

# Targets for Inhibition of HIV Replication: Entry, Enzyme Action, Release and Maturation

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## Key Words

Antiretroviral therapy · Retroviral targets · HIV replication inhibition · Drug resistance

## Abstract

Inhibition of HIV replication initially targeted viral enzymes, which are exclusively expressed by the virus and not present in the human cell. The development of reverse transcriptase (RT) inhibitors started with the discovery of antiretroviral activity of the nucleoside analog zidovudine in March 1987. Currently, six major classes of antiretroviral drugs are used for the treatment of HIV-infected patients: the RT inhibitors, nucleoside inhibitors and nonnucleoside inhibitors, the protease inhibitors, the integrase inhibitor raltegravir, the fusion inhibitor enfuvirtide (T-20), and the chemokine receptor 5 antagonist maraviroc. A seventh class, the maturation inhibitors, has not yet been approved as their effectiveness is impaired by HIV-1 polymorphisms naturally occurring in 30–40% of HIV-1 therapy-naïve isolates. The use of antiretroviral combination therapy has proven to be effective in delaying progression to AIDS and to reconstitute the immune system of HIV-infected individuals. During the last 5 years, the introduction of the newest antiretrovirals has increased treatment efficacy tremendously. However, the development and accumulation of resistance to all antiretroviral drug

classes are still a major problem. Additional targets will have to be defined to achieve the ultimate goal: the eradication of the virus from the infected human body.

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## Introduction

After the first description of the novel acquired immune deficiency syndrome (AIDS) and identification of its causal agent HIV, the urgent need for antiretroviral therapy was obvious. At that time, options for antiviral therapy were generally limited: there was amantadine against influenza virus A or the nucleoside analog aciclovir against alpha herpes viruses, but no antiretrovirals had been identified yet.

The first ideas to target HIV aimed to inhibit viral enzymes, which are exclusively expressed by the virus and not present in the human genome: this is true for the reverse transcriptase (RT) and the integrase (IN) only. The development of RT inhibitors started with the discovery of the antiretroviral activity of the nucleoside analog zidovudine [AZT (azidothymidine)], quickly followed by other nucleoside analogs like didanosine (ddI), stavudine (d4T), and lamivudine (3TC). However, treatment with nucleosides was challenged by fast resistance develop-

ment by the virus either in months, or, as in the case of 3TC, in approximately 80% of all patients after only 2 weeks of monotherapy [1]. Therefore, two major rules of antiretroviral treatment have become evident: the inhibition of HIV replication by treatment is an achievable goal, and a resistant virus is selected in a very short time [2].

Supported by the reconstitution of AZT susceptibility in 3TC-resistant virus [2], the additive and synergistic effects of drug combinations could be observed. Antiretroviral therapy was further improved by the development and final approval of the first allosteric RT inhibitor [nonnucleoside RT inhibitor (NNRTI)] in the mid-1990s [3]. Several members of both drug classes were approved and are still in use today as a successful component of antiretroviral combination therapy.

Another major idea to inhibit HIV replication was to block the viral protease (PR), identified as essential for HIV replication [4]. In 1995, the approval of the first PR inhibitor (PI), saquinavir (SQV), led to a substantial enrichment of antiretroviral therapy. Combination therapy of two nucleoside analogs with a PI or an NNRTI was found to be superior in order to achieve sustained viral suppression [5, 6]. A new era began, initially characterized by immune reconstitution in the majority of treated patients. However, this was limited by a high pill burden and side effects [7, 8]. Today PIs are generally coadministered with low-dose ritonavir (RTV) as a pharmaceutical booster, which leads to increased and more stable drug levels, and to a drastic reduction of viral resistance development from approximately 50% of all observed therapeutic failures to 0–15% against all drugs [9, 10].

In 2003, enfuvirtide (T-20), the first and up to now only member of the fusion inhibitor class, was approved. Blocking viral entry should substantially reduce the loss of activated cells and the reseeded of cellular reservoirs. T-20 is a peptide mimicking the viral fusion part of gp41 and interfering with trimer formation during an intraprotein conformation. Although the drug is quite effective and still successfully in use, its subcutaneous application limits clinical use to deep salvage regimens.

In 2007, maraviroc (MVC), the first chemokine receptor 5 (CCR5) antagonist, was approved. MVC is the first antiretroviral drug that does not bind to a viral but to a host protein. Development of drug resistance occurs in vivo by selection of X4-tropic variants. Nonetheless, the selection of R5 viruses resistant to CCR5 antagonists does rarely occur [11]. Therefore, it remains unclear if the choice of any cellular target is a sensible route to generally avoid resistance development. CCR5 antagonists have brought back an old puzzle still to be elucidated: the

role of X4 viruses for disease progression. Are X4 viruses more pathogenic and does their presence contribute to causing AIDS, or do X4-tropic viruses appear preferentially when the immune system is already exhausted? In spite of the presence of minor X4 variants during early infection only detectable by ultradeep sequencing [12], most individuals progressed to late stages of infection harboring predominantly R5 viruses [8–10]. Additionally, the selection of X4 variants by CCR5 antagonists did not lead to disease progression, indicating that X4-tropic variants are not necessarily more pathogenic than R5 viruses. This was further supported by the reappearance of R5 viruses when MVC was removed. Obviously, a competitive replication between R5 and X4 variants occurs.

In contrast to RT inhibitors, the development of inhibitors of the second specific viral enzyme, the IN, was more complicated [13, 14]. The first IN inhibitor, the strand transfer inhibitor raltegravir (RAL) [15, 16], was approved in 2008.

Since 1995, the concept of combining several antiretrovirals has been proven to be effective in suppressing viremia and to enable patients to partially reconstitute their immune systems [17].

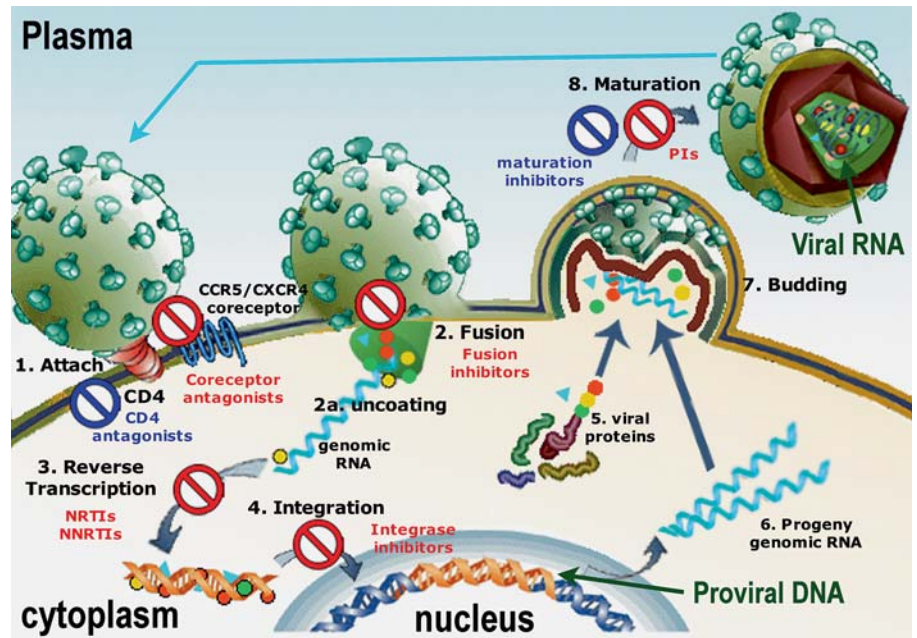
## Antiretroviral Therapy

### *Viral Entry Inhibitors*

HIV enters target cells with the help of the two envelope glycoproteins, gp120 and gp41. Both proteins have trimeric structures and together form spikes on the surface of the virions. The HIV entry process into a target cell is divided into three steps (fig. 1, 2):

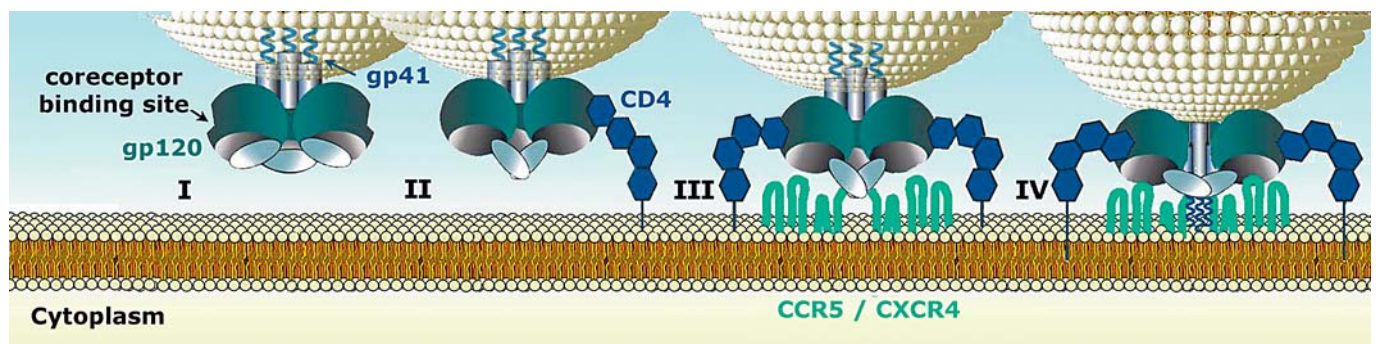
First, the virus attaches to the cellular membrane through interaction between the viral envelope protein gp120 and the first extracellular domain of the cellular CD4 receptor (D1 domain) [18–20]. Initial binding to CD4 causes a first change of gp120 conformation, which allows the presentation of the coreceptor binding site, a discontinuous epitope comprising the third hypervariable loop (V3), the  $\beta$ -19 strand and the bridging sheet of the gp120.

Second, the newly exposed gp120 region binds to the CCR5 or the CXCR4 coreceptor. Viral entry in the absence of CD4 expression has been reported and may occur rarely, but the presence of one of the coreceptors is crucial for the entry process [21]. Which coreceptor will be bound by the gp120 is mainly mediated by V3 loop sequence and glycosylation. A further conformational rearrangement then takes place, exposing certain domains of the second Env protein gp41. Gp41 is a highly conserved



**Fig. 1.** HIV-1 life cycle and current targets for antiretroviral therapy. The virus attaches to the cell membrane via CD4 (1) and co-receptor binding, thereby initiating the fusion of viral and cellular phospholipid bilayers (2). After the entry, uncoating of the viral core begins (2a), soon followed by reverse transcription (3). The freshly formed preintegration complex is transported into the nucleus, where integration of the viral genome into the host genome occurs (4). The initially slow transcription results in translation of multiple-spliced viral mRNAs. The early proteins Tat and Rev optimize translation and nuclear export of single and unspliced viral

RNAs resulting in translation of viral glycoproteins and gag-(pol) precursor proteins (5). After the transfer of viral proteins and the unspliced viral genome (6) to the cell membrane, matrix-oriented parts of gag-(pol) precursors anchor in the membrane forming a spheric particle (7) budding from the cell membrane. Released particles are immature. The viral PR processes the gag and gag-pol proteins to originate a capsid, nucleocapsid and viral enzymes producing a mature virion able to infect a new cell. Main steps in the HIV-1 life cycle are indicated in black, approved antiretroviral drugs in red and drugs still undergoing clinical trials in blue.



**Fig. 2.** Cell attachment. The viral gp120 (I) binds to the cellular receptor CD4 (II). This binding triggers a conformational change in gp120 that allows the exposition of the coreceptor binding site and interaction with the coreceptor CCR5 or CXCR4 (III). After

binding to the coreceptor, a further conformational rearrangement exposes certain domains of the gp41 (IV) leading to the fusion of both cellular and viral membranes and release of the capsid into the cytoplasm.

protein with a structure predominantly based on alpha helices and broad-sequence homologies to the type-1 fusion proteins of other viruses [22].

Third, the helical regions HR1 and HR2 of gp41 anchor in the cellular membrane and the N-terminal peptide initiates the fusion of both cellular and viral membranes and the liberation of the nucleocapsid to the cytoplasm [23–25].

#### *Inhibitors of CD4 Binding*

Blocking the interaction of CD4 and gp120 has been considered to develop the so-called attachment inhibitors. Initial approaches tried to develop neutralizing antibodies or soluble CD4 receptors that could prevent the virus from binding to the target cells [26]. Today it is known that none of the neutralizing antibodies exhibit an activity against a broad variety of HIV-1 isolates and that soluble CD4 receptors cannot reduce viral replication in vivo [27–29].

Some compounds are still under development and undergoing clinical trials [reviewed in 30]. These are humanized CD4-monoclonal antibodies binding to the cellular receptor and capable of hindering the interaction with gp120 or the conformational rearrangement necessary for the entry process. TMB-355 (TNX-355, Ibalizumab®, Tanox Biosystems), PRO-542 (Progenics Pharmaceuticals) or BMS-806 (BMS-488043, Bristol Myers Squibb) are examples of CD4 antagonists [31–37] (see also <http://clinicaltrials.gov/ct2/show/NCT00784147> or <http://clinicaltrials.gov/ct2/show/NCT00055185>).

#### **Inhibition of Coreceptor Binding**

Depending on the structure and charge of the coreceptor binding site of gp120, one or both of the chemokine receptors CCR5 or CXCR4 can be recognized and docked [19, 20]. Depending on this tropism, viruses were classified as R5 (those using exclusively CCR5 as a coreceptor), X4 (those using exclusively CXCR4 as a coreceptor) and R5X4 or dual-tropic viruses (able to use both coreceptors). Since HIV, like all RNA viruses, is present as a quasispecies in each infected individual [38, 39], mixtures of viruses with different tropism are frequently found (dual-mixed viruses). However, phenotypic methods cannot discriminate between R5X4 and mixed viral populations; therefore, results were termed as dual-mixed viruses. Meanwhile, ultradeep sequence analyses derived from plasma samples have shown the presence of all kinds of tropic viruses in the quasispecies of an individual [40].

Current studies address the relevant maximum percentage of viruses in plasma, with tropism other than R5 that can lead to clinical therapy failure [41]. The standard diagnostic method for tropism testing is the bulk sequencing of the V3-loop sequence followed by an interpretation using the geno2pheno<sub>[coreceptor]</sub> tool ([www.genafor.org](http://www.genafor.org)).

#### *CCR5 Antagonists*

Blocking the CCR5 receptor significantly hinders HIV replication and does not lead to any major side effects, as its functioning seems to be dispensable for normal immune cell activity in vivo. Approximately 1% of Caucasians are homozygous for a nonfunctional CCR5 receptor, carrying the so-called CCR5-Δ32-gene mutant alleles; they are healthy and partially resistant to HIV-1 R5 infection [42].

CCR5 antagonists interact with the host coreceptor, alter its structure and therefore hinder the recognition and binding of the viral gp120. As CCR5 antagonists only interact with the CCR5 molecule, they are only effective against R5 viruses. The study A4001029 which analyzed the effect of MVC on X4 viruses showed no major differences in HIV-1 RNA decrease between the MVC and the placebo arms [43]. Therefore, a tropism test is mandatory before CCR5 antagonist administration occurs.

MVC (Selzentry®, Celsentri®, ViiV Healthcare) is the first CCR5 antagonist approved for the treatment of R5-carrying therapy-experienced patients [reviewed in 44]. MVC is primarily metabolized by CYP3A4, so its pharmacokinetics are affected by the inducers/inhibitors of this system, such as PIs (excluding tipranavir and fosamprenavir), efavirenz, etravirine or rifampicin, with recommendation for a dose adjustment when coadministered with these drugs [45, 46]. The efficacy of MVC against R5 virus has been tested in 3 trials [43, 47–53]. In very few cases, treatment failure has been described as a consequence of certain mutations in the V3 region that permit the virus to recognize the altered CCR5 and lead to resistance [54, 55]. Usually, MVC therapy failure is associated with viral tropism switch.

Two other compounds of this drug class, aplaviroc and vicriviroc, that had reached clinical studies, have been withdrawn recently: aplaviroc (GlaxoSmithKline) due to severe side effects [56] and vicriviroc (Schering-Plough) because the primary efficacy endpoints have not been met. INCB9471 (Incyte), Pro-140 (Progenics Pharmaceuticals), and CCR5mAb004 (Human Genome Sciences) are other drugs under development [57–61] (see also <http://clinicaltrials.gov/ct2/show/NCT00243230>). In addition, aprepitant (Emend®, Merck), an antiemetic be-

longing to the neurokinin 1 (NK1) receptor blockers, has been tried for HIV-infected patients as it additionally downregulates the CCR5 coreceptor expression [62–64].

#### *CXCR4 Antagonists*

There is no genetic defect that leads to the absence of CXCR4 in humans. Contrary to the CCR5 receptor, the block of the CXCR4 leads to serious problems as the chemokine SDF1 can only interact with CXCR4, and currently, CXCR4 knockout mice are nonviable. The CXCR4 antagonist AMD3100 showed very promising results in cell culture experiments, but produced such severe side effects that the clinical studies had to be aborted. AMD887, AMD070 or AMD1170, other drugs from this series, are currently being tested [65–68].

#### *Fusion Inhibitors*

The helical regions HR1 and HR2 of the viral gp41, exposed after coreceptor binding of gp120, anchor the cellular membrane and lead to the fusion of both cellular and viral membranes. The fusion inhibitor enfuvirtide (T-20, Fuzeon<sup>®</sup>, Hoffmann-La Roche) is an HR2-analog 36-amino-acid-long peptide which binds to the HR1 sequence and inhibits the fusion event [reviewed in 69]. It is active against HIV-1 R5 and X4 viruses, but not against HIV-2. The peptide sequence is derived directly from HXB2, one of the first laboratory-adapted HIV-1 strains [70]. Similar fusogenic peptides are found in other viruses like Ebola and SARS [22]. The peptidic nature of T-20 constrains its method of administration. The lyophilized T-20 powder is reconstituted and must be administered twice daily by subcutaneous injection. Apart from the occurrence of injection site reactions, it is generally well tolerated. Mutations in the gp41 amino acids 36–45 (corresponding to the HR1 domain) have been related to T-20 resistance and therapy failure [71]. Other fusion inhibitors such as TRI-1144 [TR-0291144 (Trimeris)], sifuvirtide (FusoGen), CP32 or SC34EK are under development [44, 72] (see also <http://www.trimeris.com/300Pipeline.aspx>).

### **Reverse Transcription Inhibitors**

After capsid liberation into the cytoplasm, capsid and nucleocapsid disassemble (uncoating), yet the precise mechanism is still not known [73]. Genomic RNA is associated with viral tRNA<sup>Lys</sup> and with several viral proteins like RT, IN, PR, Vpr and MA constituting the reverse transcription complex (RTC) [74–76]. The RTC uses the microtubule system for transport through the cyto-

plasm [77, 78]. Within the RTC, reverse transcription of viral RNA into DNA takes place, conducted by the viral RT, although the efficacy of reverse transcription is highly dependent on the presence of all components of the RTC. For instance, in the absence of IN protein, the reverse transcription is completely blocked [79].

The RT is an RNA-dependent DNA polymerase which produces double-stranded DNA from single-stranded RNA. This process starts with the synthesis of one single-stranded DNA in minus orientation copied from the viral RNAs, which is used as template for the subsequent synthesis of the second DNA strand. RT is a heteromeric enzyme that comprises a regulator subunit (p51) and a catalytic subunit (RNase H – p15) building the p66 molecule.

The p66 resembles a right hand, where the subdomains are designated fingers, palm and thumb. The catalytic site lies in the palm and comprises the amino acids D185–D186 and D110, a very conserved motif also in other RTs and polymerases [80]. It includes the viral ribonuclease H activity, responsible for the degradation of the template RNA from the DNA/RNA hybrid.

Since HIV-1 RT is reported not to maintain sustained replication longer than for approximately 100–200 bases, reverse transcription is the replication step with the highest probability for recombination events between the two strains of HIV-1 RNA in each particle [79, 81]. Similar to all RNA polymerases, HIV RT has a high error rate when transcribing RNA into DNA since it has no proofreading ability [82]. This high error rate, in combination with the high recombination rate, allows mutations to accumulate at an accelerated rate, resulting in important implications for immune escape, drug resistance development and tropism switch, among others [83].

#### *Nucleoside/Nucleotide RT Inhibitors ('Nukes')*

Both nucleoside and nucleotide RT inhibitors (NRTIs and NtRTIs) (table 1) are analogs of the natural substrates used to synthesize viral DNA, and they compete with them for incorporation into the growing viral DNA chain. However, NRTIs and NtRTIs lack a 3'-hydroxyl group on the deoxyribose moiety, so subsequent incorporation of nucleotides into the nascent DNA is blocked.

Resistance to NRTIs/NtRTIs can be achieved by selection of HIV strains with accumulated mutations in the RT coding region of the *pol* gene [reviewed in 84]. Two main mechanisms of resistance to NRTIs/NtRTIs are described: (1) mutations such as A62V, K65R, L74V, V75T/I, F77L, Y115F, F116Y, V118I, Q151M and M184V that reduce the RT affinity for the drugs favoring the incorporation of the natural substrates [85–87], and (2) the so-

**Table 1.** NRTIs and NtRTIs

Drug	Abbreviation	Trade name	Company
Zidovudine	AZT, ZDV	Retrovir	ViiV Healthcare (GlaxoSmithKline)
Stavudine	d4T	Zerit	Bristol-Myers Squibb
Lamivudine	3TC	Epivir	ViiV Healthcare (GlaxoSmithKline)
Didanosine	ddI	Videx	Bristol-Myers Squibb
Zalcitabine	ddC	Hivid	Hoffmann-La Roche
Abacavir	ABC	Ziagen	ViiV Healthcare (GlaxoSmithKline)
Tenofovir	TDF	Viread	Gilead
Emtricitabine	FTC	Emtriva	Gilead

**Table 2.** NNRTIs

Drug	Abbreviation	Trade name	Company
Delaviridine	DLV	Rescriptor	Pfizer
Nevirapine	NVP	Viramune	Boehringer Ingelheim
Efavirenz	EFV	Sustiva/Stocrin	Bristol-Myers Squibb
Etravirine (TMC 125)	ETR	Intence	Tibotec
Rilpivirine (TMC 278)	RPV	–	Tibotec

called thymidine analog mutations (TAMs) 41L, D67N, K70R, L210W, T215Y and K219Q/E that increase the excision of already-incorporated NRTIs/NtRTIs [86, 88–94].

There are also certain mutations in the *pol* gene that appear significantly more frequently in NRTI/NtRTI-exposed patients than in naive ones, although no direct correlation between these mutations and NRTI/NtRTI therapy failure has been detected. These mutations, located in the p6\* region (immediately 5' of the PR), increase the incorporation of RT molecules in the progeny viruses [95].

#### *Nonnucleoside RT Inhibitors*

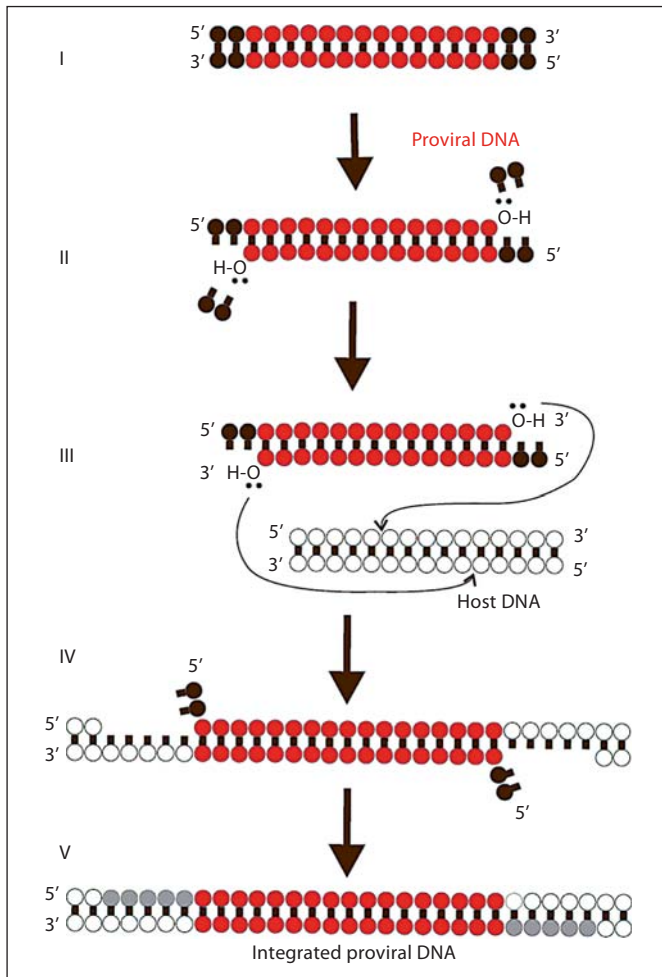
NNRTIs (table 2) block RT by binding at a hydrophobic pocket in the HIV-1 p66 unit, close to the active center of the enzyme. NNRTIs are not incorporated into the viral DNA (noncompetitive inhibitors of the RT), but instead inhibit the movement of RT domains needed to synthesize the DNA. NNRTIs are generally inactive against HIV-2 RT enzymes due to naturally occurring amino acid polymorphisms. The same substitutions, which are not present in the HIV-1 viruses of untreated individuals, can be selected under drug pressure leading to resistance to NNRTIs. Mutations leading to resistance to NNRTIs affect the tertiary structure or charge of the RT [reviewed in 84, 96]. Since the lipophilic binding pocket is built mainly by three sites of the amino acid primary sequence,

all but one of the NNRTI resistance mutations observed can be found in these regions (aa 98–108, 178–190, 225–238). The only exception is located at RT amino acid position 138 of the RT enzyme, which is associated with rilpivirine resistance and may be involved in etravirine resistance [71].

NNRTIs display a low genetic barrier so their high effectiveness can be impaired by one mutation [97]. However, resistance to the two members of the second-generation NNRTIs etravirine (ETR) or rilpivirine (RPV), diarylpyrimidin-(DAPY)-analogs designed to avoid cross-resistance with nevirapine (NVP) and efavirenz (EFV), requires the development of a higher number (2–4) of resistance mutations [98]. NNRTIs have a long plasma half-life, which allows a once-daily administration but represents a problem when therapy is discontinued. In this situation, suboptimal concentrations of the drugs may remain in the plasma for up to several weeks, favoring a rapid emergence of NNRTI resistance mutations [99].

#### **Integration Inhibitors**

Once DNA is synthesized in the RTC, the complex is named the preintegration complex. The preintegration complex docks to the nuclear membrane via viral Vpr and most likely some cellular factors [100–103]. Subse-



**Fig. 3.** HIV-1 integration. HIV-1 DNA (I); IN-catalyzed 3' processing: 2 nucleotides are removed from one or both 3' ends of the viral DNA to expose the invariant cytosine-adenosine dinucleotides at both 3' ends of the viral DNA (II); IN-catalyzed strand transfer: the processed 3' ends of the viral DNA are covalently ligated to the host chromosomal DNA (III); product of strand transfer (IV); DNA repair by cellular enzymes (V) [from 150].

quently, MA, IN and other proteins direct the preintegration complex to enter the nucleus through the nuclear pore [104, 105]. There, the viral IN, which also has an endonuclease activity, inserts the proviral DNA genome into the chromosomal host DNA [reviewed in 106]. The integration of viral DNA is an essential step required to complete the life cycle of HIV. It also makes the host cell a permanent carrier of the viral genome, responsible for the persistence of retroviral infection. The integration process comprises two main steps (fig. 3): priming of the DNA ends by generating two bases of single-stranded

DNA at each end, and transfer of the proviral DNA into the host genome [reviewed in 106].

HIV-1 IN comprises three canonical domains, connected by flexible linkers: (1) the N-terminal domain (amino acids 1–50), including an HH-CC zinc-binding motive, (2) the catalytic core domain (aa 51–212), containing the active centre (DDE motif, D64, D116 and E152), and (3) the C-terminal domain (aa 213–288), which allows the binding to the cellular DNA. The active form of the enzyme is believed to be a tetramer, a dimer of homodimers [107].

#### *IN Inhibitors*

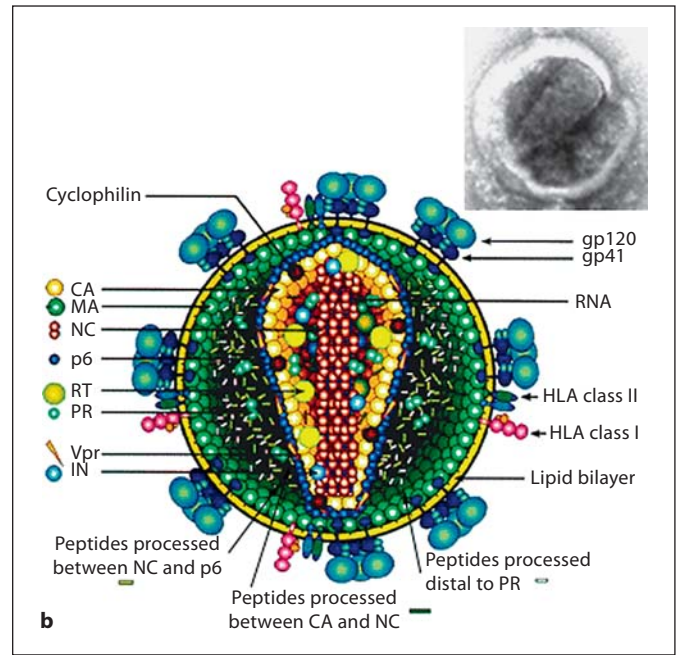
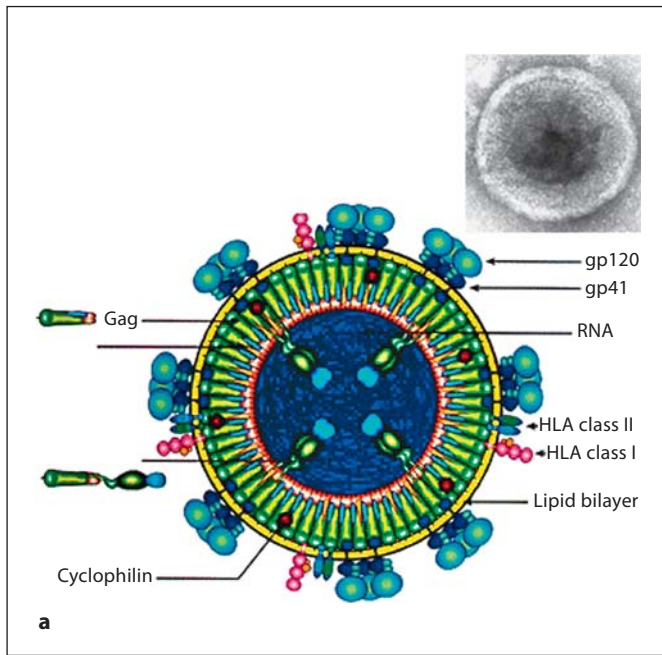
Different substances are currently under development but only one licensed for clinical use belongs to the so-called strand transfer inhibitors. These drugs bind to the IN close to the DDE motif in the active site and competitively block the IN activity. The proviral DNA cannot be inserted into the host genome and is circularized by cellular repair enzymes, stopping the viral replication irreversibly [108, 109].

Raltegravir (RAL, Isentress®, Merck) is a strand transfer inhibitor with potent activity against HIV-1 and HIV-2 [110]. RAL is administered orally twice daily, does not require boosting with RTV and is well tolerated. Results from clinical trials indicate that RAL is safe and highly effective in the treatment of both antiretroviral-naïve and antiretroviral-experienced patients [111–114]. Resistance to RAL has been associated with amino acid substitutions at three key positions in the IN protein: Y143R/C, Q148H/R/K or H155H, alone, or accompanied by other mutations such as T66I, L74M, E92Q, T97A, E138K+G140S/A GY143H, V151I and G163R [115–122].

Elvitegravir (EVG, GS-9137, Gilead), a second strand transfer inhibitor, is undergoing a phase III clinical trial ([http://www.gilead.com/pr\\_1177855](http://www.gilead.com/pr_1177855), accessed August 2010). It is also active against HIV-1 and HIV-2 [123–127]. EVG presents the advantage of an oral once-daily dosage, when boosted with RTV [125]. Resistance to EVG is associated with the mutations T66I/A/K, E92Q, E138K, Q146P, S147G, Q148R/H/K and N155H [128], which are close to the resistance mutations selected by RAL; therefore, cross-resistance for both drugs is expected [119, 129]. Other drugs under current development are reviewed in Serrao et al. [130].

#### *Inhibitors of the Viral Maturation*

If the integrated viral DNA does not lie dormant, it is transcribed by cellular RNA polymerase II and the resulting mRNAs are translated by the cellular ribosomes, under



**Fig. 4.** Models for retroviral structure: immature (**a**) and mature (**b**) HIV-1 virions. **a** The Gag and Gag-Pol proteins are shown in different colors to suggest the domains corresponding to the mature proteins formed from these precursors. The surface and transmembrane components of Env are shown jutting out from the lipid membrane, as are HLA host proteins selectively incorpo-

rated into HIV particles. Cyclophilin is also a host protein specific for HIV-1 group M virions. **b** Major Gag (CA = capsid protein; MA = matrix protein) and Pol proteins and the cone-shaped core characteristic of viruses of this genus. Vpr is an HIV accessory protein [adapted from 151].

coordination by cellular factors as well as viral accessory proteins [131]. The viral proteins, as well as tRNA<sup>Lys</sup>, genomic RNA and a number of cellular proteins start to assemble at the plasma membrane in a process orchestrated mainly by Gag [76, 132, 133]. The core of immature virions consists of uncleaved, radially arranged Gag polyproteins which interact with the Env proteins (fig. 4). Therefore, correct proteolytic Gag processing and core assembly are essential for viral infectivity [134–138]. The maturation process can be pharmacologically targeted via two different approaches: by interacting with the viral PR or with the Gag(-Pol) polyproteins (maturation inhibitors).

#### Protease Inhibitors

HIV-1 PR is an aspartic protease which is active as a homodimer. The active site lies between the two 99-amino-acid-long subunits and has the characteristic DTG (D25, T26 and G27) sequence common to aspartic PRs. The two D25 residues (one from each chain) act as the catalytic residues. PIs (table 3) are substrate analogs binding highly specific and reversibly to the active site of the enzyme. Resistance to PIs is achieved through mutations

located in the substrate-binding pocket (D30N, V32I, L33F, M46I/L, I47A/V, G48V, I50L/V, V82A/F/L/S/T and I84V) leading to conformational changes in the PR that reduce PI incorporation or binding. However, due to these conformational changes the overall fitness of the virus is reduced by these mutations, so compensatory (or secondary) mutations are needed to hold on to viral fitness [139, 140]. Last-generation PIs like tipranavir (TPV) and darunavir (DRV) were designed with the additional aim of avoiding class-embracing drug resistance, which was severely limiting 'classic' PI therapy. Designing such PIs followed certain criteria, e.g. interaction with peptidic chain-building atoms of viral amino acids only, instead of those in amino acid residues, in order to make the drug less susceptible to resistance. Although already the first-generation PIs were described as having relatively high genetic barriers to drug resistance, the last-generation PIs are drugs with very high genetic barriers, requiring the accumulation of 3–10 resistance mutations for the development of clinically relevant drug resistance [141]. In clinical practice, drug resistance to PIs is achieved gradually, after the accumulation of several PI resistance mutations.



**Table 3.** PIs

Drug	Abbreviation	Trade name	Company
Saquinavir	SQV	Invirase	Hoffmann-La Roche
Ritonavir	RTV	Norvir	Abbott
Indinavir	IDV	Crixivan	Merck
Nelfinavir	NFV	Viracept	Pfizer
Amprenavir	APV	Agenerase	ViiV Healthcare (GlaxoSmithKline)
Fosamprenavir	FPV	Telzir	ViiV Healthcare (GlaxoSmithKline)
Lopinavir/Ritonavir	LPV/r	Kaletra	Abbot
Atazanavir	ATV	Reyataz	Bristol-Myers Squibb
Tipranavir	TPV	Aptivus	Boehringer Ingelheim
Darunavir (TMC114)	DRV	Prezista	Tibotec

The impressive success of recent PIs cannot be explained only by the high genetic barrier, but also by their high efficacy. Both efficacy and the high genetic barrier could be improved by a third effect, boosting. Most PIs are metabolized by cytochrome P-450 CYP3A4 [142]. While being metabolized, PIs can induce and block the enzyme complex effectively and therefore cause severe drug interactions. Both are true for RTV, an effective PI itself but with displeasing side effects in most of the patients. RTV is used today in low dosages (100–200 mg) to reduce the liver metabolism of concomitantly administered, P-450 CYP3A4-degraded PIs (boosting). RTV boosting diminishes drug level variability in patients' plasma, eliminates food requirements and permits easier dosing schedules of PIs.

#### *Maturation Inhibitors*

Maturation inhibitors are drugs targeting one or more cleavage sites within the Gag precursor proteins or inhibiting capsid protein interactions required for core condensation. Bevirimat (PA-457; Myriad Pharmaceuticals) is the first compound in the class, although the drug has not yet been approved by the FDA and EMEA. Bevirimat is inactive against HIV-2. HIV-1 mutations conferring resistance to bevirimat were located in the cleavage site P24/P2 (H358Y, L363M/F, A364I/M/V and A366V/T) and in P2 peptide (Q369H, V370A/M/del and T371del), either increasing the cleavage rate at site P24/P2 by the viral PR or interfering with the binding of the drug [143–145]. Unfortunately, the effectiveness of bevirimat therapy is impaired by HIV-1 polymorphisms in P2 (amino acids 369–371), naturally occurring in 30–40% of HIV-1 therapy-naïve isolates. In addition, coevolution of HIV PR and Gag mutations has been observed during PI ex-

posure [146], and PI treatment failures increase the prevalence of resistance to bevirimat and reduce clinical outcome during bevirimat therapy [147].

#### **Conclusions**

The use of antiretroviral combination therapy has proven to be effective against the progression to AIDS in HIV-infected individuals. During the last 5 years, the introduction of two new PIs (DRV and TPV) with broad activities against PI-resistant viral strains, the CCR5 antagonist maraviroc, the IN inhibitor RAL, and the second-generation NNRTI ETR, has tremendously increased the efficacy of antiretroviral treatment [148, 149]. Meanwhile, successfully treated HIV infection can be considered a chronic disease instead of a deadly infection. However, the success of antiretroviral therapy is limited by high costs, viral resistance development and side effects. Eradication of the virus from the infected body by antiretroviral combination therapy or a cure of HIV infection is still not possible. Additional targets will have to be defined to achieve the goal of medical intervention in HIV infection: a worldwide perspective to survive an HIV infection to a normal life expectancy.

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