

Review

Update and latest advances in antiretroviral therapy

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Since the first cases of AIDS appeared in 1981, human immunodeficiency virus type 1 (HIV-1) infection has reached pandemic proportions. Forty years later, research has led to the approval of more than 30 antiretroviral drugs, while combination therapies have turned HIV-1 infection into a chronic, but manageable disease. Still, drug toxicity and acquired and transmitted drug resistance remain as major threats to therapy success. In this review, we provide an overview on currently available anti-HIV drugs and the latest developments in antiretroviral therapy, focused on new antiretroviral agents acting on known and unexploited antiviral targets, prevention therapies aimed to improve available drug combinations, and research on new long-acting therapies, particularly those involving novel drug candidates such as lenacapavir or islatravir.

Historical perspective and current HIV/AIDS situation

Forty years ago, in June and July 1981, the Centers for Disease Control and Prevention in the USA reported clusters of Kaposi's sarcoma and *Pneumocystis* pneumonia among gay men in California and New York City. Patients were immunosuppressed and affected by a deadly disease whose etiological agent was a retrovirus identified 2 years later and designated as HIV [1].

Although azidothymidine (3'-azido-3'-deoxythymidine, zidovudine, or AZT) was approved in 1987 as the first anti-HIV drug, the lethality of the disease remained high until therapies based on drug combinations emerged in the mid 1990s [1]. These combination therapies included three or more drugs directed against at least two different targets. With the introduction of **highly active antiretroviral therapy (HAART)**, the number of AIDS-related deaths in the USA and Europe decreased by half in the span of 3 years. Although HAART was lifesaving, the early regimens were far from perfect. The side effects were burdensome, and their daily dosing was complex. Drug toxicities, the difficulties of maintaining long-term adherence, and the development of drug resistance, frequently led to virological failure and clinical progression of the disease. In 2006, the approval of a combination of tenofovir disoproxil fumarate (TDF), emtricitabine, and efavirenz (Atripla) as the first once-daily single tablet regimen was a major therapeutic advance (reviewed in [2]). The efficacy of **combination antiretroviral therapy (cART)** (see [Glossary](#)), currently referenced instead of HAART, increased from 43% in the mid 1990s to 78% in 2010, measured as their ability to maintain undetectable **viral loads** for a minimum of 48 weeks, when administered to patients with less than 100 000 copies/mL at the initiation of treatment [3]. These data, obtained from a large meta-analysis, reflect the impact of Atripla and other novel therapies developed after the introduction of HAART.

Today, HIV infection has become a manageable chronic health condition, enabling people living with HIV, and under **virological suppression**, to live a good quality of life that is in many aspects comparable to the general population. However, HIV/AIDS remains a major global health threat.

Highlights

Current antiretroviral therapies are highly efficacious in maintaining undetectable viral loads for a long period of time.

Efforts to improve current therapies focus on treatment simplification (i.e., reducing the number of antiretroviral drugs and their doses) and the development of long-acting drugs for treatment and pre-exposure prophylaxis.

Lenacapavir (a capsid-binding agent) and islatravir (a nucleoside reverse transcriptase inhibitor) are currently in advanced clinical trials and are suitable for extended-release formulations.

Low toxicity, high potency, and efficiency against wild-type and drug-resistant strains of HIV, and convenient dosing are desirable properties to be met by newly developed antiretroviral drugs.

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Worldwide, an estimated 27.2–47.8 million people have already died of AIDS (<https://www.unaids.org/en/resources/fact-sheet>). About 38 million are living with HIV/AIDS, but half of them do not know their HIV status. The World Health Organization estimated that in 2020 there were 680 000 AIDS-related deaths and 1.5 million new infections worldwide (<https://www.unaids.org/en/resources/fact-sheet>). At present, HIV/AIDS is the leading cause of death among young people (aged 10–24 years) in Africa, and after road injuries, remains as the second largest cause of death in the world for the 25–49 year age group [4].

A cure for HIV infection, meaning that the virus is completely eradicated in the patient, is still an elusive goal. However, sustained antiretroviral therapy-free remission (also known as ‘functional cure’) could be a feasible accomplishment in coming years, although HIV persistence due to the proliferation of infected cells constitutes a major challenge for a successful intervention [5,6].

Combination therapies with at least three different antiretroviral medicines is standard treatment today, for all people newly diagnosed with HIV. In addition, potent dual therapies are also gaining support, most notably as a choice for maintenance treatment. Currently, there are 27.5 million people globally on HIV therapy (<https://www.unaids.org/en/resources/fact-sheet>). Although promising, these numbers are still far from the goals needed to achieve HIV eradication across the world. In addition, adverse side effects, long-term drug toxicity, and the potential appearance of resistance remain as major threats to current therapies.

In this review, we provide an update on available antiretroviral therapies, and recent efforts aimed at the development of long-acting drugs, treatment simplification, and HIV prevention often associated with novel antiretroviral drugs with different mechanisms of action.

Approved antiretroviral drugs

The aim of HIV treatments is to reduce viral loads to undetectable levels. Antiretroviral drugs act by different mechanisms of action, while targeting various steps in the HIV replication process (Figure 1). Thus, fostemsavir is a precursor of temsavir, a drug that binds the HIV envelope glycoprotein gp120 and blocks virus attachment to CD4+ T cells [8]. Fostemsavir was found to be effective in patients with multidrug-resistant HIV-1 infection and limited therapy options [9]. In addition, it shows no *in vitro* cross-resistance with currently available antiretroviral drugs. Binding to the cellular receptor CD4 can be also inhibited by ibalizumab. This is a non-immunosuppressive humanized monoclonal antibody with broad specificity that neutralizes many HIV-1 strains [10]. Chemokine receptors CXCR4 and CCR5 function along with CD4 to facilitate viral entry into target cells. Maraviroc is a negative allosteric modulator of the CCR5 receptor, and unique among antiretroviral drugs as a molecule targeting a host protein. A fourth mechanism of action among drugs blocking HIV entry is represented by enfuvirtide. This is a synthetic peptide (of 36 amino acids) with an acetylated N terminus and a carboxamide at the C terminus. Enfuvirtide blocks the conformational changes affecting the HIV complex **gp120/gp41** that facilitate fusion of the viral envelope and the host cell membrane.

More than half of the approved drugs are **reverse transcriptase (RT)** inhibitors. These molecules block the conversion of the viral single-stranded genomic RNA into double-stranded DNA (dsDNA), necessary for integration into host genomic DNA. HIV-1 RT is a heterodimeric enzyme composed of subunits of 66 and 51 kDa. The large subunit contains catalytic sites of DNA polymerase and ribonuclease (RNase) H activities [11,12]. RTs can use RNA or DNA as templates during DNA polymerization.

Glossary

Allosteric inhibitors: molecules that bind to an enzyme changing its shape and activity, by interacting in a location different from that of the catalytic site. In antiretroviral therapy, NNRTIs are allosteric inhibitors of HIV-1 RT.

Boosting: in combination antiretroviral therapies, this term describes the use of a small amount of a drug (typically ritonavir or cobicistat) that competes in the liver for the degradation of protease inhibitors, thereby increasing the concentration of antiretroviral drugs in the bloodstream, by reducing the speed at which the drugs are metabolized and cleared.

Combination antiretroviral therapy (cART): a synonym of HAART, implying the use of at least three antiretroviral drugs directed against two or more molecular targets.

gp120/gp41: envelope glycoprotein of HIV-1. It is a homotrimer composed of subunits gp120 (surface glycoprotein) and gp41 (transmembrane glycoprotein).

Highly active antiretroviral therapy (HAART): term used since the mid 1990s to describe the combination of antiretroviral drugs to achieve high antiviral effect.

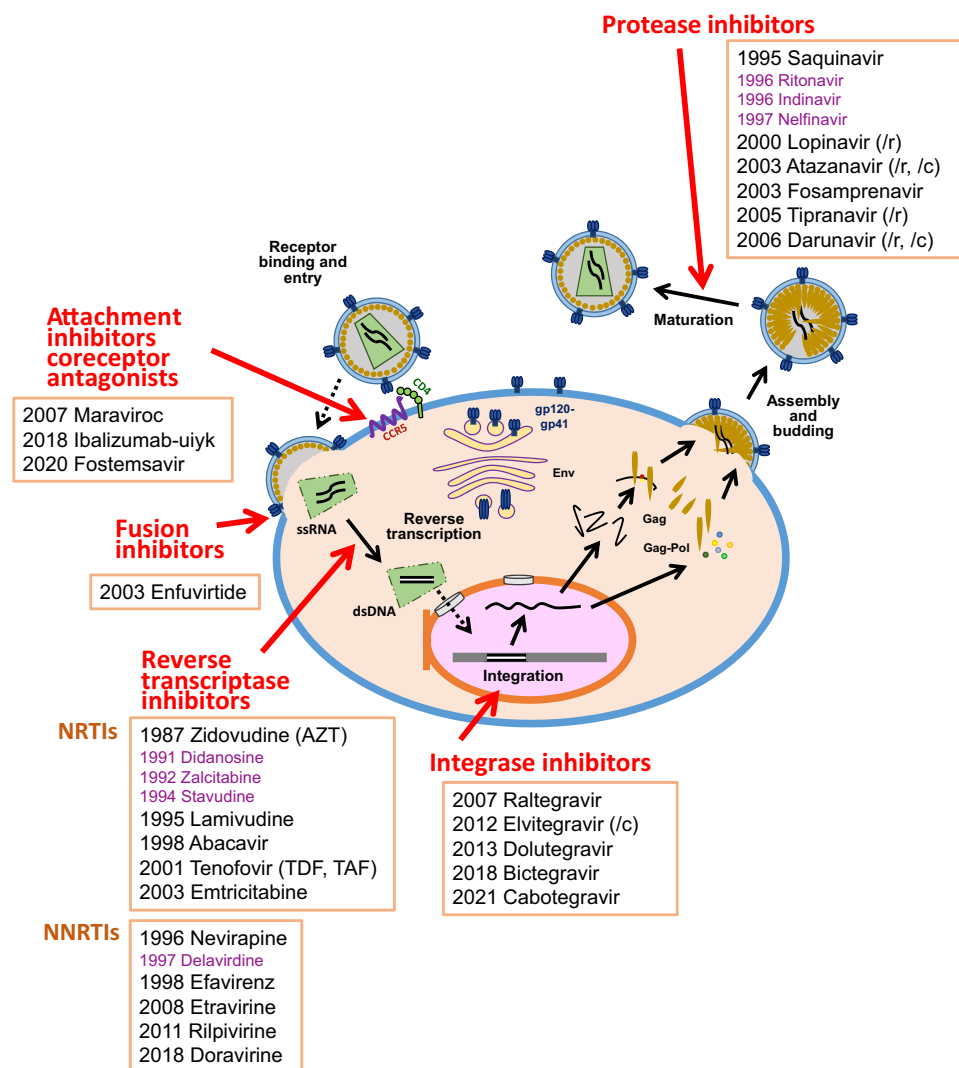
Latency reversal agents: agents that activate HIV latently infecting resting CD4 lymphocytes, contributing to their destruction and subsequent reduction of the latent HIV reservoir after administration of conventional antiretroviral therapies.

Neutralizing antibodies: immunoglobulins that bind to the viral particle, typically to the envelope glycoprotein, in such a way that cell infection is blocked.

Nexplanon: single, radiopaque, rod-shaped implant, traditionally used in birth control for women when containing contraceptives. The implant can be loaded in the needle of a disposable applicator.

Preintegration complex: ribonucleoprotein formed after viral entry and reverse transcription, that is transported into the nucleus for integration. Although its specific composition and structure is still unknown, it contains double-stranded DNA, integrase, and viral proteins, most notably, matrix protein (MA), Vpr, and probably CA and NC proteins.

Proviral DNA: the genetic information of retroviruses, integrated as DNA in the



chromosome of infected cells with the capability to be activated and produce new infective viral particles.

Reverse transcriptase (RT): retrovirus-encoded enzyme responsible for the production of double-stranded proviral DNA from single-stranded genomic RNA.

Viral load: term used to indicate the quantity of genetic material, commonly RNA, of a virus present in the blood. Usually, in HIV-infected patients, the measurement is given as HIV-1 RNA copies per ml of plasma.

Virological suppression: in treated HIV-infected individuals, it describes the achievement of complete inhibition of viral replication, measured by viral load in plasma as less than 50 HIV-1 RNA copies/mL.

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Figure 1. Approved antiretroviral drugs and HIV replication cycle. Less-frequently used or discontinued drugs are shown in purple and smaller font size. Cobicistat and ritonavir are pharmacokinetic enhancers administered with specific protease inhibitors or the integrase inhibitor, elvitegravir, to optimize their therapeutic concentrations. Their mechanism of action involves the inhibition of cytochrome P450 3A (CYP3A4) enzymes. Adapted from reference [7] with permission from the Royal Society of Chemistry. Abbreviations: AZT, azidothymidine; /c, boosted with cobicistat; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; /r, boosted with ritonavir; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

Approved drugs targeting the RT's DNA polymerase activity include nucleoside analogues (i.e., nucleoside RT inhibitors (NRTIs)) and non-nucleoside RT inhibitors (NNRTIs). NRTIs are prodrugs that have to be converted to triphosphorylated derivatives before being incorporated into **proviral DNA**. However, approved NRTIs lack a 3'OH in the ribose ring and act as chain terminators, blocking DNA synthesis. Within this class, tenofovir, an acyclic analogue of deoxyadenosine 5'-monophosphate, is usually included in clinical guidelines as part of recommended first-line therapies [13]. Currently, the prodrug TDF is being substituted by tenofovir alafenamide (TAF), which ensures higher active metabolite concentrations in peripheral blood

mononuclear cells (PBMCs) and lower plasma tenofovir exposure [14]. NNRTIs are small hydrophobic compounds that inhibit DNA polymerization through binding to an allosteric site located a few angstroms away from the polymerase catalytic site (reviewed in [15]). Nevirapine was the first drug of this class approved for clinical use, and efavirenz has been widely used for many years. First-generation NNRTIs have been replaced in recent formulations by second-generation inhibitors such as etravirine, rilpivirine, and doravirine.

Retroviral integrases catalyze the insertion of viral dsDNA into the host cell's DNA. Four identical copies of the integrase grab the two ends of the viral DNA, creating a stable complex called an intasome. The intasome binds to the cellular DNA and performs an integration reaction. First, two or three nucleotides are removed from one or both 3' ends of the proviral DNA to expose a conserved CA dinucleotide at both 3' ends (3'-processing), and then a strand transfer reaction ligates the processed 3' ends of the viral DNA to the host chromosomal DNA [16]. The HIV-1 integrase (32 kDa) has three domains and the central core contains the active site. The N- and C-terminal domains of the enzyme contain an HH-CC zinc-binding domain and a DNA-binding domain with an SH3 fold, respectively. Approved HIV-1 integrase inhibitors (raltegravir, elvitegravir, dolutegravir, cabotegravir, and bictegravir) bind to the catalytic domain and block the strand transfer reaction [17,18].

Antiretroviral drugs acting in the late phase of the replicative cycle of HIV are represented by HIV protease inhibitors [saquinavir, lopinavir, darunavir, and others, see (Figure 1)]. Although ritonavir, indinavir, and nelfinavir have been replaced by less toxic and more convenient to take protease inhibitors, ritonavir is widely used at a low dose as a pharmacokinetic enhancer (booster) of protease inhibitor-based combination therapies. HIV protease cleaves viral precursor polyproteins (i.e., Gag and Gag-Pol) to generate the mature protein components of an HIV virion. The HIV-1 protease is a homodimer containing subunits of 99 amino acids each. All approved protease inhibitors except tipranavir are peptidomimetics that contain a nonhydrolyzable hydroxyethylene core instead of a peptide linkage which would otherwise be cleaved by hydrolysis [19].

Combination antiretroviral therapies

After the approval of saquinavir in 1996 and the introduction of HAART regimens, combination therapies including three or more drugs directed against at least two different targets, were widely adopted for treating HIV infection. Ten years later pharmacological advances led to the approval of the first one-pill combination therapy, that contained TDF, emtricitabine and efavirenz (i.e., two NRTIs and one NNRTI). During the past 15 years, new one-pill combinations have been approved (Table 1). In general, new formulations show an improved safety profile (less adverse effects), more potent antiviral effects, and more resilience to the acquisition of drug resistance. Examples illustrating these advances include the replacement of TDF by TAF that reduces renal and bone toxicity [14], the incorporation of second-generation NNRTIs (e.g., rilpivirine or doravirine) effective against efavirenz-resistant viruses [15], or the introduction of integrase inhibitors such as raltegravir, dolutegravir, or bictegravir that do not require pharmacological **boosting**. In addition, dolutegravir and bictegravir show a very high genetic barrier to resistance development [15]. Clinical trials demonstrated that recently approved first-line regimens achieve viral suppression rates close to 90% after 48 weeks of treatment [20,21]. However, major threats to the efficacy of antiretroviral therapies are toxicity and drug resistance, and major efforts in drug design and discovery are focused in these areas of research.

Drug resistance and antiretroviral drug toxicity as threats to therapy success

Despite the rapid turnover and high mutation rate of HIV (around 10^{-4} to 10^{-5} mutations per nucleotide and cycle of replication), emergence of resistance has been largely contained in the

Table 1. FDA-approved single-tablet drug combinations of two or more antiretroviral drugs for antiretroviral therapy^a

Commercial name	Combination	FDA approval date
Three-drug combinations for initial therapy		
Atripla	TDF/emtricitabine/efavirenz	July 12, 2006
Complera	TDF/emtricitabine/rilpivirine	August 10, 2011
Stribild	TDF/emtricitabine/elvitegravir (boosted with cobicistat)	August 27, 2012
Triumeq	Abacavir/lamivudine/dolutegravir	August 22, 2014
Genvoya	TAF/emtricitabine/elvitegravir (boosted with cobicistat)	November 5, 2015
Odefsey	TAF/emtricitabine/rilpivirine	March 1, 2016
Biktarvy	TAF/emtricitabine/bictegravir	February 7, 2018
Symfi Lo and Symfi	TDF/lamivudine/efavirenz	February 5/March 22, 2018
Symtuza	TAF/emtricitabine/darunavir (boosted with cobicistat)	July 17, 2018
Delstrigo	TDF/lamivudine/doravirine	August 30, 2018
Two-drug combinations for maintenance therapy ^b		
Juluca	Dolutegravir/rilpivirine	November 21, 2017
Dovato	Dolutegravir/lamivudine	April 8, 2019
Two-drug combinations for initial therapy		
Cabenuva	Cabotegravir/rilpivirine (both supplied as extended-release injectable suspensions)	January 22, 2021

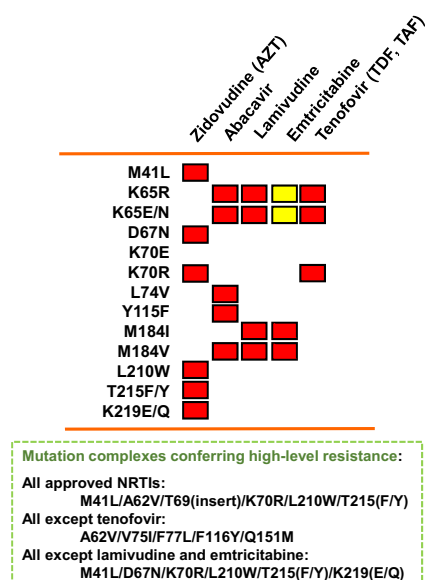
^aInformation taken from: <https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/21/58/fda-approved-hiv-medicines> (accessed September 30, 2021).

^bTo be used only in patients with undetectable viral load in blood (less than 50 copies/mL) for at least 6 months.

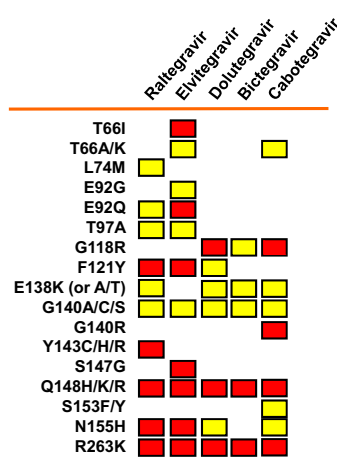
clinical setting due to the increased potency of current combination regimens. However, high levels of transmitted drug resistance have been reported for antiretroviral drugs with low genetic barriers of resistance, but prescribed for many years [22,23]. Examples are M184I/V, associated with NRTI resistance, and K103N/S, Y181C/I, and G190A/S, associated with resistance to first-generation NNRTIs [15] (Figure 2). The prevalence of these mutations seems to be decreasing as dolutegravir and new drugs with high genetic barriers to resistance development are included in the prescribed regimens. Medicinal chemistry efforts are currently focused on the discovery of new drugs targeting unexploited antiviral mechanisms of action and/or their effectiveness against drug-resistant HIV strains [7].

Drug toxicity is also very important considering the life-long duration of antiretroviral therapies. Despite being safe, current treatments are not benign. Mentioned previously are the effects of TDF on bone and renal toxicity, and how TAF ensures lower plasma tenofovir exposure [14]. Novel prodrugs (e.g., octadecyloxyethyl benzyl tenofovir) facilitating a slow release of the active metabolite could improve safety in tenofovir-containing regimens. Conversely, recently developed integrase inhibitors induce adipogenesis, lipogenesis, oxidative stress, fibrosis, and insulin resistance, resulting in weight gain and obesity [26,27]. This has been shown in clinical trials measuring the efficacy of dolutegravir-containing regimens, particularly in patients receiving TAF as part of the antiretroviral treatment [28]. For dolutegravir, obesity and metabolic disturbances are a matter of concern for patients from African descent which would be at a higher risk of cardiovascular disease [29]. Among integrase inhibitors, dolutegravir also bears a burden of neuropsychiatric side effects [30]. Preliminary data from clinical trials and non-trial settings show that bictegravir has lower rates of adverse events in comparison with dolutegravir [29,31,32].

RT inhibitors (NRTIs and NNRTIs)



Integrase inhibitors



Entry inhibitors

Fostemsavir

Resistance to temsavir associated with amino acid substitutions in gp120:
S375H/I/N/M/T, M426L/P, M434I/K and M475I.

Ibalizumab-uiyk

Resistance acquired through the loss of glycosylation sites in the V5 region of gp120 (i.e. N460Q and N464Q alone or in combination).

Maraviroc

Resistance through selection of viruses using the CXCR4 coreceptor, or mutations in V2, V3 and V4 loops of gp120.

Enfuvirtide

Resistance conferred by several amino acid substitutions in the Env transmembrane subunit gp41: G36D/E/S, I37T/N/V, V38A/E/M, Q39R, Q40H, N42T and N43D/K/S.

Protease inhibitors

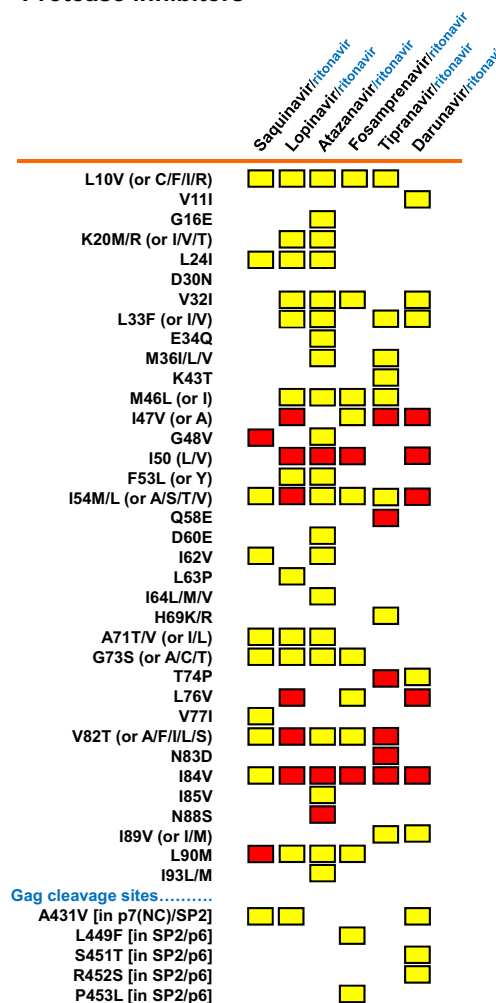


Figure 2. Amino acid substitutions associated with resistance to approved antiretroviral drugs. Major resistance mutations for each drug are indicated by red boxes, while accessory mutations are highlighted with yellow boxes. Data were taken from [24], and the Stanford University HIV Drug Resistance Database (<http://hivdb.stanford.edu>), with additional data on doravirine susceptibility from [25]. Abbreviations: AZT, zidovudine; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; RT, reverse transcriptase; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

Treatment simplification and long-acting antiretroviral drugs

Apart from being highly effective and strong suppressors of HIV replication, novel antiretroviral drugs should be safe, tolerable, and facilitate medication adherence through convenient dosing and administration. Simplifying an HIV regimen can include reducing the number of antiretroviral drugs. This is acceptable as a maintenance therapy for virologically suppressed people (i.e., those patients with a viral load in blood below 50 copies/mL for at least 6 months). Suitable two-drug combinations for maintenance therapy include dolutegravir and rilpivirine (Juluca) [33], dolutegravir and lamivudine

(Dovato) [34], boosted darunavir and lamivudine, and boosted atazanavir and lamivudine [35]. Combinations containing lamivudine should be avoided if there is evidence of resistance (M184I/V detection or virologic failure while on lamivudine), as well as in patients suffering chronic hepatitis B.

Interestingly, one of those therapies (dolutegravir/lamivudine) has been approved by regulatory agencies in Europe and the USA for the initial treatment of HIV infection, based on the results of the GEMINI 1 and 2 clinical studies that enrolled more than 1400 people around the world [36]. However, lamivudine resistance can be relatively frequent and if the M184I/V mutation is present, development of dolutegravir resistance could be facilitated, ruling out future treatments with integrase inhibitors.

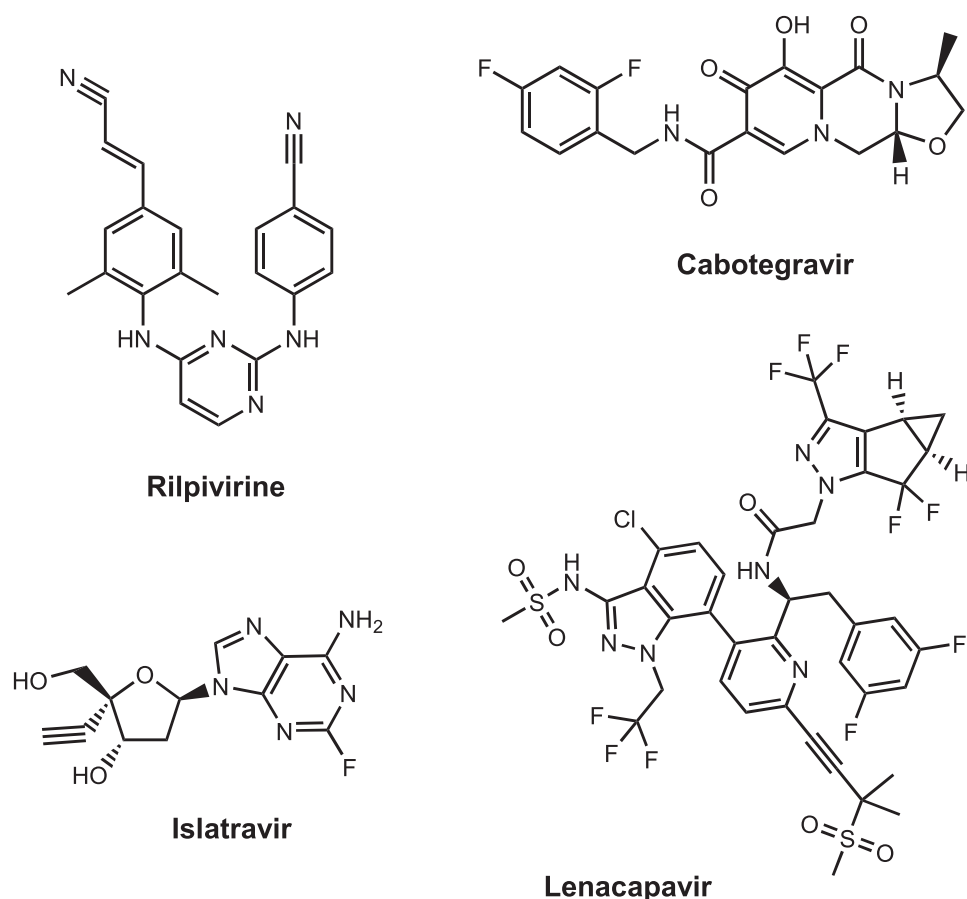
Long-acting or extended release nanoproducts of antiretroviral therapies can be administered monthly or less frequently. These formulations can be oral, parenteral (intramuscular or subcutaneous), transdermal, or implantable technologies [37]. In January 2021, CabenuvaTM became the first FDA-approved injectable intended as a complete antiretroviral regimen for adults [38]. It contains cabotegravir and rilpivirine both as extended-release injectable suspensions, that should be given intramuscularly every month. Cabotegravir is an integrase inhibitor similar in structure to dolutegravir, while rilpivirine is a second-generation NNRTI (Figure 3). At the same time, the regulatory agency also approved a single-pill cabotegravir/rilpivirine combination (named VocabriaTM). VocabriaTM should be administered for 1 month prior to starting treatment with CabenuvaTM, in order to ensure that medications are well-tolerated before switching to the extended-release injectable formulation. In addition, ATLAS and FLAIR clinical trials showed that once-monthly intramuscular injections of long-acting cabotegravir/rilpivirine was non-inferior to conventional oral antiretroviral therapy for maintenance of HIV-RNA suppression [39,40].

Pre-exposure prophylaxis (PrEP) and HIV prevention

PrEP has been shown to be highly effective in preventing HIV infections. Currently recommended formulations to be used as PrEP for HIV/AIDS include combinations of NRTIs such as TDF/emtricitabine, TDF/lamivudine, and TAF/emtricitabine. Unfortunately, use of PrEP is still low among women, particularly in vulnerable populations, and recent efforts have been concentrated on the development of strategies to improve adherence in both men and women.

In this scenario, HIV topical microbicides applied to the vagina or anus and rectum have been developed to prevent HIV infection through sexual intercourse. A flexible silicone matrix vaginal ring containing dapivirine (an NNRTI), worn for a month at a time, was found to reduce HIV infection in women by ~30% compared to placebo, in two Phase III placebo-controlled trials [41,42], and in the past year the World Health Organization and the European Medicines Agency supported its use in HIV prevention for women in high HIV burden settings [43]. Besides, novel long-acting and extended-release formulations of antiretroviral drugs are expected to facilitate a widespread use of PrEP, particularly in the most vulnerable populations.

The safety and efficacy of a cabotegravir injection every 8 weeks for HIV prevention among cisgender women has been compared to daily oral TDF/emtricitabine in a large clinical study carried out in several African countries. Although both drugs were highly effective at preventing HIV acquisition, long-acting cabotegravir injection showed statistical significant superiority in the study population [44]. Long-acting cabotegravir has low aqueous solubility and a high melting point, but requires a 2ml dosing volume to elicit injection site reactions. Its approval by the USA FDA is anticipated by the end of 2021. Animal studies showed that nanocrystals of lipophilic fatty acid ester derivatives of cabotegravir improve the pharmacokinetic properties of the drug, thereby extending its apparent half-life and making annual dosing feasible [45].



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Figure 3. Chemical structures of antiretroviral drugs approved as long-acting formulations or in advanced clinical trials.

Other antiretroviral therapies considered in PrEP studies and HIV prevention include the combination of dapivirine and maraviroc [46], and **neutralizing antibodies** such as VRC01. Although the results of a recent clinical trial showed that this antibody alone did not prevent overall HIV-1 acquisition more effectively than placebo, interestingly it showed 75% protection against VRC01-sensitive HIV-1 isolates, providing proof-of-concept that neutralizing antibodies prophylaxis can be effective [47].

Novel antiretroviral drugs in advanced clinical trials

High potency, low toxicity, and effectiveness against drug-resistant variants, are desirable characteristics for newly developed antiretroviral drugs, that should be also amenable to long-acting therapy and increased dosing intervals. Islatravir (a novel NRTI) and lenacapavir (an HIV capsid assembly inhibitor) are the most advanced candidates, and their antiretroviral potential is being evaluated in Phase III clinical trials, both in classical and long-acting formulations [48,49].

Islatravir

Islatravir (4'-ethynyl-2'-fluoro-2'-deoxyadenosine, EFdA, or MK-8591) is an investigational NRTI (Figure 3), in development for extended administration in a subdermal drug-eluting implant [50]. After conversion to islatravir-triphosphate, the nucleotide analogue is incorporated into the DNA chain. Unlike currently approved NRTIs, islatravir-triphosphate acts as a translocation inhibitor

due to its 4'-ethynyl group, that in combination with the 3'-hydroxyl group, results in chain termination [51,52]. Islatravir inhibited HIV-1 replication in activated PBMCs with an EC_{50} of 0.05–0.3 nM depending on the viral strain, a potency several orders of magnitude better than any of the currently prescribed NRTIs. It also shows a strong effect on multidrug-resistant strains [51,53] (Box 1). The active triphosphate of islatravir has an intracellular half-life of up to 128 hours [54] that allows for flexible-dose treatment including once per week dosing or more spaced administration intervals. In clinical trials, single doses of 0.5 mg of islatravir suppressed HIV-1 RNA by more than one log at day 7, in treatment-naïve adults with HIV-1 infection, and were generally well tolerated with no evidence of emergence of drug-resistance [54].

In Phase IIb clinical trials, daily administration of islatravir in combination with doravirine/lamivudine or doravirine/TDF showed high levels of virological suppression in HIV-infected individuals [55]. In the context of this trial, researchers are evaluating the possibility of using islatravir and doravirine as a maintenance two-drug regimen, for those individuals achieving an HIV-1 RNA concentration lower than 50 copies/mL after 24 weeks of treatment. Due to its long half-life, islatravir is also an excellent candidate for long-acting formulations. The efficacy of islatravir in PrEP has been shown in the rhesus macaque simian/human immunodeficiency virus (SHIV) rectal challenge model [56]. Moreover, islatravir-eluting implants for yearly HIV PrEP have been evaluated for safety, tolerability, and pharmacokinetics in healthy HIV-negative men and women. The implants inserted with a **Nexplanon** applicator were well tolerated, and specifically the 54 and 62 mg implants were found to be effective in maintaining islatravir-triphosphate concentrations above the pharmacokinetics threshold [57].

Box 1. Islatravir and CA-binding inhibitors

Islatravir resistance

The combination of amino acid substitutions I142V/T165R/M184V was selected *in vitro* after successive passage of HIV-1 in the presence of 2'-deoxy-4'-ethynyl-adenosine, a nonfluorinated analogue of islatravir. M184V, the first mutation that appeared in those experiments, conferred a 7.5-fold reduction in islatravir susceptibility. M184V contributes to islatravir resistance particularly in combination with P119S and T165A (reviewed in [83]). The relatively high prevalence of M184I/V in heavily-treated populations due to the extensive use of lamivudine and emtricitabine in combination therapies is a matter of concern for the use of islatravir. However, the drug candidate retains activity against a wide range of NRTI-resistant mutants, including M41L/T69-insertion/T215Y mutants characterized by their very high-level resistance to zidovudine (AZT). Furthermore, common drug resistance mutations found in the HIV-1 reverse transcriptase (RT)-coding region (e.g., K65R, L74V, and Q151M) confer hypersusceptibility to the drug, and many studies have confirmed that M184I/V have a negative impact on the HIV-1 replication capacity [15]. So far, emergence of resistance in patients has not been reported in ongoing clinical trials for the treatment of HIV-1 infection with islatravir in combination with other antiretroviral drugs, as well as for pre-exposure prophylaxis (PrEP) of HIV-1 infection as a single agent [54].

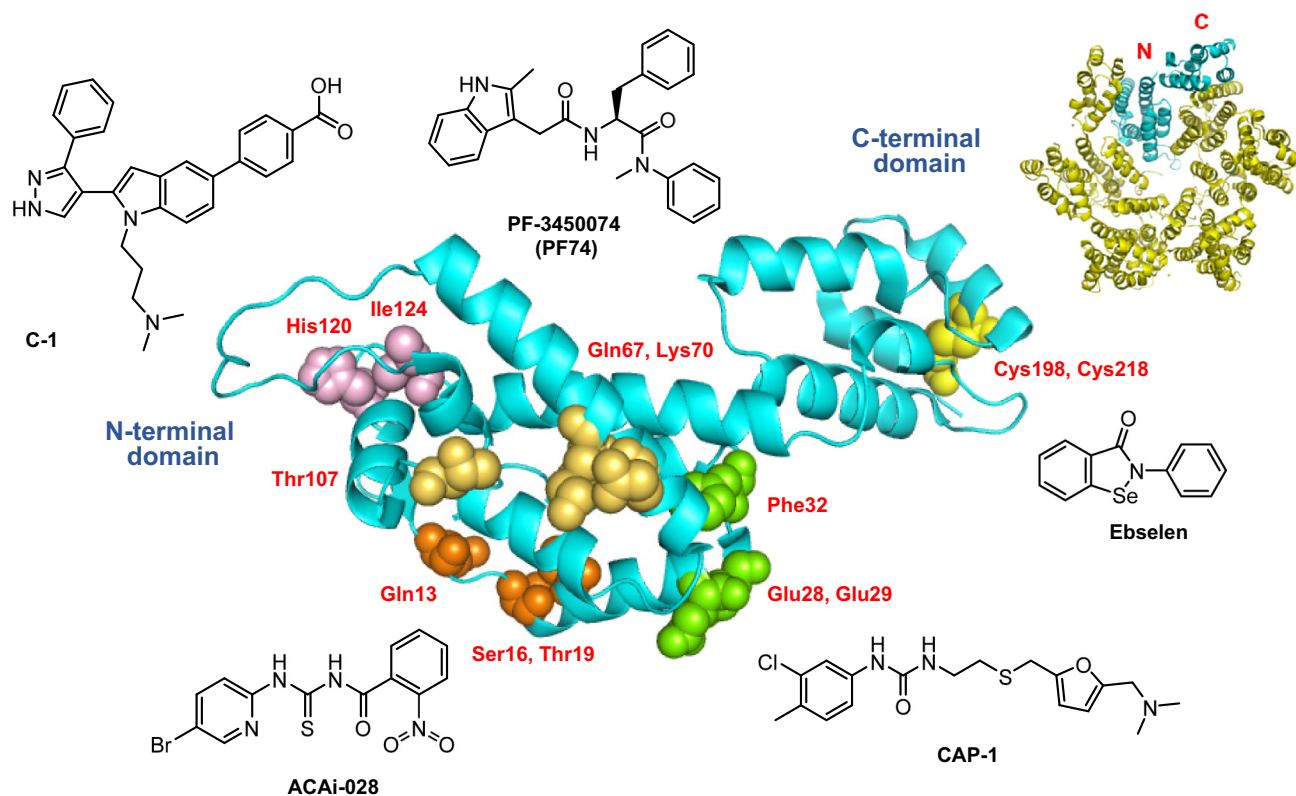
HIV-1 CA structure and inhibitor-binding sites

HIV-1 CA originates from cleavage of the 55-kDa Gag polyprotein by the viral protease. It has two independently folded domains connected by a 5-residue flexible linker (see Figure 4 in main text). The N-terminal domain includes residues 1–145, while the C-terminal domain contains amino acids 151–231. Less than two dozen CA-targeting compounds have been described, including small molecules, peptides, and a specific antibody [49]. PF74 was extensively studied and is a lead compound of lenacapavir. It inhibits both early and late steps of viral replication. According to their mechanism of action and target CA-targeting compounds can be classified in different groups: (i) molecules promoting CA multimerization and targeting the N-terminal domain, around positions 67, 70, and 107 (e.g., lenacapavir, PF74, and GS-CA1); (ii) covalent agents targeting the C-terminal region, at Cys198 and Cys218, and promoting multimerization, such as ebselen; (iii) compounds decreasing multimerization and acting in the late phase of the viral replication cycle (e.g., CAP-1, BD-1, and BM-1, binding around residues 28–32); and (iv) agents that decrease CA multimerization through binding a pocket defined by residues Glu98, His120, and Ile124 (e.g., C-1 and I-XW-053) [84]. ACAI-028 represents a fifth class of compounds binding to the N-terminal domain of CA. This compound, identified after virtual screening using *in silico* docking simulations, interacts with amino acid residues Gln13, Ser16, and Thr19 through hydrogen bonds. ACAI-028 acts in the early phase of HIV-1 replication and reduces CA multimerization [85].

Lenacapavir

The HIV CA forms a conical shell that protects the viral genomic RNA (Box 1). Lenacapavir (Figure 3), formerly known as GS-6207 or GS-CA2, inhibits HIV-1 replication mainly by stabilizing and thereby preventing capsid disassembly in infected cells [58]. Currently in Phase III clinical trials, lenacapavir is the most advanced HIV capsid binding inhibitor. Its development resulted from the optimization of PF-3450074 (PF74), a peptidomimetic compound built around a phenylalanine core and capped with indole-3-acetic acid and aniline moieties at the amino and carboxylate ends, respectively (Figure 4). PF74 interferes with capsid assembly and disassembly, as well as binding to the cellular HIV-1 cofactors Nup153 and CPSF6 that mediate nuclear import of **preintegration complexes** [59].

Lenacapavir is a tight inhibitor that binds two contiguous capsid subunits while promoting distal intra- and inter-hexamer interactions that stabilize the curved capsid lattice [58]. The compound inhibits HIV-1 replication in T cells and PBMCs with picomolar activity. Resistance to lenacapavir is associated with HIV-1 CA amino acid substitutions L56I, M66I, Q67H, K70N, N74D, N74S, and T107N (alone and in different combinations). These substitutions confer reduced susceptibility to the inhibitor, as well as reduced viral fitness [60,61]. Q67H and N74D are the most frequently selected mutations in HIV-1 grown in cell culture, while in some patients Q67H was identified after 1-week exposure to low doses of lenacapavir [62]. These experiments showed no evidence of cross-resistance with protease, RT, or integrase inhibitors. Furthermore, mutations



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Figure 4. HIV-1 CA monomer showing the location of binding sites of representative assembly and disassembly inhibitors in preclinical development. The upper right panel shows a cartoon representation of the structure of an hexameric complex. One of the subunits is represented in cyan. Images were obtained with PyMol using PDB coordinates 6VKV and 6OBH.

at the Gag polyprotein cleavage sites (as found in response to protease inhibitor therapies or bevirimat and other maturation inhibitors) had no effect on lenacapavir susceptibility [63].

Lenacapavir has a half-life of 7 to 11 weeks when administered subcutaneously [64], and evidence collected from experiments with non-human primates support its further development as a long-acting agent for PrEP [65]. Lenacapavir can be administered orally [55] and its highly favorable antiviral properties have stimulated efforts towards the development of second-generation inhibitors, including compounds with different binding sites in HIV-1 CA (Figure 4).

Other targets and drug candidates in development

The most advanced antiviral agents in clinical trials are maturation inhibitors and neutralizing antibodies. Maturation inhibitors block a late protease cleavage event occurring between CA and the spacer polypeptide SP1. When CA-SP1 is not properly processed, an abnormal eccentric capsid is formed and the virus is not infectious [66]. The first representative of this class of agents was bevirimat, a betulinic acid-like compound, whose development was interrupted due to numerous resistance-related problems. Common Gag polymorphisms such as V362I, and variability at positions 369–370 of the polyprotein precursor, limit the efficacy of bevirimat and second-generation maturation inhibitors (e.g., GSK3532795). GSK3640254 is active against viruses containing these polymorphisms. Currently in clinical trials, this compound showed favorable pharmacokinetics when combined with TAF/emtricitabine [67]. Still, emergence of resistance remains as a major concern in the development of these drugs.

Broadly neutralizing antibodies (bNAbs) provide a new approach to HIV-1 prevention and treatment, as inhibitors of viral entry. Clinical trials have shown the efficacy of some of those bNAbs in reducing viremia and maintaining viral suppression. Combination of bNAbs demonstrated antiviral activity in small clinical trials, but their dose, route of administration, and bioavailability are important concerns hampering their further development [68]. Besides, bNAbs could be used alone or in combination as long-acting antiretroviral drugs, since their administration every 3–6 months could be feasible. Unfortunately, the evaluation of VRC01 as a PrEP agent in a clinical trial has shown that the bNAb did not prevent overall HIV-1 acquisition more effectively than placebo [47]. Nevertheless, new clinical trials with antibodies showing increased potency and breadth, also in combination with other antiretroviral drugs such as long-acting cabotegravir or lenacapavir, are currently in progress (e.g., clinical trials NCT03739996 and NCT04811040, respectively).

Other antiviral agents in development (mostly at preclinical stage) include molecules expanding the currently available antiretroviral drugs, as well as compounds directed against unexploited viral targets. In the first group, there are representatives of different classes of antiretroviral agents: (i) NRTIs, such as rosofosvir etalafenamide (GS-9131), a prodrug of GS-9148 [(5-(6-amino-purin-9-yl)-4-fluoro-2,5-dihydro-furan-2-yloxymethyl)phosphonic acid] that after phosphorylation acts as a chain terminator of DNA polymerization [69]; (ii) NNRTIs (e.g., elvitegravir, IQP-0528, MIV-150, and MK-8507 among others [70]); (iii) protease inhibitors (e.g., GRL-001, GRL-003, GRL-121, and GRL-142, some of them effective against highly resistant HIV mutants [19,71]); (iv) integrase inhibitors (targeting the catalytic site such as MK-2048 [72,73], or **allosteric inhibitors** that affect integrase multimerization and HIV morphogenesis) [16,74,75]; and (v) entry inhibitors (including novel bNAbs, the humanized IgG1 monoclonal antibody UB-421, and other agents interfering with CD4 binding, CXCR4 antagonists, and fusion inhibitors) [76].

Conversely, other druggable targets include the ribonuclease activity of HIV-1 RT [77], the viral nucleocapsid protein [78], HIV-1 Nef [79], and relevant interactions between host factors and viral proteins (e.g., HIV-1 Vpu and host BST-2, or HIV-1 Vif-APOBEC3 complexes) [80]. Despite

efforts of the scientific community, suitable drug candidates targeting those biological processes are still unavailable.

Concluding remarks

More than 30 antiretroviral drugs have been licensed, and drug combinations have demonstrated high potency and minimal toxicity. However, long-term toxicity and acquired and transmitted drug resistance remain as major threats to therapy success. Continued efforts are needed to improve current formulations and facilitate adherence. Newly developed antiviral agents have interesting advantages towards this goal: low toxicity, high potency (often very efficient against resistant HIV strains frequently found in the infected population), and convenient dosing. There are multiple options for administration while very long-acting therapies facilitate their dosing every 3, 6, or even 12 months. Although drug resistance might still be problematic for islatravir, its combination with lenacapavir as a dual long-acting combination treatment holds promise for future convenient treatment of HIV infection. Besides, although not covered in this review, a number of efforts towards eradication of HIV [81], including the use of **latency reversal agents** [81,82], are being addressed and we will probably witness significant advances in the coming years (see [Outstanding questions](#)).

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Declaration of interests

L.M.-A. declares that he has no conflicts of interest with the contents of this article. R.D. has received conference fees from GSK, Gilead, and MSD.

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Outstanding questions

Could long-acting strategies for pre-exposure prophylaxis (PrEP) have a significant global impact on the AIDS pandemic?

Could the new highly active antiretroviral drugs circumvent the inexorable development of resistance mutations?

Are there any new antiviral targets still worth exploring for the treatment of HIV infection?

Could monoclonal neutralizing antibodies be part of standard prophylaxis or therapies against HIV-1 infection?

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