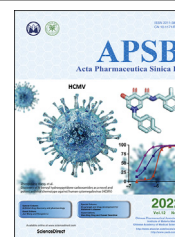




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REVIEW

Approved HIV reverse transcriptase inhibitors in the past decade



Guangdi Li^a, Yali Wang^{a,*}, Erik De Clercq^{b,*}

^aHunan Provincial Key Laboratory of Clinical Epidemiology, Xiangya School of Public Health, Central South University, Changsha 410078, China

^bRega Institute for Medical Research, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven B-3000, Belgium

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Abstract HIV reverse transcriptase (RT) inhibitors are the important components of highly active antiretroviral therapies (HAARTs) for anti-HIV treatment and pre-exposure prophylaxis in clinical practice. Many RT inhibitors and their combination regimens have been approved in the past ten years, but a review on their drug discovery, pharmacology, and clinical efficacy is lacking. Here, we provide a comprehensive review of RT inhibitors (tenofovir alafenamide, rilpivirine, doravirine, dapivirine, azvudine and elvitegravir) approved in the past decade, regarding their drug discovery, pharmacology, and clinical efficacy in randomized controlled trials. Novel RT inhibitors such as islatravir, MK-8504, MK-8507, MK8583, IQP-0528, and MIV-150 will be also highlighted. Future development may focus on the new generation of novel antiretroviral inhibitors with higher bioavailability, longer elimination half-life, more favorable side-effect profiles, fewer drug–drug interactions, and higher activities against circulating drug-resistant strains.

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Abbreviations: 3TC, (–)-2',3'-dideoxy-3'-thiacytidine (common name, lamivudine); ABC, abacavir; ATV, atazanavir; AZT, 3'-azido-2'-deoxy-thymidine (common name, zidovudine); BIC, bictegravir; CAB, cabotegravir; CC₅₀, the 50% cytotoxic concentration; COBI, cobicistat; DOR, doravirine; DPV, dapivirine; DRV, darunavir; DTG, dolutegravir; EACS, European AIDS Clinical Society; EC₅₀, half maximal effective concentration; EFV, efavirenz; ESV, elvitegravir; EVG, elvitegravir; F, bioavailability; FDA, US Food and Drug Administration; FTC, (–)-2',3'-dideoxy-5-fluoro-3'-thiacytidine (common name, emtricitabine); HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IAS-USA, International Antiviral Society-USA; IC₅₀, half maximal inhibitory concentration; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; MSM, men who have sex with men; RPV, rilpivirine; *t*_{1/2}, elimination half-life; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

*Corresponding authors. Tel./fax: +32 1637 3755.

E-mail addresses: liguangdi.research@gmail.com (Guangdi Li), wangyali@csu.edu.cn (Yali Wang), erik.declercq@kuleuven.be (Erik De Clercq).
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1. Introduction

In 2020, 37.7 million people were living with HIV infections according to the recent update from The Joint United Nations Programme on HIV/AIDS (www.unaids.org). Although HIV cure is still unavailable, antiretroviral therapies were accessible to 27.5 million patients living with HIV in 2020, and nearly 66% of patients living with HIV had virological suppression (www.unaids.org). Due to the large population of new cases (1.5 million) and untreated patients (10.2 million), it remains important to develop effective therapies to control HIV infections and transmission.

Highly active antiretroviral therapy (HAART) is the current standard of care for the management of HIV infections in clinical practice. To interrupt multiple steps of the HIV life cycle, HAARTs are mostly comprised of three compounds (Fig. 1), including two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI), one integrase inhibitor, or one protease inhibitor (boosted by ritonavir or cobicistat)^{1–7}. As shown in Fig. 2, NRTIs and NNRTIs target different binding pockets near the catalytic site of HIV reverse transcriptase (RT) to block the viral transcription of a double-stranded viral DNA genome from a single-stranded viral RNA genome^{1,4,5,8}. NNRTIs can inhibit the reverse transcriptase initiation complex even during the early viral transcription⁹. Integrase inhibitors such as bictegravir (BIC), dolutegravir (DTG), and elvitegravir (EVG) target the catalytic site of HIV integrase to inhibit the viral integration of proviral DNA into host genomes¹. Protease inhibitors such as darunavir (DRV) compete with natural substrates of HIV protease to inhibit the protease-mediated cleavage of *gag* and *gagpol* precursors¹. Among these four drug classes, NRTIs are the key backbones of combination regimens according to the recent update of the International

Antiviral Society-USA (IAS-USA) guidelines¹⁰, the European AIDS Clinical Society (EACS) guidelines¹¹, the NIH HIV/AIDS guidelines (<https://hivinfo.nih.gov>), and the WHO guidelines on HIV/AIDS (www.who.int).

From 1987 to 2010, eight NRTIs and four NNRTIs were officially approved to combat HIV infections. In the drug class of NRTIs, the US Food and Drug Administration (FDA) approved zidovudine (AZT) in March 1987, followed by didanosine (ddI) in October 1991, zalcitabine (ddC) in June 1992, stavudine (d4T) in June 1994, lamivudine (3TC) in November 1995, abacavir (ABC) in December 1998, tenofovir disoproxil fumarate (TDF) in October 2001, and emtricitabine (FTC) in July 2003. In the drug class of NNRTIs, nevirapine (NVP), delavirdine (DLV), efavirenz (EFV), and etravirine (ETR) were approved by the FDA^{4,12–14}. The “old” generation of the preceding RT inhibitors is often challenged by the emergence of drug resistance and adverse events. For example, (i) zalcitabine, stavudine, and delavirdine were discontinued because of severe adverse effects, inconvenient administration (three times daily), and low genetic barrier to resistance¹. (ii) Zidovudine, didanosine, stavudine, and nevirapine are no longer recommended due to their toxicity, low efficacy, and severe drug resistance^{10,11}. (iii) Abacavir-based therapies may increase the risk of cardiovascular diseases compared with abacavir-free regimens¹⁵. (iv) Efavirenz is associated with a high incidence of neuropsychiatric complications¹⁶. (v) TDF may cause renal impairment and reduce bone mineral density¹⁷. Drug discovery, drug resistance, and pharmacological features of the above NRTIs and NNRTIs have been reviewed by previous studies^{1–6,8,18–24}.

From 2010 to October 2021, the FDA approved three RT inhibitors: rilpivirine (RPV, Edurant®, approval date: 2011-05-20), tenofovir alafenamide (TAF, Vemlidy®, approval date: 2016-11-

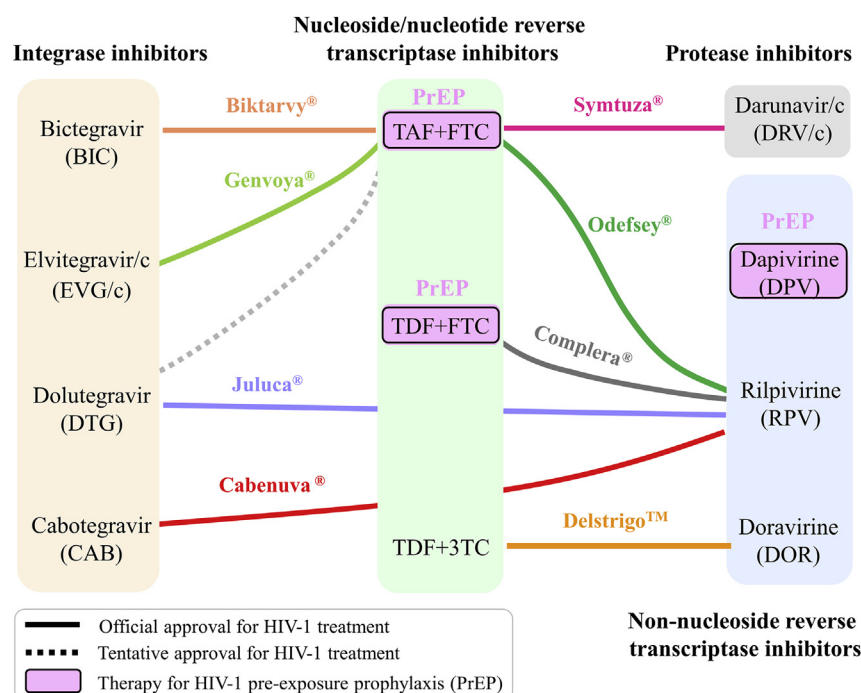


Figure 1 Approved antiretroviral regimens in the past decade (2010 to present). Most combination regimens are composed of two HIV nucleoside/nucleotide reverse transcriptase inhibitors plus one integrase inhibitor, one non-nucleoside reverse transcriptase inhibitor, or one protease inhibitor. Dapivirine vaginal ring 25 mg was approved by the European Medicines Agency on 24 July 2020, while the other regimens have been (tentatively) approved by the FDA.

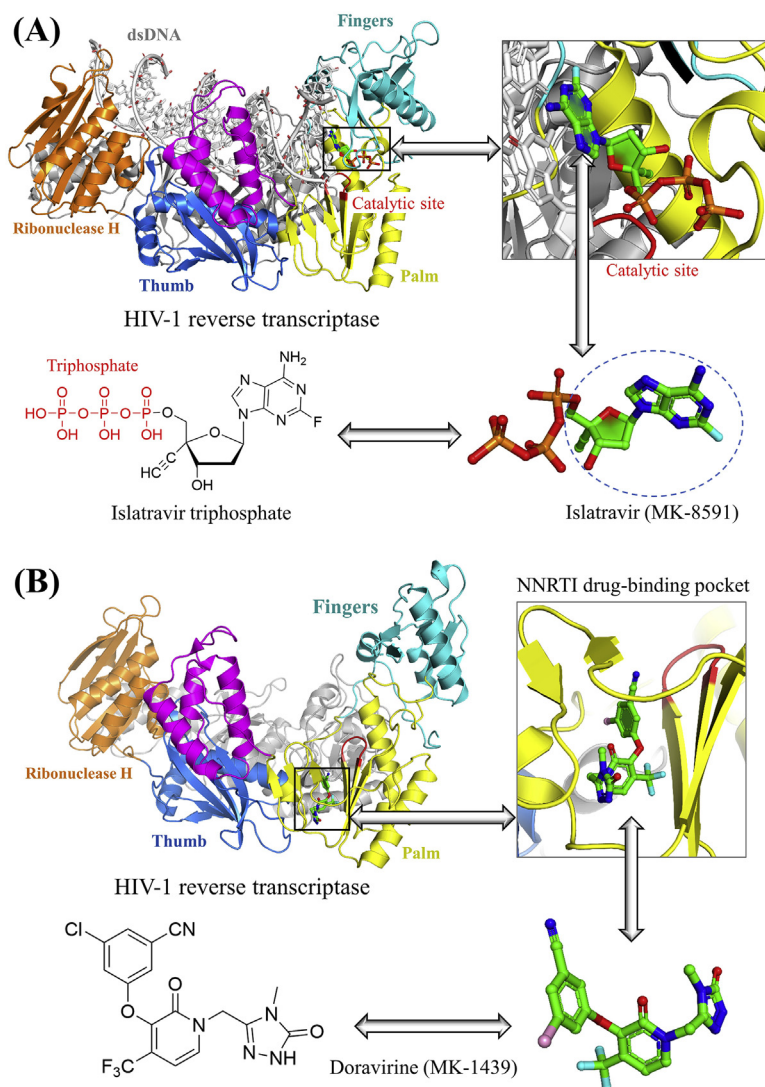


Figure 2 Structural basis of NRTI (islatravir) and NNRTI (doravirine). (A) Chemical and 3D structures of islatravir. The drug binding pocket of islatravir is highlighted in HIV-1 reverse transcriptase (PDB code: 5J2M). Islatravir triphosphate interferes with the translocation of HIV reverse transcription on the nucleic acid substrate to slow down the viral DNA synthesis. (B) Chemical and 3D structures of doravirine. Drug binding pocket of doravirine is highlighted at the palm domain of the P66 subunit in HIV-1 reverse transcriptase (PDB code: 4NCG). PDB codes were obtained from the RCSB protein data bank (www.rcsb.org/). Protein 3D structures were visualized by PyMOL V1.7 (www.pymol.org/).

10), and doravirine (DOR, Pifeltro™, approval date: 2018-08-30). These RT inhibitors are further integrated into 10 fixed-dose combination regimens that have been (tentatively) approved by the FDA (Table 1). In addition to these FDA-approved drugs, elvitegravir (Elpida®) was approved by the Russian Ministry of Health in June 2017. A vaginal ring containing dapivirine 25 mg was approved by the European Medicines Agency in July 2020. On 21 July 2021, azvudine was conditionally approved by the National Medical Products Administration in China.

To the best of our knowledge, no review has comprehensively characterized the drug discovery, clinical efficacy, and pharmacological profiles of newly-approved HIV RT inhibitors in the past decade. Based on a large body of randomized clinical trials, this review will summarize the clinical efficacy and pharmacological profiles of approved HIV RT inhibitors (tenofovir alafenamide, dapivirine, rilpivirine, doravirine, azvudine, elvitegravir) and

their combination regimens (Descovy®, Biktarvy®, Genvoya®, Acriptega®, Symtuza®, Complera®, Juluca®, Cabenuva®, Delstrigo™).

This review is organized as follows. First, our strategies for literature search and selection are described. Second, for each approved RT inhibitor, we will highlight the (i) drug discovery; (ii) antiviral activity, drug resistance, and pharmacokinetics; and (iii) clinical efficacy and safety of their approved combination regimens in randomized clinical trials. Third, novel regimens will be highlighted. Our drug movies and teaching slides that highlight HIV RT inhibitors are shared online (www.virusface.com).

2. Search strategy and selection criteria

To collect relevant literature, we searched PubMed, Google Scholar, and Journal websites using the keywords of individual

drug names. We extracted clinical trial data from [ClinicalTrials.gov](https://clinicaltrials.gov) (<https://clinicaltrials.gov>), drug labeling resources from the FDA database (www.accessdata.fda.gov), and drug resistance mutations from the IAS-USA guidelines²⁵. To summarize the clinical efficacy of approved combination regimens, we collected clinical studies that fulfilled four conditions: (i) randomized, controlled clinical trials must be at the stage of phases 2, 3, and/or 4; (ii) studies recruited adults in the absence of pregnant women; (iii) recruited adults were confirmed with HIV infections without other infectious diseases (*e.g.*, tuberculosis, HBV²⁶, HCV^{27,28}) or non-communicable diseases such as liver/kidney impairment; (iv) patients were treated with the exact dosage of approved regimens according to the drug labeling; and (v) viral suppression of plasma HIV-1 RNA <50 copies/mL was measured at Week 48.

3. Tenofovir alafenamide (Vemlidy®)

In 2015, the FDA approved Genvoya®—the first combination regimen that contains tenofovir alafenamide (GS-7340) for the treatment of HIV-1 infections. Since then, five TAF-based combination regimens (Genvoya®, Odefsey®, Biktarvy®, Symtuza®, Descovy®) have been approved by the FDA and the European Medicines Agency (Table 1). The tentative approval of dolutegravir, emtricitabine, and tenofovir alafenamide tablets was granted by the FDA in December 2020, and this regimen is currently marked with the trade name of Acriptega® in India.

3.1. Drug discovery

As shown in Fig. 3A, the discovery of tenofovir alafenamide began with the synthesis of an adenosine nucleoside analogue called tenofovir [(*R*)-9-(2-phosphonylmethoxypropyl)adenine] at the Rega Institute in Belgium²⁹. Tenofovir showed potent activities against the replications of HIV-1 (EC_{50} : $5.9 \pm 0.45 \mu\text{mol/L}$)²⁹, HIV-2 (EC_{50} : $4.9 \pm 0.45 \mu\text{mol/L}$)²⁹, and HBV (EC_{50} : $6.5 \pm 1.1 \mu\text{mol/L}$) in cell cultures³⁰. However, tenofovir carries negative charges on the phosphonate moiety,

resulting in poor cellular permeability and limited oral bioavailability^{31,32}. Many prodrugs of tenofovir such as TDF were subsequently developed to enhance the permeability across the intestinal wall³³. For instance, phosphoramidate prodrugs of tenofovir could be designed by the “ProTide” strategy that offers the efficient intracellular delivery of monophosphates and monophosphonates of nucleoside analogues across the cell membrane by passive diffusion^{34,35}.

A series of aryl phenoxy-amidate derivatives of tenofovir was synthesized and one potent candidate, designated as GS-7171, showed promising anti-HIV activity *in vitro* and oral bioavailability in dogs³⁶. Further purification of GS-7171 unexpectedly led to a 1:1 diastereomeric mixture of GS-7339 and GS-7340 due to the asymmetric center at the phosphorus atom of GS-7171 and the non-stereoselective synthetic process³⁷. Each stereoisomer was isolated using batch elution chromatography³⁷, and *in vitro* evaluations suggested that the anti-HIV-1 activity and pharmacokinetic profiles of GS-7340 were better than that of GS-7339³⁸. In fasted dogs, a single oral dose of TAF 10 mg-eq/kg preferentially produced a high concentration of tenofovir in lymphatic tissues and the peripheral blood mononuclear cells (PBMCs)—the primary location for HIV replication and latency³⁸. The intracellular concentrations of tenofovir, tenofovir-monophosphate, tenofovir-diphosphate, and TAF were respectively 138 ± 55 , 89 ± 8 , 73 ± 15 , and $0 \mu\text{mol/L}$ in monocytes that were isolated from human whole blood after the incubation with TAF $17.4 \mu\text{mol/L}$ for 1 h³⁸. Based on the above findings, GS-7340 was selected as a potent anti-HIV inhibitor for further evaluations in clinical trials.

As the prodrugs of tenofovir, both TAF and TDF efficiently delivers tenofovir into lymphoid cells and tissues, and their structural differences can be traced to the phosphonate masking groups of TAF that harbor the phenol and alanine isopropyl ester (Fig. 3B). Compared with TDF, TAF is not only more stable in blood and plasma, but also maintains antiviral potency for a longer time³⁹ and leads to higher levels of triglycerides, low-density and high-density lipoprotein cholesterol⁴⁰. Because the prolonged exposure to TDF 300 mg may increase the risk of renal

Table 1 List of approved HIV RT inhibitors and their regimens in the past decade.

Antiretroviral drug or regimen	Usage	Trade name	Region	First approval
Tenofovir alafenamide (TAF) 25 mg	With other drugs	Vemlidy®	US, EU	2016-11-10
TAF 25 mg + Emtricitabine (FTC) 200 mg	Pre-exposure prophylaxis	Descovy®	US, EU	2019-10-03 ^b
TAF 25 mg + FTC 200 mg + RPV 25 mg	Complete regimen	Odefsey®	US, EU	2016-03-01
TAF 25 mg + FTC 200 mg + BIC 50 mg	Complete regimen	Biktarvy®	US, EU	2018-02-07
TAF 25 mg + FTC 200 mg + DTG 50 mg	Complete regimen	Acriptega® ^a	US ^a , India	2020-12-04 ^a
TAF 10 mg + FTC 200 mg + EVG 150 mg + COBI 150 mg	Complete regimen	Genvoya®	US, EU	2015-11-05
TAF 10 mg + FTC 200 mg + DRV 800 mg + COBI 150 mg	Complete regimen	Symtuza®	US, EU	2018-07-17
Rilpivirine (RPV) 25 mg	With other drugs	Edurant®	US, EU	2011-05-20
RPV 25 mg + TDF 300 mg + FTC 200 mg	Complete regimen	Complera®	US, EU	2011-08-10
RPV 25 mg + DTG 50 mg	Complete regimen	Juluca®	US, EU	2017-11-21
RPV 300 mg/mL + CAB 200 mg/mL	Complete regimen	Cabenuva®	US, EU	2021-01-21
Doravirine (DOR) 100 mg	With other drugs	Pifeltro™	US, EU	2018-08-30
DOR 100 mg + TDF 300 mg + 3TC 300 mg	Complete regimen	Delstrigo™	US, EU	2018-08-30
Elsulfavirine 20 mg	With other drugs	Elpida®	Russia	2017-06-30
Dapivirine 25 mg vaginal ring	Pre-exposure prophylaxis	—	EU	2020-07-24
Azvadine 3 mg	With other drugs	Azvadine Tablet	China ^c	2021-07-21

^aThe TAF + FTC + DTG regimen is marked as the trade name of Acriptega® in India. The FDA granted the tentative approval of dolutegravir, emtricitabine, and tenofovir alafenamide tablets on 2020-12-04.

^bDescovy® was approved by the FDA for HIV-1 treatment and pre-exposure prophylaxis in 2016 and 2019, respectively.

^cAzvadine was conditionally approved in China.

impairment and a loss of bone mineral density¹⁷, TAF 25 mg offers lower systemic tenofovir exposure at reduced doses, thereby reducing renal and bone impairments^{41,42}. TAF 10 or 25 mg/day is currently considered to replace TDF 300 mg/day in fixed-dose combinations.

3.2. Antiviral activity, drug resistance, and pharmacokinetics

TAF exhibited a strong potency against broad-spectrum isolates from HIV-1 groups/subtypes (EC_{50} range: 0.1–12 nmol/L) and HIV-2 (EC_{50} range: 0.91–2.63 nmol/L)³⁹. In MT-2 cells infected with HIV-1 IIIb strain, the EC_{50} value of TAF (0.005 ± 0.002 μ mol/L) was lower than that of TDF (0.05 ± 0.03 μ mol/L) and tenofovir (5.0 ± 2.6 μ mol/L)³⁸. Despite its potency against HIV and HBV strains, TAF does not inhibit HCV, HSV-1, HCMV, dengue, or influenza A virus³⁹. According to the recent update of IAS-USA guideline, TAF and TDF share similar drug-resistance profiles that include four primary amino acid substitutions: K65 R/E/N and K70E²⁵.

In a phase 1b study of treatment-naïve adults who received the monotherapy for 10 days, TAF 25 mg was quickly absorbed with the median T_{max} of 0.5 h⁴³. At the steady-state, the plasma exposure of tenofovir on Day 10 was much lower in the treatment of TAF 25 mg (AUC_{tau} : 267.7 ng·h/mL) compared with TDF 300 mg (1918.0 ng·h/mL)⁴³. At approximately one-tenth of TDF 300 mg, TAF 25 mg offered a higher concentration of tenofovir-diphosphate in intracellular peripheral blood mononuclear cells (AUC_{tau} : 21.4 vs 3.0 μ mol/L·h) (Fig. 3B). Due to its high oral bioavailability and solubility, TAF generates sufficient mass flux across the intestine to saturate efflux transport and reduce intestinal metabolisms⁴⁴. In HIV-negative patients receiving TAF 25 mg, the pharmacokinetic parameters of C_{max} and AUC_{inf} were two times higher in patients with severe renal impairment than those with normal renal function (C_{max} : 364 vs 199 ng/mL, AUC_{inf} : 513 vs 267 ng·h/mL)⁴⁵. The plasma exposure of its active metabolite tenofovir was significantly increased in individuals with severe renal impairment *versus* those with normal renal function (C_{max} : 26.4 vs 9.5 ng/mL, AUC_{inf} : 2070 vs 343 ng·h/mL)⁴⁵.

The plasma concentration of TAF and its metabolites can be altered by the coadministration of other drugs that affect P-glycoprotein and/or breast cancer resistance protein transporters⁴⁶. Pharmacokinetic boosters such as ritonavir and cobicistat inhibit the intestinal P-glycoprotein transporter to improve the intestinal absorption of TAF from the gastrointestinal tract⁴⁷. In the presence of cobicistat, TAF could be reduced from 25 to 10 mg due to the boosting effects⁴⁸. In the absence of any booster, TAF and TDF offered similar clinical efficacies and safety profiles in virologically suppressed adults with HIV-1⁴⁸, and both exert an equivalent impact on the immune activation and inflammation in treatment-naïve adults⁴⁹.

3.3. Efficacy and safety of TAF-based combination regimens

3.3.1. TAF 25 mg + emtricitabine 200 mg (Descovy®)

In October 2019, the once-daily, fixed-dose, single-tablet of TAF 25 mg plus FTC 200 mg was approved by the FDA for at-risk HIV-negative adults (body weight ≥ 35 kg) to reduce the risk of HIV-1 infection from sexual acquisitions. The efficacy and safety of TAF + FTC in HIV-negative or transgender men who have sex with men (MSM) were evaluated by a phase three study called DISCOVER⁵⁰. This randomized, double-blind, non-inferiority

study reported the comparable incidence rates of HIV-1 infection between the TAF + FTC arm and the TDF + FTC arm (0.16 *versus* 0.34 infections per 100 person-years, P -value > 0.05), but the TAF-based regimen offered better safety on the bone mineral density and renal function⁵⁰. Of note, the efficacy and safety of TDF + FTC *versus* the placebo group were previously confirmed⁵¹ (Table 2).

Due to the success of TDF + FTC in the pre-exposure prophylaxis of HIV-1 infections, TAF was considered for the replacement of TDF to develop the TAF + FTC regimen. Similar to TDF + FTC, TAF + FTC cannot be used in HIV-1-infected adults with the estimated creatinine clearance < 30 mL/min. Ongoing clinical trials (*e.g.*, NCT04616963, NCT04742491) will evaluate the efficacy and safety of TAF + FTC to prevent HIV-1 transmission in transgender-identifying or gender non-binary individuals.

3.3.2. TAF 25 mg + emtricitabine 200 mg + rilpivirine 25 mg (Odefsey®)

In March 2016, the FDA approved the three-drug, once-daily, fixed-dose tablet of TAF + FTC + RPV as the initiating therapy for treatment-naïve patients or maintaining therapy for virologically suppressed patients. According to the drug labeling, TAF + FTC + RPV alone is not recommended for HIV-1-infected adults with HBV or creatinine clearance < 30 mL/min.

The clinical efficacy and safety of TAF + FTC + RPV were evaluated by two phase 3b, non-inferiority studies (NCT02345226, NCT02345252) (Table 3, Supporting Information Table S1). Based on the merged data from the two studies above, TAF + FTC + RPV maintained the virological suppression (HIV-1 RNA < 50 copies/mL at Week 48) in virologically suppressed adults infected with HIV-1. The drug-associated adverse events (*e.g.*, upper respiratory tract infection, nasopharyngitis, headache, and diarrhea) were observed in 10.1% (76/754) of patients who received TAF + FTC + RPV.

3.3.3. TAF 25 mg + emtricitabine 200 mg + bictegravir 50 mg (Biktarvy®)

In February 2018, the FDA approved the three-drug fixed-dose regimen of TAF + FTC + BIC as initiating therapy for treatment-naïve patients or maintaining therapy for virologically suppressed patients. According to the drug labeling, TAF + FTC + BIC alone is not recommended for the treatment of HIV-1-infected adults with HBV, severe hepatic impairment, or creatinine clearance < 30 mL/min.

The clinical efficacy and safety of TAF + FTC + BIC were evaluated by one phase two study and seven phase three studies (Table 3). For treatment-naïve adults, 91.4% (639/699) achieved the virological suppression after the 48-week treatment of TAF + FTC + BIC, and drug-associated adverse events were reported in 21.9% (139/634) participants. The 48-week treatment of TAF + FTC + BIC maintained HIV-1 RNA < 50 copies/mL in 93.6% (1020/1090) patients of treatment-experienced adults who had been virologically suppressed for at least 3 months, while drug-associated adverse events were reported in 12.7% (138/1090) participants. In HIV-1-treatment-naïve patients, the pre-existing resistance substitutions did not affect the virological outcomes of TAF + FTC + BIC, and no treatment-emergent resistance was observed after the 144-week treatment of TAF + FTC + BIC⁵². Ongoing clinical trials will evaluate the efficacy and safety of Biktarvy® in adolescents (NCT02881320), elderly patients

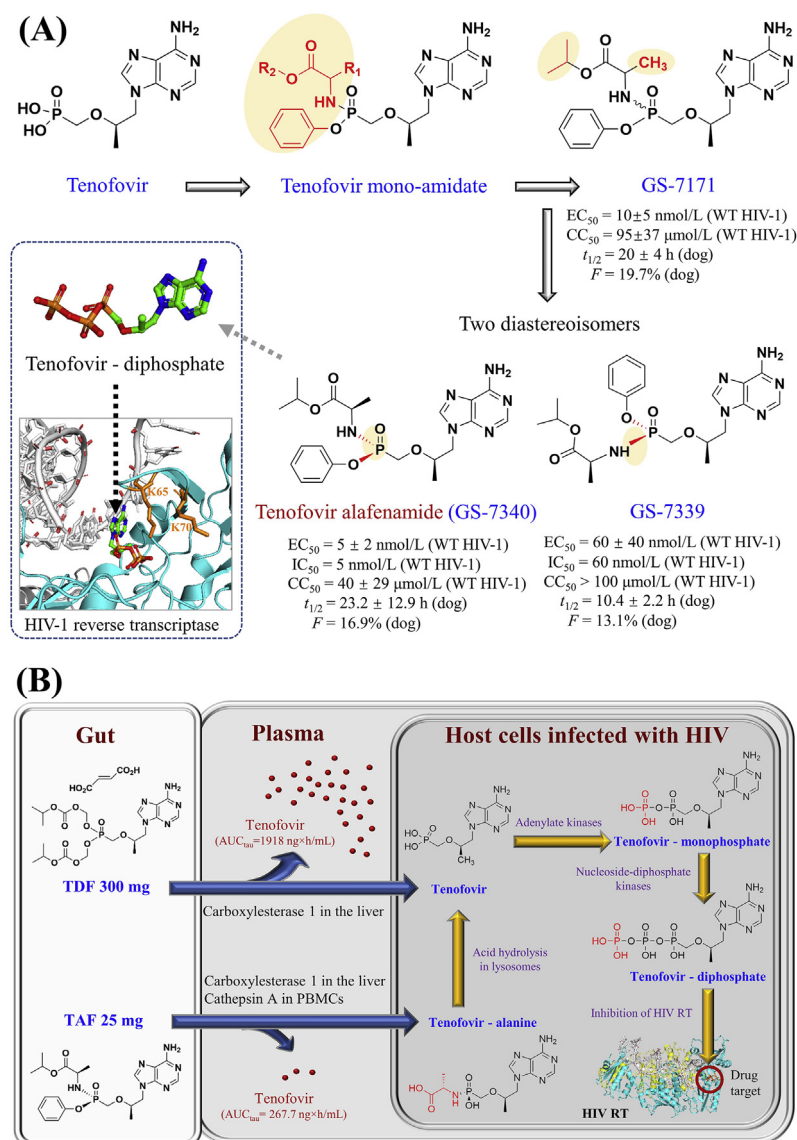


Figure 3 Discovery and metabolic pathway of tenofovir alafenamide. (A) Discovery of tenofovir alafenamide. Anti-HIV-1 parameters and pharmacokinetic values are extracted from³⁸. (B) Metabolic pathways of TDF 300 mg and TAF 25 mg from the gut to the blood plasma and lymphoid cells infected with HIV. At the steady-state, the plasma exposure of tenofovir at Day 10 was lower in the treatment of TAF 25 mg (AUC_{tau}: 267.7 ng·h/mL) compared with TDF 300 mg (1918.0 ng·h/mL)⁴³. TAF in blood enters primary hepatocytes and undertakes hydrolysis primarily by carboxylesterase one and cathepsin A that produce tenofovir–alanine conjugates within lymphocytes. TDF and TAF are converted to tenofovir and then phosphorylated to the intracellular active metabolite tenofovir diphosphate that blocks the catalytic site of HIV reverse transcriptase.

(NCT04222283), HIV-positive renal transplant patients (NCT04530630), patients within 100 days post HIV infections (NCT04483674), and patients co-infected with tuberculosis (NCT04734652).

3.3.4. TAF 25 mg + emtricitabine 200 mg + dolutegravir 50 mg
 The tentative approval of TAF + FTC + DTG was granted by the FDA in December 2020, and its fixed-dose, single-tablet is now commercialized with the brand name of Acriptega® in India. Although it has not been marketed in the USA, TAF + FTC + DTG is currently recommended by the IAS-USA and the EACS guidelines^{10,11}. Of note, TAF + FTC + DTG

alone is not recommended for HIV-1-infected patients with body weight <40 kg, HBV infections, or creatinine clearance <30 mL/min.

The efficacy and safety of TAF + FTC + DTG were reported by four clinical studies (Table 3). In treatment-naïve patients who received TAF + FTC + DTG, 88.3% (626/709) achieved the virological response of HIV-1 RNA <50 copies/mL at Week 48. In virologically suppressed patients who received TAF + FTC + DTG, 91% (256/281) maintained the virological response of HIV-1 RNA <50 copies/mL at Week 48. The drug-associated adverse events were reported in 10% (28/281) patients (Table S1).

Table 2 Efficacy of approved therapies for HIV-1 pre-exposure prophylaxis.

Clinical trial (phase)	Recruited individuals	Trial arm	HIV infection no./patient no.	Person-years ^a	Incidence rate ^b	P-value	Ref.
DISCOVER (phase 3)	HIV-negative or transgender MSM	TAF + FTC	7/2694	8756	0.16%	>0.05	50
iPrEx (phase 3)	HIV-negative or transgender MSM	TDF + FTC	15/2693	3324	0.34%	NA	51
MTN-020–ASPIRE (phase 3)	HIV-negative women (18–45 years)	Placebo	38/1251	4280	NA	0.046	77
Ring (phase 3)	HIV-negative women (18–45 years)	Dapivirine vaginal ring	72/1248	1888	NA	0.04	78
		Placebo	71/1313		4.5%		
		Dapivirine vaginal ring	97/1316		4.1%		
		Placebo	77/1307		6.1%		
			56/652				

MSM: men who have sex with men.

^aThe person-years of follow-up are summarized for individual studies.

^bIncidence rate equals the number of HIV-1 infections divided by person-years of follow-up. *P*-value indicates the statistical difference of incidence rates between the intervention arm and the placebo arm.

3.3.5. TAF 10 mg + emtricitabine 200 mg + elvitegravir 150 mg + cobicistat 150 mg (Genvoya®)

In November 2015, the FDA approved the once-daily, fixed-dose, single-tablet of TAF + FTC + EVG/c as initiating therapy and maintaining therapy to treat HIV-1-infected patients with no history of resistance to TAF, FTC, or EVG. According to the drug labeling, TAF + FTC + EVG/c alone is not recommended for HIV-1-infected patients with HBV infections, severe hepatic impairment, or creatinine clearance <30 mL/min. The clinical efficacy and safety of TAF + FTC + EVG/c were evaluated by one phase two study and four phase three studies (Table 3). The virological response of HIV-1 RNA <50 copies/mL at Week 48 was observed in 91.9% (899/978) of treatment-naïve patients who received TAF + FTC + EVG/c (Table 3). By merging data from three phase three studies, this regimen offered the virological response up to 96.5% (1184/1227) of virologically suppressed patients (Table 3). Drug-associated adverse events were observed in 19.9% (244/1228) of patients receiving TAF + FTC + EVG/c.

A switch from TDF-based regimens to Genvoya® improved the bone mineral density in virologically suppressed patients ≥60 years (Table 3). Genvoya® also improved the bone mineral density in HIV-infected adults with renal impairment⁵³ or with end-stage renal diseases on chronic hemodialysis⁵⁴. In a retrospective study of 6704 treatment-naïve patients, HIV-1 RT amino acid substitutions such as T215 A/C/D/E/G/H/I/L/N/P/Q/R/S/V showed no measurable impact on virological responses to TAF + FTC + EVG/c⁵⁵. A low prevalence of resistant RT mutations, including K65 R/N (2 cases, 0.2%) and M184V/I (11 cases, 1.3%) was observed in 866 treatment-naïve adults who received TAF + FTC + EVG/c for 144 weeks⁵⁶.

3.3.6. TAF 10 mg + emtricitabine 200 mg + darunavir 800 mg + cobicistat 150 mg (Symtuza®)

In July 2018, the FDA approved the once-daily fixed-dose regimen of TAF + FTC + DRV/c as initiating therapy and maintaining therapy to treat HIV-1-infected patients with no history of resistance to TAF, FTC, or DRV. According to the drug labeling, TAF + FTC + DRV/c alone is not recommended for HIV-1-infected patients with body weight <40 kg, HBV infections, severe hepatic impairment, or creatinine clearance <30 mL/min.

The clinical efficacy and safety of TAF + FTC + DRV/c were evaluated by one phase two study and two phase three studies: AMBER and EMERALD (Table 3, Table S1). After the 48-week

treatment with TAF + FTC + DRV/c, the virological response of HIV-1 RNA <50 copies/mL was observed in 88.2% (410/465) of treatment-naïve adults and 95% (724/763) of virologically suppressed adults with virological suppression ≥6 months (Table 3). The most common drug-associated adverse event was upper respiratory tract infection (10.6%, 81/763) and nasopharyngitis (10.6%, 81/763) (Table S1).

4. Dapivirine

Dapivirine is a potent NNRTI against HIV-1 infections. On 24 July 2020, the European Medicines Agency approved dapivirine vaginal ring (a silicone elastomer dapivirine vaginal ring containing dapivirine 25 mg) for the pre-exposure prophylaxis of HIV-1 infection. The monthly application of dapivirine vaginal ring can be administered to reduce the risk of HIV-1 infection through vaginal intercourse in sexually active HIV-negative women (≥18 years) in combination with safer sex practices when oral pre-exposure prophylaxis is not/cannot be used or is not available.

4.1. Drug discovery

As shown in Fig. 4, the discovery of dapivirine can be traced back to the development of the alpha-anilinophenylacetamide (α-APA) derivatives⁵⁷, the imidoyl thiourea (ITU) derivatives⁵⁸, the diaryl triazine (DATA) derivatives⁵⁹, and the diarylpyrimidine (DAPY) derivatives⁶⁰ based on the multidisciplinary collaborations between our Rega Institute in Belgium⁶¹, Janssen Pharmaceutica in Belgium, and Rutgers University in the USA⁶². In 1991, Pauwels et al.⁵⁷ from our Rega Institute used a high-flux screening system to screen the Janssen library of 2000 different compounds for their cytotoxicity and capacity to inhibit HIV-1 replication in MT-4 cells. This led to the discovery of an alpha-anilinophenylacetamide lead called R15345⁵⁷. Further optimization led to R89439 (loviride, loveride) that effectively inhibited HIV-1 strains *in vitro* at the nanomolar concentration (IC₅₀: 13 nmol/L)⁵⁷. However, R89439 was later discontinued because it failed to show sufficient potency in clinical trials. Stemming from alpha-anilinophenylacetamide derivatives, imidoyl thiourea analogues with a unique diarylated imidoylthiourea structure was subsequently synthesized⁵⁸. However, these candidates were not further pursued due to the hydrolytic instability of the imidoyl thiourea functionality⁵⁸. During the synthesis of the imino-*N*-cyanoguanidine derivatives of imidoyl thiourea analogues,

Table 3 Clinical efficacy of approved anti-HIV regimens in phase 2/3 clinical trials.

Brand name	Prior treatment	Arm	Efficacy ^a	P-value	Study (phase)	Ref.
Biktarvy®	Treatment-naïve	TAF + FTC + BIC	97% (63/65)	0.17	NCT02397694 (phase 2)	178
		TAF + FTC + DTG	91% (30/33)			
	Treatment-naïve	TAF + FTC + BIC	89% (286/320)	0.12	NCT02607956 (phase 3)	179
		TAF + FTC + DTG	93% (302/325)			
	Treatment-naïve	TAF + FTC + BIC	92% (290/314)	0.78	NCT02607930 (phase 3)	180
		ABC + 3TC + DTG	93% (293/315)			
	Virologically suppressed	TAF + FTC + BIC	94% (264/282)	0.59	NCT02603120 (phase 3)	181
		ABC + 3TC + DTG	95% (267/281)			
	Virologically suppressed	TAF + FTC + BIC	92% (267/290)	0.20	NCT02603107 (phase 3)	182
		PI-based regimen	89% (255/287)			
Odefsey®	Virologically suppressed	TAF + FTC + BIC	96% (224/234)	1.00	NCT02652624 (phase 3)	183
		Baseline regimen	95% (225/236)			
	Virologically suppressed	TAF + FTC + BIC	93% (265/284)	0.28	NCT03110380 (phase 3)	184
		TAF + FTC + DTG	91% (256/281)			
	Virologically suppressed	TAF + FTC + RPV	90% (394/438)	0.35	NCT02345226 (phase 3)	185
		TDF + FTC + EFV	92% (402/437)			
	Virologically suppressed	TAF + FTC + RPV	94% (296/316)	1.00	NCT02345252 (phase 3)	186
		TDF + FTC + RPV	94% (294/313)			
	Treatment-naïve	TAF + FTC + DTG	84% (294/351)	0.07	NCT03122262 (phase 3)	187
		TDF + FTC + DTG	85% (298/351)			
Symtuza®	Treatment-naïve	TDF + FTC + EFV	79% (276/351)	0.41	NCT01565850 (phase 2)	188
		TAF + FTC + DRV/c	77% (79/103)			
	Treatment-naïve	TDF + FTC + DRV/c	84% (42/50)	<0.0001	NCT02431247 (phase 3)	189
		TAF + FTC + DRV/c	91% (331/362)			
	Virologically suppressed	TDF + FTC + DRV/c	88% (321/363)	0.39	NCT02269917 (phase 3)	190
Genvoya®	Virologically suppressed	TAF + FTC + DRV/c	95% (724/763)	0.39	NCT02269917 (phase 3)	190
		TDF + FTC + PI	94% (354/378)			
	Treatment-naïve	TAF + FTC + EVG/c	88% (99/112)	0.84	NCT01497899 (phase 2)	191
		TDF + FTC + EVG/c	88% (51/58)			
	Treatment-naïve	TAF + FTC + EVG/c	92% (800/866)	0.17	NCT01780506 (phase 3)	40
		TDF + FTC + EVG/c	90% (784/867)			
	Virologically suppressed	TAF + FTC + EVG/c	97% (932/959)	0.0002	NCT01815736 (phase 3)	192
		TDF-based regimen	93% (444/477)			
	Virologically suppressed	TAF + FTC + EVG/c	94% (150/159)	0.13	NCT01705574 (phase 3)	193
		TDF + FTC + ATV/r	87% (46/53)			
	Virologically suppressed	TAF + FTC + EVG/c	94% (102/109)	1.0	NCT02616783 (phase 3)	194
		TDF + FTC + EVG/c	95% (52/55)			

^aClinical efficacy was defined by HIV-1 RNA <50 copies/mL at Week 48.

a ring closure was unexpectedly identified⁶², leading to the first compound of the DATA derivatives with improved stability⁵⁹. However, the DATA derivatives such as R106168 showed suboptimal activities against HIV-1 strains with the double mutant L100I + K103N⁵⁹. After the exploration of the structure–activity and structure–metabolism relationships, the replacement of the central aminotriazine ring of DATA with a pyrimidine ring was evaluated, leading to the class of diarylpyrimidine derivatives such as dapivirine (TMC120, R147681)⁶³ and etravirine (TMC125, R165335, Intelence®, approved by the FDA in 2008)⁶⁴.

A vaginal microbicide of dapivirine was later considered because dapivirine has long half-life time, high potency, and low cytotoxicity^{65,66}. Although the applications of vaginally administered gel, film, tablet, soft gel capsules, and ring were considered to deliver dapivirine, the vaginal ring was eventually selected due to its easy use and a low frequency of the monthly administration⁶⁷. To prevent the male-to-female transmission of HIV-1 infections, the silicone elastomer intravaginal ring could provide the sustained and controlled delivery of dapivirine into cervicovaginal fluids and vaginal tissues⁶⁸. Other drug delivery methods such as

gel products, fast-dissolving insert, lubricants and douches are still under investigation⁶⁹.

4.2. Antiviral activity, drug resistance, and pharmacokinetics

Dapivirine effectively prevented the HIV-1-induced syncytium formation (EC₅₀: 1 nmol/L, IC₅₀: 24 nmol/L) in CEM T cells infected with HIV-1 strain HTLV-III_B⁶³. A 24-h treatment of dapivirine to the MO–DC/CD4+ T-cell cocultures also prevented the HIV-1 proviral integration (EC₅₀: 4 nmol/L, range: 0.5–15 nmol/L)⁷⁰. Over a 28-day use of the vaginal ring containing dapivirine 25 mg, dapivirine distributed throughout the lower genital tract of HIV-negative women at a concentration of 4000 times higher than the required EC₅₀ against wildtype HIV-1 strains in MT4 cells⁷¹. After the ring insertion for 1.5 h, the C_{max} value of dapivirine was the highest near the ring (79.90 ± 23.20 µg/g), followed by the cervix (66.61 ± 20.12 µg/g) and the introitus (31.38 ± 10.95 µg/g) in healthy women⁷².

A high concentration of dapivirine in the genital tract maintained a strong inhibition against HIV-1 resistant viruses including those with

K103N and/or Y191C⁷³. However, L100I and/or K103N mutations may cause significant resistance to dapivirine⁷⁴. Under suboptimal concentrations *in vitro*, drug resistance mutations were less frequent in the dapivirine + tenofovir group than in the dapivirine alone⁷⁵. A significant decrease of fasting lipids (*e.g.*, cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol) was observed in doravirine-treated patients⁷⁶.

4.3. Efficacy and safety of dapivirine vaginal ring

Based on two large-scale phase three studies^{77,78}, the monthly application of dapivirine vaginal ring was evaluated for the pre-exposure prophylaxis of HIV-1 transmission in sexually active HIV-negative women (Table 2). The ASPIRE study, which was conducted between August 2012 and June 2015, reported the reduced incidence of HIV-1 infections at month 33 in the dapivirine-ring arm compared with the placebo arm (3.3 *versus* 4.5 seroconversions per 100 person-years, *P*-value = 0.046)⁷⁷. Adverse events were 14% (180/1313) in the dapivirine-ring arm⁷⁷. A follow-up study called HOPE was conducted between 16 July 2016 and 10 October 2018, and this study reported the reduced HIV-1 incidences

of 2.7 per 100 person-years in the dapivirine-ring arm compared with the expected incidence of 4.4 per 100 person-years from the ASPIRE study⁷⁹. Moreover, the dapivirine ring made of the flexible silicone matrix polymers unlikely caused cervical cytology abnormalities⁸⁰. In the ring study, the overall incidence rate of HIV-1 infections in the dapivirine arm was significantly lower than that in the placebo arm (4.1 *versus* 6.1 seroconversions per 100 person-years, *P*-value = 0.04)⁷⁸. Three NNRTI resistance mutations were identified, including E138A (11.7%, 9/77), A98G (3.9%, 3/77), and K103N (3.9%, 3/77)⁷⁸. As an open-label extension of the Ring study, the DREAM study supported the clinical use of the dapivirine ring with low HIV-1 incidences and improved adherence⁸¹. Ongoing open-label studies will evaluate the dapivirine-containing vaginal ring in young women between 16 and 21 years (NCT03593655), pregnant women (NCT03965923), and women who are breastfeeding (NCT04140266).

5. Rilpivirine (Edurant®, Rekambys®)

Rilpivirine (other names: TMC278, R278474) is a diarylpyrimidine NNRTI approved by the FDA in 2011. Rilpivirine tablets

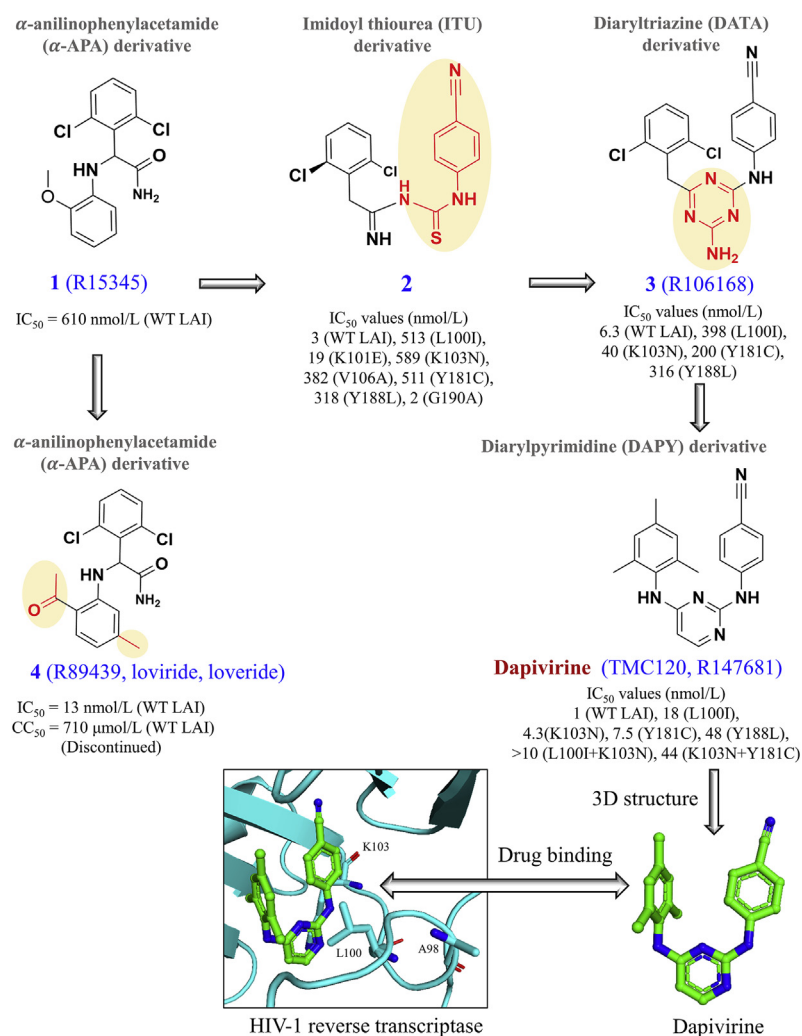


Figure 4 Discovery of dapivirine from the alpha-anilinophenylacetamide (α-APA) derivatives to imidoyl thiourea derivatives, the diaryltriazine derivatives, and the diarylpyrimidine derivatives. Drug binding pocket of dapivirine is highlighted in HIV-1 reverse transcriptase (PDB code: 1S6Q). Amino acid positions with drug resistant residues are highlighted. Three amino acid substitutions (L100I, K103N, A98G) may cause resistance to dapivirine^{74,78}.

(Edurant®) and injection (Rekambys®) are both marketed. According to the FDA labeling, the once-daily tablet of rilpivirine 25 mg in combination with other antiretroviral drugs can be orally taken with a meal to treat HIV-1 infections in treatment-naïve patients (>12 years) with bodyweight ≥ 35 kg, CD4⁺ cell count >200 cells/mm³, and HIV-1 RNA $\leq 100,000$ copies/mL. According to the drug labeling, rilpivirine should not be coadministered with cytochrome P450 inducers (e.g., rifabutin, rifampin, rifapentine) or drugs (e.g., cimetidine, famotidine, nizatidine) that increase gastric pH, because these drugs may reduce the plasma concentration of rilpivirine and cause the reduced virologic response and possible drug resistance.

5.1. Drug discovery

Rilpivirine is the *E*-isomer of the *p*-cyanovinyl analogue of dapivirine. As shown in Fig. 5, the discovery of rilpivirine began with the prototype of the diarylpyrimidine dapivirine⁶². Molecular modeling analyses suggested that the *para* substitutions on the trisubstituted phenyl ring of compound **5** may improve the drug interactions with the conserved W229 region in the drug-binding pocket of HIV-1 RT, therefore benefiting its antiviral activity against HIV-1 mutants⁸². This hypothesis led to the introduction of a spacer group G between the trisubstituted phenyl ring and the cyano group in the 4-position of compound **6** (Fig. 5). A series of substitutions at the G position was subsequently evaluated, leading to the discovery of rilpivirine with the promising potency against single- and double-mutant HIV-1 strains *in vitro*⁸².

5.2. Antiviral activity, drug resistance, and pharmacokinetics

Rilpivirine exhibits broad-spectrum antiviral activities against viral strains from HIV-1 genotypes (A1, C, D, F1, G, H) and CRFs (AE, AG, BG) with the EC₅₀ values ranging from 0.13 to 0.44 nmol/L^{83,84}. Based on *in vitro* experiments, rilpivirine actively inhibits HIV-1 group O isolates (EC₅₀: 2.88–8.45 nmol/L)⁸³. According to the IAS-USA guideline, resistance mutations to rilpivirine include L100I, K101 E/P, E138 A/G/K/Q/R, V179L, Y181C/I/V, Y188L, H221Y, F227C, and M230I/L²⁵. Importantly, common NNRTI-associated resistance mutations (e.g., V106 and G190) do not decrease the sensitivity to rilpivirine⁸³.

In a phase 2a study of treatment-naïve patients ($n = 9$) who received rilpivirine 25 mg for 7 days, the pharmacokinetic parameters of rilpivirine included mean $C_{\max}$: 263 ng/mL, $T_{\max}$: 4 h, $t_{1/2}$: 34–55 h, and AUC_{24 h}: 3659 ± 885 ng h/mL⁸⁵. Rilpivirine is largely insoluble in water and oils, supporting its development as a nanosuspension⁸⁶. In HIV-infected adults who received the monthly injection of rilpivirine plus cabotegravir, the total drug concentration was 134 (83–187) ng/mL of rilpivirine and 3.02 (2.37–5.10) µg/mL of cabotegravir in plasma that were collected 7 (± 3) days post-injection⁸⁷.

5.3. Efficacy and safety of rilpivirine-based combination regimens

5.3.1. Rilpivirine 25 mg + TDF 300 mg + emtricitabine 200 mg (Complera®)

In August 2011, the FDA approved the regimen of RPV + TDF + FTC as the initial therapy for treatment-naïve patients and the maintaining therapy for virologically-suppressed patients.

According to the drug labeling, TDF + FTC + RPV alone is not recommended for HIV-1-infected adults with HBV, bodyweight <35 kg, or creatinine clearance <50 mL/min. The efficacy and safety of TDF + FTC + RPV were evaluated by at least three phase three studies (Table 4). When TDF + FTC + RPV was used as the initial therapy, 84.5% (625/740) of treatment-naïve patients achieved HIV-1 RNA <50 copies/mL at Week 48. When this regimen was applied as the maintaining therapy, 93.9% (294/313) of virologically-suppressed patients maintained HIV-1 RNA <50 copies/mL at Week 48. The drug-associated adverse events were reported in 11.8% (37/314) of patients who received TDF + FTC + RPV.

5.3.2. Rilpivirine 25 mg + dolutegravir 50 mg (Juluca®)

In November 2017, the two-drug complete regimen of RPV + DTG was approved as the maintaining therapy to treat virologically suppressed patients who had no history of treatment failure and no drug resistance profiles associated with RPV or DTG. The efficacy and safety of RPV + DTG were reported by one phase three study⁸⁸. The virological suppression of HIV-1 RNA <50 copies/mL at Week 48 was identified in 94.7% (486/513) of virologically suppressed patients (Table 4). The drug-associated adverse events were reported in 19% (97/513) of patients who received RPV + DTG.

5.3.3. Rilpivirine 300 mg/mL + cabotegravir 200 mg/mL (Cabenuva®)

In January 2021, the FDA approved the monthly intramuscular injection of rilpivirine (RPV) plus cabotegravir (CAB) extended-release injectable suspension for virologically-suppressed patients with no history of treatment failure and with no known resistance to RPV or CAB. According to the drug labeling, the tolerability of RPV and CAB should be assessed before the initiating therapy of RPV + CAB. Due to drug–drug interactions, the coadministration of RPV + CAB plus other drugs with a known risk of Torsade de Pointes should be cautious.

The efficacy and safety of the RPV + CAB injection was evaluated by one phase two study called LATTE-2 and three phase three clinical trials called FLAIR, ATLAS, and ATLA-2M (Table 4). By merging the data from the four studies above, the virological response of HIV-1 RNA <50 copies/mL at Week 48 was maintained in 93.1% (1144/1229) of virologically suppressed patients who received the monthly intramuscular injection of RPV + CAB. The drug-associated adverse events were reported in 28.3% (167/591) of patients who received RPV + CAB. The 96-week findings from the FLAIR study reaffirmed the durability of RPV + CAB in virologically suppressed adults⁸⁹. Ongoing clinical trials will evaluate the oral and long-acting injectable regimen of rilpivirine plus cabotegravir in virologically suppressed children and adolescents (NCT03497676), patients across European countries (NCT04399551), and patients treated with the RPV + CAB injection every 2 months (NCT03299049).

6. Doravirine (DOR, Pifeltro™)

Doravirine, also named MK-1439, is an FDA-approved NNRTI with the brand name Pifeltro™ (Table 1). According to the drug labeling, the oral use of doravirine 100 mg should be combined with other antiretroviral drugs for the treatment of HIV-1 infections in treatment-naïve adults.

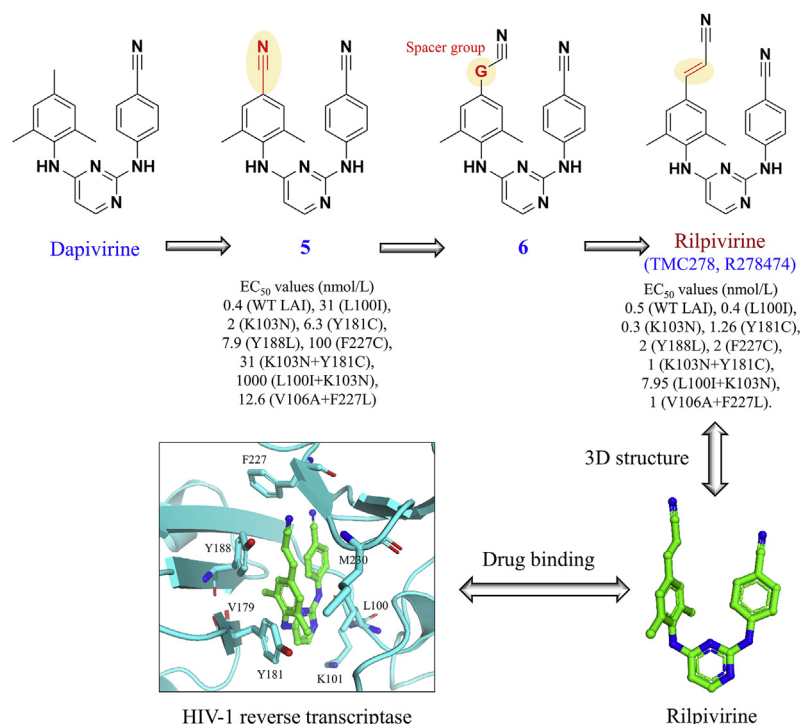


Figure 5 Discovery of rilpivirine based on the template of dapivirine. EC₅₀ values are obtained from Ref. 82. Drug binding pocket of rilpivirine is highlighted in HIV-1 reverse transcriptase (PDB code: 3MEG). Amino acid positions with known resistant residues are highlighted.

6.1. Drug discovery

As shown in Fig. 6, the discovery of doravirine began with the high-throughput screening that identified the lead tetrazole thioacetanilide inhibitor (compound 7) of HIV-1 RT using a cell-based assay⁹⁰. Subsequent optimizations were conducted by the substitution of the labile 2-nitroanilide with the more stable anilide structure (compound 8), thereby improving the oral bioavailability and decreasing the plasma clearance⁹⁰. However, these tetrazole thioacetanilide analogues showed suboptimal pharmacokinetic profiles ($t_{1/2} \leq 1.1$ h) in animal models⁹¹. Inspired by the structural comparisons of compounds 8 and 9, a series of diaryl ether analogues such as compound 10 was synthesized because the diaryl ether may improve the drug binding to inhibit HIV-1 mutant strains with better pharmacokinetic results⁹¹. Further analysis of structure–activity relationship led to the optimization of a diaryl ether/indazole compound 11 that inhibited HIV-1 wildtype and mutant strains with L100I, K103N, Y181C and/or G190 A/S (IC₅₀ range: 2.6–171 nmol/L)⁹¹.

Due to the low solubility and poor oral bioavailability of compound 11 (F : 3.3% in rats), a pyridine ring was considered to modify the phenyl ring of the indazole moiety in compound 11, leading to the development of compound 12 with better pharmacokinetic profiles (e.g., F : 52%)⁹². Next, the aryl ether core of compound 12 was replaced by a pyridone ring to increase the polarity and the binding affinity, thereby identifying the pyridone compound 13 with better antiviral activity against HIV-1 strains with the K103N + Y181C mutation⁹³. To improve the plasma half-life, the replacement of chlorine in compound 13 ($t_{1/2}$: 2 h) with the strong electron withdrawing CF₃ group led to compound 14, resulting in a longer elimination half-life ($t_{1/2}$: 7 h in rats)⁹⁴. The alkylated pyridone in compound 14 was modified to disrupt the strong donor–acceptor hydrogen bonds to improve aqueous

solubility, eventually leading to the discovery of doravirine with potent pharmacokinetic and antiviral activities against HIV-1 strains with the K103N + Y181C mutation⁹⁴.

6.2. Antiviral activity, drug resistance, and pharmacokinetics

Drug resistance to doravirine is mostly associated with three primary RT mutations (V106 A/M, Y188L) and 11 secondary RT mutations (V106T/I, Y188 C/H, G190E, P225H, F227 C/L/R, M230L, L234I)²⁵. *In vitro* assays supported the antiviral potency of doravirine against a broad range of different HIV-1 subtype strains and mutant viruses with K101E, K103N, E138K, Y181C, M184V/I, and/or G190A^{95–98}. Doravirine-associated resistance mutations were very rare (1.4%; 137/9764) according to a large-scale cohort of 9764 treatment-naïve patients from Greece, Italy, and France⁹⁹. A significant increase of doravirine-associated resistance mutations (V106 A/M, V108I, Y188 L/C/H, G190E, F227L, M230L, K103N + P225H) was observed in a large cohort of 6893 patients treated with other NNRTIs (delavirdine, efavirenz, nevirapine, etravirine, rilpivirine)¹⁰⁰.

Doravirine 100 mg/day can be orally taken with or without food and its clinical potency is unlikely altered by host factors (e.g., gender, age, race)¹⁰¹, severe renal impairment¹⁰², and modest hepatic impairment¹⁰³. In six healthy males, the pharmacokinetic profiles of single-dose doravirine 100 mg were estimated, including mean C_{max} : 1713.17 nmol/L, $C_{24\text{ h}}$: 593.43 nmol/L, AUC_{inf} : 38.32 $\mu\text{mol/L}\cdot\text{h}$, and $AUC_{24\text{ h}}$: 22.84 $\mu\text{mol/L}\cdot\text{h}$ ¹⁰⁴. In 12 healthy males who received the intravenous injection of a single-dose doravirine 100 μg , doravirine metabolites were mostly distributed in feces (84.1% of the dose recovered up to 168 h) and urine (2.2% of the dose recovered up to 96 h)¹⁰⁵.

Table 4 Clinical efficacy of approved anti-HIV regimens in phase 2/3 clinical trials.

Brand name	Prior treatment	Arm	Efficacy ^a	P-value	Study (phase)	Ref.
Complera®	Treatment-naïve	RPV + TDF + FTC	83% (287/346)	0.17	NCT00540449	195
		EFV + TDF + FTC	83% (285/344)		(phase 3)	
Juluca®	Treatment-naïve	RPV + TDF + FTC	86% (338/394)	0.12	NCT01309243	196
		EFV + TDF + FTC	82% (320/394)		(phase 3b)	
	Virologically suppressed	RPV + DTG	95% (486/513)	0.90	NCT02429791	88
		ART regimen	95% (485/511)		NCT02422797	
Cabenuva®	Virologically suppressed	RPV + CAB (monthly)	91% (105/115)	0.82	NCT02120352	197
		RPV + CAB (bimonthly)	92% (106/115)		(phase 2b)	
		ABC + 3TC + CAB	89% (50/56)			
	Virologically suppressed	RPV + CAB (monthly)	94% (265/283)	0.87	NCT02938520	198
		ABC + 3TC + DTG	93% (264/283)		(phase 3)	
	Virologically suppressed	RPV + CAB (monthly)	93% (285/308)	0.13	NCT02951052	199
		ART regimen	96% (294/308)		(phase 3)	
	Virologically suppressed	RPV + CAB (monthly)	93% (489/523)	0.61	NCT03299049	200
Delstrigo™	Treatment-naïve	RPV + CAB (bimonthly)	94% (492/522)		(phase 3b)	
		DOR + TDF + 3TC	84% (307/364)	0.24	NCT02403674	201
	Treatment-naïve	EFV + TDF + FTC	81% (294/364)		(phase 3)	
		DOR + TDF + 3TC	83% (278/333)	0.34	NCT02275780	202
		DOR + ABC + 3TC	86% (43/50)		(phase 3)	
		PI-based regimen	80% (306/383)			
	Virologically suppressed	DOR + TDF + 3TC	91% (406/447)	0.12	NCT02397096	203
		Baseline regimen	95% (211/223)		(phase 3)	

^aClinical efficacy was defined by HIV-1 RNA <50 copies/mL at Week 48.

According to the drug labeling, doravirine should not be co-administered with strong CYP3A4 inducers (*e.g.*, efavirenz, rifampin) that significantly decrease the plasma concentration of doravirine, resulting in the reduced virological response and possible drug resistance. However, doravirine is unlikely involved with other CYP enzymes (*e.g.*, CYP3A5, CYP1A2, CYP2B6, CYP2C8) and drug transporters (*e.g.*, organic anion transporting polypeptide 1B1)^{106,107}. Moreover, doravirine unlikely interacts with other drugs such as TDF, lamivudine, atorvastatin, methadone, elbasvir, grazoprevir, ledipasvir, and sofosbuvir^{108,109}.

6.3. Efficacy and safety of doravirine-based combination regimens

6.3.1. Doravirine 100 mg + TDF 300 mg + lamivudine 300 mg (Delstrigo™)

In August 2018, the FDA approved the once-daily fixed-dose regimen of TDF + 3TC + DOR for treatment-naïve adults infected with HIV-1 infections. According to the drug labeling, TDF + 3TC + DOR alone is not recommended for HIV-1-infected adults with HBV or creatinine clearance <50 mL/min. The clinical efficacy and safety of TDF + 3TC + DOR were evaluated by one phase two study¹¹⁰ and three phase three studies: DRIVE-AHEAD, DRIVE-FORWARD, and DRIVE-SHIFT (Table 4 and Table S1). In treatment-naïve patients who received TDF + 3TC + DOR for 48 weeks, the clinical efficacy of HIV-1 RNA <50 copies/mL at Week 48 was 83.5% (278/333) in the DRIVE-FORWARD study, 84.3% (307/364) in the DRIVE-AHEAD study, and 100% (8/8) in the single-arm, open-label, phase two study¹¹⁰. Drug-associated adverse events were observed in 31% (113/364) of patients receiving TDF + 3TC + DOR. The most common adverse events include dizziness, abnormal dreams, and nausea. In the DRIVE-SHIFT study of treatment-experienced patients with the virological suppression for ≥6 months, treatment switching from the baseline

regimen to TDF+3TC + DOR maintained the virological suppression of HIV-1 RNA <50 copies/mL at Week 48 in 90.8% (406/447) patients (Table 4). Ongoing clinical trials will report the potential application of doravirine 100 mg plus TAF 25 mg and FTC 200 mg to maintain the viral suppression in treatment-experienced adults (NCT04079452, NCT04097925).

6.3.2. Doravirine 100 mg + islatravir 0.75 mg + lamivudine 300 mg (novel regimen)

Doravirine was evaluated with the once-daily combination of lamivudine and islatravir for the treatment of previously untreated adults with HIV-1 infections based on a phase 2b, randomized, double-blind study¹¹¹. At Week 48, the virological suppression of HIV-1 RNA <50 copies/mL was observed in 90% (27/30) of participants receiving the regimen of doravirine 100 mg + islatravir 0.75 mg + lamivudine 300 mg¹¹¹. The clinical efficacy was 84% (26/31) in the control group of doravirine 100 mg + TDF 300 mg + lamivudine 300 mg¹¹¹. The ongoing phase three studies will evaluate doravirine 100 mg plus islatravir 0.75 mg in treatment-naïve adults (NCT04233879), virologically suppressed adults (NCT04223778, NCT04223791), pediatric patients (NCT04295772), and heavily treatment-experienced adults (NCT04233216).

7. Azvudine (FNC)

On 2021 July 21, azvudine was conditionally approved by the National Medical Products Administration in China. As shown in Fig. 7, the discovery of azvudine (2'-deoxy-2'-β-fluoro-4'-azidocytidine) began with the initial search of antiviral inhibitors that target HCV RNA-dependent RNA polymerase¹¹². In 2001, 2'-C-methylcytidine (compound 15, NM107) was discovered as a potent nucleoside inhibitor of Flaviviridae virus in cell cultures, but its further development was hampered by its low oral bioavailability¹¹³. Subsequent design of 4'-substituted cytidine analogues led to the synthesis of 4'-azidocytidine

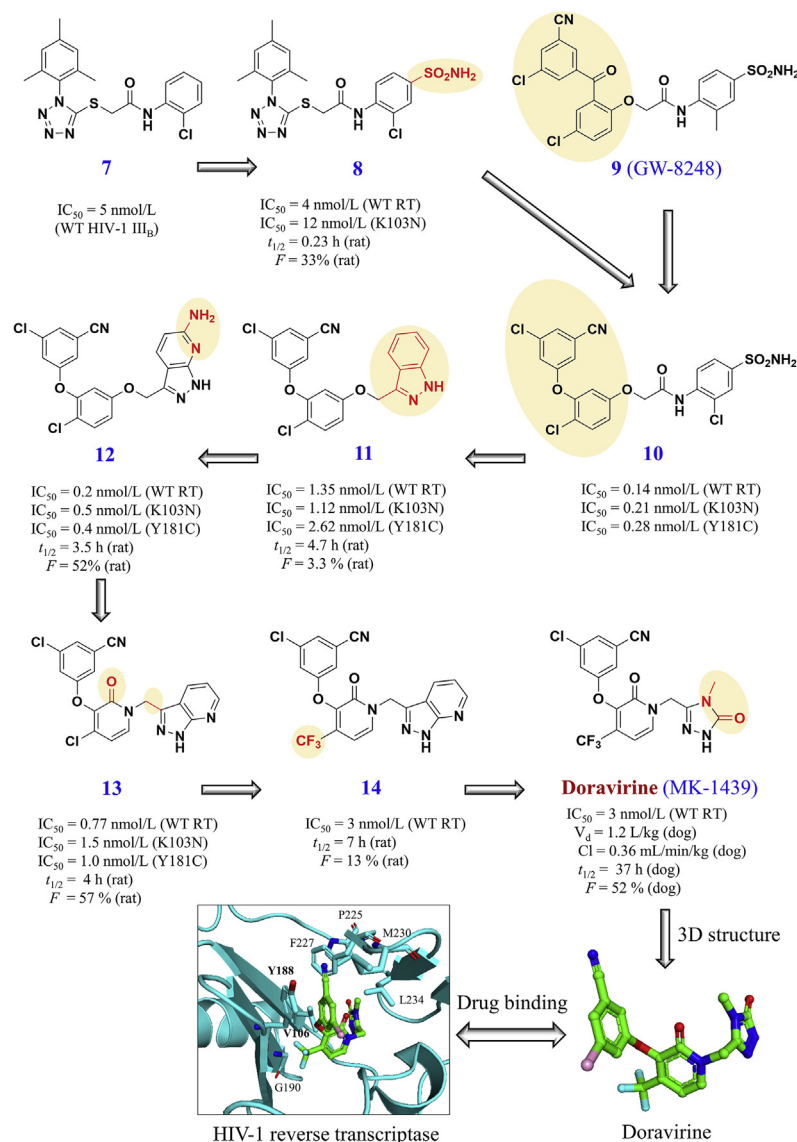


Figure 6 Discovery of doravirine based on a template of the lead tetrazole thioacetanilide inhibitor **7**. Drug binding pocket of rilpivirine is highlighted in HIV-1 reverse transcriptase (PDB code: 4NCG). Amino acid positions with known resistant residues are highlighted.

(compound **16**, R1479) to inhibit HCV chain elongation¹¹². With the inversion of the 2'-hydroxyl group, the 4'-azido-arabincytidine (compound **17**, RO-9187) showed better phosphorylation efficiency than compound **16**¹¹⁴. Because fluoronucleosides are often good substrates of RNA polymerases, the monofluoro and difluoro analogues of 4'-azidocytidine were subsequently designed, leading to the optimization of azvudine (RO-0622) and compound **18**¹¹³. In the HCV replicon system, azvudine (EC_{50} : 24 nmol/L) showed better antiviral potency than compound **18** (EC_{50} : 4020 nmol/L)¹¹³. Moreover, azvudine exhibited broad-spectrum antiviral activities against HIV¹¹⁵, HBV¹¹⁶, and SARS-CoV-2¹¹⁷, but not respiratory syncytial virus or influenza A virus¹¹⁸. In cell cultures, azvudine inhibited various strains from HIV-1 subtype B and recombinants B/C and CRF01_AE (EC_{50} : 0.03–6.92 nmol/L)¹¹⁵. Regarding its mechanisms of action, azvudine may inhibit HIV-1 RT and target the Vif-containing E3 ubiquitin ligase complex to block the Vif-induced degradation of APOBEC3G¹¹⁹. Moreover, azvudine is mainly metabolized by

CYP3A¹²⁰, and its absolute oral bioavailability is 82.7% in dog plasmas¹²¹.

A phase two study evaluated the safety and efficacy of azvudine (NCT04109183, completion date: 6 March 2019), but its clinical results have not been published as of today. An ongoing phase three trial will evaluate the efficacy and safety of azvudine 3 mg plus TDF 300 mg and EFV 200 mg in treatment-naïve patients infected with HIV-1 (NCT04303598). Future studies also need to address the advantage of azvudine over FDA-approved NRTIs such as TAF.

8. El sulfavirine (Elpida®)

El sulfavirine, the prodrug of the active NNRTI VM1500A (Fig. 8A), was approved by the Russian Ministry of Health in 2017. Although the discovery of el sulfavirine has not been documented yet in peer-reviewed literature, pharmacokinetic

features of elsofavirine 20 mg were characterized by a pre-clinical study¹²². In four healthy individuals treated with a single-dose of elsofavirine 20 mg, the VM-1500A metabolite was measured with a mean half-life $t_{1/2}$: 8.9 ± 2 days, $T_{\max}$: 0.15 ± 0.04 days, $C_{\max}$: 98 ± 74.3 ng/mL, and AUC_{inf} : 829 ± 507 ng·h/mL¹²². In HIV-infected patients receiving elsofavirine 20 mg for 7 days, the pharmacokinetic parameters of the VM-1500A metabolite included (i) $t_{1/2}$: 7.4 ± 1.6 days, (ii) $T_{\max}$: 6.3 ± 0.5 days, (iii) $C_{\max}$: 148 ± 8 ng/mL, and (iv) AUC_{inf} : 2123 ± 266 ng·h/mL¹²².

Elsufavirine 20 mg is now considered with the once-daily combination of TDF 300 mg and emtricitabine 200 mg. A phase 2b study evaluated the efficacy and safety of elsofavirine 20 mg *versus* efavirenz 600 mg in combination with TDF + FTC¹²³. In treatment-naïve patients, the efficacy of HIV-1 plasma RNA <50 copies/mL at Week 48 was higher in the elsofavirine arm (81%, 44/55) than in the elsofavirine arm (74%, 35/47)¹²³. A follow-up study reported the virological suppression of HIV RNA <50 copies/mL at week 96 in 83.9% (73/87) of treatment-naïve patients who continued the treatment of elsofavirine 20 mg plus TDF + FTC¹²⁴. The drug-associated adverse events were reported in 23.3% (14/60)¹²⁴. Ongoing trials will report the drug–drug interactions (NCT03709355) and the feasibility of the once-weekly administration of elsofavirine (NCT03730311).

9. Development of novel NRTIs and NNRTIs

A large number of novel NRTIs and NNRTIs have been reported in the literature^{6,125–128}, but only a few candidates are currently evaluated by clinical trials. Most candidates were discontinued because of their limited efficacy, unfavorable side effects, and/or high toxicity. For example, (i) lersivirine (UK-453061) was discontinued in 2013 because lersivirine showed no advantage over the existing NNRTIs; (ii) fosdevirine was halted in 2014 due to unexpected side effects such as seizures; (iii) censavudine was discontinued in 2015 due to the success of tenofovir alafenamide with better clinical efficacy and safety profiles¹²⁹; and (iv) apricitabine was discontinued in 2016 due to a lack of funding and interests from its sponsor. Here, we summarize the recent development of three NRTIs (islatravir, MK-8504, MK-8583) and three NNRTIs (MK-8507, IQP-0528, MIV-150) that have been evaluated by phase one clinical trials.

9.1. Islatravir (MK-8591, EFdA)

Islatravir, known as MK-8591 or EFdA (4'-ethynyl-2-fluoro-2'-deoxyadenosine), is a deoxyadenosine nucleoside analogue (Fig. 8B) that targets the reverse transcriptase of HIV-1, HIV-2, and SIV^{130–133}. Unlike the conventional chain-terminating NRTIs, islatravir acts as an effective immediate or delayed chain terminator depending on the sequence of the nucleic acid substrate¹³⁴. The biocatalytic cascade synthesis of islatravir could be efficiently made by five engineered enzymes with four auxiliary enzymes¹³⁵. Islatravir offers potent antiviral activities ($EC_{50} \leq 11$ nmol/L) against broad-spectrum viral isolates with multi-drug resistant mutations (e.g., K65R, Q151M, M184V)¹³³. The EC_{50} values of islatravir were less than 21 nmol/L against a variety of HIV-1 strains (e.g., HIV-1_{NL4-3}, HIV-1_{MDR}, HIV-1_{Ba-L})¹³⁶. In cell-based assays, an apparent synergism of islatravir plus rilpivirine may enhance the antiviral and

prophylactic activity against HIV-1¹³⁷. Pharmacokinetics features such as $C_{\max}$: 3.2 nmol/mL, $T_{\max}$: 0.5 h, and $AUC_{12\text{ h}}$: 9.5 nmol/h/mL were measured after the oral administration of islatravir in 25 humanized mice infected with HIV-1_{NL4-3}¹³⁸.

The once-weekly oral dosing of islatravir (1.3 or 0.43 mg/kg/week for 6 weeks) protected all male rhesus macaques ($n = 8$) from intrarectal challenges with SIV and HIV¹³⁹. A single subcutaneous administration of islatravir-eluting implants in rodents and rhesus macaques sustained the drug release at clinically relevant concentrations for at least 6 months¹⁴⁰. In a phase one study (NCT02217904), a single dose of islatravir 0.5 mg suppressed HIV-1 RNA levels by more than one log on Day 7 in 30 treatment-naïve adults¹⁴¹. The prophylaxis activity of islatravir is currently evaluated by phase three clinical studies of cisgender women (NCT04644029) and men and transgender women who have sex with men (NCT04652700).

9.2. MK-8504 and MK-8583

Similar to TAF, MK-8504 and MK-8583 are prodrugs of tenofovir that can be intracellularly converted to the active form of tenofovir-diphosphate¹⁴². Two phase one studies evaluated the single-dose pharmacokinetics, antiviral activities, and safety of MK-8504 (NCT03188523) and MK-8583 (NCT03552536). In HIV-negative patients, MK-8504 and MK-8583 were rapidly absorbed ($T_{\max}$: 0.5 h) and eliminated from plasma¹⁴². Both compounds were well-tolerated with only mild to moderate adverse events¹⁴². The efficacy and safety of MK-8503 and MK-8583 in once-weekly regimens are yet to be clarified.

9.3. MK-8507

As a novel NNRTI with two trifluoromethyl groups, MK-8507 could be a potential once-weekly oral regimen against HIV-1 infection¹⁴³. In healthy adults without HIV infection, a long terminal half-life of MK-8507 ($t_{1/2}$ range: 58–84 h) was observed across different doses and MK-8507 was unlikely a strong inducer of CYP3A4¹⁴⁴. In treatment-naïve patients infected with HIV-1, the single-dose monotherapy of MK-8507 (80 mg) reduced the plasma HIV-1 RNA by -1.5 (-1.8 to -1.19) log₁₀ copies/mL at 7-day post-dose and its plasma pharmacokinetics were measured as follows: terminal $t_{1/2}$: 69.4 h, $T_{\max}$: 2.0 h, $C_{\max}$: 2.11 μmol/L and $AUC_{0-\text{inf}}$: 129 μmol/L·h¹⁴⁵. *In vitro* IC_{50} of MK-8507 was 51.3 nmol/L based on the Monogram's PhenoSense assay¹⁴⁵. Moreover, MK-8507 maintained the *in vitro* potency against NNRTI resistant-associated variants such as K103N, Y181C and G190A¹⁴⁵. A phase 2 study (NCT04564547) was planned to evaluate the once-weekly two-drug regimen of MK-8507 (100, 200, 400 mg) plus islatravir 20 mg for the treatment of HIV-1 infection. On 18 November 2021, however, the development of MK-8507 was temporarily paused by Merck due to side effects (e.g., decreases in total lymphocyte and CD4⁺ T-cell counts) in the phase 2 study.

9.4. IQP-0528

IQP-0528 (SJ-3991) is a novel NNRTI that inhibits the viral reverse transcription and the viral entry of HIV-1 and HIV-2¹⁴⁶. A dual chamber vaginal/rectal microbicide gel called DuoGel™ inhibited the CCR5-tropic subtype B strain HIV-1_{US/92/727} with the EC_{50} values of 4.11–4.79 nmol/L¹⁴⁷. IQP-0528 gel is a

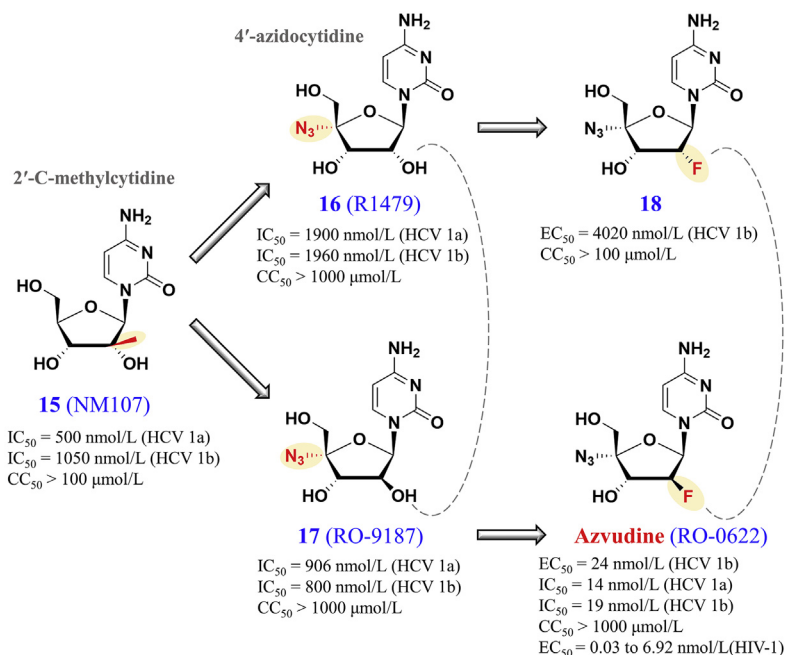


Figure 7 Discovery of azvudine. Synthesis pathways and *in vitro* results can be found in Refs. 112–115.

promising candidate to protect human polarized ectocervical tissues against HIV-1 infections, because of its dual mechanisms of action and its minimal toxicity to vaginal cells and natural flora¹⁴⁸. In a phase one study, seven HIV-negative individuals received one 10 mL rectal dose of 1% IQP-0528 gel, and no drug-associated adverse event was observed (NCT03082690). However, the application of IQP-0528 is limited because of its rapid clearance and its inability to penetrate vaginal tissues following the rectal dosing¹⁴⁹.

9.5. MIV-150

MIV-150 (Fig. 8B) is a novel NNRTI with antiviral activities against HIV-1 replications⁷³. Although the oral use of MIV-150 was discontinued because of its poor systemic absorption and rapid systemic clearance¹⁵⁰, a novel core-matrix intravaginal ring called PC-1005 was proposed in the combination of (i) MIV-150 plus zinc acetate to prevent HSV-2 infections; (ii) MIV-150 plus carrageenan to prevent the infections of HSV-2 and human

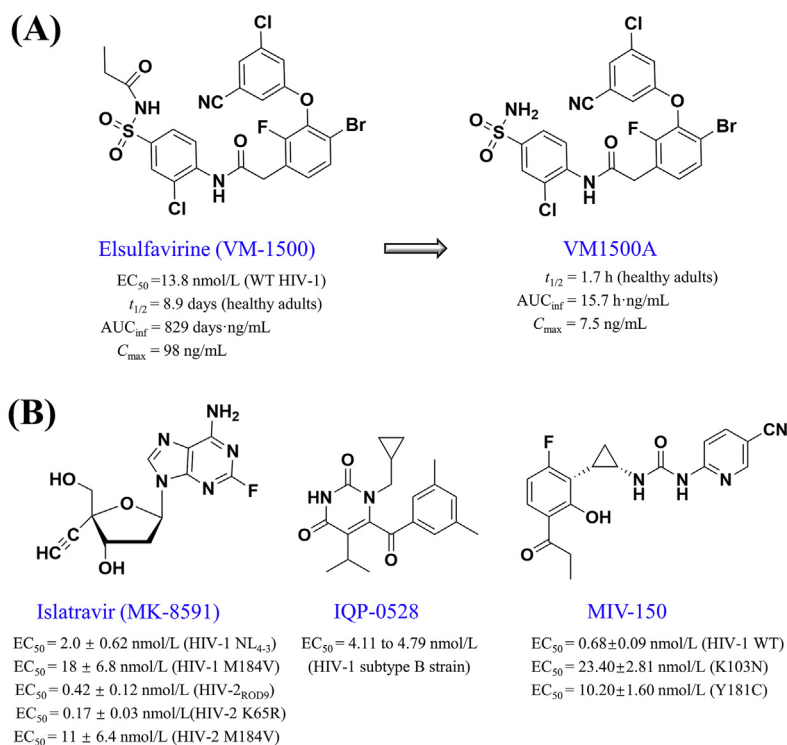


Figure 8 Chemical structures of el sulfavirine, islatravir, IQP-0528, and MIV-150.

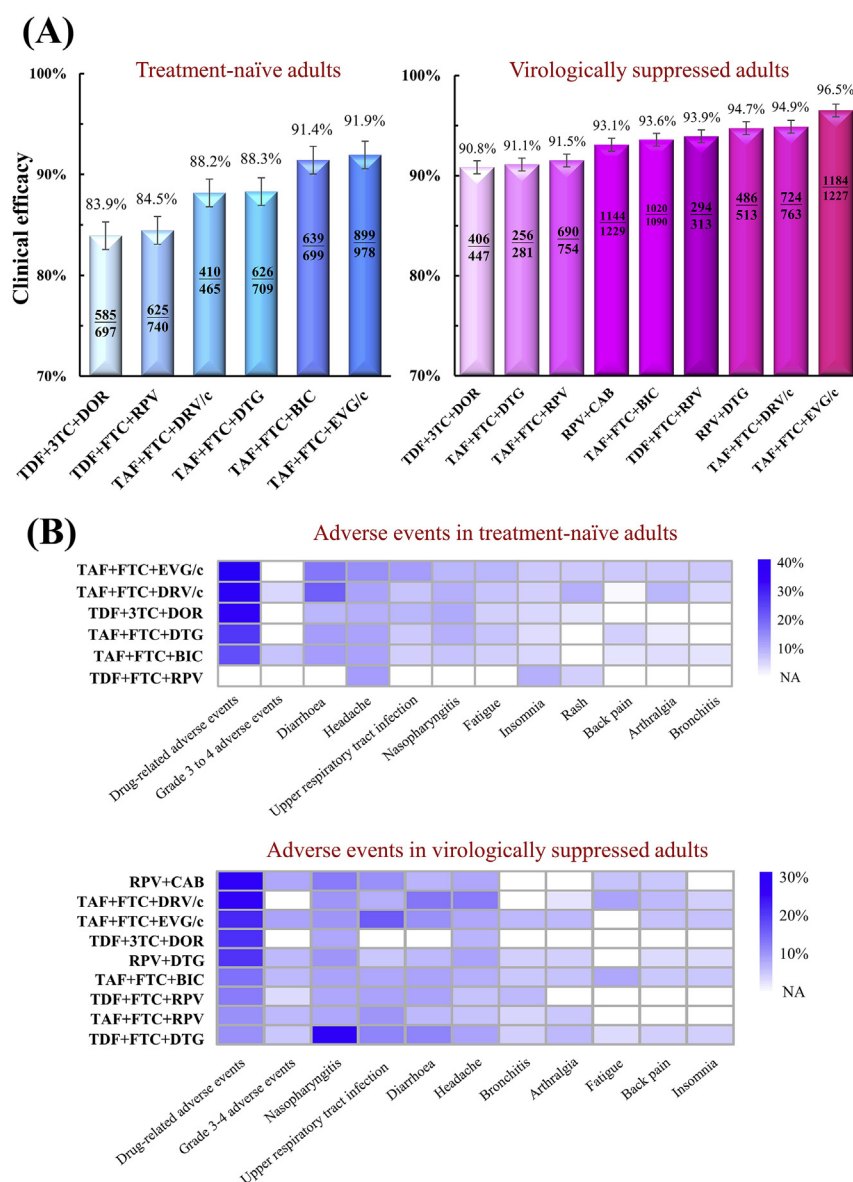


Figure 9 Efficacy and safety of approved antiretroviral regimens in the treatment of treatment-naïve and virologically-suppressed adults with HIV-1 infections. (A) Clinical efficacy was defined by the proportion of patients achieving HIV-1 RNA <50 copies/mL at Week 48. Data from Tables 3 and 4 were merged to demonstrate the clinical efficacy of approved antiretroviral regimens. (B) Proportions of adverse events during the treatment of approved antiretroviral regimens in randomized clinical trials. Missing data are indicated by white-colored cells. Original data are available in Table S1.

papillomavirus; and (iii) MIV-150 plus levonorgestrel to prevent the HIV-1, HSV-2, human papillomavirus, and unintended pregnancy¹⁵¹. In a phase one study (NCT02033109), the vaginal use of PC-1005 was well-tolerated with low systemic levels in sexually abstinent, HIV-negative women¹⁵². Another phase one study will evaluate PC-1005 as a rectal microbicide in HIV-negative adults with a history of consensual anal intercourse (NCT03408899).

10. Conclusions

In this study, we provide a comprehensive review of approved RT inhibitors and their combination regimens in the past decade. Although the development of each approved RT inhibitor had a different story, their drug discovery all requires coordinated multidisciplinary efforts from medicinal chemists,

virologists, pharmacists, crystallographers, and many others¹⁵³. Tenofovir alafenamide was discovered as the phenyl monoester isopropyl alaninyl phosphoramidate of tenofovir based on an unexpected observation of the mixture GS-7171, while its clinical approval was only granted 15 years later after its discovery. Although rilpivirine and dapivirine were both originated from the α -APA derivatives, rilpivirine was developed as once-daily oral tablets and dapivirine was applied as the vaginal ring application due to their different oral bioavailability and half-life time. Elvitegravir has been approved only in Russian, while its clinical advantage requires further investigations. Moreover, many drugs (e.g., doravirine) were developed with a series of structure-guided optimizations, thereby highlighting the importance of crystallography in antiviral drug discovery.

Our review summarized the clinical efficacy and safety of approved anti-HIV regimens based on randomized controlled trials. In clinical studies of HIV-1 pre-exposure prophylaxis, TAF + FTC and dapivirine vaginal ring significantly reduced the incidence rate of HIV infections (Table 2). To treat HIV-1 infections in treatment-naïve patients, the highest efficacy was observed in the initial therapy of TAF + FTC + EVG/c (91.9%, 899/978), followed by 3TC + DTG (91.5%, 655/716), TAF + FTC + BIC (91.4%, 639/699), ABC+3TC + DTG (90.1%, 657/729), TDF + FTC + EVG/c (89.5%, 1456/1626), TAF + FTC + DTG (88.3%, 626/709), TAF + FTC + DRV/c (88.2%, 410/465), TDF + FTC + RPV (84.5%, 625/740), and TDF+3TC + DOR (83.9%, 585/697) (Fig. 9A). Drug-associated adverse events of preceding regimens included diarrhea, headache, and nasopharyngitis (Fig. 9B). In the treatment of virologically suppressed patients with HIV-1 infections, the highest efficacy was observed in the maintaining therapy of TAF + FTC + EVG/c (96.5%, 1184/1227), followed by TAF + FTC + DRV/c (94.9%, 724/763), RPV + DTG (94.7%, 486/513), TDF + FTC + EVG/c (94.5%, 52/55), TDF + FTC + RPV (93.9%, 294/313), TAF + FTC + BIC (93.6%, 1020/1090), RPV + CAB (93.1%, 1144/1229), 3TC + DTG (93.0%, 384/413), TAF + FTC + RPV (91.5%, 690/754), TAF + FTC + DTG (91.1%, 256/281), TDF+3TC + DOR (90.8%, 406/447), and ABC+3TC + DTG (90.3%, 758/839) (Fig. 9A). The drug-associated adverse events of the preceding regimens include nasopharyngitis, diarrhea, and headache (Fig. 9B). Among these regimens, only two NRTI-free regimens (RPV + DTG, RPV + CAB) have been approved, but they are often used as maintaining therapies rather than initial therapies.

In clinical practice, once-daily fixed-dose regimens of two NRTIs (TAF + FTC, TDF + FTC, TDF+3TC) plus another drug (e.g., integrase inhibitors) have been widely applied as the standard of care for most patients infected with HIV-1 infections. Of note, TAF is now gradually replacing TDF as the backbone in the once-daily, fixed-dose, single-tablet regimens such as Odefsey®, Genvoya®, and Descovy® because TAF offers high potency and safety profiles in the life-long treatment of HIV infections¹⁵⁴. NNRTIs are now gradually replaced by HIV integrase inhibitors in the first-line combination regimens according to the IAS-USA and the EACS guidelines^{10,11}. First, NNRTIs are only limited to the treatment of HIV-1 but not HIV-2¹. Second, many NNRTIs have clinically significant drug–drug and drug–food interactions since they are the substrates (e.g., doravirine, rilpivirine) or inducers (e.g., efavirenz) of cytochrome P450 3A4 (CYP3A4)—an important oxidizing enzyme in humans. Third, NNRTIs usually have low genetic barriers to resistance and there is an increasing prevalence of NNRTI-associated transmitted drug resistance in treatment-naïve patients from North America, Latin America/Caribbean, Sub-Saharan Africa, South/Southeast Asia, and Upper-Income Asian Countries¹⁵⁵. Fourth, transmitted NNRTI resistance before first-line treatment is potentially associated with the long-term failure of first-line regimens containing integrase inhibitors such as dolutegravir¹⁵⁶. Fifth, NNRTIs may cause neuropsychiatric adverse effects and these drug-related adverse effects should be closely monitored in clinical practice¹⁵⁷.

Despite the success of many approved RT inhibitors, HIV strains evolve fast especially under the drug selective pressure^{158,159} and mutated residues near the drug-binding pocket of HIV RT may reduce the drug-target binding affinity⁵. To encounter emerging drug resistance mutations, many strategies

have been proposed to develop the next generation of RT inhibitors with better drug resistance profiles^{5,6,8,160}. (i) Multi-target strategy that designs novel RT inhibitors to target evolutionarily conserved binding pockets, divalent metal binding pockets, the substrate-envelope pocket, and/or the RNase H domain⁸. (ii) Conformation-based strategy that designs novel RT inhibitors with conformational flexibility to target ever-changing binding pockets with multiple drug positioning, covalent bonding, multivalent binding, and van der Waals forces^{5,6,8,161}. (iii) RT inhibitors without overlapping resistance profiles could be combined to act synergistically for the maximize suppression against diverse strains of the highly mutable HIV^{5,6,8}. Additionally, novel anti-retroviral agents such as lenacapavir (GS-6207) that targets the viral capsid¹⁶² may provide alternative options in the long-acting fixed-dose regimens.

HIV RT inhibitors can be repurposed for the treatment of other human diseases. (i) Three NRTIs (TAF, TDF, lamivudine) have also been approved and recommended for the treatment of HBV infections^{163–165}. (ii) Anti-inflammasome NRTIs such as lamivudine may improve insulin sensitivity and prevent the development of type two diabetes¹⁶⁶. (iii) NRTIs may reduce the risk of age-related macular degeneration that causes blindness in humans¹⁶⁷. (iv) NRTIs such as lamivudine might be repurposed as anti-cancer drugs¹⁶⁸. (v) HIV RT inhibitors have been initially proposed to treat coronavirus disease 2019 (COVID-19)^{168,169}, which has caused a global pandemic with severe morbidity and mortality since the end of 2019^{170–174}. As of today, no anti-HIV drug has been approved for the treatment of COVID-19¹⁷⁵, whereas TDF + FTC could reduce the risk of COVID-19 and hospitalization in HIV-positive patients¹⁷⁶.

Future development of antiretroviral regimens could be conceived as follows: (i) the development of novel inhibitors with better potency and safety profiles, and longer half-life for long-acting application; (ii) new applications of approved inhibitors in two-drug regimens, long-acting formulations, and once-daily, fixed-dose, single-tablet regimens; and (iii) weekly or monthly applications of novel regimens in the HIV pre-exposure prophylaxis. Novel extended-release delivery devices such as vaginal rings, nanoformulations, and subcutaneous implants also provide new options to deliver antiretroviral drugs weekly, monthly, and maybe even yearly¹⁷⁷. In the foreseeable future, antiretroviral regimens will still play a significant role in saving the lives of millions of human beings worldwide.

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Author contributions

Guangdi Li and Yali Wang performed the literature search and data collection. Erik De Clercq conceived the concept of the study. Guangdi Li, Yali Wang and Erik De Clercq prepared and revised the manuscript. All authors read and approved the final paper.

Conflicts of interest

Prof. Dr. Erik De Clercq was the co-inventor of tenofovir. We declare no competing interests.

Appendix A. Supporting information

Supporting information to this article can be found online at <https://doi.org/10.1016/j.apsb.2021.11.009>.

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