

Advances in Nucleotide Antiviral Development from Scientific Discovery to Clinical Applications: Tenofovir Disoproxil Fumarate for Hepatitis B

Lillian Lou

Nexus Development PA

Abstract

Exploration of naturally occurring chemical structures for medicinal uses has received significant interest in drug discovery and development research in the past few decades. None have had more success or products of greater clinical efficacy than synthetic analogs of nucleosides and nucleotides, especially as antiviral drugs. Nucleos(t)ide antivirals are synthetic analogs of the natural building blocks of DNA or RNA. This review focuses on the developmental path of tenofovir disoproxil fumarate (TDF), a prodrug of a nucleotide analog and its clinical applications as a first-line antiviral for chronic hepatitis B (CHB).

Tenofovir is a potent antiviral compound, but has poor oral availability. The disoproxil fumarate (DF) prodrug moiety greatly enhances intestinal absorption allowing it to become an oral medication. Tenofovir is activated intracellularly, and the incorporation into HBV DNA prevents further elongation thus terminating replication. In patients with CHB, TDF has demonstrated broad, potent and sustained virologic response. Maintenance of viral suppression for up to 5 years resulted in regression of fibrosis and cirrhosis. No tenofovir-resistant HBV variants have been detected in patients after long-term use. The efficacy and safety profiles reported from cohort studies of clinical practices were consistent with those observed in registration trials.

Continuous development includes a new oral prodrug, tenofovir alafenamide fumarate (TAF), which has enhanced delivery of tenofovir to target cells compared to TDF.

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Introduction

Nucleotide analog drugs have advantages over nucleosides as the former bypass the first of three phosphorylation steps, often the most challenging gate-keeping requirement for activation in cells. In addition, nucleotides are negatively

charged so they have extended intracellular retention compared to the nucleosides. The nucleotide analogs are not simple chemical derivatives of natural metabolites as the nucleobase portion of the molecules is not modified. However, the sugar and phosphate portions are drastically altered from the natural structures. Medicinal chemists in collaboration with virologists have invented biologically active molecules by considering the steric and electronic properties of nucleotides, and by synthesizing series of analogs for testing. The landmark publication in 1986 by Erik De Clercq and Antonin Holý suggested the tremendous potential for the acyclic nucleotide analogs such as tenofovir as antiretroviral agents.¹

TDF (Viread[®]; Gilead) has been approved for the treatment of HIV-1 infection in combination with other antiretrovirals since 2001, and for CHB as monotherapy since 2008. In the most recent updates, approval was extended to include pediatric HIV patients of 2 years and older, and CHB patients of 12 years and older.²⁻⁴ Currently, TDF is licensed for the treatment HIV/AIDS in 103 countries and for CHB in 56 countries worldwide. Although not the first nucleotide antiviral to be licensed, TDF is commonly recommended as a first-line drug for CHB.⁵⁻⁷ TDF-containing regimens especially those in fixed dose combination with emtricitabine (FTC) have become the most preferred backbone of first-line antiretroviral therapies.^{8,9} The fixed dose combination of FTC/TDF (Truvada[®]; Gilead) was recently granted approval by the US FDA for pre-exposure prophylaxis to reduce the risk of sexually acquired HIV-1 in high-risk adults.^{10,11} This present review focuses on the past and ongoing development of TDF and its clinical applications in patients with CHB. This manuscript is intended to provide an overview rather than a comprehensive review of the clinical literature on the treatment of CHB with TDF.

Delivery of tenofovir and activation in target cells

TDF is a prodrug of tenofovir, an acyclic nucleoside-phosphonate (nucleotide) analog of deoxyadenosine monophosphate (dAMP). Tenofovir has been shown to be a potent antiviral compound against HIV and HBV in cell cultures. However, it is poorly absorbed after oral administration presumably due to the negative charges on the phosphonyl group. Through structural optimization and laboratory testing of a series of chemical candidates, a novel DF prodrug moiety added to tenofovir was found to greatly enhance its intestinal absorption and uptake into target cells.¹²⁻¹⁴ In preclinical studies, oral administration of TDF delivered more tenofovir

Keywords: Nucleotide antiviral; Tenofovir; Prodrug; Hepatitis B.

Abbreviations: TDF, tenofovir disoproxil fumarate; CHB, chronic hepatitis B; DF, disoproxil fumarate; TAF, tenofovir alafenamide fumarate; dATP, deoxyadenosine triphosphate; LAM, lamivudine; ETV, Entecavir; HBIg, hepatitis B immunoglobulin.

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Correspondence to: Lillian Lou, PO Box 1345, Palo Alto, CA 94302, USA. Tel.: 650-245-7094, E-mail: LLou@NexusDevPA.com

than subcutaneous injection of tenofovir.¹⁵ The DF prodrug innovation and the long intracellular half-life (12–15 h in activated and ~50 h in resting lymphocytes)¹⁴ of tenofovir allowed it to become a once daily oral medication. A convenient dosing schedule is especially favorable for adherence in patients being treated for chronic infection. Once absorbed into the circulation, the prodrug moiety is rapidly removed by serum esterases to produce tenofovir. Both TDF and tenofovir can be taken up by target cells. Tenofovir is converted through cellular phosphorylation by host enzymes to the pharmacologically active metabolite tenofovir-diphosphate (DP), which is an analog of deoxyadenosine triphosphate (dATP) (Fig. 1),¹⁴ a natural precursor of DNA. Unlike human polymerases, HBV and other viral reverse transcriptase/polymerases (rt/pol) are less specific in selecting the correct nucleotide precursors for building DNA. This error-prone characteristic of the viral enzymes forms the basis of developing drug-resistance through mutations, but also makes it possible for certain synthetic analogs to substitute for the naturally existing precursors during viral replication. Because of this promiscuous property, HBV rt/pol recognizes tenofovir-DP, and allows it to compete with the cognate dATP and utilize tenofovir-DP as a building block in place of dATP for viral DNA synthesis. Once incorporated, tenofovir prevents the HBV DNA chain for further elongation because the structure of tenofovir renders it chemically impossible to incorporate subsequent incoming nucleotides to the growing viral DNA. This chain-terminating mechanism of action is highly effective in preventing viral replication. Tenofovir-DP binds to HBV rt/pol at the enzyme active site, whose structure is conserved between viral genotypes, and is reflected in the broad genotypic activity of TDF.¹⁶

TDF superseded Adefovir dipivoxil

Adefovir dipivoxil (ADV, Hepsera[®]; Gilead) was the first nucleotide analog approved for the treatment of CHB. In the initial stages of development, ADV was investigated for treatment of HIV/AIDS. Adefovir required a prodrug to improve oral absorption and chemical optimization resulted in ADV (dipivoxil ester). However, at the high doses investigated for

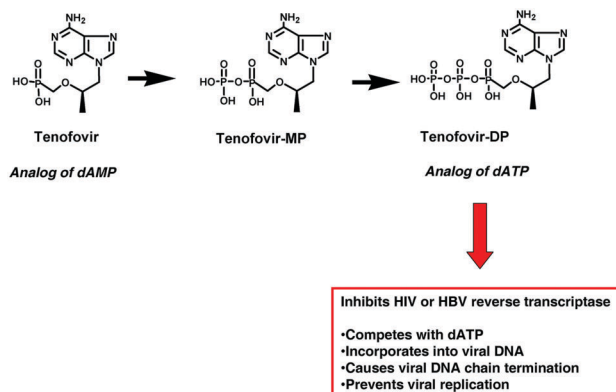


Fig. 1. A scheme depicting intracellular activation, and the antiviral mechanism of action of tenofovir. After transport into cells, tenofovir is phosphorylated in two steps by host nucleotide kinases to tenofovir-monophosphate (MP) and then tenofovir-DP, the active metabolite. Tenofovir inhibits viral replication by mechanisms indicated in the box.

HIV/AIDS, the particular prodrug group caused depletion of serum carnitine so it was necessary for patients to receive daily carnitine supplement concomitantly.¹⁷ More importantly, during this stage, unexpected renal safety concerns became apparent at doses of 60 mg and 120 mg in patients infected with HIV. These findings triggered the withdrawal of the New Drug Application to the US FDA in 1999. Kahn and colleagues reported that after 24 weeks of treatment, 120 mg of ADV taken daily was associated with delayed-onset of renal toxicity manifested by serum creatinine elevation or hypophosphatemia in HIV-infected patients.¹⁸ Kaplan-Meier analysis estimated 35% of patients would develop serum creatinine elevation of more than 0.5 mg/dL from baseline by week 48 and 50% by week 72.

ADV was found to be more effective for treating HBV than HIV infection, and was studied at lower doses. In phase 2 studies in patients with CHB, daily doses of 30 mg or above were more efficacious than 10 mg.¹⁹ However, the higher doses were associated with an increased frequency of adverse events and renal laboratory abnormalities, while the 10 mg dose showed a favorable safety profile.²⁰ ADV at 10 mg was approved in 2002 based on two pivotal studies demonstrating favorable efficacy, safety and resistance profile for the treatment of CHB.^{20,21} ADV had a better drug resistance profile than lamivudine (LAM); it was also found to be effective for treating patients who developed resistance to LAM and, therefore, fulfilled an important unmet need at that time.

ADV has now been superseded by TDF primarily because the latter has superior efficacy at the approved dose (300 mg), and better safety and resistance profiles. Of interest, tenofovir and adefovir are differentiated structurally by the critical placement of a pivotal methyl group, which defines virological and biological activities. Although the two are similar in antiviral potency in cell culture systems, they are distinct based on their recognition by the human mitochondrial DNA polymerase γ (pol- γ). In a biochemical analysis, tenofovir-DP was minimally incorporated by pol- γ into mitochondrial DNA (0.06% versus the natural nucleotide) while adefovir-diphosphate had 100 times more incorporation (6% versus the natural nucleotide).²² For comparison, LAM-triphosphate was incorporated at 0.13%. This propensity towards interference with mitochondrial DNA synthesis might be, in part, related to the associated, but low level, renal safety concerns of ADV during long-term treatment.²³ The structural differences of TDF compared to ADV provide the former with a more favorable efficacy, safety and resistance profile.

TDF in treatment-naïve CHB patients

Head-to-head comparison with ADV and multi-year follow up

TDF registration was based on two double-blind, randomized, phase 3 trials comparing TDF with ADV for 48 weeks. Viral suppression (reduction of HBV DNA to less than 400 copies/mL) occurred in more HBeAg-negative (93% vs. 63%) and HBeAg-positive patients (76% vs. 13%) receiving TDF than patients receiving ADV.¹⁶ Baseline viral load was lower in HBeAg-negative than HBeAg-positive patients reflecting the ability of the former to achieve viral suppression after 48 weeks. HBeAg seroconversion rates were 21% for TDF, and 18% for ADV. HBsAg loss after 48 weeks with TDF occurred in 3% of patients who were HBeAg-positive at baseline. This

rate is the highest among anti-HBV nucleos(t)ides, and was comparable to that of pegylated interferon. In contrast, no patients receiving ADV experienced HBsAg loss. Nausea was the only adverse event that consistently occurred more frequently in patients receiving TDF than ADV. Among 641 patients (426 TDF; 215 ADV), only 1 patient, who received ADV, had confirmed serum creatinine elevation of more than 0.5 mg/dL from baseline. No patient developed resistance to tenofovir, while 1 patient developed the signature substitution rtN236T resistance to adefovir, and 3 patients developed an rtA181T substitution in the background of an rtM204I (signature LAM-resistance) mutation.

Patients who completed the double-blind phase of the phase 3 trials continued with an open-label phase receiving TDF. Of the 641 patients who received randomized treatment, 585 (91%) entered the open-label phase and 489 (76%) completed 240 weeks. The multi-year follow-up design, and high rate of patient retention allowed evaluation of the safety and efficacy of TDF treatment in a large cohort of CHB patients over several years. The open-label phase is still ongoing and is planned to continue for a total of 9 years. The treatment with TDF from week 48 onward for all enrolled patients provided the two important observations: 1) sub-optimal response with ADV was rapidly circumvented with switching to TDF; 2) addition of FTC was not more efficacious than TDF alone in those patients who remained viremic, and took the option to add FTC after 72 weeks.²⁴ At week 240, 75% of patients in the intent-to-treat analysis had HBV less than 400 copies/mL. Of those patients on treatment, 98% had HBV DNA less than 400 copies/mL, and 49% and 40% had HBeAg loss and seroconversion, respectively. HBsAg loss and seroconversion was 10% and 8%, respectively in patients who were HBeAg-positive at baseline based on Kaplan-Meier estimation. Only one patient (<1%) who was HBeAg-negative at baseline had HBsAg seroconversion by week 240. TDF treatment was well tolerated. Nine (2%) patients experienced treatment-related serious adverse events over the 240 week duration. Eight (1%) discontinuations were due to adverse events. Laboratory abnormalities including those indicative of renal events were infrequent during the 240 week.²⁵

Resistance testing for HBV rt/pol gene sequence mutations was conducted on the small number of patients who were viremic at every 48 week time point after treatment initiation, experienced viral breakthrough during the study period or who had an early discontinuation while viremic. No mutations associated with resistance to tenofovir were detected during 240 weeks of treatment.²⁵ These data suggest that TDF's high barrier to genotypic resistance and potent antiviral efficacy may together result in durable viral suppression.

To date, the effectiveness of TDF in treating CHB patients in everyday clinical practice has been reported in four real-life cohorts in Europe.²⁶⁻²⁸ High rate of durable viral suppression, absence of drug resistance, and favorable tolerability were reported, and were consistent with the results of the registration trials.

Clinical benefit of long-term viral suppression

In small subsets of patients, it has previously been reported that reversal of fibrosis and cirrhosis occurred after prolonged treatment with anti-HBV agents. In the TDF phase 3 studies, 348 patients had liver biopsy results at both baseline and

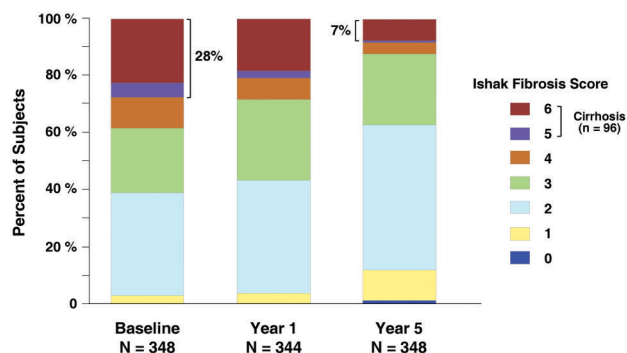


Fig. 2. Regression of liver fibrosis over 5 years of treatment with TDF. Distribution of Ishak fibrosis scores is shown for 348 patients with baseline and year 5 data, and 344 with data for all three time points.²⁵

week 240. Marcellin and colleagues reported that 304 (87%) of the 348 patients had histological improvement, and 176 (51%) had regression of fibrosis at week 240 ($P < 0.0001$). Fig. 2 shows the time course of histologic improvement over 5 years.²⁵

At baseline, 96 (28%) of 348 patients had cirrhosis (Ishak score 5 or above). At week 240, 71 (74%) had a reduction in fibrosis, and no longer had cirrhosis. All, but one had a reduction of Ishak scores by 2 units or more. Three of 252 patients who had no cirrhosis at baseline progressed to cirrhosis at year 5.

Nearly all patients who participated in the histology analysis achieved viral suppression. This significant histologic improvement observed in such large sample size with 5 years of TDF treatment definitively supports the notion that long-term viral suppression should be the treatment goal for anti-HBV therapeutics.

TDF in CHB patients previously treated with nucleos(t)ides

Effectiveness of TDF for the treatment of LAM-resistant hepatitis B was recognized in HIV/HBV co-infection before regulatory approval was granted for CHB.²⁹⁻³¹ Similar to treatment-naïve patients, TDF has higher antiviral activity for treating LAM-resistant hepatitis B than ADV.³²⁻³⁴

The nucleoside antivirals LAM and telbivudine (LdT) have high propensities to select for HBV rt/pol variants highly resistant to the drugs. Some of the most common substitutions resistant to LAM, rtM204V and rtL180M, are also resistant to LdT. Entecavir (ETV) has a high barrier for developing genotypic resistance, except if LAM-resistant substitutions are pre-existing, such as in patients who failed LAM treatment or were infected with LAM-resistant HBV. The LAM-resistant substitutions can serve as a trigger, lowering ETV's barrier, to readily select for ETV resistance. Fortunately, HBV variants resistant to LAM, LdT and ETV are all fully susceptible to tenofovir.^{6,35}

Fung and colleagues recently reported a prospective, randomized, double-blind study comparing TDF with the FTC/TDF combination in patients with documented LAM-resistant substitutions and who were viremic at baseline. It should be noted that FTC/TDF fixed-dose combination is not an FDA-approved treatment of CHB. After 96 weeks, viral suppression (HBV DNA <400 copies/mL) was reached in 89% of patients treated with TDF, and 86% with the

combination. Both treatments were well tolerated with favorable safety profiles. There were no clinically significant changes in renal function, and no evidence of clinically relevant bone loss through the treatment period.³⁴ Another randomized, placebo-controlled study enrolled adolescent patients with CHB including two-thirds of whom had prior exposure to LAM or ADV. At week 72, 89% of patients who received TDF and none on placebo reached virologic suppression.⁴ Increases in bone mineral density were significantly less in the TDF-treated than the placebo group. Bone safety with long-term TDF use in adolescents is not well understood and monitoring will continue.⁴

Adefovir-resistant variants, especially the double substitution rtN236T ± rtA181T/V, have slightly reduced susceptibility to tenofovir in cell-based studies.^{6,35,37} Several studies in patients who failed treatments with ADV and/or LAM showed that TDF was effective in viral suppression; the presence of baseline mutations conferring resistance to LAM and/or adefovir did not influence treatment response.³⁸⁻⁴² However, variability in the time to virologic response was apparent.

In a randomized, double-blind study in CHB patients who remained viremic after treatment with ADV (58% had previous treatment with LAM), compared TDF and FTC/TDF. At week 48, 81% from both groups had HBV DNA less than 400 copies/mL; inclusion of FTC had no added benefit.³⁸ In a prospective open-label multicenter study that enrolled suboptimal responders receiving ADV ± LAM, 48% and 55% had substitutions resistant to adefovir and LAM, respectively. ADV was replaced by TDF as mono- or add-on therapy according to the regimen at enrollment for individual patients, and after 48 weeks, 82% reached HBV DNA levels less than 69 IU/mL.³⁹ A retrospective study was conducted to assess the effectiveness of TDF in patients with prior failure or resistance to anti-HBV nucleos(t)ides. After 23 months, 100% of patients with LAM-resistance or suboptimal response to ADV without genotypic adefovir-resistance reached HBV DNA levels <400 copies/mL, while only 52% of those with genotypic adefovir-resistant substitutions at baseline reached the virologic endpoint. However, within the adefovir-resistant group, the patients with lower viral load at baseline had an increased chance of reaching the endpoint.⁴⁰ A prospective study evaluated TDF rescue therapy in heavily-pretreated CHB patients who had prior failure with LAM and remained viremic while receiving ADV. Only 64% reached HBV DNA less than 15 IU/ml after 96 weeks of TDF treatment. However, continuous follow-up showed that 81% (per protocol) reached the endpoint after 5 years.^{41,42} Although there was no association between virologic response and adefovir-resistant substitutions at baseline, patients who harbored the rtN236T adefovir-resistant substitution tended to have slower rates of viral decline on TDF than those without rtN236T. It was also noted that the patients with adefovir-resistant substitutions had higher viral loads at baseline.^{41,42}

These results taken together indicate that higher baseline viral loads might be associated with slower declines in DNA levels in those with adefovir-resistance. Other factors in addition to the changes at the HBV rt/pol sequences, may work in concert to define the evolution, selection and suppression of the resistant variants by antivirals. To understand why TDF has good efficacy in many patients with adefovir resistance, but has diminished efficacy in others will likely require a deeper understanding of host and viral interactions.

TDF for CHB patients with severe liver disease

The efficacy of TDF in CHB was first reported in patients suffering from LAM-resistant hepatitis B. In the early 2000's, clinical investigators noticed that TDF lowered HBV viral loads in patients co-infected with HIV/HBV who developed LAM-resistant hepatitis B while receiving LAM-containing antiretroviral regimens.²⁹⁻³¹ Also, during this time, TDF and ADV were used as new rescue agents for patients who had HBV breakthroughs while receiving LAM ± hepatitis B immunoglobulin (HBIG) post-liver transplantation.⁴³

Prevention of hepatitis B recurrence post-liver transplantation was further confirmed in a randomized controlled study. Transplant recipients who were stable on HBIG prophylaxis were enrolled to receive FTC/TDF plus HBIG for 24 weeks, and then randomized to continue the dual therapy or receive only FTC/TDF. Final results have shown no recurrence of hepatitis B up to 72 weeks after randomization with or without HBIG.⁴⁴ FTC/TDF was well tolerated.

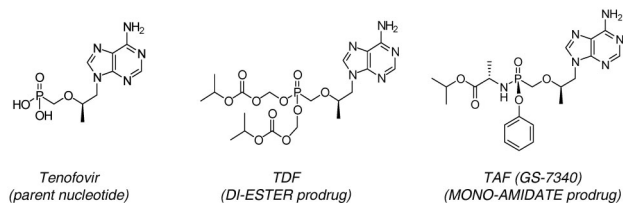
Safety and tolerability of TDF in patients with decompensated CHB liver disease was assessed in a randomized, double-blind study. Interim results at week 48 showed good virologic response, and more importantly, that adverse events and laboratory profiles were consistent with advanced liver disease and complications, with no unexpected safety signals.⁴⁵

For more than a decade, the number of patients on lists waiting to receive liver transplants has been decreasing in the US. Especially noticeable is the rapid decline by 47% from 1999 to 2006 of incidence rate of waiting list registration for end stage liver disease due to hepatitis B compared to that due to hepatitis C or non-viral liver disease.⁴⁶ The temporal correlation of the trend and the availability of anti-HBV nucleos(t)ides, starting with use of LAM in 1998, suggests that broad application of these medications, in part, contributed to the decreased incidence of decompensated CHB disease.

Tenofovir alafenamide fumarate – a new tenofovir prodrug

The TDF prodrug enhanced oral absorption and allowed tenofovir to be dosed conveniently by mouth. The prodrug is also resulted in better cellular uptake than unmodified tenofovir. However, the DF prodrug moiety is rapidly hydrolyzed by serum esterases leaving tenofovir in the circulation.⁴⁷ Continuing medicinal research aimed to improve cellular delivery of tenofovir by decreasing prodrug susceptibility to serum esterases in the circulation, and increasing conversion to tenofovir once inside cells. Tenofovir alafenamide fumarate (TAF) or GS7340 is a new prodrug of tenofovir selected for these properties, and is currently undergoing clinical investigation for HIV/AIDS and CHB.

TAF is more stable than TDF in circulation, and yet is more rapidly converted to tenofovir inside target cells (Fig. 3).⁴⁷ A recent dose-ranging study in patients with HIV-infection showed that 8 mg of TAF produced the same, and 25 mg produced more than 7 times the active metabolite tenofovir-DP in PBMCs as 300 mg of TDF. The more efficient delivery of the active metabolite was reflected in the antiviral potency in which 8 mg TAF was similar, and 25 mg TAF was more efficacious in HIV viral load reduction than 300 mg TDF.⁴⁸ A recent non-clinical study showed that first-pass hepatic extraction of TAF was 65% after oral administration suggesting high hepatic uptake.⁴⁹ Since TAF and TDF are similar in



Compound	Stability $T_{1/2}$ (min)	
	Human plasma	T-cell extract
Tenofovir	stable	stable
TDF	0.4	71
TAF (GS-7340)	90	28

Fig. 3. TAF is a new prodrug of tenofovir. TDF and TAF are oral prodrugs of tenofovir. Both can be hydrolyzed in serum and are taken up into target cells. However, TAF is more efficient in delivering tenofovir into cells. TAF is more stable in the circulation allowing more efficient transport across cell membrane; it is more readily converted to tenofovir once inside target cells.⁴⁷

anti-HBV potency in cell-based assays, it is possible that compared to TDF, a similar efficacy at lower dose of TAF can be achieved in patients with CHB. This would result in a decrease in systemic exposure of tenofovir in patients.

Expanding access

To date, more than 4 millions individuals take TDF daily for the treatment of HIV/AIDS and/or hepatitis B. Of note, patients living in the developed world only account for less than 1 million of this population. More than 3 million patients in the developing world receive TDF at no-profit or drastically reduced costs. The distribution to lower-income countries is made possible through a company-run operationally innovative program aimed to provide sustainable access of drugs for life-threatening disease to patients who need them, regardless where they may live.⁵⁰ Such a developing-world access program involves partnerships between international stakeholders such as the patent-holding company, licensed generic drug manufacturers, global health organizations, regional medical communities, health ministries in developing countries, and others.

Nevertheless, only a minute proportion of CHB patients who are eligible for treatment are currently receiving TDF in lower-income countries in the world. Continuation of efforts and collaborations are much needed to further expand access of effective medications to the needed populations.

Conclusions

Scientific innovations optimized nucleotide analogs such as tenofovir to be a potent and selective antiviral compound. Medicinal research endeavor transformed tenofovir into TDF for clinical applications, thus advancing the drug to become a critical medicine for treating life-threatening infections suffered by millions worldwide. In order to make a population-wide impact on CHB-associated liver disease, improvements in screening, diagnosis, and access to the knowledge of treatment and prevention are as important as access to the drugs themselves. CHB is a global public health challenge. Support and participation from national governments are much needed to have a significant impact on CHB on a global level.

Conflict of interest

L. Lou is a former employee of Gilead Sciences, Inc. This review was written without funding support from Gilead, and was entirely self-funded by the author.

Author contributions

Conceiving and writing the review (LL).

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