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State of the ART (antiretroviral therapy): Long-acting HIV-1 therapeutics

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Abstract: Human immunodeficiency virus (HIV) impacts millions of individuals worldwide, and well over 2/3 of those living with HIV are accessing antiviral therapies that are successfully repressing viral replication. Most often, HIV treatments and prevention are administered in the form of daily pills as combinations of multiple drugs. An emergent and effective strategy for suppressing viral replication is the application of long-acting antiretroviral therapy (LAART), or antivirals that require less-frequent, non-daily doses. Thus far, the repertoire of LAARTs includes the widely used antiviral classes of non-nucleoside reverse transcriptase inhibitors (NNRTIs) and integrase strand transfer inhibitors (INSTIs) and has recently expanded to include a capsid-targeting antiviral. Possible future additions are nucleoside reverse transcriptase inhibitors (NRTIs) and nucleoside reverse transcriptase translocation inhibitors (NRTTIs). Here, we discuss the different strategies of using long-acting compounds to treat or prevent HIV-1 infection by targeting reverse transcriptase, integrase, and capsid.

***Keywords*:** human immunodeficiency virus (HIV), antiretroviral therapy (ART), pre-exposure prophylaxis (PrEP), long-acting formulations, acquired immunodeficiency syndrome (AIDS)

Introduction

In 2023, around 20% of people living with human immunodeficiency virus (HIV) were not treating their infection (*1*). If left untreated, HIV infection progresses to acquired immunodeficiency syndrome (AIDS), which causes over 650,000 deaths annually (*1-4*). While there has been great success in HIV-related healthcare, with 75% of HIV-positive individuals virally suppressed, multiple global regions are underserved in terms of healthcare access and availability (*1,5,6*). Certain regions have a large burden of infections, especially South and West Africa, which comprise 70% of global cases. Other regions have a large proportion of untreated individuals, including the Middle East and Eastern Europe with approximately 50% untreated (*1,4,6-8*) (Figure 1).

An emerging and exciting method of treating HIV infection is the use of a long-acting antiretroviral therapy (LAART) with infrequent doses compared to daily pills (*4,9-13*). LAART can decrease the burden of acquiring and taking a daily medication, and it has received great patient reception for those switching to and initiating LAART to treat or prevent HIV type 1 (HIV-1) infection (*6,9,13-16*). The main utility of LAART is in adding another tool to patient treatment. While it is not expected that all long-acting formulations are favored equally within high-incidence populations, there is a reported bias in LAART uptake toward highly informed individuals and those who practice sex with the use of preventative tools (*6,14,16,17*).

At present, all HIV-1 treatments are administered in combinations of at least two drugs in order to prevent the emergence of antiviral resistance. The coadministration of multiple drugs decreases the chance of antiviral resistance due to the volume of simultaneous mutations needed to escape the drugs' activities (*18-21*). It is possible that LAART helps decrease the occurrence of antiviral resistance by having unwavering continuity of treatment, but this also requires that patients have the opportunity to replenish the LAART at the recommended interval (*10,12,15,22,23*).

Opportunities introduced by LAART include less frequent dosing, avoidance of "pill fatigue", oral dosing being bypassed (with bioavailability near 100%), less adverse events, fewer drug-drug interactions, as well as protection of health privacy, avoidance of HIV-related stigma, and improved consistency of care. Challenges include large limitations due to injection volume restrictions, management of missed doses, pharmacokinetic considerations, possible development of drug resistance, management of drug-drug interactions, management of serious adverse events, and unknown dosing for children and pregnant

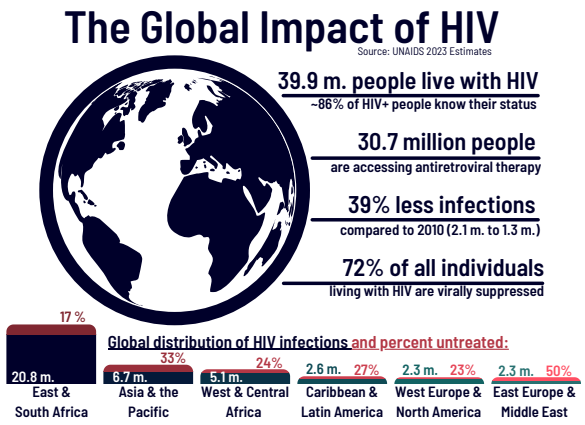

Figure 1. Global statistics of HIV-1 infections, treatment, and access in 2023. Regional-specific data below, showing the total estimated infections (in millions, blue) and percent of untreated individuals (red). Data from the UNAIDS 2023 report, "The urgency of now: AIDS at a crossroads" (*1*).

women. Delivery routes of long-acting antivirals are oral, parenteral, and an implant/device with respective dosing frequencies of more than one week, more than one month, and more than six months (*6,22,24*).

Currently, the approved delivery methods for LAART are intramuscular (Cabenuva) or subcutaneous (Sunlenca) injections (Figure 2). This method of administration has been highly effective; however, there are complications and limitations to receiving injections due to patient anxiety, injection site reactions, the need for a professional healthcare worker for administration/ lack of clinical support, high cost, accessibility, and difficulty in discontinuation once the treatment is injected (*10,14-17,22,25*). Because of these reasons and more, other long-acting treatment options are being developed. These include subdermal implants, intravaginal rings (IVRs), microneedle array patches, long-acting hydrogels, and oral regimens that are dosed

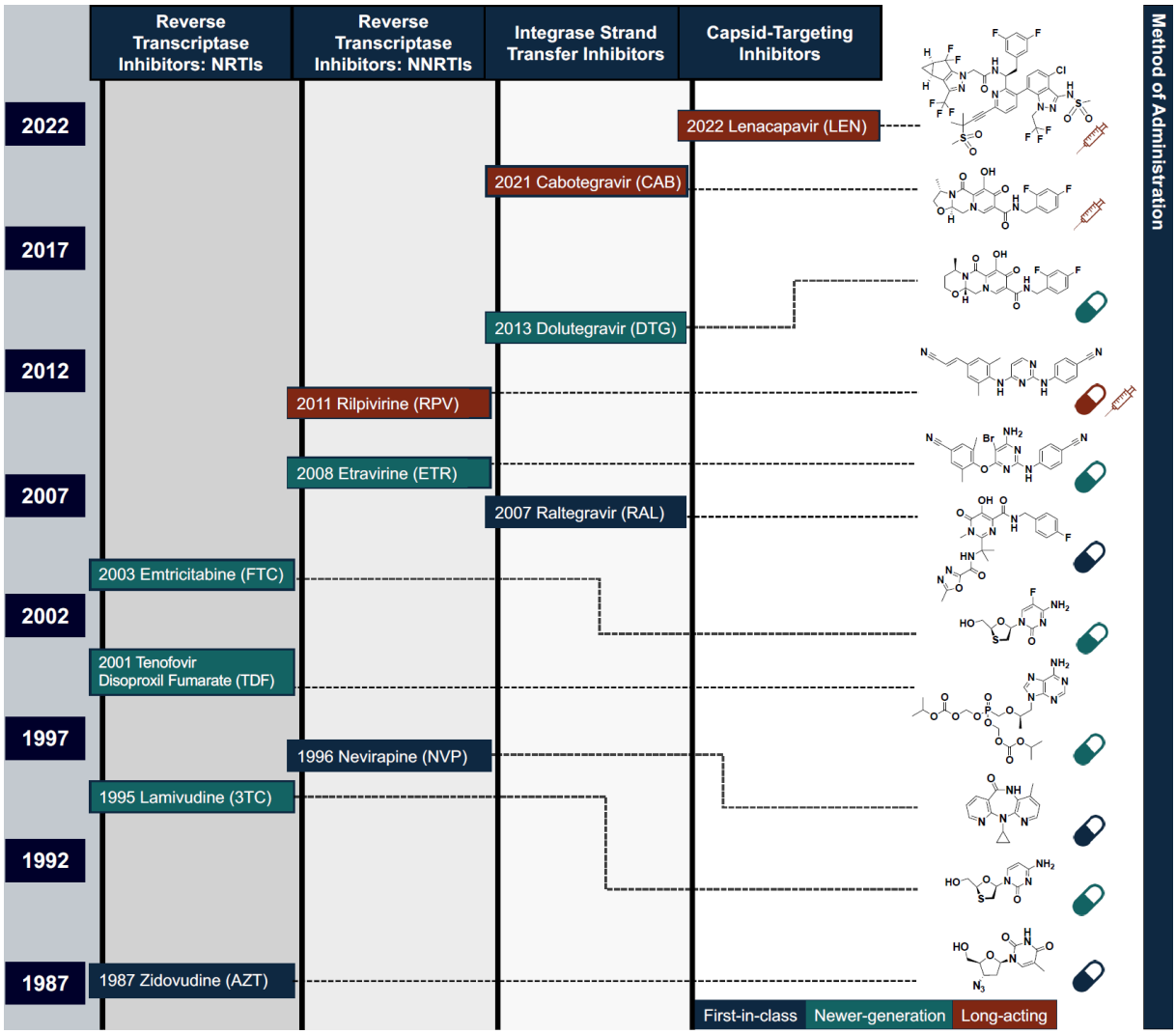

Figure 2. Timeline of some important discoveries, approvals, and advancements leading to long-acting HIV-1 therapeutics. Compounds are categorized as first-in-class (blue), newer-generation (green), and long-acting therapeutics (red) for treating and preventing infection. Chemical structure and delivery method(s) included for each antiviral on the right. Represented inhibitor classes are nucleoside reverse transcriptase inhibitor (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase strand transfer inhibitors (INSTIs), and capsid-targeting antivirals. Pill and syringe icons designed by Freepik.

less often. Other delivery strategies have been deployed in specific areas, like the ring approved for use in Africa but not by the US FDA (*25*). Of note, adverse reactions can be challenging for LAART, especially for irreversible administration through subcutaneous injections; however, entry periods into the therapy with low and oral lead-in doses, when available, can be useful to test patient tolerance to the new medication before a long-term treatment is in place (*13,23,25,26*).

Thus far, LAART has been successfully employed in clinical trials and as approved medications for HIV-1 treatment and as a preventative measure for pre-exposure prophylaxis (PrEP) by targeting viral proteins required for an HIV-1 infection: reverse transcriptase, integrase, and capsid.

Reverse transcriptase

HIV-1 reverse transcriptase (RT) inhibitors have been used to treat infection since 1987, with the first FDA-approved HIV-1 antiviral zidovudine (AZT). Since the late 1980s, several other RT-targeting antivirals have been developed and approved (*27*) (Figure 2). Currently, there are five non-nucleoside reverse transcriptase inhibitors (NNRTIs) and nine nucleoside reverse transcriptase inhibitors (NRTIs) in the US market, although only five of these NRTIs are recommended (*28*). Another class of RT inhibitors being developed, but not yet FDA-approved, is called nucleoside reverse transcriptase translocation inhibitors (NRTTIs). These inhibitors include islatravir (ISL, EFdA, or MK-8591) and MK-8527.

All of these classes – NNRTIs, NRTIs, and NRTTIs – target HIV-1 RT. This viral enzyme is essential for the HIV-1 replication cycle as it is responsible for converting the positive-sense, single-stranded RNA genome into double-stranded DNA, which is the product that is integrated into the host genome (*29*). NNRTIs, NRTIs, and NRTTIs act through distinct mechanisms of action, though. NNRTIs are allosteric inhibitors that do not target the polymerase active site and instead bind to a region called the NNRTI binding pocket (NNIBP) located at the base of the thumb of RT. When these inhibitors bind, they cause conformational changes to the thumb and surrounding areas of RT, reducing the ability of RT to polymerize (*30,31*). NRTIs are nucleoside analogs and bind at the polymerase active site of RT (*29*). These inhibitors lack a 3'-OH, so once incorporated into the elongating DNA strand, another nucleotide cannot be added. Thus, NRTIs are termed immediate or obligate chain terminators (*32,33*). Differentially, NRTTIs retain the 3'-OH group, allowing another nucleoside to be added (*34*). Thus, NRTTIs can act through multiple mechanisms of action, effectively inhibiting the translocation step of reverse transcription through immediate chain termination, delayed chain termination, or increased misincorporation (*35*).

Non-nucleoside reverse transcriptase inhibitors

Most antiretroviral therapy (ART) treatments, including those based on RT inhibitors, are administered as once-daily oral medications. Rilpivirine (RPV), an NNRTI, was the first RT inhibitor to be approved in long-acting therapies such as Cabenuva. Initially approved in 2011, RPV is considered a next-generation NNRTI due to its capacity to overcome both resistance and safety concerns associated with earlier NNRTIs (*36*). With its extended half-life, RPV allows for monthly dosing using non-oral methods (*37*). Another promising NNRTI, doravirine (DOR), marketed as Pifeltro by Merck & Co., Inc., received FDA approval in 2018 (*38*). While currently available as a once-daily oral medication, efforts are underway to develop its formulation as a long-acting injectable (*39*). VM-1500A, another potent NNRTI, is currently in development by Viriom Inc. Elsulfavirine (ESV), the prodrug of VM-1500A, was approved in Russia in 2017 under the brand name Elpida (ESV 20 mg) as a once-daily oral medication. ESV has a long half-life (*40*) and is being formulated as a once-weekly oral medication and as depulfavirine in a once-monthly nanosphere drug formulation (*41*).

Nucleoside reverse transcriptase inhibitors

Tenofovir, an NRTI, has been used in first-line therapy worldwide for the last twenty years. It has been formulated as tenofovir alafenamide fumarate (TAF), which increases its potency and safety profile. This antiviral is currently being used as a once-daily oral medication but is in preclinical formulation as a long-acting implant for treatment up to six months (*12*). There are currently several implant variations being tested, which include a TAF-filled subcutaneous silicone implant with a polyvinyl alcohol (PVA) coating and orthogonal delivery channels (*42*), a reservoir-style biodegradable implant filled with a TAF/oil formulation (*43*), an implant loaded with TAF pellets sealed in a polyether urethane tube (*44*), and poly(ε-caprolactone) (PCL) reservoir-style implant with a TAF core formulation (*45-47*). Long-acting microspheres are also in development (*48*).

Nucleoside reverse transcriptase translocation inhibitors

ISL is a highly potent NRTTI licensed by Merck & Co. This antiviral has the potential to be long-acting due to its long half-life and favorable selectivity index. It is currently being tested in several clinical trials as a once-daily, weekly, and monthly oral medication partnered with DOR and lenacapavir (LEN) (*49-70*). Preclinical studies of ISL delivered as an injection, implant, and micro patch for long-acting therapies are also underway; ISL has paved the way for other NRTTI development, including MK-8527, a 7-deazadeoxyadenosine analog

that is currently in phase I/II clinical trials with the potential to be used as a once-monthly oral treatment for HIV-1 (*71-74*).

Integrase

HIV-1 integrase protein (IN) is a target of ART formulations that contain integrase strand transfer inhibitors (INSTIs). Currently, there are five FDA-approved INSTIs used in HIV-1 treatments – first generation: raltegravir (RAL), elvitegravir (EVG); second generation: dolutegravir (DTG), bictegravir (BIC), and cabotegravir (CAB) (*75-77*). HIV-1 requires integration of virus-encoding nucleic acid into the host's genome as part of its replication cycle, and IN is responsible for integrating the double-stranded viral DNA (vDNA) resulting from reverse transcription of the viral positive-sense, single-stranded RNA genome (*78,79*). Hence, the integration process has emerged as another ART target, with multiple classes of integration-targeting compounds arising as potential treatments (reviewed in (*80*)). IN was first discovered within an avian retrovirus as a nucleic acid-associating protein that later was found to possess both 3'-processing and strand transfer enzymatic activities (*81-86*). During integration, IN oligomerizes and complexes with vDNA to form the intasome, which contains a conserved integration core (CIC) (*87*). Within the CIC, the IN multimer exposes 3'-OH groups on the vDNA ends, which can then catalyze a strand transfer reaction into the target host DNA (*88,89*). To prevent this from happening, INSTIs contain key structural moieties that block host DNA capture immediately preceding this step. These antiretrovirals have a β-diketo acid-containing a dicyclic or tricyclic pharmacophore to chelate Mg^{2+} ions required to catalyze reactions in the IN active site (*87*). Moreover, INSTIs contain a halogenated benzyl group that performs π-π stacking with the terminal vDNA base, thus preventing its interaction with host DNA (*87*).

RAL became the first FDA-approved INSTI in 2007, followed by EVG in 2012. In building upon these first-generation INSTIs, second generation INSTIs have seen widespread adoption due to their increased tolerability, high barrier to resistance, and low cross-reactivity (*75*). INSTI-containing ARTs typically administer it with two NRTIs, but these combinations can alternatively apply one NRTI and one NNRTI instead. Of these INSTIs, CAB is the only approved long-acting (LA) agent, either in combination with RPV LA as an ART (*90-92*) or on its own for PrEP (*93,94*). CAB is a structural analog of DTG and similarly has a high genetic barrier to resistance, yet it possesses a much longer half-life than DTG does (*95,96*). It also has potent activity at low concentrations, minimal adverse side effects, and little cross-reactivity (*97-99*).

CAB LA, owned by ViiV Healthcare, was approved in early 2021 in combination with Janssen's RPV LA for HIV-1 treatment under the trade name Cabenuva (*100*), becoming the first injectable LAART. This came after the success of the 2020 phase III Antiretroviral Therapy as Long-Acting Suppression (ATLAS) and First Long-Acting Injectable Regimen (FLAIR) studies; these trials confirmed non-inferiority of CAB LA/RPV LA against standard ART in treating and suppressing HIV-1 infection (*90,91*). Following this, the 2020 phase IIIb ATLAS dosed every two months (ATLAS-2M) trial established the non-inferiority of bimonthly CAB LA/RPV LA administration when compared to monthly treatment, leading to the approval of a bimonthly regimen as well (*92,101*). In late 2021, CAB LA itself was FDA-approved as the first LA injectable PrEP and was released under the trade name Apretude (*102*). This announcement resulted from the phase IIb/III HPTN 083 and phase III HPTN 084 studies showing non-inferiority of CAB LA against conventional PrEP treatment (*93,103*).

INSTIs can be given either as oral drugs or as intramuscular injections in the case of CAB LA. INSTI-containing ARTs are orally administered daily, while CAB LA/RPV LA involves a ventrogluteal injection once either monthly or bimonthly (*104*). However, before this treatment starts, individuals may be advised to undergo an oral lead-in period (OLI) to assess their tolerance to CAB and RPV. During OLI, individual tablets of CAB (brand name Vocabria) and RPV (brand name Edurant) are taken daily for at least four weeks. For patients enacting this optional OLI, an immediate switch to CAB and RPV initiation injections (trade names Vocabria and Rekambys, respectively) takes place on the final OLI day; otherwise, the treatment initiation period can directly begin at this step.

Initiation injection schedules for HIV-1 treatment differ between patients undergoing a monthly versus a bimonthly CAB LA + RPV LA dosing timeline. For monthly treatment, initiation injections of CAB LA and RPV LA are each given once in the month prior to the start of their monthly injection schedule at doses higher than during treatment (*104*). For those on a bimonthly schedule, the same monthly initiation injections are administered, though instead for two months before their bimonthly schedule begins (*104*). Upon successful completion of this period, the patient will then begin their prescribed injection schedule (*105*).

Capsid

The HIV-1 virion contains the HIV-1 capsid, the most recent molecular target of an antiviral compound, LEN (previously called GS-6207), approved in 2022 for highly treatment-experienced (HTE) patients (Figure 2). LEN targets the HIV-1 capsid protein (CA) and inhibits viral replication by perturbing the capsid core stability, assembly, and maturation (*19,106-112*); it was developed by Gilead Sciences to disrupt the intricately-

tuned kinetics of capsid assembly by interacting with CA at the phenylalanine-glycine (FG)-binding site (*3,106,113-119*). The mature capsid is essential for the replication of HIV-1 in numerous ways, for example acting as a reaction vessel for RT activity and as a shuttle to carry the viral genome through the cytoplasm and to or through the nuclear pore complex (*114,116,120-127*). The disassembly or "uncoating" of the capsid core needs to be perfectly timed, as early or late uncoating decreases viral fitness (*106,123,124,128-134*). Interfering with post-entry and pre-integration events prevents the establishment of a viral infection, making the mature capsid a great target for preventing or treating HIV-1 (*107,135*). In fact, an interim update of the PURPOSE1 clinical trial (NCT04994509) was recently released, stating that twice-yearly LEN was shown to prevent HIV-1 infection with 100% efficacy as a PrEP regimen in cisgender women in South Africa and Uganda, with 0 reported infections among 2,134 participants who received LEN (*136,137*). The press release also stated that PURPOSE1 is the first phase III HIV-1 prevention trial to report zero infections (*136*).

Interestingly, the first compound reported to target the same site as LEN, the FG-binding site, was PF-3450074 (PF74). Similar to LEN, PF74 contains an amide group and FG scaffold to mimic the FG-containing host factors that bind to the same pocket in CA (*i.e.* Nup153 and CPSF6) (*3,114-119,131,132,134*). Due to these chemical bonds, PF74 has an exceptionally short metabolic half-life and is quickly degraded by cellular enzymes, preventing its further drug development (*3,132,138,139*), although more potent and more stable analogs of PF74 have been published (*139,140*). Intriguingly, this amide bond and general FG structure is still within the LEN molecule, and LEN maintains its exceptionally long-lasting half-life (*3,106,113,141*).

LEN is only approved for use in HTE patients as of June 2024, and not yet as a coformulation (*109*). Specifically, the patients approved for LEN treatment are already taking an ART regimen that is not successfully repressing viral replication. In fact, the CAPELLA trial (NCT04150068) with LEN found that most HTE participants that had established infections with drug-resistant viruses were able to initially suppress viremia (21 of 24), although multiple LEN-associated resistance mutations have been reported shortly after use in CA, including M66I and N74D (*110,142-144*). Multiple clinical trials are ongoing to investigate LEN as a form of treatment or prevention against HIV-1 with either oral formulations or subcutaneous injections in combination with BIC, ISL, emtricitabine/tenofovir disoproxil fumarate (F/TDF), F/TAF, and broadly neutralizing antibodies (bNAbs) (*19,108,111,145*). LEN is typically administered in 927 mg subcutaneous injections in 26-week increments and/or as 300–600 mg tablets as OLI (*111,112,145,146*). Overall, LEN as a first-in-class inhibitor is an exceptionally potent and long-lasting antiretroviral, though its apparent tendency to rapidly select resistance mutations in CA may be a challenge for its future applications.

Conclusions

From many oral doses administered daily and limited drug options to non-daily injections and over 30 FDA-approved antivirals with various delivery strategies, the treatment of HIV-1 infection has evolved and is becoming increasingly accessible to more patients. Many challenges remain to overcome barriers in HIV-1 treatment and prevention. With more and varied treatment options, and numerous clinical and pre-clinical trials underway to use LAARTs, clinicians will be more able to take patient preferences into account and build applicable strategies together to effectively combat HIV-1. Some of the developed LAART compounds expand the existing treatments in antiviral classes like NNRTIs and INSTIs; other novel classes of inhibitors have been reported with long-acting applications, like the NRTTIs and capsid-targeting compounds. As with all antiviral compounds, LAARTs create a selective pressure on the virus and thus, there are known resistance mutations associated with the independent use of a single antiretroviral. Therefore, more and alternative strategies are required to expand the LAART field and optimize combinations of antiviral drugs with applications that meet patient needs.

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