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HIV-2 Susceptibility to Entry Inhibitors

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Abstract

Currently, there is a growing interest in using entry inhibitors to treat HIV-2-infected patients because, among the available drugs, few are fully active against HIV-2. Recent studies indicate that maraviroc and other experimental entry inhibitors, including new CCR5 and CXCR4 antagonists, inhibit primary isolates of HIV-2 as well as HIV-1 and may, therefore, expand the existing therapeutic armamentarium against HIV-2. There are, however, significant differences between the evolution of HIV-1 and HIV-2 envelope glycoproteins during infection that can lead to differences in the response to therapy with entry inhibitors over the course of the infection. Here, we review the available data on the susceptibility of HIV-2 to entry inhibitors in the context of the evolution of the sequence, structure, and function of envelope glycoproteins during infection. **(AIDS Rev. 2013;15:49-61).**

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Key words

HIV-2. HIV envelope. Viral entry. Entry inhibitors. CCR5 antagonists. Fusion inhibitors.

Introduction

Both HIV-2 and HIV-1, the causative agents of AIDS, were introduced into the human population by zoonotic transmission from distinct simian immunodeficiency viruses (SIV) that naturally infect nonhuman primates[1,2]. Worldwide, strains of HIV-1 have evolved into four very divergent phylogenetic groups: M, N, O, and P[3]. HIV-1 group M, the only pandemic group, has diversified into nine divergent subtypes (A, B, C, D, F, G, H, K, J), six sub-subtypes (F1, F2, A1-A5) and multiple circulating recombinant forms[4,5]. As for HIV-2, there are eight genetic groups, named A to H; only groups A and B seem to be spreading, with group A being much more common than group B[6-8].

HIV-2 and HIV-1 infections lead to very different immunological and clinical outcomes. Compared to HIV-1 patients, the majority of HIV-2-infected individuals has reduced general immune activation, normal $CD4^+$ T-cell counts, low or absent plasma viremia, and longer disease-free survival[9-12]. Nevertheless, with disease progression $CD4^+$ T-cell depletion becomes similar in HIV-1 and HIV-2 infections[10], most of the immunological differences are lost, and the mortality risk is equivalent[7,13,14]. The transmission rate of HIV-2 is significantly lower than that of HIV-1[15,16] and this is probably due to the markedly lower plasma viremia[16] and reduced viral shedding in the genital tract[17]. Consequently, and in contrast to the HIV-1 pandemic, HIV-2 is restricted to West Africa (e.g. Guinea-Bissau, Cape Verde, Ivory Coast)[18-20] and a few other countries (e.g. Portugal, France, Brazil, India)[7,21], affecting an estimated one to two million people[14].

There are major differences in the susceptibility of HIV-2 and HIV-1 to the currently available drugs. HIV-2 is naturally resistant to nonnucleoside reverse transcriptase inhibitors (NNRTI) and it presents a diminished sensitivity to some protease inhibitors (PI)[22]. Importantly, most combination antiretroviral therapy (cART) regimens used in HIV-1 patients are unable to fully suppress HIV-2 replication, or to increase the number of CD4 cells or prevent accumulation of drug-resistant mutations[23-29]. Currently there is a growing interest in using maraviroc to treat HIV-2-infected patients[30,31]. Recent *in vitro*

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studies indicate that primary isolates of HIV-2 are as inhibited by maraviroc and other experimental CCR5 and CXCR4 antagonists as HIV-1[32-34]. There are, however, significant differences between the evolution of HIV-1 and HIV-2 envelope glycoproteins during infection that can lead to significant differences in the response to therapy with entry inhibitors over the course of the infection[35-37]. Here, we review the available data on the susceptibility of HIV-2 to entry inhibitors in the context of the evolution of the sequence, structure, and function of envelope glycoproteins during infection.

Structural organization of the HIV envelope and the mechanism of viral entry

The entry of HIV into host cells is mediated by the envelope glycoproteins. The *env* gene encodes for a polyprotein precursor, which is heavily glycosylated in the Golgi apparatus ($Pr160^{Env}$ in HIV-1 and $Pr140^{Env}$ in HIV-2) and subsequently cleaved by a cellular protease into the surface (SU: gp120 in HIV-1; gp125 in HIV-2) and transmembrane (TM: gp41 in HIV-1; gp36 in HIV-2) glycoproteins. Mean HIV-1 and HIV-2 genetic diversity in *env* is about 0.5 nucleotide substitutions per site (0.48 ± 0.18 standard error), if comparing only the most prevalent HIV-1 group M and HIV-2 group A using the maximum composite likelihood method in MEGA 5[38].

The SU and TM glycoproteins are attached by non-covalent bonds and are assembled as trimers (3x[SU/TM]) on the surface of the mature virion[39]. The SU is composed of five hypervariable regions, V1 to V5, separated by five more conserved regions, C1 to C5 (Fig. 1). Hypervariable regions tend to form loops, stabilized by disulfide bridges. In its native trimeric conformation, SU has two domains: (i) an internal, hydrophobic in nature, and (ii) an external, highly glycosylated; both are linked by a small binding domain, the bridging sheet[40,41]. The TM glycoprotein consists of one extracellular ectodomain, one transmembrane region, and one intracytoplasmic domain (Fig. 1). The fusion peptide at the hydrophobic N-terminal end of the ectodomain is followed by two α-helices containing leucine zipper-like motifs, HR1 and HR2, separated by a cysteine bridge (CC)[39,42].

Binding of the SU glycoprotein to the CD4 cellular receptor generally marks the first stage of the multistep process of HIV entry. The CD4 receptor is a transmembrane protein with 58 kDa that exists on the surface of several cell types, like T helper cells, monocytes, and macrophages[43]. Upon SU – CD4 attachment, the V3 loop in the envelope glycoprotein is projected into close proximity to the cellular membrane where it can interact with the coreceptor[44]. *In vivo*, the major coreceptors for HIV entry are CCR5 and CXCR4, natural receptors for α and β chemokines[45]. The CCR5 is predominantly expressed on the surface of memory T lymphocytes, activated T lymphocytes, and macrophages, whereas CXCR4 is mainly found in T lymphocytes, monocytes, dendritic cells, and B lymphocytes. The engagement of the SU with its receptors brings the envelope and cellular membrane to close proximity and promotes additional structural rearrangements of the TM glycoprotein. As a result, the fusion peptide becomes exposed and is inserted into the cytoplasmic membrane, thus creating a pre-hairpin intermediate configuration of TM[46-48]. Then the HR2 trimer folds back in an anti-parallel fashion towards the HR1 trimer, forming a six-helix bundle structure (final hairpin state), stabilized by the hydrophobic interactions between the internal trimeric HR1 in the centre (central coiled-coil) and the HR2 domains outside[49]. During this process, the viral envelope and the cellular membrane are brought together, leading to the formation of the fusion pore through which the viral capsid enters the target cell.

HIV-1 can also gain entry into the cells by endocytosis[50,51] and by cell-to-cell fusion through viral synapses or membrane nanotubes[52]. It seems that HIV-1 infection by cell-to-cell fusion is more efficient than by cell-free virus[53,54] and permits ongoing HIV-1 replication even in the presence of the reverse transcriptase inhibitors tenofovir and efavirenz[55].

Kinetic studies have shown that Env-mediated fusion is faster in HIV-2 than in HIV-1[56]. Moreover, the rate at which the coreceptor binding site in Env becomes exposed after CD4 binding is faster in HIV-2 than in HIV-1[56]. These results indicate that CD4-induced changes in envelope conformation differ in HIV-1 and HIV-2. In fact, unlike HIV-1, some HIV-2 strains have the ability to infect cells via CCR5 and CXCR4 independently of CD4[57], suggesting that in its native state, the HIV-2 surface glycoprotein may sometimes adopt a CD4-induced conformation. This conformation may be stabilized by interactions between the cysteine residues of the V1/V2 regions in the hydrophobic cavity of gp125[58].

Coreceptor usage in HIV-2 infection

As with HIV-1, CCR5 and CXCR4 are the most important HIV-2 coreceptors[34,45,57,59-61]. However, some primary isolates of HIV-2 from asymptomatic patients may infect peripheral blood mononuclear cells (PBMC) independently of these two major coreceptors[62]; others may even enter into CD4-negative cells via CCR5 or CXCR4[57]. Most chronically infected asymptomatic HIV-2 patients

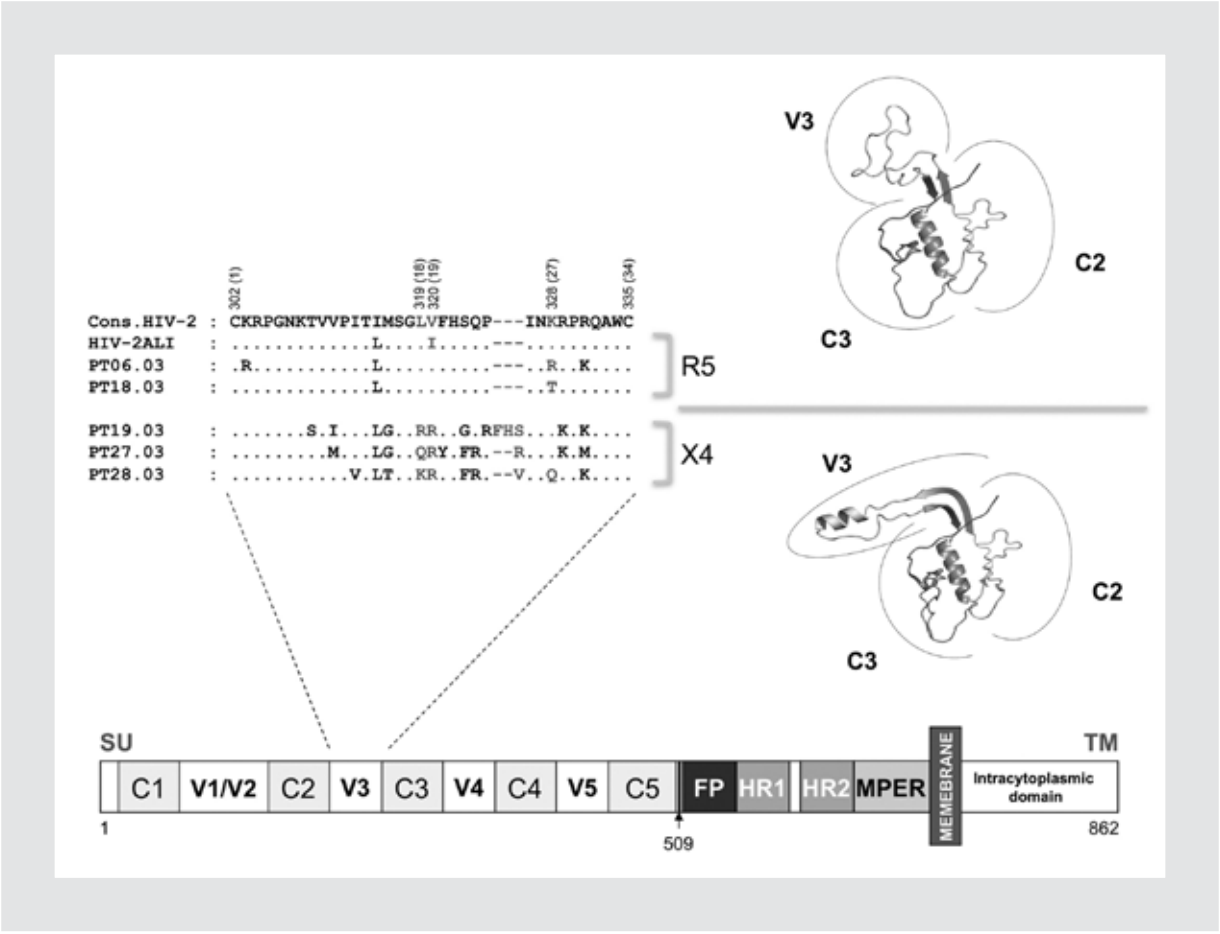

Figure 1. *Structural and functional domains of HIV-2 envelope glycoproteins. The SU glycoprotein (gp125) is composed by five conserved (C1-C5) and five variable (V1-V5) domains. The TM glycoprotein (gp36) contains the N-terminal fusion peptide (FP), two heptad repeats (HR1 and HR2), the membrane proximal transmembrane region (MPER), one transmembrane region and the intracytoplasmic domain. The amino acid sequence alignments show the variability of the V3 region between R5 and X4 HIV-2 strains. It includes an HIV-2 consensus sequence, and sequences from the reference R5 strain HIV-2ALI and from other primary isolates reported by us in a previous study[37]. Amino acid positions 18, 19, 24, and 27 that are associated with coreceptor usage are highlighted in grey in the alignments[37,60,67,70,71] (Cheila R, unpublished). In HIV-2, X4 tropism is associated with the presence of any mutation at residue 18, positively charged residues at positions 19 and 27, and insertions at position 24, thus leading to a significant change in conformation compared to the V3 loop of R5 isolates[37,183]. The 3-D models show the differences in V3 conformation of R5 and X4 isolates and were constructed by homology modeling[184]. Sites were numbered according to codon* env *position of HIV-2ALI reference strain.*

harbor CCR5-using (R5) strains which, unlike R5 HIV-1 isolates, may also infect cells expressing one or more of other chemokine receptors such as CCR1, CCR2b, CCR3, GPR15 (BOB), and CXCR6 (BONZO)[63,64]. CXCR4-tropic (X4) HIV-2 isolates have only been found in patients with advanced disease and low $CD4^+$ T-cell counts and are strongly associated to faster disease progression compared to R5 isolates[37,60,63,64]. Of note, most X4 isolates also use one or more alternative coreceptors, such as CCR1, CCR2b, CCR3, CCR5, BOB, and BONZO, though less efficiently than CXCR4[37,60,65,66]. Notably, there is a close association between HIV-2 tropism and susceptibility to antibody neutralization since X4 isolates are significantly more resistant to antibody neutralization than R5 isolates[37,67]. This is in contrast to HIV-1 where X4 variants are more sensitive to neutralization than R5 variants[68,69], and suggests that in HIV-2-infected individuals, R5-to-X4 changes in viral tropism might be associated to escape from the neutralizing antibody response.

As in HIV-1, the V3 loop in the SU glycoprotein is the major determinant of HIV-2 tropism and its charge, size, and structural conformation directly influence interaction with CCR5 or CXCR4 (Fig. 1)[37,60,67,70,71]. Visseaux, et al. recently described an algorithm to infer HIV-2 CXCR4 usage from V3 sequences[71]. They found that CXCR4 usage is associated to two of the following characteristics: V3-loop net charge > +6; any mutation at residue L18; V19R/K mutation; and insertions at

Table 1. Recommended regimens for the treatment of HIV-2 infection[30,31,73,74,76,185]

	First-line	Second-line
Nucleoside reverse transcriptase inhibitors	Tenofovir or zidovudine plus emtricitabine or lamivudine	Zidovudine or tenofovir plus emtricitabine or lamivudine or other active agent (e.g. abacavir)
Protease inhibitors boosted with ritonavir	Plus lopinavir or darunavir	Plus saquinavir or lopinavir or darunavir
Integrase inhibitors		Plus raltegravir
Entry inhibitors*		Plus maraviroc (could be considered as part of a third-line regimen for treatment-experienced patients infected with R5 viruses)

*Additional potentially active agents may include P3, CADA, cyanovirin-N or others, but their clinical safety and efficacy are yet to be evaluated.

residue 24. Based on these and other rules[70], new computational tools are currently being developed to infer coreceptor use and susceptibility of HIV-2 isolates to CCR5-antagonists such as maraviroc.

Antiretroviral therapy

Highly active antiretroviral therapy (HAART), significantly increases the number of $CD4^+$ T-cells and decreases the morbidity and mortality of HIV-1-infected patients[72]. For HIV-2, the benefit of antiretroviral therapy is not so clear since the number of $CD4^+$ T-cells usually does not increase to the same level as in HIV-1 infection[10,24,25,27,29]. This is not surprising since all antiretroviral drugs were specifically designed to suppress HIV-1 replication. Currently, only tenofovir, zidovudine, lamivudine and emtricitabine (NRTI), raltegravir (integrase inhibitors, INI), and saquinavir, lopinavir and darunavir (PI) are as effective against HIV-2 infection[22,73,74]. However, there is still little information on the clinical use of these inhibitors in HIV-2 patients and treatment management often relies on HIV-1-based evidence[73-75].

Recent HIV-2 treatment guidelines recommend the use of tenofovir, emtricitabine, and lopinavir/ritonavir or darunavir/ritonavir as a good first-line regimen[74], saving saquinavir/ritonavir as a second-line option[73] (Table 1). Raltegravir may also be combined with second-line regimens[76], but the long-term effectiveness of this drug in HIV-2 infection is still under evaluation[77,78]. At the moment, clinical evidence supporting the use of maraviroc, a CCR5 antagonist, in the treatment of HIV-2 has only been provided by two case reports (see CCR5 antagonists)[30,31].

Compared to HIV-1, the genetic barrier to resistance is lower in HIV-2 for some NRTI[73,79], PI[80] and probably for INI[81]. The positions involved in drug resistance generally match the ones of HIV-1, but there are some natural polymorphisms in HIV-2 that are associated with resistance in HIV-1 (219Q/E in RT[73], 46I in PR[82,83]). Moreover, several reports suggest that the pathways of resistance are also different between both viruses. For instance, HIV-2 has a higher propensity to develop the resistance mutation V47A in lopinavir-based regimens[84]. Also, high level resistance to NRTI can be conferred just by two mutations (Q151M/ K65R or Q151M/M184V)[85], whereas in HIV-1 it usually involves a combination of thymidine-associated mutations. Genotypic drug resistance interpretation algorithms are already available for HIV-2 (e.g. ANRS v21 and REGA v8.0.2)[73,75].

HIV entry inhibitors

Inhibition of viral entry is one of the most attractive targets in the search for new anti-HIV molecules. Enfuvirtide (Fuzeon®, Roche, Switzerland; T-20) was approved for clinical use in HIV-1 patients in 2003 and represented the first entry inhibitor[86]. Four years later, maraviroc (Selzentry®, Pfizer, USA) was approved by the US Food and Drug Administration (FDA)[87]. Entry inhibitor molecules can be classified in three groups according to the step of viral entry that they target: attachment inhibitors, coreceptor binding inhibitors, and fusion inhibitors[88,89]. Enfuvirtide is a fusion inhibitor, while maraviroc is a CCR5 antagonist. This section will focus on the comparison between HIV-1 and HIV-2 susceptibilities to some representative molecules of each class. The concentrations necessary to inhibit 50% of HIV-1 and HIV-2 infectivity (IC_{50}) are summarized in table 2.

Table 2. Comparison between HIV-1 and HIV-2 susceptibilities to different classes of entry inhibitors

Mechanism of action	Name	Type of molecule	Target	Assay used to measure antiviral activity	IC_{50}		Reference
					HIV-1	HIV-2	
Attachment inhibitors	rsCD4	Recombinant soluble CD4	SU	RT activity	37.5 nM	950 nM	90, 91
	BMS-378806	Small molecule	SU	p24 production and RT activity	0.9-743.0 nM	> 300,000 nM	94
	Cyclotriazadisulfonamide	Small molecule	CD4	P24 production	0.3-1.5 µM	0.2 µM	102
Coreceptor binding inhibitors	TAK-779	Small molecule	CCR5	p24 production (HIV-1); RT activity and single cycle reporter gene (HIV-2)	1.6-100 nM	0.6-128.3 nM	34, 122, 123
	Maraviroc	Small molecule	CCR5	RT activity (HIV-1); Viral load, RT and single cycle reporter gene (HIV-2)	0.1-4.5 nM	0.04-5.5 nM	34, 125
	AMD3100	Small molecule	CXCR4	Single cycle reporter gene	0.9-5.2 nM	1.0-4.6 nM	34, 144
	AMD3451	Small molecule	CCR5/CXCR4	p24 production	1,200-26,500 nM	9,000 nM	147
Fusion inhibitors	Enfuvirtide (T-20)	Peptide	TM	Single cycle reporter gene	0.7-228 nM	35.6-2,857 nM	34, 155, 156
	T-1249	Peptide	TM	MAGI/cMAGI (HIV-1) Single cycle reporter gene (HIV-2)	0.08-10.3 nM	0.9-21.9 nM	34, 165
	Sifuvirtide	Peptide	TM	p24 production and single cycle reporter gene (HIV-1) Syncytia formation (HIV-2)	0.96-209.77 nM	256.4-745.3 nM	167-169
	HR212	Recombinant protein	TM	Cell-to-cell fusion and syncytia formation	3.9-6.6 nM	103-5,927 nM	172
	P3	Peptide	TM	Single cycle reporter gene	0.3-62.2 nM	13.3-369.4 nM	174
	2,4(1H,3H)-pyrimidinedione	Small molecule	Undetermined	MAGI/cMAGI (HIV-1) Cytopathic effect (HIV-2)	6-2,390 nM	100-28,200 nM	176
Natural products	Cyanovirin-N	Protein	SU	RT activity	0.1-5.8 nM	2.3-7.6 nM	179
	Mannose-specific plant lectins from *Amaryllidaceae* family	Protein	SU	Cytopathic effect	0.3-4.7 µg/ml	0.12-0.25 µg/ml	180
	Galactan sulfate	Polysaccharide	SU	Cytopathic effect	0.6 µg/ml	0.5 µg/ml	181
	Prostratin	Small molecule	Cellular receptors	p24 production	0.06 -0.07 µg/ml	0.02-0.03 µg/ml	182

SU: surface; TM: transmembrane; RT: reverse transcriptase.

Attachment inhibitors

Several strategies have been pursued in order to block the interaction between SU and CD4. So far none has resulted in a clinically useful anti-HIV drug. One of the earliest was the development of recombinant soluble CD4 (rsCD4) molecules, which function as a molecular decoy, inhibiting the ability of SU to attach to cell-associated CD4. *In vitro,* rsCD4 inhibits the replication of cell line-adapted HIV-1 isolates at 37.5 nM; a 25-fold higher concentration (950 nM) is needed to inhibit HIV-2[90,91], which is consistent with the lower binding affinity of the HIV-2 gp125 to CD4[58]. Despite potent activity against laboratory strains of HIV-1 *in vitro*, 200-2,700 higher concentrations of rsCD4 are needed to neutralize clinical isolates *in vivo*, precluding its use in the treatment of HIV infection[92]. These molecules might, however, be useful as microbicides[93].

BMS-378806 is a small-molecule that binds with great affinity to gp120 and seems to prevent CD4-induced conformational changes[94,95]. It has strong antiviral activity against HIV-1 (median IC_{50} 12 nM, range 0.9-743), but not against HIV-2 (IC_{50} > 300 μM)[94]. The development of this molecule has stopped at phase I trials. However, BMS-378806 and its analogs are attractive alternatives to monoclonal antibodies as vaginal microbicides[96].

Ibalizumab (TNX-355) is an anti-CD4 monoclonal antibody that binds to the D2 domain of CD4[97]. It acts as a post-attachment inhibitor by decreasing the flexibility of CD4 and hindering the access of CD4-bound SU to the coreceptors[89]. The antiviral activity (IC_{50}) of TNX-355 against lab-adapted and primary HIV-1 isolates ranges from 0.13 to 2.0 μg/ml (mean, 1.02 μg/ml) *in vitro*[98]. Promising results were obtained *in vivo* against HIV-1 in early clinical trials[99,100]. However, preliminary experiments suggest that TNX-355 activity against HIV-2 is limited since its precursor (MAb 5A8) does not block rsCD4 enhancement of HIV-2 fusion in $CD4^+$ T-cells (as reported by Moore, et al.[97]).

Cyclotriazadisulfonamide (CADA) and its analogs are CD4-targeted small molecules that inhibit HIV entry by down-modulation of CD4 at the cell surface[101,102]. CADA is a broad spectrum anti-HIV agent and inhibits the replication of HIV-2 and HIV-1 strains at similar concentrations (IC_{50} of 0.2 μM and 0.3-1.5 μM, respectively)[102]. It acts synergistically in combination with other entry inhibitors as well as with several RTI and PI *in vitro*[103]. CADA is being proposed as a potential microbicide candidate[104].

Coreceptor binding inhibitors

The rationale for developing drugs that block the SU-coreceptor interaction was supported by the genetic restriction of HIV-1 infection and delayed disease progression observed in individuals that carry a defective *ccr5* allele (Δ32-*ccr5*), without suffering any significant clinical consequences for bearing such mutation[88,105,106]. Generally, coreceptor binding inhibitors are either CCR5 or CXCR4 antagonists.

The CCR5 antagonists can be divided according to the type of molecule: anti-CCR5 antibodies, such as PRO-140; derivatives of natural CCR5 ligands, like AOP-RANTES; and small-molecules, like TAK-779 and maraviroc[88,107].

PRO 140 is a humanized CCR5 monoclonal antibody that binds to a complex epitope involving the second extracellular loop and the amino-terminal domain of CCR5 and blocks this receptor[108]. PRO 140 is a potent subtype-independent inhibitor of HIV-1 R5 replication[109], with IC_{50} concentrations ranging from < 5.3 to 42 nM in macrophage cultures (median IC_{50}, 16 nM). Preliminary data indicates that this molecule can also inhibit HIV-2 replication in PBMC with similar efficiency to HIV-1[110]. Currently, PRO 140 is under development and has demonstrated potent and prolonged antiretroviral activity after both subcutaneous[111] and intravenous[112] administrations in phase II clinical trials.

RANTES, MIP-1α, and MIP-1β are naturally occurring ligands of CCR5 with major HIV-suppressive activity[113]. Compared to HIV-1, the higher production of these chemokines in response to HIV-2 infection may contribute to the longer survival of HIV-2-infected patients[114,115]. Recombinant human RANTES, MIP-1α, and MIP-1β efficiently block both HIV-1 and HIV-2 infection *in vitro* by two mechanisms: receptor down-regulation and direct competition with the viral envelope for CCR5 binding[116,117]. However, these molecules might also have a potential agonist activity on CCR5. Therefore, a number of analogs have been developed to reduce such undesirable effects, e.g. AOP-RANTES, NNY-RANTES and PSC-RANTES. The antiviral activity of AOP-RANTES has been characterized both against HIV-1 and HIV-2, but IC_{50} values were only measured for HIV-1[118-120]. The AOP-RANTES inhibits HIV-1 replication in PBMC, with IC_{50} ranging between 0.04 to 1.3 nM[120]. This molecule completely blocks HIV-2 replication at a minimal concentration of 4 nM[119]. Currently, recombinant RANTES analogs are also being formulated as vaginal microbicides[121].

Small molecules that block the CCR5 coreceptor and hinder the SU-CCR5 interaction have demonstrated potent inhibition of HIV-1 replication *in vitro*[89]. TAK-779 was one of the first compounds of this group[122]. It binds to residues lining a cavity formed by the 1, 2, 3, and 7 transmembrane helices of CCR5 extracellular loops[123]. It is highly selective to CCR5 and is a potent antiviral agent, inhibiting HIV-1 replication in PBMCs with IC_{50} ranging between 1.6 and 100 nM[122,123]. TAK-779 also inhibits HIV-2 cell entry at similar concentrations (IC_{50} range for HIV-2: 0.6-128.3 nM)[33,34]. However, its clinical development was discontinued due to its toxicological profile and poor bioavailability[124].

As mentioned above, maraviroc is the only coreceptor antagonist approved for clinical use in HIV infection. It acts as a functional antagonist of CCR5[125] and interacts with residues lining a cavity formed by the 2, 3, 6, and 7 transmembrane helices of CCR5 extracellular loops[126]. Maraviroc has potent antiviral activity (IC_{50} range: 0.1-4.5 nM) against HIV-1 groups M and O[125]. The efficacy of maraviroc in HIV-1-infected patients was originally confirmed in a pair of phase III clinical trials, MOTIVATE 1 and 2[127,128]. Maraviroc is administered orally twice daily and in combination with other antiretroviral agents for the treatment of HIV-1 infection in treatment-experienced patients infected with CCR5-tropic viruses. As it is only active against viruses using exclusively the CCR5 coreceptor[125], it is necessary to test for coreceptor usage before starting therapy[129]. The successful results obtained in vaginal topical applications are also encouraging the formulation of maraviroc as a microbicide[130,131].

Two case reports have described the use of maraviroc in salvage therapy of HIV-2 patients who have failed all other therapeutic regimens[30,31]. Neither a genotypic nor a phenotypic tropism test has been carried out before initiation of maraviroc. It was shown that patients taking either regimens containing maraviroc and raltegravir[30] or maraviroc and foscarnet[31] increased their $CD4^+$ T-cell counts and maintained undetectable viral load. However, the nature of these studies prevents any firm conclusion regarding the efficacy of maraviroc in HIV-2 patients. Moreover, until very recently there were no studies on the *in vitro* activity of maraviroc on primary isolates of HIV-2. Recent estimates of the baseline susceptibility of HIV-2 to maraviroc indicate that it inhibits entry and replication of HIV-2 R5 primary isolates with similar IC_{50} compared to HIV-1 (IC_{50} range for HIV-2: 0.9-5.5 nM)[32-34]. However, maraviroc inhibits HIV-2 entry with significantly higher IC_{90} (42.7 nM for HIV-2 and 9.7 nM for HIV-1) and lower curve slope values (0.7 vs. 1.3) than HIV-1, suggesting that higher dosages of maraviroc might be required for the effective treatment of HIV-2-infected patients[34]. Clinical trials are warranted to determine the most effective maraviroc dosage on HIV-2 patients.

Altered CCR5 use may evolve during the course of HIV-2 and HIV-1 infection such that R5 variants isolated from late-stage disease patients with low levels of $CD4^+$ cells have reduced sensitivity to C-C chemokines, TAK-779, and maraviroc[34,132-134]. These results suggest that maraviroc could be a more useful drug in early HIV-1 and HIV-2 infection.

The importance of CXCR4 blockage as a strategy to prevent HIV infection was first highlighted by the potent anti-HIV activity of SDF-1 chemokine, the natural ligand of CXCR4[135,136]. Stromal cell-derived factor-1 (SDF-1) prevents syncytia formation and inhibits HIV-1 and HIV-2 infection at the nanomolar range[33,135]. While there are numerous CCR5 antagonists with different structures, the array of CXCR4 antagonists is scarce and their structure is similar to AMD3100, one of the first small molecules of this group to enter in clinical trials[137,138]. Like SDF-1, these antagonists are positively charged and basic in nature. AMD3100 anchors to the negatively charged Asp^{171} and Asp^{262} located in transmembrane domains 4 and 6 of the CXCR4 coreceptor, hampering its interaction with the viral SU glycoprotein[139,140]. AMD3100 is a strong inhibitor of X4 strains *in vitro*, but is completely inactive against R5 strains[34,141]. Viral cytopathic effect (syncytia) is blocked at equivalent concentrations for both HIV-1 and HIV-2 isolates (IC_{50} range: 0.8-5.0 nM for HIV-1 and 3-17 nM for HIV-2)[142,143]; inhibition of viral entry also occurs at similar IC_{50} for both viruses (IC_{50} range, 0.9-5.2 nM for HIV-1 and 1.0-4.6 nM for HIV-2)[34,144]. Further development of AMD3100 as an antiviral was discontinued early on due to cardiac abnormalities during an open-label clinical trial[145], but its use has been approved as an hematopoietic stem cell mobilizer (plerixafor; Mozobil®, Genzyme, USA)[146].

AMD3451 is a small molecule with specific dual CCR5/CXCR4 antagonistic properties that has demonstrated antiviral activity *in vitro* against both HIV-1 and HIV-2 isolates[147]. It inhibits the replication of a broad range of R5, R5X4 dual-tropic, and X4 strains in different cells (cell lines, PBMC, and monocytes/macrophages) at IC_{50} ranging from 1.2 to 26.5 μM in HIV-1 and 9 μM in HIV-2. The mode of interaction between AMD3451 and the receptor proteins is still unclear, but it seems to be different for CCR5 and CXCR4.

Fusion inhibitors

Peptides derived from HR1 and HR2 sequences in the TM glycoprotein can inhibit HIV infection by competitive binding to their complementary regions. T-20 was the first fusion inhibitor approved for clinical use under the generic name enfuvirtide. It is a linear peptide with 36 amino acids and its sequence corresponds to amino acids 638-673 in the HR2 region of HIV-1 LAI isolate[148,149]. According to the currently accepted model, enfuvirtide inhibits virus entry by binding to the HR1 core exposed at the pre-hairpin intermediate state of TM, thereby blocking the subsequent formation of the six-helix bundle structure and viral fusion[48,49,150,151]. Because enfuvirtide alone is a weak inhibitor of the six-helix bundle formation[152], the mechanism of action of enfuvirtide seems to involve also the interaction with lipids of the target cell membrane in such a way that the cell membrane acts as enfuvirtide reservoir, enabling direct contact of the peptide with its gp41 target region[150,153].

Enfuvirtide is indicated, in combination with other antiretroviral agents, for the treatment of HIV-1 infection in treatment-experienced patients with evidence of viral replication despite ongoing HAART[154]. The recommended dosage is 90 mg twice daily by subcutaneous injection. Coadministration of enfuvirtide has even significantly improved the response rates to newer agents like maraviroc[89,127]. Baseline susceptibility of HIV-1 subtypes B and non-B to enfuvirtide is highly variable, ranging between 0.1 to 223 nM (IC_{50}) in viral entry inhibition assays[34,155-157]. This variability can be explained by the genetic heterogeneity of Env in the HR1/HR2 regions of TM and also in the CD4 binding and V3 region of SU[156,158]. Indeed, the majority of mutations associated with enfuvirtide resistance are mapped within these regions and especially between the 36-45 codons of HR1[157,159-161]. It has been suggested that the genetic variability of HR1/HR2 regions between HIV-1 and HIV-2[162] is responsible for the lack of activity of enfuvirtide on HIV-2. In fact, when compared to HIV-1, up to 100-fold higher concentrations of enfuvirtide are necessary to inhibit HIV-2 cell-free infection (IC_{50} range: 35.6-2,857 nM)[34,143,149].

Second and third generations fusion inhibitors have been developed in an attempt to improve antiviral potency, increase *in vivo* stability, and overcome enfuvirtide resistance[151,163]. T-1249 is a representative second generation 39-mer peptide derived from HR2 consensus sequence of HIV-1, HIV-2, and SIV strains[164,165]. It is a strong inhibitor of HIV-1 replication (IC_{50} range: 0.08-10.3 nM), including isolates resistant to enfuvirtide, and it also inhibits HIV-2 with similar potency[34,164,165]. Baseline susceptibility of HIV-1 primary isolates to T-1249 ranges from 0.08 to 10.3 nM (IC_{50}); for HIV-2 isolates it ranges from 0.9 to 21.9 nM. Since enfuvirtide peptide only includes the HR core and lipid-binding domain, the stronger antiviral activity of T-1249 over enfuvirtide has been attributed to the inclusion of all functional domains of HR2 region in its sequence (pocket-binding domain, HR core, and lipid-binding domain)[150,151]. However, the elevated production costs and drug formulation difficulties associated with its long size have hampered clinical development of T-1249 beyond phase I/II clinical trials[166]. Sifuvirtide is a third generation fusion inhibitor peptide with increased α-helical content (hence lower susceptibility to proteolytic degradation in the serum), which has shown promising results in phase II clinical studies, being active against a broad range of HIV-1 isolates (IC_{50} range: 0.96-209.77 nM), including enfuvirtide-resistant strains[163,167-169]. Despite its better pharmacokinetic profile than enfuvirtide, it is still administered as a subcutaneous injection. In addition, its activity seems to be significantly weaker against HIV-2, as suggested by the susceptibility of a couple of laboratory strains to sifuvirtide in syncytia assays (IC_{50} range: 256.4-745.3 nM)[167]. Both T-1249[170] and sifuvirtide[171] have also been formulated as topical gels and are potential microbicide candidates.

HR212 is a soluble and stable recombinant protein expressed in *Escherichia coli*, formed by linking the HR2-based C34 peptide to the N terminus of a HR1-HR2 segment. This configuration (C34-N34-C34) successfully inhibits HIV fusion and is moderately active against HIV-2[172]. HR212 blocks cell-to-cell fusion of the lab-adapted strains HIV-2 CBL-20 and HIV-2 ROD at an IC_{50} of 103 and 5,377 nM, respectively, and inhibits the replication (reduction of syncytia formation) of the same isolates at 170 and 5,927 nM. Overall, these IC_{50} values are 3- to 30-fold lower than the ones obtained in parallel with enfuvirtide, but noticeably higher than the activity of HR212 measured against HIV-1 IIIB (3.92 nM in the cell-to-cell fusion assay and 6.59 nM to inhibit viral replication). The clinical efficacy of HR212 peptides is yet to be confirmed.

Peptides derived from the HR2 region of HIV-2 TM were shown to potently inhibit both HIV-1 and SIVmac envelope-mediated cell fusion (IC_{50} range: 4-73 nM)[173]. In line with these observations, we have recently demonstrated that a new peptide (P3) based on HR2 ancestral sequences of HIV-2 and SIVmac blocks

cell-free HIV-1 and HIV-2 infection at the nanomolar range[174]. Remarkably, P3 is a stronger inhibitor of HIV-1 (mean IC_{50} of 11 nM for several highly diverse subtypes) than HIV-2 (63.8 nM). This peptide is also very active against HIV-1 variants resistant to enfuvirtide. Collectively, these results suggest that fusion inhibitor peptides derived from HIV-2 could be potentially useful to treat patients infected with HIV-1, including enfuvirtide-resistant strains.

A number of 2,4(1H,3H)-pyrimidinedione derivatives of SJ-3366, a potent NNRTI of HIV-1 and HIV-2[175], have the unique feature of displaying a dual mechanism of action against HIV, both as NNRTI and entry inhibitors[176]. The mechanism of entry inhibition is not well defined, but preliminary data suggests that these compounds do not prevent the attachment of virus to target cells, but recognize a pre-fusion conformational complex involving both envelope and Gag determinants. Anti-HIV-2 activity occurred only at the entry step of the replication cycle and 100 to 28,200 nM were required to inhibit HIV-induced cytopathic effect in CEM-SS cells. In contrast, HIV-1 infection was inhibited by both antiviral mechanisms; cell entry was blocked at 6-2,930 nM (MAGI assay). These pyrimidinedione compounds are awaiting further preclinical development.

Natural products

Over the last two decades, a large variety of natural molecules have demonstrated anti-HIV activity by interfering with several steps of the replication cycle. Natural products extracted from medicinal plants and marine organisms represent the vast majority of such molecules[177]. Carbohydrate-binding proteins are potent HIV entry inhibitors and are interesting microbicides candidates[178]. Cyanovirin-N, isolated from the cyanobacteria *Nostoc ellipsosporum*, is a lectin that targets N-linked high mannose oligosaccharides of the SU glycoprotein and inhibits both HIV-2 (IC_{50}: 2.3-7.6 nM) and HIV-1 (0.1-5.8 nM) infection[179]. It is under preclinical development and topical gel formulations effectively block SHIV infection in macaques[178]. Other mannose-specific lectins, like those isolated from the plants *Galanthus nivalis* and *Hippeastrum* hybrid (*Amaryllidaceae* family), also inhibit both HIV-2- and HIV-1- induced cytopathicity at similar concentrations (IC_{50}: 0.12-0.25 µg/ml for HIV-2 vs. 0.3-4.7 µg/ml for HIV-1) even when they are formulated as a gel (IC_{50}: 0.25 µg/ml for HIV-2 vs. 0.1 µg/ml for HIV-1)[180]. Galactan sulfate, a sulphated polysaccharide extracted from the red seaweed *Aghardhiella tenera*, has broad-spectrum activity against several enveloped viruses[181]. It is a polyanion, a group of compounds that are commonly formulated as microbicides[178]. Galactan sulfate binds directly to the SU glycoprotein and inhibits HIV-2 and HIV-1 entry at 0.5 and 0.6 µg/ml, respectively[181]. Prostratin is another natural HIV-2 entry inhibitor. Isolated from the tropical plant *Homalanthus nutans*, it is a 12-deoxiphorbol ester that targets HIV cellular receptors and blocks viral entry at 0.02-0.03 µg/ml in HIV-2 and 0.06-0.07 µg/ml in HIV-1 (IC_{50})[182].

Conclusion

Several entry inhibitors are highly active against HIV-2 *in vitro.* This is particularly the case of CADA an attachment inhibitor, maraviroc a coreceptor binding inhibitor, P3 a new fusion inhibitor peptide, and cyanovirin-N a natural product. Characterization of the safety and effectiveness of these entry inhibitors in clinical trials is urgently required as they may expand the limited therapeutic armamentarium currently available for HIV-2.

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