Tenofovir disoproxil fumarate for the treatment of chronic hepatitis B monoinfection

Wai-Kay Seto, Man-Fung Yuen, James Fung, Ching-Lung Lai

Department of Medicine, the University of Hong Kong, Queen Mary Hospital

Keywords: hepatitis B, tenofovir, resistance, HBeAg, HBsAg, HBV DNA

Correspondence and Reprint request:

Prof. Ching-Lung Lai, Department of Medicine, The University of Hong Kong

Queen Mary Hospital, Pokfulam Road, Hong Kong

E mail: hrmelcl@hkucc.hku.hk Fax: 852 28162863 Tel: 852 22554477

The authors declare they have participated in the preparation of the manuscript and have seen and approved the final version.

Conflicts of Interest

MF Yuen and CL Lai have given lectures at Gilead Sciences Symposia.

Role of the funding source

All the authors have nothing to disclose

Note : Serum HBV DNA levels are expressed as IU/mL in this review. If HBV DNA levels were originally quoted as copies/mL in their cited reference, they are converted from copies/mL to IU/mL in a ratio of 5.8 :1.

(3455 words)

Abstract

Resistance in nucleoside / nucleotide analogue (NA) therapy has always been a challenge in the management of chronic hepatitis B (CHB). Initially developed for the treatment of HIV infection, early *in vitro* and clinical observational studies had shown tenofovir disoproxil fumarate (TDF) to be also active against CHB. Recent data from various multicenter phase 3 and 4 clinical trials have confirmed TDF being able to achieve a high viral suppression in both NA-naïve and -experienced CHB patients. There are also emerging data on the efficacy of TDF in decompensated CHB. Although there are *in vitro* studies identifying certain mutation loci associated with a reduced susceptibility to TDF, there have so far been no reports of virologic resistance to TDF in clinical studies. TDF has a favorable safety profile, although more long-term data would be needed. In conclusion, TDF has the makings of an “ideal” first-line drug for the treatment of CHB.

Introduction

Chronic hepatitis B (CHB) infection constitutes a global health burden, with 400 million patients infected with the hepatitis B virus (HBV) (1), and an estimated 600,000 people dying annually from complications of CHB (2). The last two decades have seen the introduction of various nucleoside / nucleotide analogues (NA) (3-7). There is increasing medical evidence supporting long-term NA therapy in order to maintain a permanent virologic suppression (8), since this could result in histologic improvement with reversal of fibrosis (9) and reduce the incidence of cirrhotic complications and hepatocellular carcinoma (HCC) (10, 11).

The prerequisites for long-term therapy include a simple dosing regimen to ensure drug compliance, satisfactory tolerability, minimal toxicity, favorable potency, and minimal drug resistance. Drug resistance is an important issue in NA therapy, especially with lamivudine, adefovir and telbivudine (12). Resistance to lamivudine can be up to 67% and more than 75% after 4 and 5 years of therapy respectively (11, 13). The cumulative resistance rate of telbivudine is 34% after 3 years (14). Both lamivudine and telbivudine have a low genetic barrier to resistance, with only one mutation at the rtM204V/I loci required. The acyclic structure of adefovir has a high molecular flexibility, resulting in effective drug action even when there are minor alterations in the binding pocket as a result of viral mutations (15). Despite this molecular advantage, the resistance rate of adefovir is still 22% after 2 years of therapy (16), with mutations at the rtA181T and rtN236T loci.

An ideal NA would need to have a durable antiviral potency and also a high genetic barrier of resistance. Entecavir was the first drug with favorable properties, with

only a resistance rate of 1.2% after 5 years of therapy in treatment-naïve CHB patients (17, 18). However the results in lamivudine-resistant patients are suboptimal, with a resistance rate of 51% after 5 years of therapy (17, 19). This review discusses another potent NA recently available for CHB treatment: tenofovir disoproxil fumarate (TDF).

Early observational studies

TDF is an orally bioavailable prodrug of tenofovir (20), which belongs to a class of drugs known as acyclic nucleoside phosphonates (21), known for their potent antiviral activity against both DNA and RNA viruses through direct binding with the viral polymerases. TDF was first approved by the US Food and Drug Administration (FDA) for the treatment of human immunodeficiency virus (HIV) infection in 2001, and has since become the cornerstone for anti-HIV combination treatment regimens. TDF is also active against HBV, with *in vitro* studies showing potent antiviral activity against both wild-type and lamivudine-resistant HBV(22). Tenofovir is converted to its active form through a two-step phosphorylation, and terminates chain elongation catalyzed by the HBV DNA polymerase.

The initial clinical evidence of TDF in the treatment of CHB arose from HIV-HBV co-infected individuals. In 2002, van Bommel et al treated 5 lamivudine-resistant patients with HBV and HIV co-infection with TDF 300 mg daily, resulting in a 4.5 log reduction in HBV DNA by week 24 (23). Two similar studies by Ristig et al (24) and Benhamou et a l(25) in 6 and 10 co-infected individuals respectively also had similar findings, with serum HBV DNA decreasing by 4.3 logs and 3.8 logs respectively at week 24. The first comparative study in 12 co-infected individuals also found TDF achieving

better viral suppression when compared to placebo (26). TDF was also found to achieve potent viral suppression in a small study of CHB patients with no HIV co-infection (27), as well as in lamivudine-resistant patients following liver transplantation (28).

The initial success of TDF led to comparative studies with adefovir dipivoxil, also an acyclic nucleoside phosphatase, which was approved by the US FDA for the treatment of CHB infection in 2002. The main use of adefovir was as “rescue” therapy in lamivudine resistance (29, 30), but its usage is hindered by the increased frequency of adefovir resistance mutations when compared to NA-naïve patients (31). In the first study comparing TDF and adefovir (32), 53 lamivudine-resistant patients were treated with TDF 300 mg daily or adefovir 10 mg daily for up to 130 weeks. The majority of patients had HIV co-infection. TDF was shown to achieve a faster HBV DNA reduction by week 4 when compared to adefovir, with the HBV DNA reduction becoming significantly better than adefovir by week 12. All patients on TDF achieved undetectable HBV DNA (less than 69 IU/mL) at week 48, compared to 44% of patients on adefovir. There was no evidence of phenotypic viral resistance to TDF up to week 130. Despite the similar molecular structure of TDF and adefovir, TDF was also effective in patients with sub-optimal virologic response to adefovir (33).

Clinical trials of tenofovir disoproxil fumarate in chronic hepatitis B

From the above observational studies, based on its antiviral potency, minimal resistance and effectiveness in lamivudine-resistant patients, TDF has the potential to become the “ideal” drug for CHB. This had led to the commencement of different trials in both treatment-naïve and NA-experienced CHB patients (Table 1).

Marcellin and Heathcote et al conducted the first randomized double-blind study concerning TDF, with both HBeAg-positive (study 103) and -negative (study 102) patients with compensated liver disease randomly assigned to receive either TDF (n=426) or adefovir (n=215) for 48 weeks (34). A small proportion of patients were previously treated with lamivudine, emtricitabine or interferon. The primary endpoint was a serum HBV DNA level of less than 69 IU/mL (or 400 copies/mL). By weeks 4 to 12, there was already an obvious difference in viral suppression between the two treatment groups. By week 48, significantly more patients on TDF achieved HBV DNA undetectability (76% for HBeAg-positive, 93% for HBeAg-negative) when compared to adefovir (13% and 63% respectively). Three percent of HBeAg-positive patients on TDF achieved hepatitis B surface antigen (HBsAg) seroclearance, while histologic improvements were similar in both treatment groups. Tenofovir produced similar viral suppression in patients with prior lamivudine treatment.

Patients from the above studies, irrespective of their therapeutic response, were then enrolled in a follow-up study to receive open-label TDF for 7 years (35). Patients from both groups with detectable serum HBV DNA at week 72 were eligible to receive tenofovir together with emtricitabine at the discretion of the investigators. The primary endpoints were the serologic and virologic outcomes and the resistance profile at week 144 (n=542). Up to 72% and 87% of HBeAg-positive and -negative patients respectively achieved undetectable HBV DNA (less than 69 IU/mL) by week 144. Patients who were originally on adefovir and subsequently switched to TDF at week 48 also had similar outcomes. No resistance to tenofovir was detected. A cumulative 8% of HBeAg-positive patients (the majority being genotype A and D) achieved HBsAg seroclearance,

although there were no cases of HBsAg loss in HBeAg-negative patients. When the same patient cohort was followed up to week 192, rates of undetectable HBV DNA were 77% and 86% in HBeAg-positive and -negative patients respectively. HBsAg seroclearance rate among HBeAg-positive patients increased to 10%, with 7.5% seroconverting to serum antibody to HBsAg (anti-HBs) (36, 37). In a sub-group analysis of patients with high baseline viral load (more than 8.3 log IU/mL), more than 95% achieved undetectable HBV DNA and 15.2% of HBeAg-positive patients achieved HBsAg seroclearance (38) at week 192. Another subgroups analysis of 198 Asian CHB patients also found 79% (74% HBeAg-positive, 84% HBeAg-negative) achieving HBV DNA undetetectability at week 192, although there were no Asian patients achieving HBsAg seroclearance (39). Of the 51 patients eligible for receiving combination of TDF and emtricitabine, 34 had combination therapy while 17 remained on TDF monotherapy. There was no difference in viral suppression between those receiving combination therapy and those whom continued to receive TDF monotherapy up to year 3 (35).

TDF is also effective in patients with prior exposure to different NAs. A randomized double-blind study by Berg et al. in Europe (40) recruited patients with an incomplete virologic response to adefovir (n=105) with 31 having proven adefovir resistance mutations and 25 having lamivudine resistance mutations. They were randomly assigned to either TDF or TDF plus emtricitabine. At 48 weeks, 81% of patients in both treatment groups had HBV DNA levels of less than 69 IU/mL. A continued follow-up of the same patient cohort showed similar viral suppression (86%) up to week 168 (41). Another prospective study by Patterson et al in Australia (42) enrolled 60 patients with incomplete virologic response to both lamivudine and adefovir.

After 96 weeks of TDF or combination TDF and lamivudine, 64% of patients achieved HBV DNA levels of less than 15 IU/mL. Viral suppression was similar regardless of the addition of lamivudine. Both the European and Australian studies found treatment response to be independent of baseline mutations. An preliminary Italian study also showed similar findings (43).

However, the above findings were not entirely reproduced in a retrospective multicenter study from Germany (44), with 131 NA-experienced patients having previous treatment failure with lamivudine, adefovir, combination lamivudine and adefovir or entecavir. After a mean treatment duration of 23 months, 79% achieved a HBV DNA level below 69 IU/mL. HBsAg seroclearance occurred in 3% of patients. Unlike the previous three studies, the presence of adefovir mutations impaired TDF efficacy when compared to the presence of lamivudine mutations (52% versus 100% respectively achieving viral suppression). The outcome of adefovir-resistant patients in different trials is depicted in Table 2. For all 4 studies, the number of patients with proven genotypic adefovir resistance is actually small. In addition, different methods of resistance methods were used. Two of the four studies (42, 44) only used direct sequencing for resistance detection, a method which could only detect mutations if there are at least 20-30% of mutants circulating in the total viral population (45). The other 2 studies (40, 43) used a line probe assay (LiPA), which is able to detect mutations constituting 5% of the total viral population (46). Further studies with a larger study population are needed to determine the efficacy of TDF in adefovir-resistant patients.

Tenofovir in decompensated chronic hepatitis B

The use of NAs in decompensated CHB had previously shown mixed results. Lamivudine was able to improve liver function in decompensated CHB (47), but evidence on its effect in improving survival has been mixed (48, 49). The use of adefovir in decompensated CHB also improved liver function significantly, however 14% of patients died within the first year and 33% still required liver transplantation for long-term survival (50). Entecavir in decompensated CHB achieved an 87% to 91% 1-year patient survival (51, 52). However there were reports of 5 cases of lactic acidosis according to a study with limited number of patients (53) The same center subsequently describe similar cases of lactic acidosis with other NAs There was also a possible increase in short-term mortality when used in acute exacerbation of CHB in a retrospective study comparing entecavir and lamivudine in 36 and 117 patients respectively (54).

A recent study compared TDF (n=45), emtricitabine / TDF (n=45) and entecavir (n=22) in patients with decompensated CHB for 48 weeks (52). All 3 treatment arms had comparable biochemical and virologic improvements. Patients on TDF or emtricitabine / TDF were more likely to achieve HBeAg loss. There were no cases of lactic acidosis reported for any treatment group.

Tenofovir in acute on chronic liver failure

A recent prospective study randomized patients diagnosed with acute-on-chronic liver failure due to CHB to either TDF (n=14) or placebo (n=13) (55). Patients were followed up for 3 months, with no liver transplantation offered due to its

unavailability. Fifty-seven percent of patients on TDF survived compared to 15% of the placebo patients. One patient on TDF developed HBsAg seroclearance. Patients on TDF also showed marked virologic and biochemical improvements, with a 2 log HBV DNA reduction within 2 weeks independently predicting survival. Despite its small sample size, the authors concluded that responders to TDF could be identified within 2 weeks, thus optimizing patient selection for liver transplantation. Prospective studies on a larger scale are needed to confirm this finding.

Tenofovir in solid organ transplant recipients

The use of TDF in solid organ transplant recipients has not been extensively studied, with most studies to date focusing on lamivudine.

A recent small study on 7 transplant recipients with prior suboptimal response to other NAs found TDF to be both efficacious and safe (56). Another small study followed up 8 patients with HBV recurrence after liver transplantation. Four patients had detectable viremia despite entecavir, and eventual viral suppression was achieved with the addition of TDF (57). While more large-scale studies are required to determine efficacy and safety of TDF in this special group of patients, it would be reasonable to anticipate an optimal virologic response and low rates of resistance.

Resistance profile of tenofovir

To date, there are no reports of any virologic resistance to TDF among patients with CHB monoinfection.

A phenotypic analysis with recombinant HBV was performed using serum derived from studies 102 and 103 (34, 35). The majority of these patients were NA-naïve, and baseline genotypic mutations were only found in 1.1% of patients (all lamivudine-related rtM204V/I with or without rtL180M). Serum was chosen from patients who developed virologic breakthrough, although virologic breakthroughs were infrequent and found to be mostly associated with drug non-compliance. No patients developed amino acid substitutions associated with resistance to TDF up to week 144 (58).

The first study to suggest a mutation loci associated with TDF resistance was based on 43 CHB patients with HIV co-infection (59). The rtA194T substitution found in 2 patients was found to be associated with reduced susceptibility to TDF *in vitro* when the lamivudine mutations of rtM204V and rtL180M were present. Nevertheless, further studies concerning the rtA194T mutation showed mixed results. A subsequent *in vitro* study failed to reproduce the results of Sheldon et al (60). Another study using replicated HBV plasmid harboring rtA194T alone or with lamivudine-associated mutants found the rtA194T mutation to be associated with partial resistance to TDF; however, the effect on resistance is negated when the mutations occur together with precore or basic core promoter substitutions (61).

The clinical impact of the rtA194T mutation is still unknown. A preliminary clinical study followed up 10 CHB patients with the rtA194T mutation present before commencing on TDF. After receiving either TDF monotherapy or in combination with either lamivudine or emtricitabine for 1.5 years, all patients had at least 3 log reduction

in DNA after 12 weeks, and 9 patients achieved HBV DNA levels of less than 100 IU/mL at their last follow-up (62).

In vitro studies had also found the adefovir-associated mutations rtA181T and rtN236T to be associated with reduced susceptibility to TDF (63, 64). As mentioned previously, clinical studies on the efficacy of TDF in adefovir-resistant patients showed mixed results (40, 42-44).

It is possible that TDF results in rapid viral suppression, thus preventing resistant strains with reduced replicative capacity from taking over the majority of the viral population, making the rtA194T, rtA181T or the rtN236T mutations clinically irrelevant. More long-term studies with larger cohort of patients on TDF harboring the above mutations would be needed before definitive conclusions can be drawn.

Cost utility analysis of tenofovir

Data on the cost-effectiveness of TDF is emerging. A recent study from the United Kingdom investigated this issue, using a Markov model to compare TDF with lamivudine, adefovir and entecavir. First-line TDF was found to be the most cost-effective treatment for patients with CHB, costing £ 19,084 per quality-adjusted life-year gained compared with the next best alternative (65). Another study compared the 12-month treatment outcomes of the five NAs and pegylated interferon in various randomized control trials using a Bayesian mixed treatment comparison model. TDF was found to be most cost-effective in HBeAg-negative disease (66). Given its limited availability in Asia, similar cost utility analysis of TDF in Asia is lacking, although a Hong

Kong study on the cost-effectiveness of other NAs found TDF to be the most cost-effective if TDF was priced similarly to telbivudine (67).

Safety

An understanding of the safety profile of TDF is essential, especially when NA therapy is likely to be long-term in order to achieve permanent viral suppression. Tenofovir is primarily excreted by the kidneys, and the most important safety concern of TDF would be its association with renal dysfunction. The majority of cases with TDF-associated renal impairment were reported from the HIV population. Although less commonly seen when compared to adefovir therapy, prolonged treatment with TDF is associated with renal proximal tubular dysfunction (68-70) and hypophosphatemia, the latter secondary to reduced phosphate reabsorption and excessive loss of urinary phosphates (71-73). Fanconi syndrome and acute renal failure have also been reported (74, 75), although the nephrotoxicity is usually reversible when therapy is stopped. TDF is associated with a 4% decline in glomerular filtration rate (GFR) in HIV patients over one year (76) and a decline of 9.8 mL/min/1.37 m^2 up to 5 years(77), although given the differences in disease nature, such data might not be directly applicable in CHB.

Safety data of long-term TDF in CHB is emerging. In patients with HIV co-infection taking TDF, GFR declined by 22.19 ml/min/1.73 mm^2 from baseline over a median follow-up period of 251 weeks (78). However, clinical trials of TDF in CHB monoinfection mentioned previously did not detect any significant changes in creatinine levels or creatinine clearance up to 144 weeks (35, 42, 44). An preliminary Italian study

followed 737 CHB patients on TDF for a median period of 16 months, of which 524 had prior exposure to other NAs. Less than 1% of patients had an increase in serum creatinine of more than 44 umol/L (or 0.5 mg/dL). In terms of tubular function, 37% patients had a significant decrease in urinary phosphate reabsorption, of which the majority had prior exposure to adefovir. The authors concluded that TDF had a favorable safety profile, with only a few patients (mainly NA-experienced) showing a mild degree of renal dysfunction (79). TDF usage in decompensated liver disease is of special concern, especially since many such patients have concurrent renal impairment. In a study on patients with decompensated CHB, only 7.8% had an increase in serum creatinine of more than 44 umol/L after 48 weeks of TDF (52), which was not significantly different from the 4.5% observed with entecavir in the same study.

Dosage adjustments are required for patients with a creatinine clearance of less than 50 ml/min. TDF is taken at 300mg every 48 hours for patients with a creatinine clearance of 30 to 49 ml/min, and every 72 to 96 hours for those with a creatinine clearance of 10 to 29 ml/min. Patients on hemodialysis should take TDF every 7 days following dialysis.

Concerning non-renal side effects of TDF, there had been some concerns of TDF being associated with a low bone mineral density and fat redistribution (80, 81). However, a recent study of HIV-infected individuals with 10 years of follow-up did not find any significant changes in both bone mineral density and limb fat (82). More safety data is needed concerning the significance of non-renal side effects of TDF in CHB.

TDF is labeled by the US FDA as a category “B” pregnancy drug. Animal reproduction studies using doses up to 19 times the human dose revealed no evidence

of impaired fertility of harm to the fetus. There are currently no adequate well-controlled studies of TDF in pregnant woman. However in a follow-up of 3695 HIV-infected mothers treated with TDF or lamivudine, the incidence of congenital fetal abnormalities was 2.3% and 2.7% respectively, which was not different from the incidence of congenital abnormalities in NA-naïve mothers(83). Maternal bioavailability may be decreased in the third trimester. Concerning lactation, animal studies have demonstrated that TDF is secreted in milk, and breastfeeding is not advised for mothers taking TDF (84).

Future directions and conclusions

One interesting phenomenon with TDF is the relatively high HBsAg seroclearance noted in HBeAg-positive CHB with genotypes A and D, with a cumulative rate of 10% after 192 weeks of therapy (36). Given the low incidence of spontaneous and NA-induced HBsAg seroclearance (18, 85-87), the possibility of HBsAg seroclearance as a treatment endpoint is finally potentially achievable. Despite this optimism, preliminary studies of TDF in Asians (majority being genotypes B and C) did not achieve HBsAg seroclearance (39). This could be related to the different immunology of HBV infection in Asians compared to Caucasians, with Asians acquiring the infection at the early stages of life (8). Further long-term studies in different ethnic groups are needed to address this issue. Additional data of the efficacy of TDF in adefovir-resistant cases and long-term safety profile would also be needed.

In conclusion, with its potent viral suppression, favorable resistance profile, and superior efficacy in lamivudine rescue therapy when compared to adefovir, TDF is perhaps the “ideal” first-line drug in the treatment of CHB.

References

1. Lai CL, Yuen MF. The natural history and treatment of chronic hepatitis B: a critical evaluation of standard treatment criteria and end points. Ann Intern Med. 2007 Jul 3;147(1):58-61.

2. World Health Organization. WHO Fact Sheet N*204. Published August 2008. [cited 2011 February]; Available from: http://www.who.int/mediacentre/factsheets/fs204/en/.

3. Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. N Engl J Med. 1998 Jul 9;339(2):61-8.

4. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N Engl J Med. 2003 Feb 27;348(9):800-7.

5. Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med. 2006 Mar 9;354(10):1011-20.

6. Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med. 2007 Dec 20;357(25):2576-88.

7. Yuen MF, Lai CL. Treatment of chronic hepatitis B: Evolution over two decades. J Gastroenterol Hepatol. 2011 Jan;26 Suppl 1:138-43.

8. Lai CL, Yuen MF. Chronic hepatitis B--new goals, new treatment. N Engl J Med. 2008 Dec 4;359(23):2488-91.

9. Chang TT, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, et al. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology. 2010 Sep;52(3):886-93.

10. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004 Oct 7;351(15):1521-31.

11. Yuen MF, Seto WK, Chow DH, Tsui K, Wong DK, Ngai VW, et al. Long-term lamivudine therapy reduces the risk of long-term complications of chronic hepatitis B infection even in patients without advanced disease. Antivir Ther. 2007;12(8):1295-303.

12. Yuen MF, Fung J, Wong DK, Lai CL. Prevention and management of drug resistance for antihepatitis B treatment. Lancet Infect Dis. 2009 Apr;9(4):256-64.

13. Chang TT, Lai CL, Chien RN, Guan R, Lim SG, Lee CM, et al. Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. J Gastroenterol Hepatol. 2004 Nov;19(11):1276-82.

14. Seto WK, Lai CL, Fung J, Wong DK, Yuen JC, Hung IF, et al. Significance of HBV DNA levels at 12 weeks of telbivudine treatment and the 3 years treatment outcome. J Hepatol. 2010 Dec 10.

15. Yang H, Westland CE, Delaney WEt, Heathcote EJ, Ho V, Fry J, et al. Resistance surveillance in chronic hepatitis B patients treated with adefovir dipivoxil for up to 60 weeks. Hepatology. 2002 Aug;36(2):464-73.

16. Fung SK, Chae HB, Fontana RJ, Conjeevaram H, Marrero J, Oberhelman K, et al. Virologic response and resistance to adefovir in patients with chronic hepatitis B. J Hepatol. 2006 Feb;44(2):283-90.

17. Tenney DJ, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, et al. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naive patients is rare through 5 years of therapy. Hepatology. 2009 May;49(5):1503-14.

18. Chang TT, Lai CL, Kew Yoon S, Lee SS, Coelho HS, Carrilho FJ, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. Hepatology. 2010 Feb;51(2):422-30.

19. Reijnders JGP, Deterding K, Petersen J, Zoulim F, Santantonio T, Buti M, et al. Antiviral effect of entecavir in chronic hepatitis B: Influence of prior exposure to nucleos(t)ide analogues. Journal of Hepatology. 2010;52(4):493-500.

20. Gallant JE, Deresinski S. Tenofovir disoproxil fumarate. Clin Infect Dis. 2003 Oct 1;37(7):944-50.

21. De Clercq E, Holy A. Acyclic nucleoside phosphonates: a key class of antiviral drugs. Nat Rev Drug Discov. 2005 Nov;4(11):928-40.

22. Ying C, De Clercq E, Nicholson W, Furman P, Neyts J. Inhibition of the replication of the DNA polymerase M550V mutation variant of human hepatitis B virus by adefovir, tenofovir, L-FMAU, DAPD, penciclovir and lobucavir. Journal of Viral Hepatitis. 2000;7(2):161-5.

23. van Bommel F, Wunsche T, Schurmann D, Berg T. Tenofovir treatment in patients with lamivudine-resistant hepatitis B mutants strongly affects viral replication. Hepatology. 2002 Aug;36(2):507-8.

24. Ristig MB, Crippin J, Aberg JA, Powderly WG, Lisker-Melman M, Kessels L, et al. Tenofovir disoproxil fumarate therapy for chronic hepatitis B in human

immunodeficiency virus/hepatitis B virus-coinfected individuals for whom interferon-alpha and lamivudine therapy have failed. J Infect Dis. 2002 Dec 15;186(12):1844-7.

25. Benhamou Y, Tubiana R, Thibault V. Tenofovir disoproxil fumarate in patients with HIV and lamivudine-resistant hepatitis B virus. N Engl J Med. 2003 Jan 9;348(2):177-8.

26. Dore GJ, Cooper DA, Pozniak AL, DeJesus E, Zhong L, Miller MD, et al. Efficacy of tenofovir disoproxil fumarate in antiretroviral therapy-naive and -experienced patients coinfected with HIV-1 and hepatitis B virus. J Infect Dis. 2004 Apr 1;189(7):1185-92.

27. Kuo A, Dienstag JL, Chung RT. Tenofovir disoproxil fumarate for the treatment of lamivudine-resistant hepatitis B. Clin Gastroenterol Hepatol. 2004 Mar;2(3):266-72.

28. Neff GW, Nery J, Lau DT, O'Brien CB, Duncan R, Shire NJ, et al. Tenofovir therapy for lamivudine resistance following liver transplantation. Ann Pharmacother. 2004 Dec;38(12):1999-2004.

29. Peters MG, Hann Hw H, Martin P, Heathcote EJ, Buggisch P, Rubin R, et al. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology. 2004 Jan;126(1):91-101.

30. Lampertico P, Vigano M, Manenti E, Iavarone M, Sablon E, Colombo M. Low resistance to adefovir combined with lamivudine: a 3-year study of 145 lamivudine-resistant hepatitis B patients. Gastroenterology. 2007 Nov;133(5):1445-51.

31. Lee YS, Suh DJ, Lim YS, Jung SW, Kim KM, Lee HC, et al. Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. Hepatology. 2006 Jun;43(6):1385-91.

32. van Bommel F, Wunsche T, Mauss S, Reinke P, Bergk A, Schurmann D, et al. Comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus infection. Hepatology. 2004 Dec;40(6):1421-5.

33. van Bommel F, Zollner B, Sarrazin C, Spengler U, Huppe D, Moller B, et al. Tenofovir for patients with lamivudine-resistant hepatitis B virus (HBV) infection and high HBV DNA level during adefovir therapy. Hepatology. 2006 Aug;44(2):318-25.

34. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med. 2008 Dec 4;359(23):2442-55.

35. Heathcote EJ, Marcellin P, Buti M, Gane E, De Man RA, Krastev Z, et al. Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. Gastroenterology. 2011 Jan;140(1):132-43.

36. Heathcote EJ, Gane EJ, deMan RA, Chan S, George J, Tsai NC, et al. Long term (4 year) efficacy and safety of tenofovir disoproxil fumarate (TDF) treatment in HBeAg-positive patients (HBeAg+) with chronic hepatitis B (study 103): preliminary analysis (abstract). Hepatology. 2010;52(4 (Suppl)):556A.

37. Marcellin P, Buti M, Krastev Z, Gurel S, Di Bisceglie AM, Odin JA, et al. Continued efficacy and safety through 4 years of tenofovir disoproxil fumurate (TDF) treatment in HBeAg-negative patients with chronic hepatitis B (study 102): preliminary analysis (abstract). Hepatology. 2010;52(4 (Suppl)):555A.

38. Gordon SC, Marcellin P, Krastev Z, Horban A, Petersen J, Sperl J, et al. 4 year efficacy of tenofovir disoproxil fumarate (TDF) in chronic hepatitis B patients with high

viral load (HBV DNA ≥9log copies/mL): preliminary analysis (abstract). Hepatology. 2010;52(4 (Suppl)):388A.

39. Gane E, Lee SS, Heathcote EJ, Sievert W, Trinh H, Kaita K, et al. Efficacy / safety of tenofovir disoproxil fumarate (TDF) in Asians with HBeAg-positive / HBeAg-negative chronic hepatitis B (CHB), four year preliminary analysis (abstract). Hepatol Int. 2011;5:11.

40. Berg T, Marcellin P, Zoulim F, Moller B, Trinh H, Chan S, et al. Tenofovir is effective alone or with emtricitabine in adefovir-treated patients with chronic-hepatitis B virus infection. Gastroenterology. 2010 Oct;139(4):1207-17.

41. Manns M, Berg T, Moller B, Trinh H, Chan S, Marcellin P, et al. Week 168 tenofovir DF (TDF) versus emtricitabine and TDF in viremic patients receiving adefovir dipivoxil for chronic hepatitis B (CHB) (abstract). Hepatol Int. 2011;5:11.

42. Patterson SJ, George J, Strasser SI, Lee AU, Sievert W, Nicoll AJ, et al. Tenofovir disoproxil fumarate rescue therapy following failure of both lamivudine and adefovir dipivoxil in chronic hepatitis B. Gut. 2011 Feb;60(2):247-54.

43. Levrero M, Cimino L, Lampertico P, Vigano M, Gaeta GB, Brancaccio G, et al. Tenofovir (TDF) for chronic hepatitis B patients with suboptimal response to adefovir or adefovir / lamivudine treatment: results of the OPTIB Italian multicenter prospective open labal study (abstract). Hepatology. 2010;52(4 (Suppl)):389A.

44. van Bommel F, de Man RA, Wedemeyer H, Deterding K, Petersen J, Buggisch P, et al. Long-term efficacy of tenofovir monotherapy for hepatitis B virus-monoinfected patients after failure of nucleoside/nucleotide analogues. Hepatology. 2010 Jan;51(1):73-80.

45. Sablon E, Shapiro F. Advances in Molecular Diagnosis of HBV Infection and Drug Resistance. Int J Med Sci. 2005;2(1):8-16.

46. Niesters HG, Zoulim F, Pichoud C, Buti M, Shapiro F, D'Heuvaert N, et al. Validation of the INNO-LiPA HBV DR assay (version 2) in monitoring hepatitis B virus-infected patients receiving nucleoside analog treatment. Antimicrob Agents Chemother. 2010 Mar;54(3):1283-9.

47. Villeneuve JP, Condreay LD, Willems B, Pomier-Layrargues G, Fenyves D, Bilodeau M, et al. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. Hepatology. 2000 Jan;31(1):207-10.

48. Fontana RJ, Keeffe EB, Carey W, Fried M, Reddy R, Kowdley KV, et al. Effect of lamivudine treatment on survival of 309 North American patients awaiting liver transplantation for chronic hepatitis B. Liver Transpl. 2002 May;8(5):433-9.

49. Chien RN, Lin CH, Liaw YF. The effect of lamivudine therapy in hepatic decompensation during acute exacerbation of chronic hepatitis B. J Hepatol. 2003 Mar;38(3):322-7.

50. Schiff E, Lai CL, Hadziyannis S, Neuhaus P, Terrault N, Colombo M, et al. Adefovir dipivoxil for wait-listed and post-liver transplantation patients with lamivudine-resistant hepatitis B: final long-term results. Liver Transpl. 2007 Mar;13(3):349-60.

51. Shim JH, Lee HC, Kim KM, Lim Y-S, Chung Y-H, Lee YS, et al. Efficacy of entecavir in treatment-naïve patients with hepatitis B virus-related decompensated cirrhosis. Journal of Hepatology. 2010;52(2):176-82.

52. Liaw YF, Sheen IS, Lee CM, Akarca US, Papatheodoridis GV, Suet-Hing Wong F, et al. Tenofovir disoproxil fumarate (TDF), emtricitabine/TDF, and entecavir in

patients with decompensated chronic hepatitis B liver disease. Hepatology. 2011 Jan;53(1):62-72.

53. Lange CM, Bojunga J, Hofmann WP, Wunder K, Mihm U, Zeuzem S, et al. Severe lactic acidosis during treatment of chronic hepatitis B with entecavir in patients with impaired liver function. Hepatology. 2009 Dec;50(6):2001-6.

54. Wong VW, Wong GL, Yiu KK, Chim AM, Chu SH, Chan HY, et al. Entecavir treatment in patients with severe acute exacerbation of chronic hepatitis B. J Hepatol. 2011 Feb;54(2):236-42.

55. Garg H, Sarin SK, Kumar M, Garg V, Sharma BC, Kumar A. Tenofovir improves the outcome in patients with spontaneous reactivation of hepatitis B presenting as acute-on-chronic liver failure. Hepatology. 2011;53(3):774-80.

56. Daude M, Rostaing L, Saune K, Lavayssiere L, Basse G, Esposito L, et al. Tenofovir therapy in hepatitis B virus-positive solid-organ transplant recipients. Transplantation. 2011 Apr 27;91(8):916-20.

57. Jiménez-Pérez M, Sáez-Gómez AB, Mongil Poce L, Lozano-Rey JM, de la Cruz-Lombardo J, Rodrigo-López JM. Efficacy and Safety of Entecavir and/or Tenofovir for Prophylaxis and Treatment of Hepatitis B Recurrence Post-Liver Transplant. Transplantation Proceedings. 2010;42(8):3167-8.

58. Snow-Lampart A, Chappell B, Curtis M, Zhu Y, Myrick F, Schawalder J, et al. No resistance to tenofovir disoproxil fumarate detected after up to 144 weeks of therapy in patients monoinfected with chronic hepatitis B virus. Hepatology. 2010 Nov 18.

59. Sheldon J, Camino N, Rodes B, Bartholomeusz A, Kuiper M, Tacke F, et al. Selection of hepatitis B virus polymerase mutations in HIV-coinfected patients treated with tenofovir. Antivir Ther. 2005;10(6):727-34.

60. Delaney WEt, Ray AS, Yang H, Qi X, Xiong S, Zhu Y, et al. Intracellular metabolism and in vitro activity of tenofovir against hepatitis B virus. Antimicrob Agents Chemother. 2006 Jul;50(7):2471-7.

61. Amini-Bavil-Olyaee S, Herbers U, Sheldon J, Luedde T, Trautwein C, Tacke F. The rtA194T polymerase mutation impacts viral replication and susceptibility to tenofovir in hepatitis B e antigen–positive and hepatitis B e antigen–negative hepatitis B virus strains. Hepatology. 2009;49(4):1158-65.

62. Fung S, Mazzuli T, Sherman M, Popovic V. Tenofovir (TDF) is effective in lamivudine (LAM)-resistant chronic hepatitis B patients who harbor rtA194T at baseline (abstract). Hepatology. 2009;50(4 (Suppl)):493A.

63. Qi X, Xiong S, Yang H, Miller M, Delaney WEt. In vitro susceptibility of adefovir-associated hepatitis B virus polymerase mutations to other antiviral agents. Antivir Ther. 2007;12(3):355-62.

64. Villet S, Pichoud C, Billioud G, Barraud L, Durantel S, Trépo C, et al. Impact of hepatitis B virus rtA181V/T mutants on hepatitis B treatment failure. Journal of Hepatology. 2008;48(5):747-55.

65. Dakin H, Bentley A, Dusheiko G. Cost-utility analysis of tenofovir disoproxil fumarate in the treatment of chronic hepatitis B. Value Health. 2010 Dec;13(8):922-33.

66. Woo G, Tomlinson G, Nishikawa Y, Kowgier M, Sherman M, Wong DKH, et al. Tenofovir and Entecavir Are the Most Effective Antiviral Agents for Chronic Hepatitis B:

A Systematic Review and Bayesian Meta-analyses. Gastroenterology. 2010;139(4):1218-29.e5.

67. Lui YY, Tsoi KK, Wong VW, Kao JH, Hou JL, Teo EK, et al. Cost-effectiveness analysis of roadmap models in chronic hepatitis B using tenofovir as the rescue therapy. Antivir Ther. 2010;15(2):145-55.

68. Labarga P, Barreiro P, Martin-Carbonero L, Rodriguez-Novoa S, Solera C, Medrano J, et al. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. AIDS. 2009 Mar 27;23(6):689-96.

69. Kohler JJ, Hosseini SH, Hoying-Brandt A, Green E, Johnson DM, Russ R, et al. Tenofovir renal toxicity targets mitochondria of renal proximal tubules. Lab Invest. 2009 May;89(5):513-9.

70. Lebrecht D, Venhoff AC, Kirschner J, Wiech T, Venhoff N, Walker UA. Mitochondrial tubulopathy in tenofovir disoproxil fumarate-treated rats. J Acquir Immune Defic Syndr. 2009 Jul 1;51(3):258-63.

71. Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA. 2004 Jul 14;292(2):191-201.

72. Izzedine H, Hulot JS, Vittecoq D, Gallant JE, Staszewski S, Launay-Vacher V, et al. Long-term renal safety of tenofovir disoproxil fumarate in antiretroviral-naive HIV-1-infected patients. Data from a double-blind randomized active-controlled multicentre study. Nephrol Dial Transplant. 2005 Apr;20(4):743-6.

73. Buchacz K, Brooks JT, Tong T, Moorman AC, Baker RK, Holmberg SD, et al. Evaluation of hypophosphataemia in tenofovir disoproxil fumarate (TDF)-exposed and

TDF-unexposed HIV-infected out-patients receiving highly active antiretroviral therapy. HIV Med. 2006 Oct;7(7):451-6.

74. Rifkin BS, Perazella MA. Tenofovir-associated nephrotoxicity: Fanconi syndrome and renal failure. Am J Med. 2004 Aug 15;117(4):282-4.

75. Mathew G, Knaus SJ. Acquired Fanconi's syndrome associated with tenofovir therapy. J Gen Intern Med. 2006 Nov;21(11):C3-5.

76. Gallant JE, Parish MA, Keruly JC, Moore RD. Changes in renal function associated with tenofovir disoproxil fumarate treatment, compared with nucleoside reverse-transcriptase inhibitor treatment. Clin Infect Dis. 2005 Apr 15;40(8):1194-8.

77. de Vries-Sluijs TEMS, Reijnders JGP, Hansen BE, Zaaijer HL, Prins JM, Pas SD, et al. Long-term Therapy With Tenofovir Is Effective for Patients Co-Infected With Human Immunodeficiency Virus and Hepatitis B Virus. Gastroenterology. 2010;139(6):1934-41.

78. Tan LK, Gilleece Y, Mandalia S, Murungi A, Grover D, Fisher M, et al. Reduced glomerular filtration rate but sustained virologic response in HIV/hepatitis B co-infected individuals on long-term tenofovir. J Viral Hepat. 2009 Jul;16(7):471-8.

79. Lampertico P, Vigano M, Yurdaydin C, Idilman R, Buti M, Estaban R, et al. Effectiveness and safety of tenofovir disoproxil fumarate in field practice: a multicenter European cohort study of 737 patients with chronic hepatitis B (abstract). Hepatology. 2010;52(4 (Suppl)):503A.

80. Grund B, Peng G, Gibert CL, Hoy JF, Isaksson RL, Shlay JC, et al. Continuous antiretroviral therapy decreases bone mineral density. AIDS. 2009 Jul 31;23(12):1519-29.

81. Fontana RJ. Side effects of long-term oral antiviral therapy for hepatitis B. Hepatology. 2009;49(S5):S185-S95.

82. Cassetti I, Etzel A, Madruga J, Suleiman J, Zhou Y, Rhee M, et al. The 10 year safety and efficacy of tenofovir disoproxil fumarate (TDF)-containing once-daily highly active antiretroviral therapy (HAART) (abstract). Journal of the International AIDS Society. 2010;13(Suppl 4):P86.

83. Antiretroviral Pregnancy Registry Steering Committee. Antiretroviral Pregnancy Registry International Interim Report for 1 January 1989 through 31 January 2010. Wilmington, NC: Registry Coordinating Center2010 [cited 2011 6 March]; Available from: http://www.apregistry.com/forms/interim_report.pdf.

84. Gilead Sciences. Full prescribing information for VIREAD tablets. 2010 [updated October 2010; cited March 2011]; Available from: http://www.gilead.com/pdf/viread_pi.pdf.

85. Liu J, Yang HI, Lee MH, Lu SN, Jen CL, Wang LY, et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. Gastroenterology. 2010 Aug;139(2):474-82.

86. Yuen MF, Wong DK, Sablon E, Tse E, Ng IO, Yuan HJ, et al. HBsAg seroclearance in chronic hepatitis B in the Chinese: virological, histological, and clinical aspects. Hepatology. 2004 Jun;39(6):1694-701.

87. Gish RG, Chang TT, Lai CL, de Man R, Gadano A, Poordad F, et al. Loss of HBsAg antigen during treatment with entecavir or lamivudine in nucleoside-naive HBeAg-positive patients with chronic hepatitis B. J Viral Hepat. 2010 Jan;17(1):16-22.