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Review

Antiretroviral therapy and drug resistance in human immunodeficiency virus type 2 infection

Luis Menéndez-Arias *, Mar Álvarez

Centro de Biología Molecular "Severo Ochoa" (Consejo Superior de Investigaciones Científicas & Universidad Autónoma de Madrid), c/Nicolás Cabrera, 1, Campus de Cantoblanco, 28049 Madrid, Spain

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ABSTRACT

One to two million people worldwide are infected with the human immunodeficiency virus type 2 (HIV-2), with highest prevalences in West African countries, but also present in Western Europe, Asia and North America. Compared to HIV-1, HIV-2 infection undergoes a longer asymptomatic phase and progresses to AIDS more slowly. In addition, HIV-2 shows lower transmission rates, probably due to its lower viremia in infected individuals. There is limited experience in the treatment of HIV-2 infection and several antiretroviral drugs used to fight HIV-1 are not effective against HIV-2. Effective drugs against HIV-2 include nucleoside analogue reverse transcriptase (RT) inhibitors (e.g. zidovudine, tenofovir, lamivudine, emtricitabine, abacavir, stavudine and didanosine), protease inhibitors (saquinavir, lopinavir and darunavir), and integrase inhibitors (raltegravir, elvitegravir and dolutegravir). Maraviroc, a CCR5 antagonist blocking coreceptor binding during HIV entry, is active *in vitro* against CCR5-tropic HIV-2 but more studies are needed to validate its use in therapeutic treatments against HIV-2 infection. HIV-2 strains are naturally resistant to a few antiretroviral drugs developed to suppress HIV-1 propagation such as nonnucleoside RT inhibitors, several protease inhibitors and the fusion inhibitor enfuvirtide. Resistance selection in HIV-2 appears to be faster than in HIV-1. In this scenario, the development of novel drugs specific for HIV-2 is an important priority. In this review, we discuss current anti-HIV-2 therapies and mutational pathways leading to drug resistance.

Contents

1. Introduction

According to the latest estimates from UNAIDS, there are approximately 34 million people worldwide living with the human immunodeficiency virus (HIV), with a prevalence rate (i.e.

* Corresponding author. Tel.: +34 91 196 4494; fax: +34 91 196 4420.
E-mail address: lmenendez@cbm.csic.es (L. Menéndez-Arias).

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percentage of HIV-infected people 15–49 years old) of 0.8% (UNAIDS, 2013). Although HIV type 1 (HIV-1) is responsible for most of the global AIDS pandemic, there are about one to two million people infected with HIV type 2 (HIV-2), most of them in Senegal, Guinea Bissau, Gambia, Ivory Coast and Cape Verde (Gottlieb et al., 2008a). The highest prevalence has been observed in Guinea Bissau where up to 8–10% of the adult population could be infected (Poulsen et al., 1993). In addition to being endemic in West Africa, during the last two decades HIV-2 has spread to nineteen different countries in Europe, Asia and North America (Matheron et al., 1997; Nam et al., 2006; Barin et al., 2007; Gurjar et al., 2009; Valadas et al., 2009; Torian et al., 2010). In Europe, relatively high prevalences of HIV-2 infection have been reported in Portugal (Valadas et al., 2009) and France, where surveillance studies showed that around 2% of the new infections in 2003–2006 were caused by HIV-2 (Barin et al., 2007).

HIV-2 was first isolated in 1986 (Clavel et al., 1986, 1987). The genome organization of HIV-2 (Fig. 1) was determined from virus obtained from an AIDS patient from Cape Verde islands, and designated as the ROD isolate (Guyader et al., 1987). At present, HIV-2 strains are classified in eight groups named A through H (Damond et al., 2004; Santiago et al., 2005), although only groups A and B cause epidemics (Fig. 1). Isolates from group A are responsible for most HIV-2 infections worldwide, although it is most prevalent in Guinea Bissau (Rowland-Jones, 2006), while HIV-2 group B is more frequent in Ivory Coast and Ghana. ROD is a prototypic HIV-2 group A strain while EHO is usually considered the reference strain for group B. Phylogenetic relationships between HIV-2 groups A and B and HIV-1 clades and selected simian immunodeficiency viruses (SIV) are depicted in Fig. 2.

Recombinant HIV-2 containing sequences of groups A and B have been identified in Cameroon and the Ivory Coast (Gao et al., 1994; Yamaguchi et al., 2008), and evidence of a circulating recombinant form (HIV-2 CRF01_AB) has been recently reported in Japan (Ibe et al., 2010). Infections with groups C to G have been detected only in one or two individuals and available evidence indicates that in those cases infection did not lead to immune suppression (Gao et al., 1994). An exception is the highly divergent HIV-2 group H strain that was isolated from a man suffering immunodeficiency in the Ivory Coast (Damond et al., 2004). More recently, a large screening study in villages close to Tai National Park (in the south-west of Ivory Coast) led to the identification of a novel HIV-2 variant (HIV-2-071C-TNP03) infecting an 8-year old boy, and unrelated to any of the previously defined HIV-2 groups (Ayouba et al., 2013).

HIV-2 groups derive from independent transmissions from sooty mangabeys (*Cercocebus atys*) to humans (Santiago et al., 2005; for a recent review, see Peeters et al., 2013). Sooty

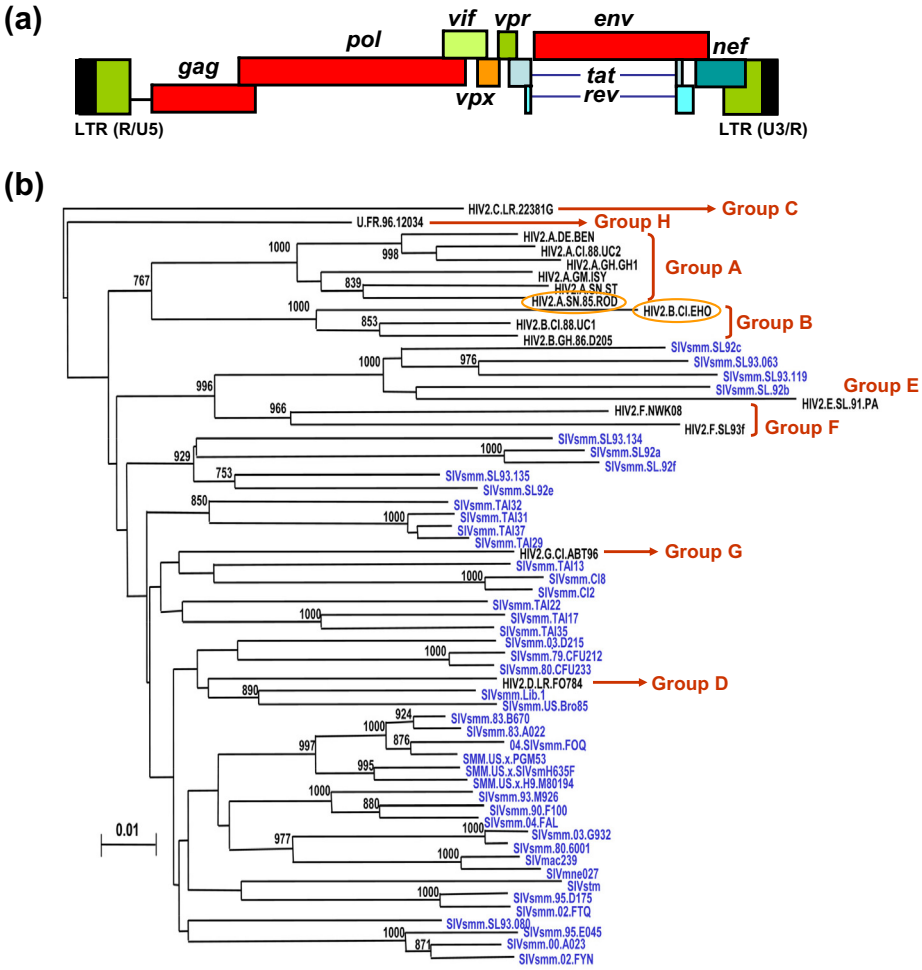


Fig. 1. HIV-2 genome organization and *gag* phylogenetic tree of HIV-2 and simian immunodeficiency virus strains. (a) Major (*gag*, *pol*, *env*) and accessory and regulatory genes (*vif*, *vpx*, *vpr*, *tat*, *rev*, *nef*) in the HIV-2 genome. Antiretroviral drug targets such as the protease, the reverse transcriptase and the integrase are encoded within the *pol* gene. (b) Phylogenetic tree based on *gag* nucleotide sequences and showing the group classification of HIV-2 isolates (adapted from Smith et al., 2008a). Reference HIV-2 strains such as ROD (group A) and EHO (group B) are indicated with an orange oval.

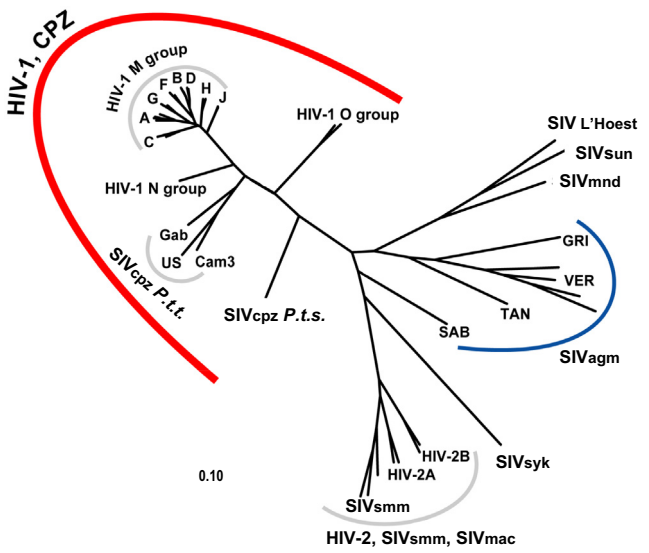


Fig. 2. Phylogenetic relationships between primate lentiviruses. The tree was obtained using complete viral genomes of HIV-1 group M, N and O and HIV-2 group A and B strains, as well as selected SIV variants of chimpanzees (*Pan troglodytes troglodytes and Pan troglodytes schweinfurthii*) (SIVcpz *P.t.t.* and *P.t.s.*, respectively), sooty mangabeys (*Cercocebus atys*) (SIVsmm), rhesus macaques (*Macaca mulatta*) (SIVmac), Sykes' monkeys (*Cercopithecus mitis*) (SIVsyk), African green monkeys (*Chlorocebus* sp.) (SIVagm), represented by the tantalus, vervet, grivet, and sabaeus species, and the SIVs of the L'Hoest group including those of L'Hoest monkeys (*Cercopithecus lhoesti lhoesti*) (SIV L'Hoest) and sun-tailed monkeys (*Cercopithecus lhoesti solatus*) (SIVsun) and a distantly related SIV from mandrills (*Mandrillus sphinx*). Adapted from Kuiken et al. (1999).

mangabeys are found in the forests of the West coast of Africa from Senegal to Ghana. Early sources of the epidemic have been located to Guinea-Bissau (as well as Ivory Coast and Senegal). Epidemiological studies have shown that HIV-2 group A spread to Portugal from Guinea-Bissau and Cape Verde islands, while isolates from Ivory Coast and Senegal have been imported into France. Within Europe, strong support has been found for further dissemination from Portugal to Luxembourg and the U.K (Faria et al., 2012).

Coinfections of HIV-1 and HIV-2 are relatively common in West Africa, where they represent 0.3–1% of all HIV-infected patients (De Silva et al., 2010; Nsagha et al., 2012). Today, the HIV-2 epidemic seems to be contained or even decreasing while HIV-1 is spreading rapidly (van der Loeff et al., 2006). Field studies in rural areas of NW Guinea-Bissau showed a drop in HIV-2 prevalence from 8.3% in 1990 to 4.7% in 2000, while the HIV-1 prevalence increased from 0.5% to 3.6% (van Tienen et al., 2010). Despite the lower impact in public health, the genetic and biological similarities of HIV-2 to HIV-1 and SIV makes HIV-2 an important model for correlative studies of HIV natural history, pathogenesis and prevention. HIV-2 is less pathogenic than HIV-1 but immune recovery is not as effective. In addition, fewer drugs are available for the treatment of HIV-2 infection compared to HIV-1, and therapy response in HIV-2 is usually worse than with HIV-1.

2. Natural history, pathogenesis and immune response

HIV-2 is transmitted in the same way as HIV-1 (i.e. through sexual contact, perinatally, by intravenous drug use or by contact with contaminated blood products). However, it shows lower rates of heterosexual transmission and is less frequently transmitted from mothers to newborns (Markovitz, 1993).

HIV-2 has a longer asymptomatic phase and shows a slower progression to AIDS in comparison with HIV-1 (Marlink et al., 1994; Whittle et al., 1994). Around 80% of the HIV-2-infected patients behave like long-term non-progressors (Thiébaut et al., 2011). Most of the HIV-2-infected patients produce broadly reactive neutralizing antibodies and control viral replication. It has been estimated that around 25–35% of the untreated patients behave as "elite controllers" (Simon et al., 1993; Kanki et al., 1994; Marlink et al., 1994). Despite the lack of consensus on the definition of an elite controller, in general, these would be infected individuals that in the absence of treatment show viral loads below the limit of detection of the clinical assay (i.e. 50 viral RNA copies/ml) for more than two years. These criteria are more restrictive than the general term "long-term non-progressor" defined on the basis of their ability to maintain high CD4 cell counts over many years in the absence of antiretroviral therapy. Long-term non-progressors may show low but detectable viremia despite controlling the infection for many years (Saag and Deeks, 2010). In recent studies authors conclude that the slower progression of HIV-2 infection is also observed in co-infections of HIV-1 and HIV-2, where HIV-1 outcompetes HIV-2, particularly in individuals with low CD4+ cell counts (Esbjörnsson et al., 2012; Raugi et al., 2013a).

Viral RNA levels in HIV-2 infection are usually around 30 times lower than those seen with HIV-1 (Popper et al., 1999; MacNeil et al., 2007). Several groups have reported similar proviral DNA levels in both infections (Berry et al., 1994; Gomes et al., 1999; Popper et al., 2000; Soares et al., 2006; MacNeil et al., 2007). However, there is at least one report showing that at CD4 levels above 300 cells/μl the proviral load is higher in HIV-1 than in HIV-2 infections (Gueudin et al., 2008). In contrast, Gottlieb et al. (2008b) found significant differences only in those patients with CD4 counts below 300 cells/μl. The differences between HIV-1 and HIV-2 proviral DNA levels could be attributed to a significant amount of ongoing viral replication that would not translate into plasma viral load. It has been shown that in HIV-2-infected individuals, reduced viremia is not associated with reduced viral transcription (e.g. production of *gag*) (Soares et al., 2011). However, in comparison with HIV-1-infected patients, the levels of *tat* mRNA transcripts were significantly lower in individuals infected with HIV-2. This observation suggests that the rate of *de novo* infection is decreased in HIV-2-infected patients (Soares et al., 2011).

One major hallmark of HIV infection is chronic immune activation that promotes viral replication and drives CD4+ T cell depletion to less than 30% of the initial count. Disease progression is characterized by an alteration in the frequencies of specific T cell subsets (e.g. Vδ1+ and Vδ2+) (Zheng et al., 2011). Systemic immune activation is characterized by elevated plasma levels of proinflammatory cytokines and increased T cell turnover, followed by a loss of T cell regenerative capacity. In addition, increased levels of soluble CD14, β2-microglobulin and neopterin have also been associated with disease progression in HIV-2-infected patients (Nyamweya et al., 2012; Thiébaut et al., 2012). The lower levels of immune activation found in HIV-2 infections in comparison to HIV-1 have been attributed to the lower levels of HIV-2 replication. Several studies have shown that with disease progression (higher viremia), the levels of immune activation are similar in both HIV-1- and HIV-2-infected patients (Michel et al., 2000; Hanson et al., 2005; Drylewicz et al., 2008; Nyamweya et al., 2012). Therefore, there is a clear relationship between HIV-2 replication and immune activation during the chronic phase of the infection (Leligdowicz et al., 2010).

Low virus replication results in lower immune activation, and as a consequence T cell turnover may be reduced. Maintenance of T cell function would in turn delay progression to immunodeficiency. The lower plasma viral load in HIV-2-infected individuals is also probably related with the lower rates of mother-to-child transmission and reduced genital tract shedding. In addition, HIV-2 is relatively more susceptible to antibody neutralization than HIV-1 (Reeves and Doms, 2002). Thus, HIV-2 Env is highly immunogenic in natural infection and potent neutralizing antibod-

ies specific for epitopes in the gp120 C2, C3, V3 and V4 regions and the CD4 binding site were shown to neutralize many HIV-2 strains (Barroso et al., 2011; Uchtenhagen et al., 2011; Kong et al., 2012). Unlike HIV-1, HIV-2 trimers expose multiple broadly cross-reactive epitopes accessible to neutralizing antibodies (Kong et al., 2012). In a recent study involving two children infected with CCR5-tropic HIV-2 at birth, it has been shown that the response by neutralizing antibodies can be elicited very early after infection and is associated with a rapid evolution of the viral envelope, particularly at gp120 variable regions V1 and V3 (Rocha et al., 2013).

HIV-2 infections are also characterized by strong cytotoxic T lymphocyte responses particularly in asymptomatic patients (Gotch et al., 1993; reviewed in Whittle et al., 1998). In these individuals, the cytolytic activity against HIV-2 *gag*-, *pol*- and *nef*-derived antigens seems to be inversely correlated with the proviral load (Ariyoshi et al., 1995).

The stronger immune response elicited by HIV-2 as compared to HIV-1 could be attributed, at least in part, to the higher efficiency of HIV-2 in infecting dendritic cells. The expression of the host factor SAMHD1 (a triphosphohydrolase) in monocytes, dendritic cells and mature macrophages restricts HIV-1 replication by reducing the dNTP pools and blocking reverse transcription (Lahouassa et al., 2012; Baldauf et al., 2012). However, the HIV-2 Vpx protein (absent in HIV-1) induces the proteosomal degradation of SAMHD1 upon viral entry, and facilitates HIV-2 replication in myeloid and resting T cells (Hrecka et al., 2011; Laguette et al., 2011). Vpx has been found in two of the major lineages of primate lentiviruses: (i) HIV-2, SIVsmm (sooty mangabey) and SIVmac (rhesus macaque) (shown in Fig. 2); and (ii) SIVrcm (red capped mangabey) and SIVmnd2 (mandrill) (Fregoso et al., 2013).

3. Treatment of HIV-2 infection

Compared to HIV-1, treatment of HIV-2 infection poses some difficulties. First, viral load is not a good tool to monitor treatment response. Second, in the absence of clinical trials targeting HIV-2-infected population and without large observational studies providing guidelines for therapy, it is important to start therapy as soon as possible, always before advanced immunodeficiency develops. Clinical guidelines for anti-HIV therapy usually recommend to initiate treatment if the CD4 count falls below 500/mm^3. A third problem in the treatment of HIV-2 infection is that some antiretroviral drugs designed against HIV-1 are not effective in inhibiting HIV-2 propagation. Thus, HIV-2 is broadly resistant to approved nonnucleoside reverse transcriptase inhibitors (NNRTIs) and fusion inhibitors. In addition, several protease inhibitors licensed to treat HIV-1 infection show weak or no inhibitory activity against HIV-2.

In this scenario, preferred initial treatments consist of combinations of two nucleoside reverse transcriptase inhibitors (NRTIs) (either tenofovir plus emtricitabine or lamivudine, or zidovudine plus lamivudine) and an appropriate boosted protease inhibitor (usually ritonavir-boosted lopinavir or darunavir) (Gilleece et al., 2010; Vandamme et al., 2011) (Fig. 3). Integrase inhibitors recently licensed for treating HIV-1 infection are also effective against HIV-2, but information available is restricted to only a few studies. In addition, there are only two reports on the use of coreceptor antagonists (i.e. maraviroc) in treating HIV-2 infection, and in both cases the drug was used in salvage therapy (Armstrong-James et al., 2010; Caixas et al., 2012). Although studies *in vitro* have shown that there are entry inhibitors active against HIV-2, there is still uncertainty about their effectiveness *in vivo*. In the following sections, we will discuss the targets for antiretroviral intervention (e.g. reverse transcriptase, protease, integrase and viral entry), antiretroviral drugs and their inhibitory activities, and the mutational pathways leading to drug resistance.

4. HIV-2 RT structure and inhibition

The genomic organization of HIV-2 is similar to that found in other lentiviruses, with three major genes, arranged in the order

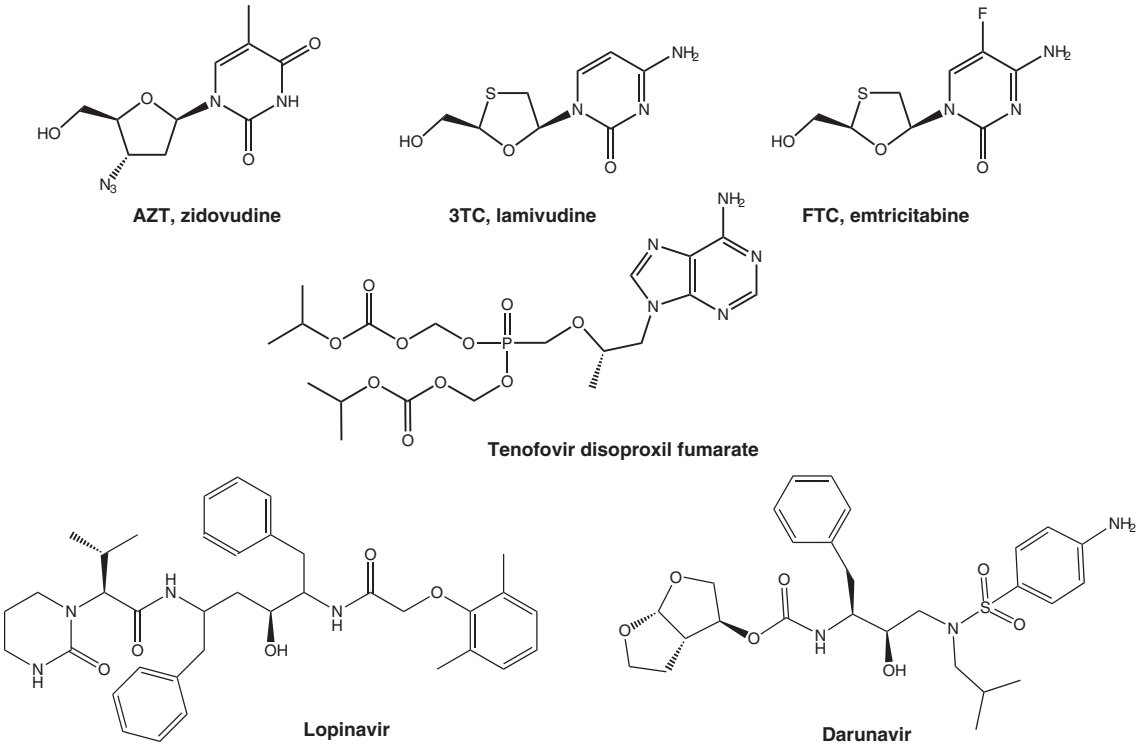


Fig. 3. Chemical structures of nucleoside RT inhibitors and protease inhibitors recommended as part of combination therapies in the initial treatment of HIV-2-infected patients. Ritonavir-boosted protease inhibitors (lopinavir or darunavir) should be given in combination with tenofovir plus emtricitabine or lamivudine, or with zidovudine plus lamivudine (although this combination may result in some toxicity).

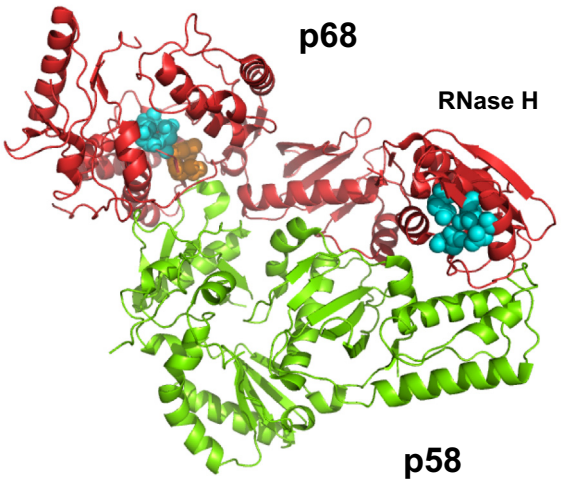


Fig. 4. Crystal structure of unliganded HIV-2 reverse transcriptase. Catalytic residues in the DNA polymerase and RNase H active sites are shown in cyan, while residues at positions 181 and 188 (in the putative NNRTI binding site) are represented with orange spheres. Atomic coordinates were obtained from Protein Data Bank (PDB) file 1MU2 (Ren et al., 2002). Structures were obtained with the PyMOL molecular graphics system (DeLano Scientific).

5′-*gag-pol-env*-3′, and a series of accessory and regulatory genes that in the case of HIV-2 are *vif*, *vpx*, *vpr*, *tat*, *rev* and *nef* (Fig. 1). The HIV-2 RT is encoded within the *pol* gene and derives from the proteolytic processing of the polyprotein precursor Gag-Pol. The RT is the enzyme that catalyzes the conversion of the single-stranded genomic RNA of the virus into a double-stranded DNA (i.e. a provirus) that integrates into a host cell chromosome.

The mature HIV-2 RT is a heterodimer composed of two subunits of around 68 and 55 kDa, designated as p68 and p58 (also designated as p54 or p55), respectively (Shaharabany and Hizi, 1992). The large subunit is 559 residues long and shows around 60% identity when compared with a prototypic RT of HIV-1 group M subtype B. As in HIV-1 RT, the smaller subunit of HIV-2 RT lacks the RNase H domain, as a result of cleavage by the viral protease. Both RTs have similar amino acid sequences around positions 440 and 441. However, studies with oligopeptide substrates showed that unlike in the case of HIV-1 RT, the Phe^{440}–Tyr^{441} bond of HIV-2 RT was not cleaved by the viral protease (Fan et al., 1995). Compositional and sequence analysis of peptides produced by cleavage of HIV-2 RT by HIV proteases led to the identification of . . .-Ala-Phe-Ala-Met^{484} as the likely C-terminus of p58 in the HIV-2 RT (Fan et al., 1995). The differences between the small subunits of HIV-1 and HIV-2 RTs have been attributed to the distinct specificities of the two viral proteases.

Crystallographic analyses of HIV-2 RT are limited to one structure of the unliganded enzyme, obtained as a heterodimer (Ren et al., 2002) (Fig. 4). However, the RT used to produce the crystals was expressed in *Escherichia coli* and had a smaller subunit of only 427 residues that resulted from the proteolytic processing of p58 by bacterial proteases (Bird et al., 2003). In the absence of crystal structures of ternary complexes containing the RT, the template–primer and the incoming dNTP, any functional or mechanistic interpretation of the role of different residues in DNA polymerization relies on information available for HIV-1 RT. Nevertheless, the HIV-2 RT structure reveals a similar domain organization than that described for HIV-1 RT, with 'fingers', 'palm', 'thumb' and 'connection' subdomains in both subunits and the RNase H domain only in p68.

4.1. HIV-2 RT inhibition by NRTIs and mechanisms of resistance

All NRTIs approved for clinical use against HIV-1 (zidovudine, stavudine, lamivudine, emtricitabine, didanosine, zalcitabine, abacavir and tenofovir disoproxil fumarate) are also effective inhibitors of HIV-2 RT (Smith et al., 2008b). These compounds are given as prodrugs that inside the cell are converted into their triphosphate form (or diphosphate in the case of tenofovir) to act as competitive inhibitors (or alternate substrates) of the RT. Once incorporated into the DNA chain, DNA polymerization is blocked due to the lack of a 3′-OH in the ribose ring of the NRTI. Studies carried out with HIV-1 RT have shown that NRTI resistance can be achieved by two different mechanisms: (i) through the acquisition of mutations affecting residues of the nucleotide binding site that increase the ability of the RT to discriminate against the triphosphate derivatives of NRTIs (e.g. M184V for lamivudine or emtricitabine), or (ii) by the acquisition of thymidine analogue resistance mutations (TAMs) (i.e. M41L, D67N, K70R, L210W, T215F/Y and K219E/Q) that in the presence of a pyrophosphate (PPi) donor (most likely, ATP) facilitate the excision of the inhibitor from the 3′ end of the growing DNA chain, in a reaction that is most efficient with zidovudine, stavudine and tenofovir (for reviews, see Menéndez-Arias, 2008, 2013).

Amino acid substitutions conferring resistance through the discrimination mechanism affect residues of the nucleotide binding site (e.g. Lys^{65}, Gln^{151} or Met^{184}), while TAMs at positions 41, 210, 215 and 219 are located away from the dNTP binding site. Clinical studies have shown that the nucleotide excision mechanism is rarely used by HIV-2 RT to acquire resistance to NRTIs (van der Ende et al., 1996; Rodés et al., 2000; Brandin et al., 2003; Castro et al., 2012) (Table 1). For example, S215Y (equivalent to T215Y in HIV-1 RT) is rarely found in HIV-2-infected patients (Brandin et al., 2003) and is difficult to select *in vitro* after successive passages of the virus in the presence of zidovudine (Reid et al., 2005). This is unexpected because the T215Y substitution occurring in HIV-1 requires two nucleotide changes while only one base substitution is needed to generate the equivalent S215Y change in HIV-2.

Sequence analysis of viral isolates from treated patients and cell culture selection experiments have shown that HIV-2 RT favors the Q151M discrimination pathway for resistance to zidovudine and

Table 1
HIV-2 RT inhibitor resistance-associated mutations.

Drugs	AA sequence differences between HIV-1 and HIV-2 RTs [a]	Major resistance-associated mutations	
NRTIs	T69N, V75I, V118I, L210N, T215S, K219E	Class-wide nucleoside analogue resistance [b]	K65R, Q151M, M184V
		All NRTIs except tenofovir	V111I, Q151M
		Lamivudine, emtricitabine	M184I/V
		Tenofovir	K65R
		Abacavir	K65R, Y115F
NNRTIs	V106I, E138A, Y181I/V, Y188L, G190A	Not active against HIV-2	

[a] Indicated amino acid substitutions are changes found in HIV-2 RT in comparison with HIV-1 RT at positions involved in resistance to RT inhibitors in HIV-1.
[b] Thymidine analogue resistance mutations (TAMs) are found less frequently and their impact has not been studied in detail. (Expected HIV-2 TAMs are M41I/L, D67N, K70R, N210W, S215Y and E219D).

other NRTIs (Ruelle et al., 2008; Gottlieb et al., 2009; Jallow et al., 2009; Ntemgwa et al., 2009). As in the case of T215F/Y in HIV-1 RT, selection of Q151M requires two nucleotide changes and additional mutations to increase the level of resistance to NRTIs. Biochemical studies showed that HIV-2 RT has a much lower ability to excise AZT monophosphate than the HIV-1 RT (Boyer et al., 2006, 2012). HIV-2 RTs with mutations S215Y or F214L/S215Y showed very poor excision activity on RNA/DNA and DNA/DNA complexes containing AZT-terminated primers (Boyer et al., 2012). These data could explain the poor relevance of the excision pathway in the acquisition of HIV-2 resistance to NRTIs. Based on the antagonistic effect of V75I on TAMs on the ATP-dependent phosphorolytic activity of mutant HIV-1 RT (Matamoros et al., 2009), it has been suggested that Ile^{75} in HIV-2 RT could be responsible in part for the lack of an excision-based resistance mechanism against AZT and other NRTIs.

In general, NRTI resistance in HIV-2 has a lower genetic barrier than in HIV-1. Apart from Q151M that confers resistance to all NRTIs except tenofovir, K65R and M184I or V are frequently found in viral isolates and confer classwide NRTI resistance (Smith et al., 2009) (Table 1). Virological studies have shown that Q151M alone contributes to high-level resistance to zidovudine (AZT), as well as the investigational drugs β-D-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (D-d4FC), (−)-(2R,4R)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)thymine (DOT) and β-D-2′,3′-dideoxy-5-fluoro-oxacytidine (D-FDOC) (Bennett et al., 2007). However, Q151M had a small impact on tenofovir resistance (Damond et al., 2005; Bennett et al., 2007). In HIV-1 RT, the Q151M mutational pathway involves additional changes around the dNTP binding site (i.e. A62V, V75I, F77L and F116Y) (reviewed in Menéndez-Arias, 2008). In HIV-2 RT, the Q151M complex contains V75I (present in the wild-type enzyme) and very often V111I. Selection of V111I together with Q151M in HIV-2 isolates resulted in decreased viral susceptibility to all tested NRTIs (i.e. zidovudine, lamivudine, stavudine, didanosine, abacavir and tenofovir), although in the case of tenofovir a smaller effect was observed (Damond et al., 2005).

Other mutations involved in classwide NRTI resistance such as K65R or M184V have been identified in HIV-2-infected patients exposed to tenofovir or abacavir (Rodés et al., 2008; Peterson et al., 2011) or in patients treated with lamivudine (van der Ende et al., 2003), respectively. Emtricitabine and tenofovir are potent inhibitors of HIV-2 replication with 50% effective concentrations (EC_{50}) in cell culture of 0.99 and 3.50 μM, respectively. Amino acid substitutions M184I and particularly M184V confer high-level resistance to emtricitabine. On the other hand K65R together with Y115F have been selected in the presence of tenofovir and confer resistance to tenofovir and emtricitabine (Andreatta et al., 2013).

Structural analysis of HIV-1 RT complexes containing double-stranded DNA and an incoming nucleotide (Huang et al., 1998) shows that Gln^{151} interacts with the nitrogenous base (thymine in the available crystal structure) and the sugar ring of the dNTP, while Lys^{65} and Val^{111} locate close to the phosphates of the dNTP (Fig. 5). Met^{184} sits at the base of the 3′-end nucleotide of the primer and Val/Ile^{75} would interact with the template strand at nucleotide position + 1.

4.2. HIV-2 RT resistance to NNRTIs

NNRTIs are hydrophobic compounds frequently containing at least one aromatic ring that binds to a hydrophobic pocket located near the DNA polymerase active site. Based on their structures, more than 30 different classes of NNRTIs have been identified since the first reports describing 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio) thymine (HEPT) and tetrahydroimidazo-[4,5,1-jk][1,4]-benzodiazepinone (TIBO) derivatives (Baba et al., 1989; Pauwels et al., 1990; reviewed in Chen et al., 2012; Yang et al., 2012). HIV-2 RT is intrinsically resistant to NNRTIs, although the IC_{50}s obtained for recently approved diarylpyrimidines etravirine and rilpivirine for the HIV-2_{ROD} strain were 5.7 μM and 5.2 μM, respectively (Azijn et al., 2010). However, those drugs were >1000 times more effective on HIV-1 than on HIV-2 (Andries et al., 2004; Azijn et al., 2010). The presence of Ile^{181} and Leu^{188} in HIV-2 RT, instead of Tyr residues at both positions as found in HIV-1 RT abolishes the effect of ring stacking interactions with many NNRTIs, most notably with nevirapine. Other amino acid substitutions found in HIV-2 that in the context of the HIV-1 RT sequence confer resistance to NNRTIs are V106I, E138A and G190A (Table 1).

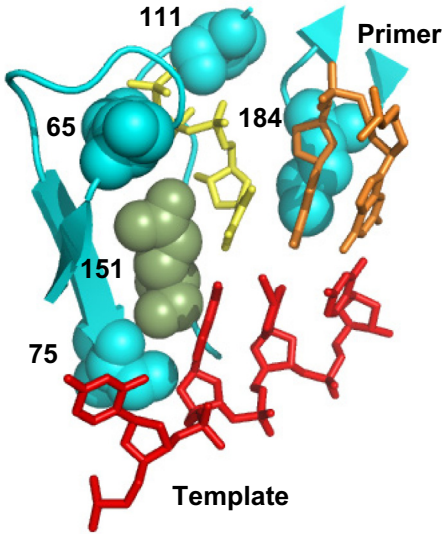


Fig. 5. Nucleotide binding site in the DNA polymerase domain of the HIV-1 p66 subunit. Template (red) and primer (orange) residues and the incoming dNTP (yellow) are represented with sticks. Residues that mutate in HIV-2 in response to therapy with nucleoside analogues are indicated with spheres. Atomic coordinates were obtained from PDB file 1RTD (Huang et al., 1998). Structures were obtained with the PyMOL molecular graphics system (DeLano Scientific).

Despite the wealth of information on NNRTIs, there is little evidence of their efficiency *in vitro* on HIV-2 strains. One remarkable exception is MSK-076, a phenylmethylthiazolylthiourea derivative that showed an IC_{50} of 0.63 μM against HIV-2, although 350 times less effective than against HIV-1 (Auwerx et al., 2004). Interestingly, *in vitro* selection experiments carried with the HIV-2_{ROD} strain in the presence of MSK-076 selected for mutations A101P and G112E that conferred 50-fold increased resistance to the drug. A101P is located at the putative NNRTI binding site of the RT, while G112E is away from that location and close to the active site, implying a different mode of action and mechanism of resistance compared to the classical NNRTIs (Auwerx et al., 2004).

4.3. Other RT inhibitors: Foscarnet and nucleotide-competing HIV RT inhibitors

Foscarnet is an inhibitor of DNA polymerases active against HIV-2 RT and used in the treatment of infections caused by herpes virus. This drug is a pyrophosphate analogue that blocks the DNA synthesis reaction by impairing PPi formation. *In vitro* studies have shown that foscarnet inhibits both HIV-1 and HIV-2 RTs with similar potencies although it is a relatively weak inhibitor (IC_{50}s around 40 μM) (Auwerx et al., 2004). However, there is at least one report on the usage of foscarnet as part of a salvage therapy given to a heavily-treated HIV-2-infected patient (Stegmann et al., 2010). In this individual, the introduction of foscarnet led to a

30-fold reduction of the viral load. The viral RT in the HIV-2 isolates found in this patient at the initiation of treatment contained RT inhibitor resistance-associated mutations such as K65R, N69S and M184V.

In addition to NRTIs, NNRTIs and pyrophosphate analogues, a new class of inhibitors known as nucleotide-competing HIV-1 RT inhibitors has been recently described (Jochmans et al., 2006; Zhang et al., 2006). These are nonnucleosidic compounds that bind to the DNA polymerase active site and compete with natural dNTPs. Indolpyridinones such as INDOPY-1 are the prototypic compounds of this class of inhibitors. They show potent activity against HIV-1 strains, and select for amino acid substitutions Y115F and M184V in the RT dNTP binding site (Ehteshami et al., 2008). Although INDOPY-1 retains activity against HIV-2, its IC_{50} is about 6–22 times higher than against HIV-1 (Jochmans et al., 2006; Zhang et al., 2006; Jegede et al., 2011).

5. Protease inhibitors and resistance

HIV proteases are composed of two identical subunits, each containing 99 amino acids. These enzymes are responsible for the proteolytic cleavage of viral polyproteins Gag and Gag-Pol rendering the mature HIV proteins (e.g. MA, CA, NC, p6 and the viral enzymes). HIV-1 and HIV-2 proteases share 39–48% amino acid sequence identity, depending on the viral strains considered in the comparison (Fig. 6).

Crystal structures of HIV-1 protease showed that the active site of the enzyme is located at the interface between both subunits and contains the characteristic Asp^{25}–Thr^{26}–Gly^{27} sequence common to aspartic proteases (reviewed in Ghosh et al., 2012). The aspartic acid residues at position 25 in both subunits have a catalytic role, and the active site is held in a rigid position by a network of hydrogen bonds, known as the 'fireman's grip'. Other structural features of the HIV-1 protease include the 'flap' regions (residues 42–58) that close down upon binding of the inhibitor or the substrate, and a four-stranded β-sheet formed by the N- and C-termini of each subunit (residues 1–5 and 95–99, respectively) that play a role in protease dimerization. There are many crystal (or NMR) structures available of wild-type or mutant HIV-1 proteases. However, the number of HIV-2 protease structures deposited in the Protein Data Bank (PDB) (http://www.pdb.org) is relatively small. Among them, three structures correspond to the protease bound to approved anti-HIV drugs such as indinavir (PDB code 1HSI), amprenavir (PDB code 3S45) and darunavir (PDB code 3EBZ).

Approved HIV-1 protease inhibitors (saquinavir, ritonavir, indinavir, nelfinavir, fosamprenavir, lopinavir, atazanavir, tipranavir and darunavir) block the conversion of the immature capsid with a round-shaped core to a mature virion containing its characteristic conical capsid. HIV-1 and HIV-2 proteases have similar substrate specificities (reviewed in Tözsér, 2010). However, there are relevant differences between HIV-1 and HIV-2 that affect substrate binding sites and therefore to the mutational patterns conferring resistance to various protease inhibitors. Amino acid sequence differences between HIV-1 and HIV-2 proteases affecting substrate binding site residues are V32I, I47V, L76M and V82I (reviewed in Menéndez-Arias and Tözsér, 2008) (Fig. 7). Additional substitutions involving secondary mutations conferring resistance to protease inhibitors are L10I/V, G16E, L33V, M36I/V, M46I/V, Q58E, A71I/V, G73A, L89I/V and F99L, as well as polymorphisms frequently found in positions harboring minor mutations associated with resistance (e.g. E34A, D60K and L63E).

Phenotypic susceptibility assays carried out with the $HIV\text{-}2_{ROD}$ reference isolate and virus obtained from infected patients showed that saquinavir, lopinavir and darunavir were the most potent protease inhibitors against HIV-2 (Fig. 8), and should be preferred as first-line options for HIV-2-infected patients (Desbois et al., 2008). HIV-2 shows natural resistance to amprenavir *in vitro* and in addition, most viral isolates are partially resistant to nelfinavir, ritonavir, indinavir, atazanavir and tipranavir (Yoshimura et al., 2002; Koh et al., 2003; Witvrouw et al., 2004; Ntemgwa et al., 2007; Desbois et al., 2008). In general, inhibitors with the lowest inhibitory constant (K_i) for the HIV-2 protease were amongst the most potent inhibitors of the HIV-1 enzyme (Brower et al., 2008).

The lower efficiencies of various HIV-2 protease inhibitors could also be related to differences between HIV-1 and HIV-2 proteases affecting the substrate binding site. Thus, amprenavir and darunavir are chemically similar, differing only in the moiety interacting with the S2 site of the protease. Darunavir has a bicyclic tetrahydrofuranyl urethane group (Fig. 3), while in amprenavir, this is a monocyclic structure. However, darunavir is an efficient inhibitor

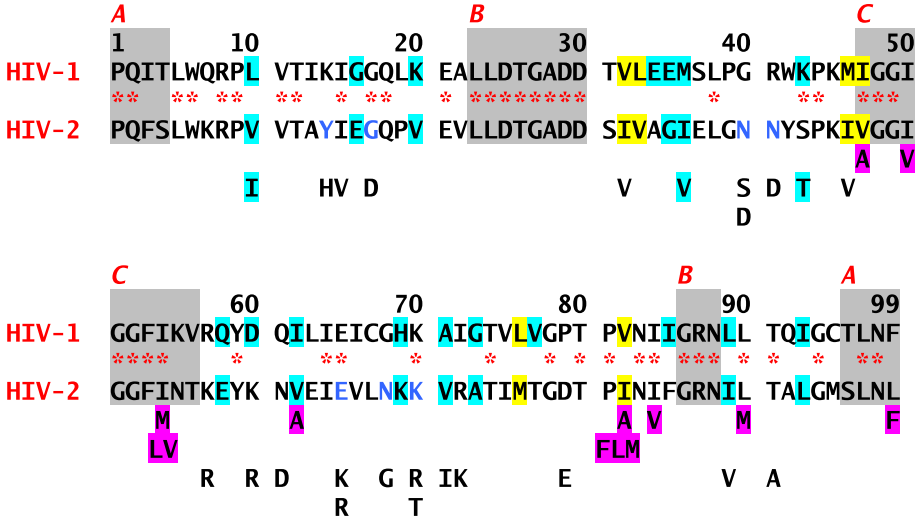


Fig. 6. Amino acid sequence comparison of HIV-1 and HIV-2 proteases of reference strains HXB2 (HIV-1) and ROD (HIV-2). Identical residues are marked with red asterisks. Conserved structurally important regions are highlighted on a grey background. Box A corresponds to the dimerization domain, B to the active site region and the catalytic triad, and C indicates the flap region. Amino acid sequence differences between HIV-1 and HIV-2 proteases at positions relevant for resistance are indicated. Major and minor protease inhibitor resistance mutations are highlighted in yellow and light blue, respectively. Highly polymorphic positions in HIV-2 subtype A isolates are indicated with blue letters. Other residues appearing frequently are shown below. Amino acid changes selected in the HIV-2 protease under treatment with approved protease inhibitors are highlighted in magenta. Adapted from Menéndez-Arias and Tözsér (2008) and reproduced with permission from Elsevier©.

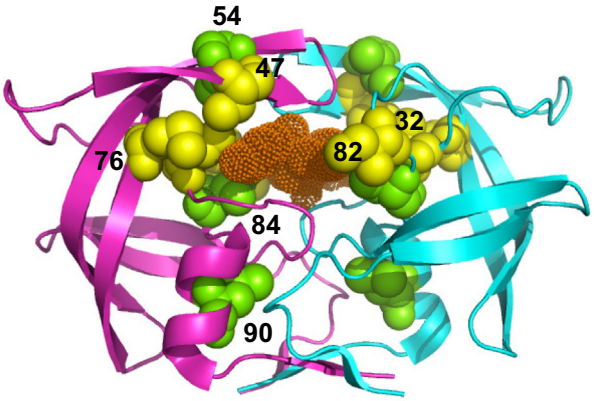


Fig. 7. Crystallographic structure of the HIV-2 protease complexed with darunavir. Residues at positions 32, 47, 76 and 82 (yellow spheres) are located in the inhibitor binding site and are different in HIV-1 and HIV-2 proteases. Green spheres are used to indicate the location of Ile^{54}, Ile^{84} and Leu^{90} that when substituted by Met^{54}, Val^{84} and Met^{90} confer resistance to multiple protease inhibitors. Atomic coordinates were obtained from PDB file 3EBZ (Kovalevsky et al., 2008). Structures were obtained with the PyMOL molecular graphics system (DeLano Scientific).

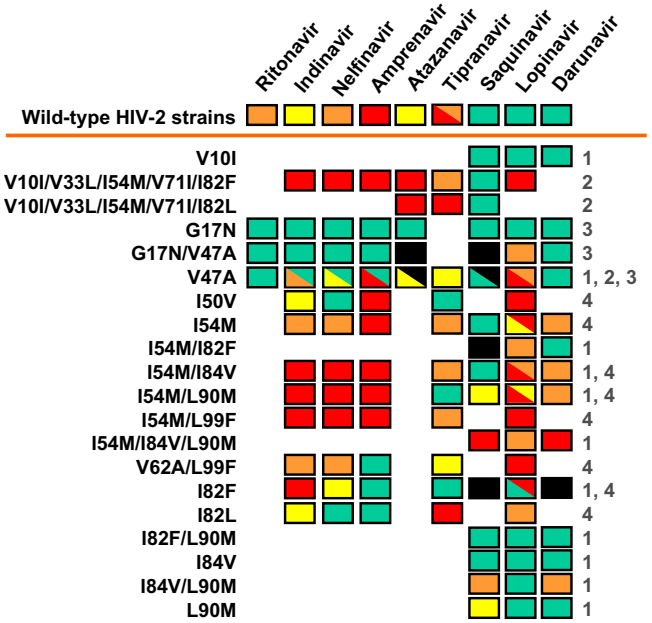


Fig. 8. Summary of phenotypic drug susceptibility data obtained with wild-type and mutant HIV-2 bearing amino acid substitutions in the protease. High-level (>20-fold increase of the IC_{50} for the inhibitor), moderate (5- to 20-fold increase) and low-level (2.5- to 5-fold increase) resistance is indicated in red, orange and yellow, respectively. Susceptible isolates are indicated in green and hypersusceptible (>5-fold reduction of the IC_{50} for the inhibitor) variants are shown in black. Double color is used in those cases where we found significant discrepancies in the literature. Relative increases in the corresponding IC_{50} values are referred to the reference strain used in each experiment. Data for wild-type HIV-2 strain were obtained from Yoshimura et al., 2002; Koh et al., 2003; Witvrouw et al., 2004; Ntemgwa et al., 2007; and Desbois et al., 2008. Numbers on the right represent the corresponding references: (1) Raugi et al. (2013b); (2) Rodés et al. (2006); (3) Masse et al. (2007); and (4) Ntemgwa et al. (2007). Fold-increases in the IC_{50}s were obtained using the HIV-2_{ROD} strain as a reference virus in Masse et al. (2007) and Raugi et al. (2013b). HIV-2 reference isolates used in Ntemgwa et al. (2007) were CBL-20, CBL-23 and MVP-1532, while Rodés et al. (2006) used a non-standard drug-susceptible clinical isolate.

of HIV-2 protease, in contrast to amprenavir that has no inhibitory activity. Structural studies showed that Ile^{32}, Val^{47} and Ile^{82} in HIV-2 protease disrupt interactions with the P2 and P2′ groups of both inhibitors (Fig. 7), explaining the differences in potency between both drugs (Tie et al., 2012).

There is little information specific for mutational pathways leading to HIV-2 resistance to protease inhibitors. Evidence has been collected from clinical studies monitoring the emergence of drug resistance-associated mutations in HIV-2-infected patients and from *in vitro* assays including the selection of mutations under drug pressure in virus grown in cell culture, or phenotypic assays with mutant HIV-2 variants. Thus, I54M has been frequently selected in cell culture when the virus was passaged in the presence of amprenavir, nelfinavir or indinavir (Ntemgwa et al., 2007). I50V has been found in virus selected in the presence of amprenavir, while I82F and L90M have emerged alone or in combination with others in HIV-2 strains grown in the presence of nelfinavir (Ntemgwa et al., 2007). On the other hand, I82L seems to be the predominant mutation that confers resistance to tipranavir (Ntemgwa et al., 2007). These and other mutations have been tested in phenotypic assays using HIV-2 infectious clones. Their impact on the IC_{50} for different drugs is shown in Fig. 8. Interestingly, the combination of I54M, I84V and L90M confers high-level resistance to the most effective protease inhibitors saquinavir, lopinavir and darunavir (Raugi et al., 2013b).

As in the HIV-1 protease, G48V and L90M are expected to be major mutations selected in patients under saquinavir therapy (Jacobsen et al., 1995; for a review see Menéndez-Arias, 2010). V47A has been selected in virus from patients treated with lopinavir-based highly active antiretroviral therapy (HAART) regimens (Rodés et al., 2006; Jallow et al., 2009), and after passage in cell culture in the presence of the drug (Masse et al., 2007). The presence of I54M together with I82F has been also associated with lopinavir therapy failure in treated patients (Rodés et al., 2006), although I54M contributed to extensive cross-reactivity (Fig. 8). In a French clinical study including 46 HIV-2 group A-infected patients receiving lopinavir, emergence of I82F (sometimes associated with Y14H) has been correlated with the presence of mutations at Gag and Gag-Pol cleavage sites (Larrouy et al., 2013). Despite differences around the NC/p1 and p1/p6 cleavage sites between HIV-1 and HIV-2, some of those mutations (e.g. DRQAG/FLGLG → DRQVG/FLGLG) occurred at equivalent positions in the viral polyproteins of both viruses. Although atazanavir is a poor inhibitor of the HIV-2 protease, the use of ritonavir-boosted atazanavir as part of a HAART therapy led to the selection of I50L, often associated with I82F and also, but less frequently with I54L, I64V and V71I (Cavaco-Silva et al., 2013).

6. Integrase inhibitors and resistance

Integration is a hallmark of retroviruses. The double-stranded DNA resulting from reverse transcription is transported into the host cell nucleus where it is integrated into the cellular DNA. This process is catalyzed by the viral integrase and involves two reaction steps. First, the integrase cleaves a dinucleotide from each viral DNA terminus to produce reactive CpA 3′-hydroxyl ends (3′-end processing), and then the 3′-end processed DNA is covalently joined to the host DNA during a strand transfer reaction (for recent reviews, see Delelis et al., 2008; Craigie and Bushman, 2012). A cellular protein, the human lens epithelium-derived growth factor (LEDGF/p75) acts as a tethering factor linking the viral integrase to the host cell chromatin (Maertens et al., 2003; Ciuffi et al., 2005; Llano et al., 2006). Approved antiretroviral drugs targeting the HIV-1 integrase (i.e. raltegravir, elvitegravir and dolutegravir) bind the catalytic site of the integrase and act as inhibitors of the strand transfer reaction. Drugs in preclinical development include novel strand transfer inhibitors and allosteric compounds interfering with the interaction between LEDGF/p75 and the viral integrase (for a review, see Menéndez-Arias, 2013).

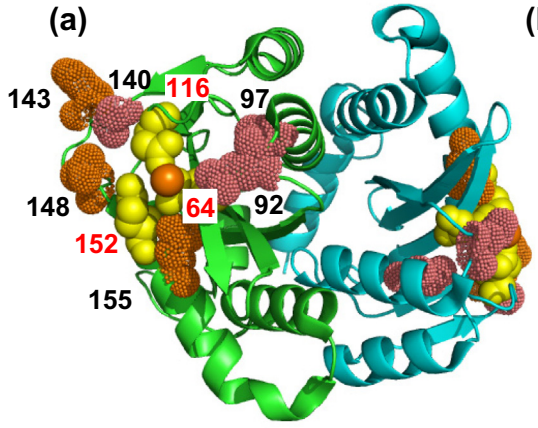
Retroviral integrases have three domains. The N-terminal domain consists of a series of α-helices coordinated with a single Zn^{2+} ion. The central domain is the catalytic core and contains the conserved DDE motif including active site residues Asp^{64}, Asp^{116} and Glu^{152} (Fig. 9). These residues coordinate with two divalent cations (Mg^{2+} or Mn^{2+}) and are required for 3′-end processing and strand transfer. The C-terminal region of the integrase binds DNA in a non-specific manner. The crystal structure of a retroviral (foamy virus) integrase containing all three domains, in complex with viral DNA has been recently solved (Hare et al., 2010; Maertens et al., 2010). Analysis of this structure revealed that all three domains are involved in multimerization, mainly through interactions between the C-terminal domains and between the N- and C-terminal domains of the integrase. For HIV-2 integrase, there are available structures for the N-terminal zinc-binding domain (PDB file 1E0E) (Eijkelenboom et al., 2000) and for a complex containing the N-terminal and catalytic core domains of HIV-2 integrase and the integrase binding domain of LEDGF/p75 (PDB file 3F9K) (Hare et al., 2009).

Despite a 40% difference in amino acid sequence between HIV-1 and HIV-2 integrases (Fig. 9), phenotypic assays carried out with reference strains or clinical isolates have shown that all approved inhibitors (i.e. raltegravir, elvitegravir and dolutegravir) are effective against HIV-2 (Roquebert et al., 2008b; Charpentier et al., 2010; Koh et al., 2011; Smith et al., 2011). There is limited information on the mutational pathways leading to HIV-2 resistance to approved integrase inhibitors. However, the available evidence indicates that mutations conferring resistance in HIV-1 and HIV-2 are essentially the same (Charpentier et al., 2011; Smith et al., 2012). In both viruses, raltegravir and elvitegravir have a low-to-moderate genetic barrier and show extensive cross-resistance. On the other hand, dolutegravir retains activity against raltegravir- and elvitegravir-resistant HIV-1 strains (reviewed in Geretti et al., 2012), but the information on its efficiency against HIV-2 strains bearing integrase inhibitor resistance mutations is still limited (Charpentier et al., 2010).

There are three major mutational pathways leading to high-level resistance to raltegravir and elvitegravir in HIV-2. Their characteristic amino acid substitutions are: (i) E92Q/Y143C or T97A/Y143C, (ii) Q148K, Q148R or the combination G140S/Q148R, and (iii) N155H or the combinations E92Q/N155H and T97A/N155H (Charpentier et al., 2011; Ni et al., 2011; Smith et al., 2012) (Fig. 9). These combinations produce a >50-fold increase in the IC_{50} for both inhibitors in phenotypic assays, although their effects might be slightly different depending on the drug. Thus, E92Q/Y143C confers higher levels of resistance to raltegravir than to elvitegravir. In contrast, HIV-2 harboring the combination T97A/Y143C showed a higher IC_{50} for elvitegravir than for raltegravir (Smith et al., 2012). These studies also showed that unlike in the case of HIV-1, Y143C had a relatively minor effect on the HIV-2 susceptibility to raltegravir (Smith et al., 2011), unless being associated with other resistance-related mutations such as E92Q (Ni et al., 2011) or T97A (Smith et al., 2011). HIV-2 resistant variants carrying Y143C have been detected in patients who previously developed N155H (Xu et al., 2009).

The selection of Q148R (Roquebert et al., 2008a; Charpentier et al., 2011; Smith et al., 2011) or N155H (Garrett et al., 2008; Salgado et al., 2009; Xu et al., 2009; Charpentier et al., 2011; Smith et al., 2011) in HIV-2 from patients treated with raltegravir has been widely documented. N155H has a negative effect on viral fitness and may appear together with mutations such as E92G or E92Q, S147G, A153G, H157R and M183I (Smith et al., 2011). However, the role of those accompanying mutations has not been studied in detail. Mutations E92A/T97A found together with N155H in clinical isolates from raltegravir-treated HIV-2-infected patients had no effect on resistance, although enzymatic assays with recombinant integrase showed an increase of its strand transfer activity mediated by T97A (Ni et al., 2011). In addition to the well-characterized mutants described above, an $HIV\text{-}2_{ROD}$ variant containing mutations Q91R/I175M has been selected *in vitro* in the presence of the drug (Perez-Bercoff et al., 2010). This variant was about 13 times less susceptible to raltegravir than the reference strain.

Dolutegravir has been recently approved as a drug with a different HIV-1 resistance profile in comparison with raltegravir or elvitegravir (for a summary, see Menéndez-Arias, 2013). However, phenotypic assays carried out with HIV-2 clinical isolates from patients treated with raltegravir showed that mutations T97A/Y143C, G140S/Q148R or G140T/Q148R/N155H conferred moderate resistance to dolutegravir (7–18-fold increase of the EC_{50}) (Charpentier et al., 2010). At this time, it is still unknown whether the combination H51Y/R263K that confers resistance to dolutegravir in HIV-1



(b)

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BH10   FLDGIDKAQD EHEKYHSNWR AMASDFNLPP VVAKEIVASC DKCQLKGEAM  50
ROD    FLEKIEPAQE EHEKYHSNVK ELSHKFGIPN LVARQIVNSC AQCQQKGEAI
EHO    FLEKIEPAQE EHEKYHNNVK ELVHKFGIPQ LVARQIVNSC DKCQQKGEAI

BH10   HGQVDCSPGI WQLDCTHLEG KVILVAVHVA SGYIEAEVIP AETGQETAYF 100
ROD    HGQVNAELGT WQMDCTHLEG KIIIVAVHVA SGFIEAEVIP QESGRQTALF
EHO    HGQVNSELGT WQMDCTHLEG KVIIVAVHVA SGFIEAEVIP QETGRQTALF

BH10   LLKLAGRWPV KTIHTDNGSN FTSATVKAAC WWAGIKQEFG IPYNPQSQGV 150
ROD    LLKLASRWPI THLHTDNGAN FTSQEVKMVA WWIGIEQSFG VPYNPQSQGV
EHO    LLKLASRWPI THLHTDNGAN FTSQDVKMAA WWIGIEQTFG VPYNPESQGV

BH10   VESMNKELKK IIGQVRDQAE HLKTAVQMAV FIHNFKRKGG IGGYSAGERI 200
ROD    VEAMNHHLKN QISRIREQAN TIETIVLMAI HCMNFKRRGG IGDMTPSERL
EHO    VEAMNHHLKN QIDRIRDQAV SIETVVLMAT HCMNFKRRGG IGDMTPAERI

BH10   VDIIATDIQT KELQKQITKI QNFRVYYRDS RNPLWKGPAK LLWKGEGAVV 250
ROD    INMITTEQEI QFLQAKNSKL KDFRVYFREG RDQLWKGPGE LLWKGEGAVL
EHO    VNMITTEQEI QFLQTKNLKF QNFRVYYREG RDQLWKGPGD LLWKGEGAVI

BH10   IQDNSDIKVV PRRKAKIIRD YG--KQMAGDD CVASRQDED
ROD    VKVGTDIKII PRRKAKIIRD YGG-RQEMDSG SHLEGARED GEMA
EHO    IKVGTEIKVI PRRKAKIIRN YGGGKELDCSA DVEDTMQAR EVAQSN
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Fig. 9. Crystal structure of the catalytic domain of HIV-2 integrase and amino acid sequence comparison of HIV-1 and HIV-2 integrases. (a) Structure of the catalytic core of HIV-2 integrase showing the location of the catalytic triad (Asp^{64}, Asp^{116} and Glu^{152}) (yellow spheres) and residues associated with resistance to approved integrase inhibitors (Glu^{92}, Thr^{97}, Gly^{140}, Tyr^{143}, Gln^{148} and Asn^{155}) (pink and orange dots). A Mg^{2+} ion in the catalytic site is shown as an orange sphere. Atomic coordinates were obtained from PDB file 3F9K (Hare et al., 2009). (b) Amino acid sequence alignment of HIV-1 (BH10) integrase versus HIV-2 (ROD and EHO) integrases, showing relevant positions for catalytic activity (highlighted in yellow) and resistance. Residues associated with major resistance mutations are highlighted in blue, while those associated with minor mutations are shown on a grey background. Amino acid sequences of $HIV\text{-}1_{BH10}$, $HIV\text{-}2_{ROD}$ and $HIV\text{-}2_{EHO}$ integrases were obtained after translation of nucleotide sequences of full viral genomes obtained from GenBank accession numbers M15654, M15390 and U27200, respectively.

(Quashie et al., 2012; Mesplède et al., 2013) could be relevant as a mutational pathway leading to HIV-2 resistance.

Recently described inhibitors of the interaction between LEDGF/p75 and HIV-1 were found to be inactive against HIV-2 EHO and ROD strains due to the presence of Met^{128} instead of Ala in their integrase (Christ et al., 2010). However, the same group has identified cyclic peptides generated through phage display that show broad specificity and antiviral activity against HIV-1_{NL4-3} and HIV-2_{ROD} strains (Desimmie et al., 2012).

7. Targeting HIV-2 entry

As in the case of HIV-1, surface (SU) and transmembrane (TM) glycoproteins are potential targets of antiviral intervention against HIV-2 infection. In HIV-2, SU and TM derive from a 140-kDa Env precursor and are designated as gp125 and gp36, respectively. The SU and TM glycoproteins are assembled into trimers on the virion surface (Liu et al., 2008; Mao et al., 2012). During HIV-2 infection, gp125 binds to the primary CD4 receptor in the host cell. As a result, there is a conformational change that favors binding of the V3 loop in the envelope glycoprotein to a chemokine receptor that acts as coreceptor of viral entry. In HIV-1 and HIV-2, major coreceptors are CCR5 and CXCR4. However, HIV-2 can use a broad range of coreceptors *in vitro*, such as CCR1, CCR2b, CCR3, CCR6, GPR15(BOB), CCR8 and CXCR6 (BONZO) (Guillon et al., 1998; Owen et al., 1998; Mörner et al., 1999; Calado et al., 2010; Islam et al., 2013). CCR5-tropic HIV-2 strains are common in chronically asymptomatic patients while CXCR4-tropic HIV-2 isolates have been found only in patients with advanced disease and low levels of CD4+ T cells (Owen et al., 1998; Guillon et al., 1998; Shi et al., 2005; Marcelino et al., 2012). Nevertheless, those studies were based on a relatively small number of patients or involved individuals receiving antiretroviral therapy.

In general, CXCR4-tropic HIV-2 isolates are more resistant to antibody neutralization. It has been argued that the higher susceptibility to neutralization of HIV-2 could be related to the predominance of CCR5-tropic isolates in HIV-2 infections (Bunnik et al., 2007; Marcelino et al., 2010, 2012). Coreceptor binding triggers fusion of the viral envelope and the host cell membrane in a reaction that in HIV-2 involves the participation of gp36 (the homologous of gp41 in HIV-1) (for recent reviews, see Eggink et al., 2010; Blumenthal et al., 2012).

Many drugs targeting viral entry have been developed for HIV-1, although maraviroc (a CCR5 antagonist) and enfuvirtide (a fusion inhibitor) are the only ones approved for clinical use (for a review, see Menéndez-Arias, 2013). Experimental drugs designed for blocking HIV-1 infection, but tested on HIV-2 include attachment inhibitors (e.g. galactan sulfate and prostratin), CD4 binding inhibitors (e.g. recombinant soluble CD4, BMS-378806 and cyclotriazadisulfonamides), coreceptor binding inhibitors (e.g. TAK-779 and AMD3100), and fusion inhibitors (e.g. sifuvirtide, tifuvirtide and others).

7.1. Blocking attachment and receptor/coreceptor recognition

A number of entry inhibitors effective against HIV-1 and HIV-2 have been identified (Table 2). Similar IC_{50} values have been obtained for both types of viruses with attachment inhibitors such as cyanovirin-N (Boyd et al., 1997), mannose-specific lectins (Balzarini et al., 2004), polyanions (e.g. cellulose sulfate, dextran sulfate and galactan sulfate) (Witvrouw et al., 1994; Witvrouw and De Clercq, 1997) or prostratin (Witvrouw et al., 2003). However, CD4 binding inhibitors specifically designed to block HIV-1 infection are much less effective against HIV-2. Examples are the recombinant soluble CD4 (Clapham et al., 1989), and the azaindole derivative BMS-378806 (Lin et al., 2003). Ibalizumab (TNX-355), a humanized IgG4 monoclonal antibody that binds CD4 also shows limited activity against HIV-2 isolates (Moore et al., 1992). Another group of drugs that interfere with the CD4 recognition are cyclotriazadisulfonamides (known as CADA). These are wide-spectrum inhibitors that block HIV propagation by down-regulating the expression of CD4. These drugs show IC_{50}s in the submicromolar range for both HIV-1 and HIV-2 isolates (Vermeire et al., 2002).

An amphotericin B derivative (i.e. MS8209) has been shown to specifically block entry of HIV-2 strains EHO and ROD_{10} as well as HIV-1 at concentrations in the range of 5–10 μM. However, MS8209 was not active against the HIV-2 strain ROD_{CEM} (Pleskoff et al., 1996). Amino acid sequence differences between ROD_{10} and ROD_{CEM} in the V3 loop of gp125 (i.e. T312I and Q329R) seem to be responsible for the loss of susceptibility shown by the ROD_{CEM} strain (Pleskoff et al., 1996). Interestingly, Gln^{329} is part of a core epitope recognized by neutralizing antibodies (Matsushita et al., 1995). The mechanism of action of MS8209 is not known but the blockage occurs after CD4 binding.

Many coreceptor binding inhibitors have been found to be active against HIV-1 and HIV-2 isolates. For example, CXCR4 antagonists such as the natural human ligand SDF-1 (or CXCL12) inhibit both types of viruses at nanomolar concentrations (Oberlin et al., 1996; Espirito-Santo et al., 2012). On the other hand, synthetic bicyclams such as plerixafor (also known as AMD3100) showed IC_{50}s of 1.0–4.2 nM in HIV-2 inhibition assays, similar to those reported for CXCR4-tropic HIV-1 strains (De Clercq et al., 1994; Labrosse et al., 1998; Borrego et al., 2012). Compared to HIV-1 IIIB (a group M subtype B strain), the EHO and ROD strains of HIV-2 showed only 1.6- and 3.4-fold reduced AMD3100 susceptibility, respectively (Witvrouw et al., 2004).

Attempts to block CCR5 binding are based on the usage of three different types of molecules: (i) antibodies recognizing the CCR5 molecule, (ii) natural or modified chemokines binding CCR5, and (iii) small drugs binding specific pockets in the CCR5 structure. In the first group, the anti-CCR5 antibody PRO 140 has shown potent and prolonged antiretroviral activity in subjects infected with CCR5-tropic (R5) HIV-1 (Jacobson et al., 2010). Preliminary data suggest that in peripheral blood mononuclear cells, PRO 140 could inhibit both HIV-1 and HIV-2 with similar efficiencies (Ketas et al., 2007). Recombinant human chemokines binding the CCR5 receptor (e.g. RANTES (CCL3), MIP-1α (CCL4) and MIP-1β (CCL5)) block HIV-1 and HIV-2 infection *in vitro*. However, CCL3, CCL4 and CCL5 were found to be weak HIV-2 inhibitors (Cocchi et al., 1995; Blaak et al., 2008). AOP-RANTES, an analogue of CCL3 blocks HIV-2 replication at a minimal concentration of 4 nM (Zhang et al., 2000). In the group of the small drugs targeting CCR5, TAK-779 is a quaternary ammonium anilide that blocks the chemokine coreceptors CCR5 and CXCR3 (Baba et al., 1999; Dragic et al., 2000). TAK-779 inhibits HIV-2 entry with IC_{50}s in the range from 0.6 to 128.3 nM, similar to those described for HIV-1 (Borrego et al., 2012; Espirito-Santo et al., 2012).

As mentioned above, maraviroc is the only coreceptor antagonist approved for clinical use against HIV-1 infection. Maraviroc binds to a pocket formed by the transmembrane helices of CCR5 (Tan et al., 2013). HIV-2 susceptibility to maraviroc has been determined *in vitro* for clinical isolates (Borrego et al., 2012; Visseaux et al., 2012), but there is little information on its clinical use. Thus, there is one report showing that an HIV-2-infected patient with resistance to protease and RT inhibitors responded positively to a raltegravir- and maraviroc-based therapy (Armstrong-James et al., 2010), while a second one described the long-term successful control of HIV-2 replication in one patient using maraviroc (Caixas et al., 2012).

CCR5-tropic HIV-1 and HIV-2 clinical isolates showed similar IC_{50}s (or EC_{50} values) for maraviroc in phenotypic assays (Borrego

Table 2
Antiretroviral drugs targeting HIV-1 and HIV-2 entry and showing similar IC_{50}s for both types of viruses.

Target	Types of drugs and names	IC_{50}[a] HIV-1	IC_{50}[a] HIV-2	References
Attachment	Aurintricarboxylic acid (ATA)	0.2 ± 0.03 μg/ml	(0.8 ± 0.5 μg/ml)	Witvrouw et al. (1994)
	Cyanovirin A	0.1–5.8 nM	2.3–7.6 nM (7.6 nM)[b]	Boyd et al. (1997)
	HB-19 (5[Kψ(CH_2N)PR]-TASP)	0.3 μM	0.2–2 μM (0.2 μM)	Nisole et al. (2000)
	Mannose-specific lectins			
	GNA (from *Galanthus nivalis*)	0.3–4.7 μg/ml	0.12–0.25 μg/ml (0.25 μg/ml)	Balzarini et al. (2004)
	HHA (from *Hippeastrum hybrid*)	0.3–3.2 μg/ml	0.18–0.25 μg/ml (0.18 μg/ml)	Balzarini et al. (2004)
	Prostratin	40–90 ng/ml	20–30 ng/ml (20 ng/ml)	Witvrouw et al. (2003)
	Sulfated polysaccharides			
	DS (dextran sulfate)	0.4–0.9 μg/ml	0.08–3.8 μg/ml (80 ng/ml)	Witvrouw and De Clercq (1997)
	GS (galactan sulfate)	0.6 ± 0.4 μg/ml	(0.5 ± 0.3 μg/ml)	Witvrouw et al. (1994)
	PS (pentosan sulfate)	0.8–0.9 μg/ml	0.01–2.7 μg/ml (10 ng/ml)	Witvrouw and De Clercq (1997)
CD4 blockers	Cyclotriazadisulfonamide (CADA)	0.3–1.5 μM	(0.2 μM)	Vermeire et al. (2002)
	Sulfonic acid polymers			
	PAMPS	1 μg/ml	(3 μg/ml)	Mohan et al. (1992)
	PVS	3 μg/ml	(1 μg/ml)	Mohan et al. (1992)
CCR5 antagonists[c]	Chemokines			
	AOP-RANTES	0.04–1.3 nM	4 nM	Torre et al. (2000); Zhang et al. (2000)
	RANTES	>480 ng/ml	100–200 ng/ml	Cocchi et al. (1995); Trkola et al. (2001)
	Maraviroc	0.1–5.63 nM	0.04–5.5 nM	Borrego et al. (2012); Visseaux et al. (2012)
	Monoclonal antibodies[d]			
	MAb 2D7	8.85 ± 0.32 nM	3.34 ± 0.017 nM	Espirito-Santo et al. (2012)
	PRO 140	2.4 μg/ml	<0.2 μg/ml	Trkola et al. (2001); Ketas et al. (2007)
	PF-227153 (maraviroc analogue)	0.57 ± 0.02 nM	0.21–0.40 nM	Espirito-Santo et al. (2012)
	TAK-779	1.6–178.4 nM	0.6–128.3 nM	Borrego et al. (2012); Espirito-Santo et al. (2012)
CXCR4 antagonists	Bicyclams			
	AMD 2763	0.37–0.5 μM	0.75–1.37 μM (0.75 μM)	De Clercq et al. (1992)
	AMD 3100 (plerixafor)[e]	1–5 nM	4–7 nM (7 nM)	De Clercq et al. (1994)
	JM 1657	0.29–0.43 μM	(0.29 μM)	De Clercq et al. (1992)
	Monoclonal antibody 12G5[d]	7.86 ± 0.41 μg/ml	3.54–16.86 μg/ml	Espirito-Santo et al. (2012)
	SDF-1α/CXCL12	2.42 ± 0.40 μg/ml	2.39–2.82 μg/ml	Espirito-Santo et al. (2012)
	Synthetic peptide T22 ([Tyr-5,12,Lys-7] polyphemusin II)	8 ± 3 ng/ml	31–71 ng/ml (31 ng/ml)	Nakashima et al. (1992)
CCR5/CXCR4 dual antagonists	Bicyclam AMD 3451	1.2–26.5 μM	(9 μM)	Princen et al. (2004)
	Suradista (distamycin analog NSC 651016)	1.5 ± 0.2 μM	(13.9 ± 1.7 μM)	Howard et al. (1998)

Abbreviations: CADA, (9-benzyl-3-methylene-1,5-di-*p*-toluenesulfonyl-1,5,9-triazacyclododecane); NSC 651016, 2,2′-[4, 4′-[[aminocarbonyl]amino]bis[*N*,4′-di[pyrrole-2-carboxamide-1, 1′-dimethyl]]-6,8-naphthalenedisulfonic acid]hexasodium salt; PAMPS, Poly (2-acrylamido-2-methyl-1-propanesulfonic acid); PVS, Poly (vinylsulfonic acid) sodium salt; TAK-779, *N*,*N*-dimethyl-*N*-[4-[[[2-(4-methylphenyl)-6,7-dihydro-5*H*-benzocyclohepten-8-yl]carbonyl]amino]benzyl]tetrahydro-2*H*-pyran-4-ammonium chloride; TASP, template assembled synthetic peptide. HB-19 is a TASP with branches containing Lys–Pro–Arg sequences.

[a] For each drug, the reported IC_{50} values were obtained in the same type of cells and monitoring the same property (e.g. cytopathic effect, infectivity, or p24 production).

[b] Numbers between parentheses are used to indicate the IC_{50}s of HIV-2_{ROD} isolates.

[c] The IC_{50} values given for CCR5 and CXCR4 antagonists correspond to inhibition of CCR5- and CXCR4-tropic strains, respectively. Maraviroc is not effective on dual-tropic and CXCR4-tropic strains of HIV. HIV-2_{ROD} is a CXCR4-tropic isolate.

[d] IC_{50} values refer only to those strains susceptible to inhibition by the indicated monoclonal antibody.

[e] Similar values have been recently reported by Borrego et al. (2012), with IC_{50} values for HIV-1 isolates in the range of 0.9–5.2 nM, and for HIV-2 isolates in the range of 1.0–4.2 nM, with an IC_{50} for HIV-2_{ROD} of 1.0 nM.

et al., 2012; Visseaux et al., 2012). However, the IC_{90}s of maraviroc required to inhibit replication of CCR5-tropic HIV-2 strains were about four times higher than for CCR5-tropic HIV-1 isolates (Borrego et al., 2012). These results suggest that higher doses of maraviroc should be used for effective treatment of HIV-2 infection. Nevertheless, we cannot exclude the possibility of HIV-2 escaping from maraviroc by using alternative coreceptors, although this has not been documented in the literature.

7.2. Fusion inhibitors

Fusion inhibitors in the clinical treatment of HIV-1 infection are represented by enfuvirtide (Fig. 10). This drug is a peptide of 36 amino acids that derives from the C-terminal region of the heptad repeat 2 (HR2) (i.e. residues 127–162), located within the N-terminal ectodomain of the HIV-1 transmembrane glycoprotein (TM, gp41). HR2-based peptides target the HR1 region and block the formation of a stable six-helix bundle during the fusion process. Enfuvirtide has to be administered twice daily by subcutaneous injection and this has limited its use to "salvage" therapy in patients with multidrug-resistant HIV-1.

HIV-2 strains show natural resistance to enfuvirtide. Thus, in phenotypic assays, reference HIV-2_{ROD} and HIV-2_{EHO} strains showed 64- and 88-fold decreased susceptibility to the drug, respectively (Witvrouw et al., 2004), while most HIV-2 clinical isolates tested *in vitro* in another study involving patients naïve to therapy, showed increases in the IC_{50} for enfuvirtide that ranged from 7- to >500-fold (Borrego et al., 2012). Interestingly, many of those clinical isolates were inhibited by an investigational fusion inhibitor known as tifuvirtide (or T1249) which is a polypeptide of 39 amino acids that derives from highly conserved domains in the TM proteins of HIV-1, HIV-2 and SIV (reviewed in Eggink et al., 2010). Tifuvirtide was about twofold less active against many HIV-2 clinical isolates in comparison with HIV-1, although the

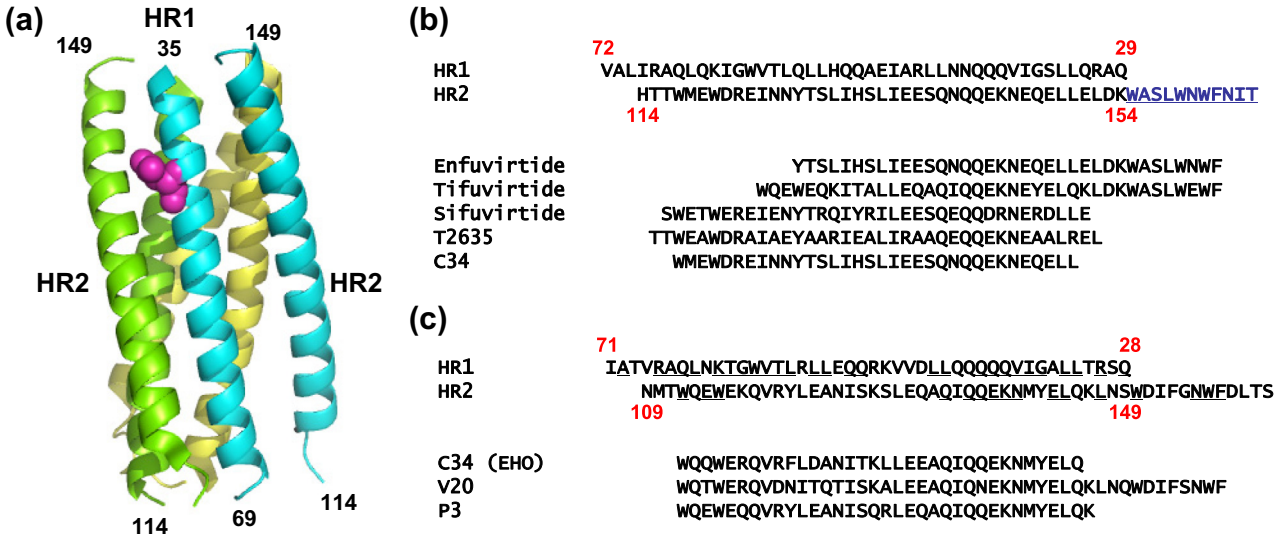

Fig. 10. Structure of the six-helix bundle structure in the TM protein and peptide sequences of fusion inhibitors. (a) Trimer of hairpins motif showing the heptad repeats HR1 and HR2 and the location of Asn[43] (purple spheres). Atomic coordinates were obtained from PDB file 1F23 (Liu et al., 2001) and correspond to the HIV-1 gp41 ectodomain. Structures were generated with the PyMOL molecular graphics system (DeLano Scientific). (b) Amino acid sequences of the HR1 and HR2 regions in HIV-1 gp41 (group M subtype B HXB2 strain) (GenBank accession number K03455), and fusion inhibitors mimicking in part the HR2 structure. The underlined sequence corresponds to the linker between HR2 and the transmembrane region of gp41. Sequences of peptide inhibitors mimicking the HR2 region of HIV-1 TM were taken from Eggink et al. (2010). (c) Amino acid sequences of the HR1 and HR2 regions in the TM protein of HIV-2 (ROD strain) (GenBank accession number M15390). Conserved residues between HIV-1 and HIV-2 are shown underlined. Peptides below are fusion inhibitors mimicking the HR2 region of HIV-2 TM (Gustchina et al., 2005; Borrego et al., 2013; Brauer et al., 2013).

observed variability was high (Borrego et al., 2012). For example the IC_{50}s obtained with CCR5-tropic HIV-1 isolates ranged from 1.4 to 8 nM, while for CCR5-tropic HIV-2 isolates, these values ranged from 2.3 to 21.9 nM (Borrego et al., 2012). CXCR4-tropic HIV-2 isolates were usually more susceptible to inhibition (IC_{50}s in the range 0.9–9.1 nM), although the HIV-1 prototypic NL4–3 strain (CXCR4-tropic) showed an IC_{50} of 0.6 nM in these assays (Borrego et al., 2012).

New HR2-based peptides that inhibit HIV-2 fusion and entry have been designed (Borrego et al., 2013). One of them (designated as P3) was a peptide of 34 amino acids that mimics the sequence of the HR2 region of the HIV-2 TM protein (Fig. 10c). This peptide shows some sequency similarity with enfuvirtide, but unlike this drug it forms an α-helical structure in solution. P3 inhibits HIV-1 clinical isolates with IC_{50}s in the range of 6.5–18.4 nM, while these values go up to 51.9–78.5 nM in the case of HIV-2. The N43K mutation in HIV-1 gp41 confers high-level resistance to the inhibitor, although selection of P3-resistant mutants was not achieved with HIV-2 isolates (Borrego et al., 2013). In agreement with these observations, a related peptide derived from the HIV-2_{EHO} strain (i.e. C34 (EHO)) (Fig. 10c) was found to be a good inhibitor of HIV-1 cell fusion (Gustchina et al., 2005). Another peptide (V20) based on the sequences of the HR2 regions of HIV-2_{EHO} and HIV-1_{HXB2} (Fig. 10c) showed broad anti-HIV and anti-simian immunodeficiency virus activity (Brauer et al., 2013). V20 was a good inhibitor of enfuvirtide-resistant HIV-1_{HXB2} strains (IC_{50} 0.26 nM), and unlike enfuvirtide it was active against the SIV_{mac251} strain (IC_{50} 5 nM). These results have opened new possibilities for the development of novel fusion inhibitors with broad spectrum against different types of immunodeficiency viruses, including effective inhibitors of HIV-2 fusion and entry. However, a limitation for such a development is the relatively low number of HIV-2-infected patients that could benefit from a therapeutic strategy based on the used of fusion inhibitors.

Recently, a new pyrimidinedione (IQP-0410, 1-(3-cyclopenten-1-ylmethyl)-5-ethyl-6-(3, 5-dimethylbenzoyl)-2,4 (1H, 3H)-pyrimidinedione) has been identified as a drug that in HIV-1 acts simultaneously as an NNRTI and as a fusion inhibitor (Buckheit et al., 2013). IQP-0410 is active against wild-type HIV-1 strains at subnanomolar concentration levels, and against clinical strains of HIV-2 at nanomolar concentrations. However, unlike other HEPT derivatives, IQP-0410 does inhibit HIV-2 RT, but at a viral entry step. HIV-2 passaged in cell culture in the presence of IQP-0410 selected for resistant virus lacking mutations in the RT-coding region but containing a truncated TM glycoprotein (Buckheit et al., 2013). This truncation involved the elimination of about 100 amino acids from the intraviral tail of HIV-2 gp36.

8. Final remarks

Despite the success of current antiretroviral therapies in controlling HIV-1 infection, treatment of individuals infected by HIV-2 poses some challenges due to the reduced armamentarium of available drugs and the faster emergence of HIV-2 resistance to NRTIs and other approved therapies. Combinations based on two NRTIs (e.g. zidovudine/lamivudine, tenofovir/lamivudine or tenofovir/emtricitabine) plus one ritonavir-boosted protease inhibitor (usually lopinavir or darunavir) are pivotal in the treatment of HIV-2 infection (see available guidelines and recommendations in Gilleece et al., 2010; Vandamme et al., 2011). The benefits of using maraviroc are still not clear, but the recent approval of integrase inhibitors (raltegravir, elvitegravir or dolutegravir) is an important addition to current therapies.

However, we believe that in the long run additional therapies will be necessary to fight HIV-2 infection and in addition to more potent drugs, other viral functions should be exploited for antiviral intervention. Despite the similarities between HIV-1 and HIV-2, it is clear that some antiviral drugs effective against HIV-1 may not work with HIV-2. We have provided compelling examples such as NNRTIs, several protease inhibitors, and enfuvirtide and other fusion inhibitors. At a preclinical stage we have examples of drugs that have similar activities on HIV-1 and HIV-2. For example, vinylogous ureas that inhibit the RNase H activity of HIV-1 and HIV-2

showed IC_{50}s around 2–4 μM (Wendeler et al., 2008). On the other hand, a small molecule known as PF-3759857 that inhibits capsid assembly, was found to be active against HIV-2 (IC_{50} around 4.7 μM), although this compound showed higher potency against HIV-1 strains (IC_{50} values in the range of 0.51–3.17 μM) (Blair et al., 2010). On the other hand, differences between HIV-1 and HIV-2 can limit the utility of some anti-HIV-1 drugs in clinical trials. Examples include allosteric inhibitors of the interaction between the integrase and the host factor LEDGF/p75 discussed above, and bevirimat, an HIV-1 maturation inhibitor in preclinical development (for reviews see Adamson and Freed, 2008; Menéndez-Arias, 2010). HIV-2 and SIV strains show natural resistance to bevirimat due to sequence differences at their CA-SP1 cleavage sites that in HIV-2 is KARLM∗AEALK (asterisk indicates the hydrolyzed bond), instead of KARLV∗AEAMS, as found in susceptible HIV-1 strains (Zhou et al., 2004).

Evidence discussed above is important not only because natural resistance reduces the therapeutic options for infected individuals, but also for the development of needed prophylactic measures such as antiretroviral drug-based microbicides. It is clear that NNRTIs and other inhibitors would not work in preventing HIV-2 infection, but also more potent drugs may be needed to avoid the likely emergence of resistance mutations if the prophylactic microbicide lacks potency or is given in suboptimal doses. Preclinical studies discussed above establish new principles and possibilities for the treatment of HIV-2 infection that with a better understanding of the molecular and cell biology of this retrovirus will warrant further research in this area.

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