IC_{50} or EC_{50} (μM)		Ref.
		M-tropic	T-tropic	
AMD3100		ND	0.002–0.02	38
AMD3465		ND	0.001–0.01	47
AMD3541		ND	1.2–26.5	47
PXA		0.36	0.26	43
AMD070		ND	0.0048	47
KRH-3955		ND	0.0003–0.001	41
Compound 1		36	52	42
Compound 2		5.3	1.4	44
Compound 3		ND	0.003	45

(continued)

Table 1. Continued.

Name of inhibitor	Chemical structure	Anti-HIV-1 IC_{50} or EC_{50} (μM)		Ref.
		M-tropic	T-tropic	
Compound 4		ND	8.01	45
Compound 5		ND	NA	48
Compound 6		ND	0.02	48
Compound 7		ND	0.0005	46

Ser283, and Arg30. Penicillixanthone A (PXA), a natural xanthone dimer from *Aspergillus* fungi, is another dual antagonist of CXCR4 and CCR5[43] with potent anti-M-tropic HIV-1 SF162 and T-tropic HIV-1 NL4-3 entry activities (IC_{50} of 0.36 and 0.26 μM, respectively).[44] A series of chloro-1,4-dimethyl-9*H*-carbazoles also exhibits promise as a class of dual antagonists of HIV-1 entry inhibitors that show encouraging potential for blocking HIV-1 entry for both R5-tropic ($IC_{50} = 5.3$ μM) and X4-tropic ($IC_{50} = 1.4$ μM) viruses (compound 2).[44]

T-tropic (X4-tropic) anti-HIV entry inhibitors

Studies on a series of T-tropic viral entry inhibitors have identified compounds 3 and 4 as promising lead candidates. These two compounds show selectivity toward blocking HIV-1 entry over CXCR4 antagonism, based on studies of HIV-1_{IIIB} MAGI entry and of CXCL12-induced calcium flux.[45] Compounds 5–7, which share N-aryl piperazines, are another series of CXCR4-antagonists. Compounds 6 and 7 exhibit high anti-HIV-1 entry activity at very low nanomolar levels (20 and 0.5 nM, respectively). Compound 7 (also a purine-based CXCR4 antagonist) interacts with some critical CXCR4 residues (Asp193, His281, and Glu288) that are essential for interaction of the HIV-1 gp120 V3 loop with CXCR4, and the inhibition of viral entry ($EC_{50} = 0.5$ nM) is 130-fold stronger with compound 7 than with AMD3100.[46] Compound 7 could strongly bind to CXCR4 at a sub-nanomolar level (16.4 nM) with high selectivity through a panel of related chemokine receptors, including CXCR2, CCR2, CCR4, and CCR5, whose binding affinities were >10,000 nM.

CXCR4-targeted peptides that function as HIV-1 entry inhibitors

The earliest anti-HIV-1 entry peptides to be designed and developed included some cationic antimicrobial peptides, such as polyphemusins,[50,51] these showed high antiviral activities but also strong toxicities. This toxicity issue led to the development of T22, an 18-residue peptide analog of polyphemusin-II with nine positive charged residues. T22 also targets CXCR4 and blocks CXCR4-mediated HIV-1 entry into host cells but with a lower cytotoxicity.[52] A structure–activity relationship (SAR) study of T22 identified its analog, T140, a 14-residue peptide that had a stronger anti-HIV-1 entry activity and maintained the antiparallel β-sheet conformation, but had a lower molecular weight.[53,54] Subsequent efforts that employed a combination of SAR analysis and sequence-based library screening resulted in the discovery of FC131, a small cyclic pentapeptide that showed high stability, CXCR4-binding affinity, and anti-HIV-1 entry activity, and again had a smaller molecular weight than T22.[55,56] Evaluation of the cocrystal structure of CXCR4 in complex with CVX15 (which shares similar binding modes to those of FC131) identified a new conjugate, MCo-CVX-5c, which was produced by grafting CVX15 amino acid sequence into MCo-TI-I scaffold. MCo-CVX-5c shows very high CXCR4-binding affinity

and anti-HIV-1 entry activity, with EC_{50} value of 2 nM.[57] Currently, cyclotide analogs have become important lead candidates against CXCR4 mediated HIV-1 entry. Some of them (e.g., 4 F-benzoyl-TN14003 [BKT140]) are being tested in clinical trials in cancer patients who have undergone hematopoietic stem cell transplantation.[58]

CXCR4 interacts with many small protein ligands, like CXCL12 (SDF-1α), viral vMIP-II, and microphage migration inhibition factor; therefore, peptides derived from the motifs of these ligands or from the mimics of natural ligand proteins represent other sources of lead candidates for developing CXCR4-targeted HIV-1 entry inhibitors. One approach has been the derivation of CXCR4-targted peptides or mimics (conjugated or non-conjugated) from viral proteins. For example, the V1 peptide derived from the N-terminus 21-residues of vMIP-II can bind to CXCR4 and inhibit HIV-1 entry. However, its synthetic analog DV1, which is an all-D-amino-acids substituted V1, shows much stronger activity in both CXCR4-binding and anti-HIV-1 entry. Chemical grafting and/or conjugating of DV1 to the other fragment of vMIP-II (e.g., RCP168) or DV1 (e.g., DV1 dimer, DV1-K-DV3, 4DV3) yields new conjugates that further enhance the CXCR4 antagonist and antiviral activities.[59–63] In addition, peptide or mimic drug leads have also been derived from the partial structures of a coreceptor CXCR4 or CCR5. Two examples of these new inhibitors are peptide 2C (which is derived from the residues 178–191 of CCR5) and its analog peptide 40–2; both are R5- and X4-tropic HIV-1 entry inhibitors. Peptide 40–2 has a better anti-HIV-1 entry activity when compared to its parental peptide 2C.[64,65] The wise application of a combination of chemical, molecular modeling, mutagenesis, and biological approaches has led to the continuous discovery and development of more potent cyclic, bivalent, and multivalent analogs (Table 2).[52,53,56,57,59,60,62–69] The molecular modeling of CXCR4 dimer–synthetic ligand interactions based on the crystal structure of CXCR4 has demonstrated that synthetic dimeric ligands are capable of interacting with the CXCR4 dimeric structure by allowing the essential amino acid residues to interact with binding or signaling pockets of CXCR4 molecules. The use of different linkers, including polyethylene glycol, poly(L-proline), or Ahx, could allow the maintenance of an appropriate distance between the two binding sites of the ligands, consistent with that of CXCR4 dimers.[66–68] This part of research will surely be expanded in the future, not only to develop drug leads, but also to elucidate how the lead ligands interact with the amino acid residues of CXCR4.

Challenges in the development of synthetic CXCR4-targeting anti-HIV-1 drugs

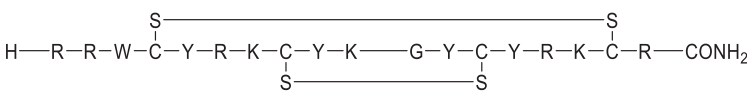
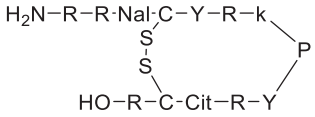
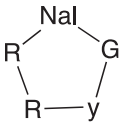
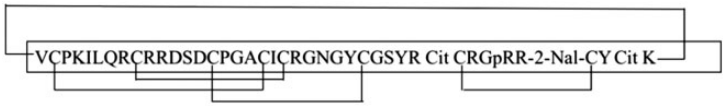
CXCR4 inhibitors that have entered clinical trials for anti-HIV-1 therapy mainly include AMD3100 derivatives.[40] New drug candidates are also emerging and have been designed based on the structural features of CXCR4 and the chemokine or viral ligands of CXCR4, such as CXCL12, the V3 loop of gp120, and vMIP-II. Some of these new inhibitors show promise as therapeutic candidates for drug development and clinical applications, not only limited to inhibit HIV-1 entry, but also hinder HIV-1-induced actin rearrangement (which facilitates viral genome integration into host chromosome), and induce apoptosis of latently infected resting memory T cells.[32,68,70,71] The use of physiological ligand sequences, the viral ligand sequences, and conjugation strategies has led to a serious of peptide–peptide, peptide–small-molecule, or small molecule–small-molecule conjugates that possess bivalent or multivalent properties with enhanced or improved CXCR4 binding and anti-HIV-1 entry activities against either sole-tropic or dual-tropic HIV-1 isolates.[32,63,68] The use of natural protease-resistant D-peptides can be advantageous for drug development, because these peptides are highly stable and resistant to proteolytic degradation.[72] Some of these peptides have potent anti-HIV-1 activities that are comparable to that of enfuvirtide (T20), a marketed drug targeting HIV-1 gp41 and entry.[32]

The challenge in the development of new CXCR4-targeted HIV-1 entry inhibitor is still overcoming the impediment of undesirable side effects of the CXCR4 antagonists. Concern arise regarding the full antagonist activity, as this affects the normal CXCR4-CXCL12 functions in HIV-1 therapy. For this reason, an increasing number of allosteric antagonists (e.g., RSVM, ASLW) are now being sought and developed.[73] These allosteric modulators are considered to modulate orthosteric ligand–CXCR4 binding affinity and/or efficacy at sites that topographically distinct from the orthosteric agonists binding sites. These allosteric modulators can be physiological or non-physiological, and they can behave either as partial agonists or super agonists. A combination or conjugation of allosteric agonists with orthosteric antagonists for anti-HIV-1 therapy represents a new drug development strategy that could specifically target therapeutically relevant binding sites of CXCR4 that are selectively implicated in HIV-1 infection, while sparing binding sites that contribute to essential non-HIV-1-related CXCR4 functions.

Bioengineered coreceptor and CD4 co-targeted HIV-1 entry inhibitors

Besides synthetic small-molecule and peptide HIV-1 entry inhibitors, other kinds of HIV-1 entry inhibitors have also been developed in recent years. Based on the participation of multiple and complexity of the HIV-1 viral proteins and host receptors, a series of CXCR4-targeting nanobodies (Nbs) have been reported that can provide effective inhibition of HIV-1 infection. Among these are VUN400, VUN401, and VUN402, which exhibit potent inhibitory activity against a wide range of T-tropic HIV-1 strain infection with IC_{50} values of 6.9, 7.5 and 6.7 nM, respectively.[74] Interestingly, the anti-HIV-1 activity of these Nbs does not always correlate with their ability to modulate CXCR4 signaling and function. This indicates that anti-HIV-1 properties and CXCR4 antagonism do not entirely overlap and may be functionally separate, so the Nbs only minimally disturb the physiological function of CXCR4.[75] VUN401 binds to the N-terminus of CXCR4, VUN400 binds to the second extracellular loop of CXCR4, and VUN402 binds to

Table 2. Representatives of CXCR4-targeted peptides that function as HIV-1 entry inhibitors.

Name of inhibitor	Amino acid sequence[a]	Ref.
T22		52
T140		53
FC131		56
MCo-CVX-5c		57
V1	LGASWHRPDKCCLGYQKRPLP	60
DV1	lgaswhrpdkcclgyqkrplp	59
Dimer DV1	lgaswhrpdkcalgyqkrplp | lgaswhrpdkcalgyqkrplp	66
DV3	lgaswhrpdk	69
DV1-K-(DV3)	lgaswhrpdkcclgyqkrplpk | lgaswhrpdk	62
4DV3	$(lgaswhrpdkk)_4$	63
AR5	lgaswhrpdkkr-Ahx-KRKGDIRQAHC	68
AR6	lgaswhrpdkkr-Ahx-KRKTNNNPRTC	68
2C	CSSHFPYSQYQFWK	64,65
40-2	YSSYFPFSQYQWWK	65

[a]Lowercase letters denote D-amino acids.

both the second extracellular loop and the top of transmembrane helices 4 and 5 of CXCR4. Constructing each of them (VUN400, VUN401, and VUN402) to bivalent Nb-Fc forms further enhances their potencies and therapeutic potentials by increasing their CXCR4 binding affinities, making these highly potent inhibitors of HIV-1 entry.

The Nbs target just one coreceptor for inhibition of HIV-1 entry, but some bispecific Nbs have been developed to block CXCR4 and CD4, thereby enhancing the inhibition of HIV-1 infection. One example is 281F12-35GS-3F11, a bispecific CD4-CXCR4 Nb construct that can bind to both CD4 and CXCR4 and that show extremely potent inhibition of HIV-1 X4-tropic virus infection, with an HIV-1 inhibitory IC_{50} of 1.5 nM in peripheral blood mononuclear cells.[76] When compared with the monovalent CXCR4 and CD4 Nbs, the bispecific CXCR4-CD4 construct (281F12-35GS-3F11) is 250–320 folds more potent. This indicates that the simultaneous binding to both CD4 and CXCR4 by the bispecific construct on HIV-1 attacked host cells could enhance the potency for prevention of HIV-1 entry. This also provides a promising strategy and principle for developing new and highly potent bivalent or multivalent HIV-1 entry inhibitors using either biological constructions or chemical conjugations. With a similar idea, certain groups have recently discovered and developed conjugated peptides and small molecules that could co-target CD4, gp120, and/or CXCR4/CCR5 to inhibit HIV-1 entry into host cells.[76–78]

Conclusion and perspective

Although HIV-1 entry inhibitors that targeting CXCR4 presently undergoing extensive research, no drug has yet been approved that targets CXCR4 to inhibit HIV-1 infection. Thus, the prevention, inhibition, and eradication of HIV-1 infection remain a challenge using only the currently available medicines. Developing new CXCR4-targeted HIV-1 entry inhibitors is still a tough mission faced by scientists.

The successful development of the CCR5 inhibitor, maraviroc, an R5-tropic HIV-1 entry inhibitor, has encouraged and accelerated the exploration of CXCR4-targeted X4-tropic HIV-1 entry inhibitors. To date, various classes of CXCR4-targeted anti-HIV-1 drug candidates have been developed, including small-molecule, peptide, and macromolecule inhibitors that are now attracting much attention (Figure 1).

Under the direction of rational medicinal chemistry strategies, more potent small-molecule HIV-1 entry

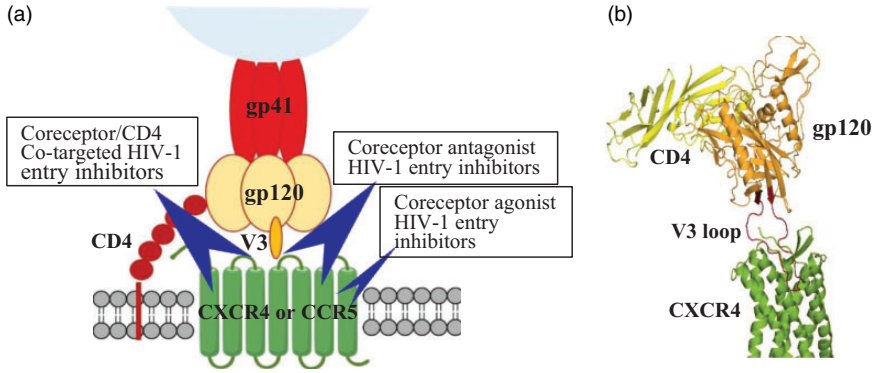


Figure 1. A cartoon illustration of coreceptor-targeted HIV-1 entry inhibitors (a) and the binding model of CD4/gp120/CXCR4 (b).[18,22,35] (A color version of this figure is available in the online journal.)

inhibitor will be discovered or developed by optimizing or linking the current compounds, either orthosterically and/or allosterically. More effective peptide inhibitors could also be developed by modifying molecular mimics of natural/physiological or viral ligands of CXCR4, CD4, moieties of gp120 (V3 loop) or gp41, or some peptide sequences of CXCR4/CCR5. Identification of multiprotein-targeted micromolecular or bispecific or bivalent inhibitors is particularly promising for the discovery of new HIV-1 entry inhibitors with enhanced CXCR4 binding affinity and HIV-1 entry inhibition. As more novel molecules are rationally designed and evaluated, more potent and effective HIV-1 entry inhibitors targeting CXCR4 will be identified and that will promote the development of efficacious HIV-1 therapeutic drugs.

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DECLARATION OF CONFLICTING INTERESTS

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REFERENCES

1. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vézinet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983;**220**:868–71
2. Hoover DR, Graham NM, Chen B, Taylor JM, Phair J, Zhou SY, Muñoz A. Effect of $CD4^+$ cell count measurement variability on staging HIV-1 infection. *J Acquir Immune Defic Syndr* 1992;**5**:794–802
3. Arts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. *Cold Spring Harb Perspect Med* 2012;**2**:a0071611–23
4. Bartlett JA, DeMasi R, Quinn J, Moxham C, Rousseau F. Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infected adults. *Aids* 2001;**15**:1369–77
5. Palella FJJ, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV outpatient study investigators. *N Engl J Med* 1998;**338**:853–60
6. Jacobo-Molina A, Arnold E. HIV reverse transcriptase structure-function relationships. *Biochemistry* 1991;**30**:6351–6
7. Patel MB, Hoffman NG, Swanstrom R. Subtype-specific conformational differences within the V3 region of subtype B and subtype C human immunodeficiency virus type 1 env proteins. *J Virol* 2008;**82**:903–16
8. Saracino A, Monno L, Brindicci G. Are the proposed env mutations actually associated with resistance to maraviroc?. *J Acquir Immune Defic Syndr* 2010;**53**:550–2
9. Ribeiro RM, Hazenberg MD, Perelson AS, Davenport MP. Naïve and memory cell turnover as drivers of CCR5-to-CXCR4 tropism switch in human immunodeficiency virus type 1: implications for therapy. *J Virol* 2006;**80**:802–9
10. Tabler CO, Lucera MB, Haqqani AA, McDonald DJ, Migueles SA, Connors M, Tilton JC. $CD4^+$ memory stem cells are infected by HIV-1 in a manner regulated in part by SAMHD1 expression. *J Virol* 2014;**88**:4976–86
11. Terahara K, Iwabuchi R, Hosokawa M, Nishikawa Y, Takeyama H, Takahashi Y, Tsunetsugu-Yokota Y. A $CCR5^+$ memory subset within HIV-1-infected primary resting $CD4^+$ T cells is permissive for replication-competent, latently infected viruses in vitro. *BMC Res Note* 2019;**12**:1–7
12. Flexner C. HIV drug development: the next 25 years. *Nat Rev Drug Discov* 2007;**6**:959–66
13. Clavel F, Hance AJ. HIV drug resistance. *N Engl J Med* 2004;**350**:1023–35
14. De Cock KM, Brun-Vezinet F, Soro B. HIV-1 and HIV-2 infections and AIDS in West Africa. *AIDS* 1991;**5 Suppl**:S21–8
15. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. *Cold Spring Harb Perspect Med* 2011;**1**:a0068411–22
16. Wilen CB, Tilton JC, Doms RW. HIV: cell binding and entry. *Cold Spring Harb Perspect Med* 2012;**2**:a0068661–13
17. Freeman MM, Seaman MS, Rits-Volloch S, Hong XG, Kao CY, Ho DD, Chen B. Crystal structure of HIV-1 primary receptor CD4 in complex with a potent antiviral antibody. *Structure* 2010;**18**:1632–41
18. Zimmermann K, Liechti T, Haas A, Rehr M, Trkola A, Gunthard HF, Oxenius A. The orientation of HIV-1 gp120 binding to the CD4 receptor differentially modulates $CD4^+$ T cell activation. *J Immunol* 2015;**194**:637–49

19. Sattentau QJ, Moore JP. Conformational changes induced in the human immunodeficiency virus envelope glycoprotein by soluble CD4 binding. *J Exp Med* 1991;**174**:407–15
20. Cocchi F, DeVico AL, Garzino-Demo A, Cara A, Gallo RC, Lusso P. The V3 domain of the HIV-1 gp120 envelope glycoprotein is critical for chemokine-mediated blockade of infection. *Nat Med* 1996;**2**:1244–7
21. Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, Berger EA. CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 1996;**272**:1955–8
22. Huang CC, Tang M, Zhang MY, Majeed S, Montabana E, Stanfield RL, Dimitrov DS, Korber B, Sodroski J, Wilson IA, Wyatt R, Kwong PD. Structure of a V3-containing HIV-1 gp120 core. *Science* 2005;**310**:1025–8
23. Berger EA, Doms RW, Fenyo E, Korber BT, Littman DR, Moore JP, Sattentau QJ, Schuitemaker H, Sodroski J, Weiss RA. A new classification for HIV-1. *Nature* 1998;**391**:240
24. Antell GC, Dampier W, Aiamkitsumrit B, Nonnemacher MR, Jacobson JM, Pirrone V, Zhong W, Kercher K, Passic S, Williams JW, Schwartz G, Hershberg U, Krebs FC, Wigdahl B. Utilization of HIV-1 envelope V3 to identify X4- and R5-specific tat and LTR sequence signatures. *Retrovirology* 2016;**13**:32–14
25. Schroeder S, Kaufman JD, Walla PJ, Lakomek NA, Wingfield PT. HIV-1 gp41 transmembrane oligomerization monitored by FRET and FCS. *FEBS Lett* 2018;**592**:939–48
26. Mamede JI, Cianci GC, Anderson MR, Hope TJ. Early cytoplasmic uncoating is associated with infectivity of HIV-1. *Proc Natl Acad Sci U S A* 2017;**114**:E7169–78
27. Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. *Science* 1998;**280**:1884–8
28. Wu L, Gerard NP, Wyatt R, Choe H, Parolin C, Ruffing N, Borsetti A, Cardoso AA, Desjardin E, Newman W, Gerard C, Sodroski J. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. *Nature* 1996;**384**:179–83
29. Myszka DG, Sweet RW, Hensley P, Brigham-Burke M, Kwong PD, Hendrickson WA, Wyatt R, Sodroski J, Doyle ML. Energetics of the HIV gp120-CD4 binding reaction. *Proc Natl Acad Sci U S A* 2000;**97**:9026–31
30. Verrier F, Borman AM, Brand D, Girard M. Role of the HIV type 1 glycoprotein 120 V3 loop in determining coreceptor usage. *AIDS Res Hum Retroviruses* 1999;**15**:731–43
31. Wu B, Chien EY, Mol CD, Fenalti G, Liu W, Katritch V, Abagyan R, Brooun A, Wells P, Bi FC, Kuhn P, Handel TM, Cherezov V, Stevens RC. Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists. *Science* 2010;**330**:1066–71
32. Choi WT, Duggineni S, Xu Y, Huang Z, An J. Drug discovery research targeting the CXC chemokine receptor 4 (CXCR4). *J Med Chem* 2011;**55**:977–94
33. Oberlin E, Amara A, Bachelerie F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, Schwartz O, Heard JM, Clark-Lewis I, Legler DF, Loetscher M, Baggiolini M, Moser B. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* 1996;**382**:833–5
34. Saini V, Staren DM, Ziarek JJ, Nashaat ZN, Campbell EM, Volkman BF, Marchese A, Majetschak M. The CXC chemokine receptor 4 ligands ubiquitin and stromal cell-derived factor-1alpha function through distinct receptor interactions. *J Biol Chem* 2011;**286**:33466–77
35. Qin L, Kufareva I, Holden LG, Wang C, Zheng Y, Zhao C, Fenalti G, Wu H, Han GW, Cherezov V, Abagyan R, Stevens RC, Handel TM. Structural biology. Crystal structure of the chemokine receptor CXCR4 in complex with a viral chemokine. *Science* 2015;**347**:1117–22
36. Pennings PS. HIV drug resistance: problems and perspectives. *Infect Dis Rep* 2013;**5**: e521–25
37. Gupta RK, Abdul-Jawad S, McCoy LE, Mok HP, Peppa D, Salgado M, Martinez-Picado J, Nijhuis M, Wensing AMJ, Lee H, Grant P, Nastouli E, Lambert J, Pace M, Salasc F, Monit C, Innes AJ, Muir L, Waters L, Frater J, Lever A, Edwards SG, Gabriel IH, Olavarria E. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. *Nature* 2019;**568**:244–8
38. Doranz BJ, Grovitferbas K, Sharron M, Mao S, Goetz MB, Daar ES, Doms RW, O'Brien WA. A small-molecule inhibitor directed against the chemokine receptor CXCR4 prevents its use as an HIV-1 coreceptor. *J Exp Med* 1997;**186**:1395–400
39. Hatse S, Princen K, De Clercq E, Rosenkilde MM, Schwartz TW, Hernandez-Abad PE, Skerlj RT, Bridger GJ, Schols D. AMD3465, a monomacrocyclic CXCR4 antagonist and potent HIV entry inhibitor. *Biochem Pharmacol* 2005;**70**:752–61
40. Stone ND, Dunaway SB, Flexner C, Tierney C, Calandra GB, Becker S, Cao YJ, Wiggins IP, Conley J, MacFarland RT, Park JG, Lalama C, Snyder S, Kallungal B, Klingman KL, Hendrix CW. Multiple-dose escalation study of the safety, pharmacokinetics, and biologic activity of oral AMD070, a selective CXCR4 receptor inhibitor, in human subjects. *Antimicrob Agents Chemother* 2007;**51**:2351–8
41. Murakami T, Kumakura S, Yamazaki T, Tanaka R, Hamatake M, Okuma K, Huang W, Toma J, Komano J, Yanaka M, Tanaka Y, Yamamoto N. The novel CXCR4 antagonist KRH-3955 is an orally bioavailable and extremely potent inhibitor of human immunodeficiency virus type 1 infection: comparative studies with AMD3100. *Antimicrob Agents Chemother* 2009;**53**:2940–8
42. Cox BD, Prosser AR, Sun Y, Li Z, Lee S, Huang MB, Bond VC, Snyder JP, Krystal M, Wilson LJ, Liotta DC. Pyrazolo-Piperidines exhibit dual inhibition of CCR5/CXCR4 HIV entry and reverse transcriptase. *ACS Med Chem Lett* 2015;**6**:753–7
43. Tan S, Yang B, Liu J, Xun T, Liu Y, Zhou X. Penicillixanthone A, a marine-derived dual-coreceptor antagonist as anti-HIV-1 agent. *Nat Prod Res* 2019;**33**:1467–71
44. Saturnino C, Grande F, Aquaro S, Caruso A, Iacopetta D, Bonomo MG, Longo P, Schols D, Sinicropi MS. Chloro-1,4-dimethyl-9H-carbazole derivatives displaying anti-HIV activity. *Molecules* 2018;**23**:E2861–8
45. Huang MB, Giesler KE, Katzman BM, Prosser AR, Truax V, Liotta DC, Wilson LJ, Bond VC. Small molecule CXCR4 antagonists block the HIV-1 nef/CXCR4 axis and selectively initiate the apoptotic program in breast cancer cells. *Oncotarget* 2018;**9**:16996–7013
46. Wu CH, Wang CJ, Chang CP, Cheng YC, Song JS, Jan JJ, Chou MC, Ke YY, Ma J, Wong YC, Hsieh TC, Tien YC, Gullen EA, Lo CF, Cheng CY, Liu YW, Sadani AA, Tsai CH, Hsieh HP, Tsou LK, Shia KS. Function-oriented development of CXCR4 antagonists as selective human immunodeficiency virus (HIV)-1 entry inhibitors. *J Med Chem* 2015;**58**:1452–65
47. Zhang H, Kang D, Huang B, Liu N, Zhao F, Zhan P, Liu X. Discovery of non-peptide small molecular CXCR4 antagonists as anti-HIV agents: recent advances and future opportunities. *Eur J Med Chem* 2016;**114**:65–78
48. Zhao HC, Prosser AR, Liotta DC, Wilson LJ. Discovery of novel N-aryl piperazine CXCR4 antagonists. *Bioorg Med Chem Lett* 2015;**25**:4950–5
49. Horuk R. Promiscuous drugs as therapeutics for chemokine receptors. *Expert Rev Mol Med* 2009;**11**:e11–16
50. Morimoto M, Mori H, Otake T, Ueba N, Kunita N, Niwa M, Murakami T, Iwanaga S. Inhibitory effect of tachyplesin I on the proliferation of human immunodeficiency virus in vitro. *Chemotherapy* 1991;**37**:206–11
51. Murakami T, Niwa M, Tokunaga F, Miyata T, Iwanaga S. Direct virus inactivation of tachyplesin I and its isopeptides from horseshoe crab hemocytes. *Chemotherapy* 1991;**37**:327–34
52. Masuda M, Nakashima H, Ueda T, Naba H, Ikoma R, Otaka A, Terakawa Y, Tamamura H, Ibuka T, Murakami T. A novel anti-HIV synthetic peptide, T-22 ([Tyr5,12,Lys7]-polyphemusin II). *Biochem Biophys Res Commun* 1992;**189**:845–50
53. Tamamura H, Arakaki R, Funakoshi H, Imai M, Otaka A, Nakashima H, Murakami T, Waki M, Matsumoto A, Yamamoto N, Fujii N. Effective lowly cytotoxic analogs of an HIV-cell fusion inhibitor, T22 ([Tyr5,12, Lys7]-polyphemusin II). *Bioorg Med Chem* 1998;**6**:231–8
54. Tamamura H, Xu Y, Hattori T, Zhang X, Arakaki R, Kanbara K, Omagari A, Otaka A, Ibuka T, Yamamoto N, Nakashima H, Fujii N. A low-molecular-weight inhibitor against the chemokine receptor CXCR4: a strong anti-HIV peptide T140. *Biochem Biophys Res Commun* 1998;**253**:877–82
55. Fujii N, Oishi S, Hiramatsu K, Araki T, Ueda S, Tamamura H, Otaka A, Kusano S, Terakubo S, Nakashima H, Broach JA, Trent JO, Wang ZX,

Peiper SC. Molecular-size reduction of a potent CXCR4-chemokine antagonist using orthogonal combination of conformation- and sequence-based libraries. *Angew Chem Int Ed Engl* 2003;**42**:3251–3

56. Tamamura H, Hiramatsu K, Ueda S, Wang Z, Kusano S, Terakubo S, Trent JO, Peiper SC, Yamamoto N, Nakashima H, Otaka AA, Fujii N. Stereoselective synthesis of [L-Arg-L/D-3-(2-naphthyl)alanine]-type (E)-alkene dipeptide isosteres and its application to the synthesis and biological evaluation of pseudopeptide analogues of the CXCR4 antagonist FC131. *J Med Chem* 2005;**48**:380–91
57. Aboye TL, Ha H, Majumder S, Christ F, Debyser Z, Shekhtman A, Neamati N, Camarero JA. Design of a novel cyclotide-based CXCR4 antagonist with anti-human immunodeficiency virus (HIV)-1 activity. *J Med Chem* 2012;**55**:10729–34
58. Peled A, Abraham M, Avivi I, Rowe JM, Beider K, Wald H, Tiomkin L, Ribakovsky L, Riback Y, Ramati Y, Aviel S, Galun E, Shaw HL, Eizenberg O, Hardan I, Shimoni A, Nagler A. The high-affinity CXCR4 antagonist BKT140 is safe and induces a robust mobilization of human CD34(+) cells in patients with multiple myeloma. *Clin Cancer Res* 2014;**20**:469–79
59. Zhou N, Luo Z, Luo J, Fan X, Cayabyab M, An J, Kaji H, Sodroski JG, Huang Z. Exploring the stereochemistry of CXCR4-peptide recognition and inhibiting HIV-1 entry with D-peptides derived from chemokines. *J Biol Chem* 2002;**277**:17476–85
60. Luo Z, Fan X, Zhou N, Hiraoka M, Luo J, Kaji H, Huang Z. Structure–function study and anti-HIV activity of synthetic peptide analogues derived from viral chemokine vMIP-II. *Biochemistry* 2000;**39**:13545–50
61. Liu D, Madani N, Li Y, Cao R, Choi WT, Kawatkar SP, Lim MY, Kumar S, Dong CZ, Wang J, Russell JD, Lefebure CR, An J, Wilson S, Gao YG, Pallansch LA, Sodroski JG, Huang Z. Crystal structure and structural mechanism of a novel anti-human immunodeficiency virus and D-amino acid-containing chemokine. *J Virol* 2007;**81**:11489–98
62. Xu Y, Duggineni S, Espitia S, Richman DD, An J, Huang Z. A synthetic bivalent ligand of CXCR4 inhibits HIV infection. *Biochem Biophys Res Commun* 2013;**435**:646–50
63. Heon Lee I, Palombo MS, Zhang X, Szekely Z, Sinko PJ. Design and evaluation of a CXCR4 targeting peptide 4DV3 as an HIV entry inhibitor and a ligand for targeted drug delivery. *Eur J Pharm Biopharm* 2019;**138**:11–22
64. Dogo-Isonagie C, Lam S, Gustchina E, Acharya P, Yang Y, Shahzad-Ul-Hussan S, Clore GM, Kwong PD, Bewley CA. Peptides from second extracellular loop of C-C chemokine receptor type 5 (CCR5) inhibit diverse strains of HIV-1. *J Biol Chem* 2012;**287**:15076–86
65. Bobyk KD, Mandadapu SR, Lohith K, Guzzo C, Bhargava A, Lusso P, Bewley CA. Design of HIV coreceptor derived peptides that inhibit viral entry at submicromolar concentrations. *Mol Pharm* 2017;**14**:2681–9
66. Choi W, Santhosh K, Madani N, Han X, Tian S, Dong C, Liu D, Duggineni S, Yuan J, Sodroski JG, Huang Z, An J. A novel synthetic bivalent ligand to probe chemokine receptor CXCR4 dimerization and inhibit HIV-1 entry. *Biochemistry* 2012;**51**:7078–86
67. Yang Y, Gao M, Zhang Q, Zhang C, Yang X, Huang Z, An J. Design, synthesis, and biological characterization of novel PEG-linked dimeric modulators for CXCR4. *Bioorg Med Chem* 2016;30679–4
68. Zhang C, Huang LS, Zhu R, Meng Q, Zhu S, Xu Y, Zhang H, Fang X, Zhang X, Zhou J, Schooley RT, Yang X, Huang Z, An J. High affinity CXCR4 inhibitors generated by linking low affinity peptides. *Eur J Med Chem* 2019;**172**:174–85
69. Kumar S, Choi WT, Dong CZ, Madani N, Tian S, Liu D, An J, Sodroski JG, Richman DD, Huang Z. SMM-chemokines: a class of unnatural synthetic molecules as chemical probes of unnatural synthetic molecules as chemical probes of chemokine receptor biology and leads for therapeutic development. *Chem Biol* 2006;**13**:69–79
70. Abraham M, Klein S, Bulvik B, Wald H, Weiss ID, Olam D, Weiss L, Beider K, Eizenberg O, Wald O, Galun E, Avigdor A, Benjamini O, Nagler A, Pereg Y, Tavor S, Peled A. The CXCR4 inhibitor BL-8040 induces the apoptosis of AML blasts by downregulating ERK, BCL-2, MCL-1 and cyclin-D1 via altered miR-15a/16-1 expression. *Leukemia* 2017;**31**:2336–46
71. Yoder A, Yu D, Dong L, Iyer SR, Xu X, Kelly J, Liu J, Wang W, Vorster PJ, Agulto L, Stephany DA, Coope JN, Marsh JW, Wu Y. HIV envelope-CXCR4 signaling activates cofilin to overcome cortical actin restriction in resting CD4 T cells. *Cell* 2008;**134**:782–92
72. Zhu Y, Shu Y, Wang J, Xu Y, An J, Huang Z. Development and validation of a LC-MS/MS assay for the determination of a novel D-peptide antagonist of CXCR4 in rat plasma and its application to a preclinical pharmacokinetic study. *J Pharm Biomed Anal* 2018;**161**:159–67
73. Sachpatzidis A, Benton BK, Manfredi JP, Wang H, Hamilton AD, Dohlman HG, Lolis E. Identification of allosteric peptide agonists of CXCR4. *J Biol Chem* 2003;**278**:896–907
74. Van Hout A, Klarenbeek A, Bobkov V, Doijen J, Arimont M, Zhao C, Heukers R, Rimkunas R, de Graaf C, Verrips T, van der Woning B, de Haard H, Rucker JB, Vermeire K, Handel T, Van Loy T, Smit MJ, Schols D. CXCR4-targeting nanobodies differentially inhibit CXCR4 function and HIV entry. *Biochem Pharmacol* 2018;**158**:402–12
75. Bobkov V, Zarca AM, Van Hout A, Arimont M, Doijen J, Bialkowska M, Toffoli E, Klarenbeek A, van der Woning B, van der Vliet HJ, Van Loy T, de Haard H, Schols D, Heukers R, Smit MJ. Nanobody-Fc constructs targeting chemokine receptor CXCR4 potently inhibit signaling and CXCR4-mediated HIV-entry and induce antibody effector functions. *Biochem Pharmacol* 2018;**158**:413–24
76. Soares H, Schols D, Vanlandschoot P, Van Rompaey P, Stortelers C. *Bispecific CXCR4-CD4 polypeptides with potent anti-HIV activity.* Patent application US20180086837A1, USA, 2018, pp.1–105
77. Tripathi S, Chaubey B, Ganguly S, Harris D, Casale RA, Pandey VN. Anti-HIV-1 activity of anti-TAR polyamide nucleic acid conjugated with various membrane transducing peptides. *Nucleic Acids Res* 2005;**33**:4345–56
78. Montefiori DC. Bispecific antibodies against HIV. *Cell* 2016;**165**:1563–4