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Commentary

Tenofovir alafenamide (TAF) as the successor of tenofovir disoproxil fumarate (TDF)

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ABSTRACT

Tenofovir alafenamide (TAF) can be considered a new prodrug of tenofovir (TFV), as successor of tenofovir disoproxil fumarate (TDF). It is *in vivo* as potent against human immunodeficiency virus (HIV) at a 30-fold lower dose (10 mg) than TDF (300 mg). TAF has been approved in November 2015 (in the US and EU), as a single-tablet regimen (STR) containing 150 mg elvitegravir (E), 150 mg cobicistat (C), 200 mg emtricitabine [(–)FTC] (F) and 10 mg TAF, marketed as Genvoya®, on 01 March 2016 in the US as an STR containing 25 mg rilpivirine (R), 200 mg F and 25 mg TAF, marketed as Odefsey®, and on 4 April 2016 in the US, as an STR containing 200 mg F and 25 mg TAF, marketed as Descovy®, for the treatment of HIV infections. STR combinations containing TAF and emtricitabine could be paired with a range of third agents, for example, darunavir and cobicistat. TAF has a much lower risk of kidney toxicity or bone density changes than TDF, and also offers long-term potential in the pre-exposure prophylaxis (PrEP) of HIV infections. TAF is specifically accumulated in lymphatic tissue, and in the liver, and hence also holds great potential for the treatment of hepatitis B virus (HBV) infections. Akin to TDF, TAF is converted intracellularly to TFV. Its active diphosphate metabolite (TFVpp) is targeted at the RNA-dependent DNA polymerase (reverse transcriptase) of either HIV or HBV.

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1. Introduction

Tenofovir (Fig. 1) was first described in 1993 under the name of (R)-PMPA [(R)-9-(2-phosphonylmethoxypropyl)adenine], as an anti-HIV agent, together with its 2,6-diaminopurine derivative (R)-PMPDAP [1]. That (R)-PMPA was far more effective than azidothymidine (AZT) as an antiretroviral agent was ascertained in 1995 by Tsai et al., who demonstrated that (R)-PMPA when infected shortly (within a few days) before or after SIV (simian immunodeficiency virus) infection in rhesus macacus monkeys completely suppressed the infection [2], an observation that later on would prove the basis for the pre-exposure prophylaxis (PrEP) of HIV infections.

To ensure the oral bioavailability of (R)-PMPA, its bis(isopropyl oxycarbonyloxymethyl) ester was conceived [3,4], and this diester of tenofovir would then be formulated with fumarate, as TDF (tenofovir disoproxil fumarate) (Fig. 1). It would be marketed at Viread® in 2001 for the treatment of HIV infections, and in 2008 for the treatment of hepatitis B virus (HBV) infections.

In combination with emtricitabine (Emtriva®, (–)FTC) (Fig. 1), TDF was approved by the US FDA in 2004 for the treatment of HIV infections, and in 2012 for the prophylaxis of HIV infections. The combination of TDF with (–)FTC has been marketed as Truvada®. This single-tablet regimen (STR) was further extended in 2006 to efavirenz (Sustiva®) (Fig. 1) in an STR, now called Atripla®; in 2011 to rilpivirine (Edurant®) (Fig. 1), in an STR, now called Complera® (US)/Eviplera® (EU), and in 2012 to elvitegravir

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(Fig. 1) and cobicistat (Fig. 1), resulting in a quadruple drug regimen, now termed Stribild®.

The route of tenofovir to Complera®/Eviplera® and Stribild® has been described in previous review articles [5,6]. Here, I describe the advent of TAF (tenofovir alafenamide) and its potential for the treatment and prevention of HIV infections. Ray et al. [7] stated in their conclusion that TAF would be a promising new therapeutic agent for the lifelong treatment of HIV (infections).

2. Tenofovir alafenamide (TAF): synthesis and anti-HIV properties

The original synthesis, stereochemical assignment, X-ray crystallography of the diastereomerically pure tenofovir alafenamide (Fig. 1), originally named GS-7340, was described by Chapman et al. [8,9] and Eisenberg et al. [10]. GS-7340 (9-[(R)-2-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphoryl]methoxy]propyl]adenine) can be considered as a phenylmonophosphoramidate prodrug of (R)-PMPA [10].

Its unique properties as a prodrug of tenofovir, that was preferentially taken up by the lymphatic tissue, were highlighted by Lee et al. in 2005 [11]. Topical administration of a low dose of GS-7340 was quoted by Van Rompay et al. [12] as not having detectable prophylactic efficacy against SIV in infant macaques. It would take another 7 years before Ruane et al. would report that TAF, as compared to TDF, demonstrated higher antiviral potency, higher peripheral blood mononuclear cell (PBMC) intracellular tenofovir diphosphate (TFVpp) levels and lower plasma tenofovir concentrations, at approximately 1/10 of the dose [13].

That TAF would be superior to TDF, both in efficacy and safety, was first demonstrated in a phase 2 study with STR regimens, where the combinations of E/C/F/TAF and E/C/F/TDF (E being elvitegravir, C, cobicistat and F (–)FTC, respectively) effected similar rates of virologic suppression (<50 HIV copies per ml at week 48 in 88.4% and 87.9% for E/C/F/TAF and E/C/F/TDF, respectively), but patients on E/C/F/TAF experienced significantly smaller changes in estimated creatinine clearance, renal tubular proteinuria, and bone mineral density [14]. The doses used in the study of Sax et al. [14] were 10 mg for TAF versus 300 mg for TDF, 150 mg for elvitegravir (E), 150 mg for cobicistat (C) and 200 mg for emtricitabine [(–)FTC] (F). In another phase I/II study [15], monotherapy with TAF (40 or 120 mg once daily for 14 days in HIV-1-infected adults) proved more antivirally effective than TDF (300 mg).

3. Metabolism of TAF

A critical step in the intracellular metabolic activation of TAF in PBMCs is mediated by the lysosomal protease cathepsin A (Cat A), which converts TAF to tenofovir-alanine (TFV-Ala) [16]. In liver cells, the conversion of TAF to TFV-Ala is driven by the Ces 1 carboxylesterase [16]. Intracellular metabolism of TAF to TDFV, and the concomitant anti-HIV potency, occurs across PBMCs from variable gender, age and ethnicity [17]. After TAF has been converted by Cat A (PBMCs) or Ces 1 (liver cells) to TFV-Ala, the latter is hydrolyzed to TFV by acidic hydrolysis in lysosomes (Fig. 2) [18]. TFV is then phosphorylated by adenylate kinases (i.e. AMP kinase) to TFVp and by NDP kinases to TFVpp [18]. TFVpp is the final active metabolite of TAF, as it is for TDF as well.

4. TAF for the treatment of chronic HBV infection

TAF was found to decrease HBV DNA levels (at week 4) to a similar extent, comparable to that of TDF (300 mg) at all doses evaluated (8, 25, 40 or 120 mg) [19]. The dose of 25 mg was selected for the clinical development of TAF for the treatment of HBV infection

[19]. At this dose TAF has been validated by the European Medicines Agency for marketing for the treatment of chronic hepatitis B [press release of 25 February 2016; <http://www.businesswire.com/news/home/20160225006065/en/>]. TAF is efficiently taken up by the liver (in dogs) [20]. In fact, higher liver TFVpp levels were observed after administration of TAF to dogs than those observed after administration of TDF [21]. Thus, TAF may supersede TDF in the treatment of chronic HBV infections.

Based on the 48-week results from 2 phase 3 studies of TAF (25 mg daily) in both HBeAg-negative and HBeAg-positive patients with chronic HBV infection a new drug application has been submitted to the US FDA [press release of 15 April 2016; <http://www.businesswire.com/news/home/20160415005142/en/>].

5. Subdermal implant of TAF for HIV prophylaxis

TAF holds particular promise as subdermal implant for pre-exposure prophylaxis (PrEP), due to the sustained release and prolonged activity of TAF [22]. A device has been designed for this purpose [22]. In dogs, the TAF implants maintained sustained plasma levels of TAF and TFV for 40 days [22], and the molar TAF:TFV plasma concentration ratio remained unchanged during the whole period, suggesting that TAF is stable in the implant for 40 days. The question was raised whether a 1-year subdermal TAF implant is feasible [22]? Equally relevant would be the question whether for PrEP the subdermal implant should contain only TAF or TAF combined with, for example, (–)FTC. The broad-spectrum anti-HIV activity of TAF offers a precious advantage for its prophylactic use: TAF has proven to be effective against all HIV-1 group M subtypes A, B, C, D, E, F, G, as well as group N, group O and HIV-2 isolates (Fig. 3) [23].

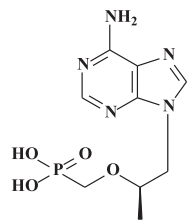
6. Resistance to TAF

Resistance, not exceeding an EC₅₀ (50% effective concentration) fold change of 5.4 toward TAF has been noted only with the multidrug resistant isolate MDR-769 (Table 1). Much higher resistance (i.e. >89-fold) was observed with other anti-HIV agents (i.e. AZT) [24]. Preliminary clinical observations (phase 2 and phase 3 studies) have shown a very low incidence of TFV genotype resistance in treatment-naïve patients treated either with TAF (one in 978) or TDF (three in 925) (unpublished data) [24]. In patients treated with the combination of elvitegravir, cobicistat, emtricitabine with either TAF or TDF, emergence of drug resistance did not exceed 1% [25].

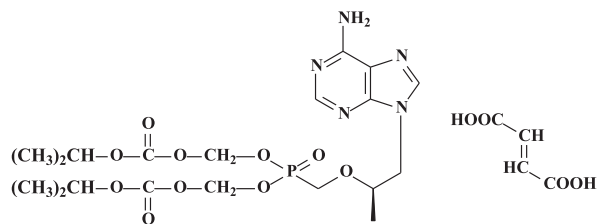
7. Combination of E/C/F/TAF

The combination of elvitegravir (E, 150 mg), cobicistat (C, 150 mg), emtricitabine [F, (–)FTC, 200 mg] and TAF (10 mg) has been marketed as Genvoya® after it had been approved in both the US (on 5 November 2015) and EU (on 23 November 2015), based on 2 phase 3 studies (no 104 and 111): they had indicated that 92.4% of patients treated for 48 weeks with Genvoya, as compared to 90.4% of patients treated for 48 weeks with Stribild had HIV-1 RNA levels less than 50 copies/mL [26]. As compared to E/C/F/TDF, E/C/F/TAF was consistently found to be associated with significant improvement of renal function and urinary markers of proximal tubulopathy, and significant improvement of bone mineral density [27].

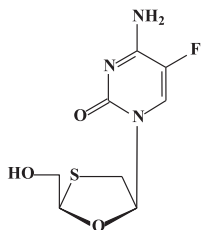
At 96 weeks, 86.6% of patients taking Genvoya and 85.2% of patients taking Stribild achieved HIV-1 RNA levels less than 50 copies/mL [28]. The rate of virologic success between the two regimens was similar across patient subgroups (age, gender, race, baseline HIV-1 RNA level and baseline CD4 cell count) (data



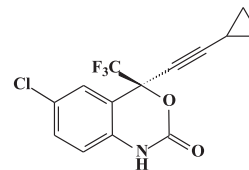
Tenofovir [(R)-PMPA]



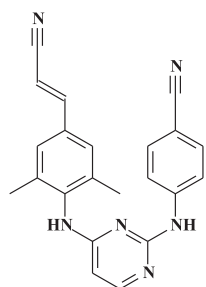
Tenofovir disoproxil fumarate (TDF), Viread®



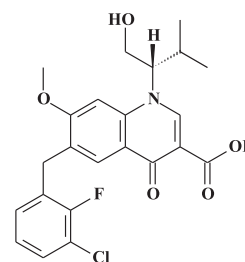
Emtricitabine [(-)FTC], Emtriva®



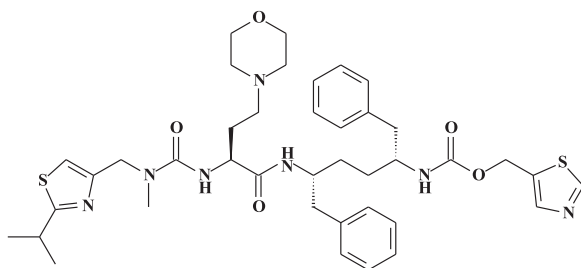
Efavirenz, Sustiva®



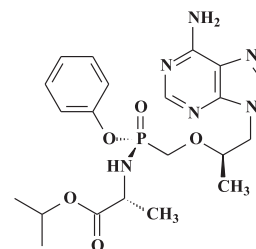
Rilpivirine, Edurant®



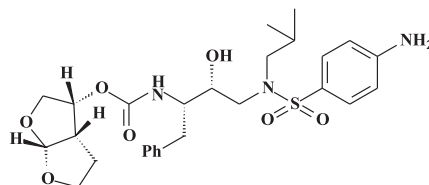
Elvitegravir



Cobicistat



Tenofovir alafenamide (TAF), GS-7340



Darunavir, Prezista®

Fig. 1. Structural formulae.

presented at the 15th European AIDS Conference (EACS) in Barcelona (session BD 01). To examine kidney function, multiple laboratory tests of renal and tubular function were conducted, all of

which statistically favored Genvoya over Stribild. This included a statistically significant difference in the median change in estimated glomerular filtration rate (eGFR) from baseline to week

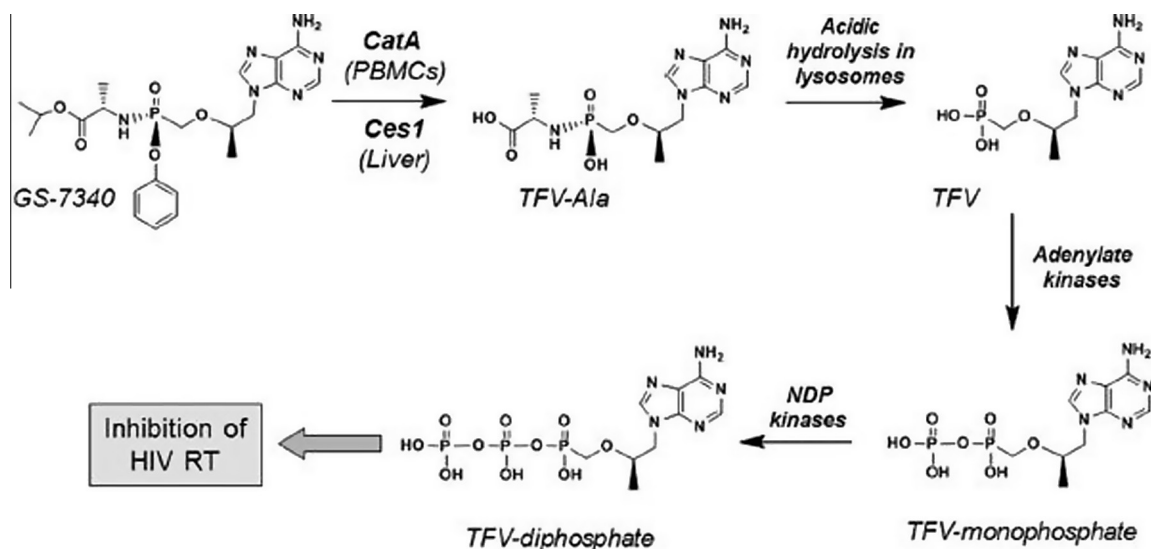


Fig. 2. Intracellular metabolism of TAF. Data taken from Birkus et al. [18].

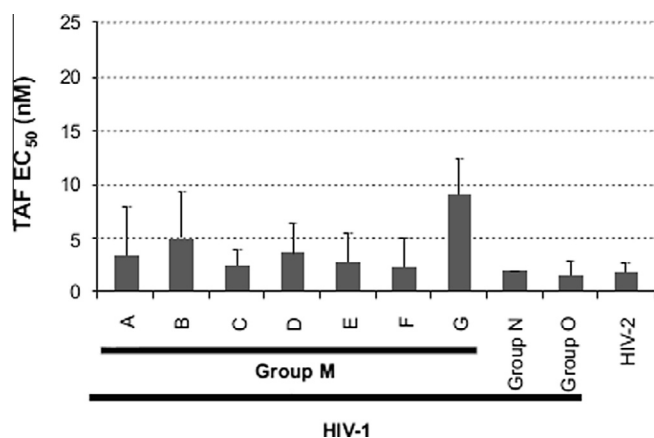


Fig. 3. Broad-spectrum anti-HIV activity of TAF. Data taken from Callebaut et al. [23].

96, favoring Genvoya (−2.0 mL/min for Genvoya versus −7.5 mL/min for Stribild). Patients had smaller declines in bone mineral density (BMD) compared to patients taking Stribild (spine: −0.96 versus −2.79; hip: −0.67 versus −3.28). There were no reports of proximal renal tubulopathy (including Fanconi Syndrome) in the Genvoya arm while there were 2 cases in the Stribild arm [press

release of 22 October 2015; <http://www.businesswire.com/news/home/20151022005728/en/>].

8. Combination of R/F/TAF

Gilead's second TAF-based single tablet regimen (Odefsey[®], containing 200 mg emtricitabine (F), 25 mg rilpivirine (R) and 25 mg TAF, or R/F/TAF, was approved by the FDA on 01 March 2016 [press release of 1 March 2016; <http://www.businesswire.com/news/home/20160301006840/en/>]. Odefsey is indicated as a complete regimen for the treatment of HIV-1 infection in patients 12 years of age, and older who have no antiretroviral treatment history and HIV-1 RNA levels less than or equal to 100,000 copies per ml. Odefsey is also indicated as replacement for a stable antiretroviral regimen in those who are virologically suppressed (HIV-1 RNA less than 50 copies per ml) for at least six months with no history of treatment failure and no known substitutions associated with resistance to the individual components of Odefsey.

9. Combination of D/C/F/TAF

The combination of D/C/F/TAF [D standing for darunavir (Fig. 1)] in a single-tablet regimen (STR) offers another promising option for initial HIV treatment, due to the high barrier of resistance to daru-

Table 1
TAF activities against drug-resistant primary HIV-1 isolates in PBMCs.

Isolate ID	Resistance class(es)	Resistance-associated amino acid substitution(s) ^b	EC ₅₀ fold change ^a						
			TAF	AZT	NVP	IDV	T20	RAL	EVG
A-17	NNRTI-R	RT: K103N Y181C	1.7	0.7	>380	–	0.2	–	–
1064-52	PI-R	RT: D67N PR: I54V V82F L90M	0.5	1.0	–	39.3	0.4	–	–
52-52	PI-R	PR: M46I I54V V82T	0.4	0.4	–	15.2	0.2	–	–
8070_1	INSTI-R	IN: G140S Y143H Q148H	0.2	0.2	–	–	–	250	222
4736_4	INSTI-R	IN: E92Q N155H	0.1	0.2	–	–	–	18.9	101
5705-72	NRTI-R, NNRTI-R	RT: D67N K70R K103N M184V K219E	2.1	33.1	279	–	0.6	–	–
MDR-769	NRTI-R, PI-R	RT: M41L A62V K65R D67N V75I F116Y Q151M L210W T215Y PR: M46L I54V V82A I84V L90M	5.4	>89	–	210	0.7	–	–

^a The fold changes calculated from the average EC₅₀ across wild-type isolates were as follows: 3.4 nM (TAF), 11.2 nM (AZT), 25.1 nM (NVP), 12.0 nM (IDV), 39.4 nM (T20), 3.1 nM (RAL), and 1.0 nM (EVG). –, not tested.

^b IN, integrase; PR, protease.

navir, and the improved long-term renal and bone safety of TAF as compared to TDF [29]. The interpretation of Bernardino et al. [30] concerning a treatment regimen containing darunavir, raltegravir or tenofovir that “patients at high risk of osteopenia or osteoporosis are not suitable for NtRTIs such as abacavir or tenofovir alafenamide” is erroneous and misleading: first, abacavir is a nucleoside analog (NRTI) and should not be called a nucleotide analog (NtRTI), second, the loss of bone mineral density was seen with TDF, not TAF.

10. Switching from TDF to TAF

What seems to be justified in the treatment of HIV infection is switching from a TDF-containing to a TAF-containing regimen, as the latter was non-inferior for maintenance of viral suppression and led to improved bone mineral density and renal function [31]. In fact, switching from E/C/F/TDF to E/C/F/TAF in HIV-positive patients with mild or moderate renal impairment, can be perfectly well rationalized, as it significantly improved proteinuria (albuminuria) and bone mineral density [32].

Patients and providers have long been averse to changing HIV treatment regimens [33], essentially based on the principle of “not changing a winning team”. However, the risk of long-term toxicity [34] and the cost-effectiveness of long-acting antiretroviral therapy [35] justify switching from TDF to TAF, as part of the newer ART combination of E/C/F/TAF.

11. TAF lifelong?

Wyatt and Baeten [36] postulated that TAF might signal yet another evolution in treatment, i.e. toward regimens designed for lifelong use, achieving maximum adherence and minimum toxicity. This would require the switch from TDF to TAF, in view of the kidney disease risk [37] and bone mineral density changes [38] associated with TDF.

12. TAF for PrEP

Besides the subdermal implant of TAF [22], pre-exposure prophylaxis of TAF could be successful for HIV prevention, when given orally [36], as has been demonstrated previously for TDF (in combination with emtricitabine [39,40]). TDF-based pre-exposure prophylaxis has been associated with some changes in glomerular kidney function among HIV-1-uninfected men and women [41]. Whether TAF would be an alternative substitute for TDF in the prevention of HIV infection remains an intriguing possibility.

13. Other combinations containing TAF

Besides the combination of E/C/F/TAF, which has already been approved (Genvoya®), and the combination of R/F/TAF, which has also been approved (Odefsey®), and the combination of D/C/F/TAF [29], other TAF-containing combinations are forthcoming, containing (–)FTC (emtricitabine) (F/TAF). Doses in these STR of F/TAF are 200 mg for emtricitabine and 25 or 10 mg for TAF. Dosing of TAF in F/TAF is dependent on the third agent: 200/10 mg with ritonavir-boosted protease inhibitors (darunavir, atazanavir and lopinavir) and 200/25 mg with unboosted third agents (raltegravir, dolutegravir, nevirapine, efavirenz, rilpivirine and maraviroc). All these F/TAF-based regimens demonstrated high rates of virologic suppression and improved renal and bone laboratory parameters compared to Truvada-based regimens [press release of 23 February 2016; <http://www.businesswire.com/news/home/20160223006478/en/>]. The fixed-dose combina-

tion of emtricitabine [(–)FTC (200 mg)] with TAF (25 mg) (Descovy®) has been approved by the US FDA on 4 April 2016. It can be paired with a range of third agents for the treatment of HIV-1 infection in adults and pediatric patients 12 years of age and older [press release of 4 April 2016; <http://www.businesswire.com/news/home/20160404005324/en/>].

Whereas TAF and (–)FTC have been marketed as Genvoya® in combination with elvitegravir and cobicistat the fixed-dose combination of emtricitabine (200 mg) with TAF (25 mg) (Descovy®) could also be combined with dolutegravir to yield an extremely potent treatment of HIV infections.

14. Conclusions

The highlights of TAF could be summarized as follows:

- (i) TAF is equally potent as an antiretrovirus agent at a 30-fold lower dose (10 mg as compared to 300 mg) than TDF. This reduces the risk of toxicity for TAF by a factor of 30-fold as well.
- (ii) In fact, TAF, as compared to TDF, has been shown to significantly reduce kidney (glomerular and tubular) disturbances and bone mineral (spine, hip) density changes.
- (iii) TAF should also offer a reduced risk of these kidney and bone side effects when used for PrEP, for which TDF in combination with (–)FTC (emtricitabine) (marketed as Truvada®) has been approved in the US since 2012.
- (iv) Akin to TDF, TAF leads to little or negligible emergence of drug resistance (to tenofovir), and this is likely to be further decreased given the lower dosage of TAF as compared to TDF.
- (v) TAF, due to its antiretrovirus potency, combined with its virtually complete safety, might form the cornerstone for long-term, or even lifelong use, in the treatment of HIV infections.
- (vi) TAF has so far been approved for clinical use in combination with elvitegravir, cobicistat and emtricitabine (marketed as Genvoya®), with emtricitabine (marketed as Descovy®) and with rilpivirine and emtricitabine (marketed as Odefsey®), and this use is likely to be extended in the future to other combinations, including, i.e., darunavir.
- (vii) TAF not only shows promise for the treatment and prevention of HIV infections, but also for the treatment of HBV infections.

Conflict of interest

The author is co-discoverer of tenofovir.

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