Original Article

Efficacy and Safety of Tenofovir Disoproxil Treatment for Chronic Hepatitis B Patients with Genotypic Resistance to Other Nucleoside Analogues: A Prospective Study

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Abstract

Background: Tenofovir disoproxil (TDF) is a promising salvage therapy for patients with chronic hepatitis B (CHB) who failed regimens of other nucleoside analogues (NAs). In this study, we aimed to investigate the clinical efficacy and safety of TDF monotherapy in Chinese CHB patients with genotypic resistance.

Methods: A total of 33 CHB patients who had failed treatment with other NAs and had genotypic resistance were switched to TDF monotherapy for 48 weeks. Patients' demographic data (age, sex, history of hepatitis B virus [HBV] therapy), laboratory testing results (hepatitis B e antigen [HBeAg] status, HBV DNA levels, alanine aminotransferase [ALT] levels, serum creatinine, urinary protein, genotypic assay), clinical symptoms, and liver color ultrasound examinations were collected for evaluation at day 0 (baseline) and the 12th, 24th, 36th, and 48th weeks after initiating treatment. Statistical analyses were carried out using rank sum test or rank correlation.

Results: With regard to efficacy, the study found that all patients who switched to TDF monotherapy had undetectable HBV DNA levels after 48 weeks. In addition, patients with lower baseline HBV DNA levels realized earlier virological undetectability ($r_s = 0.39$, $P = 0.030$). ALT levels were normal in 30 of 33 patients (91%). HBeAg negative conversion occurred in 7 of 25 patients (28%), among whom HBeAg seroconversion (12%) and HBeAg seroclearance (16%) occurred. The time of complete virological response was significantly affected by the number of resistance loci ($r_s = 0.36$, $P = 0.040$). Concerning safety, the study found that no adverse events were observed during the 48 weeks.

Conclusion: TDF monotherapy is an effective and safe salvage treatment for CHB patients who are resistant to other NAs.

Key words: Drug Resistance; Hepatitis B; Tenofovir; Therapy; Safety

Introduction

Nucleoside analogues (NAs) can rapidly inhibit the transcription of hepatitis B virus (HBV) and suppress serum HBV DNA to a very low or undetectable level, reduce hepatic inflammation, prevent the progression of liver fibrosis, and inhibit the progression of hepatocellular carcinoma. The tolerability, safety, and convenience of NAs promoted their widespread use for chronic hepatitis B (CHB) antiviral therapy. However, the development of drug resistance and the poor virological response after rescue therapy have become major limitations of NAs. These issues remain obstacles to CHB patients' long-term clinical antiviral therapies, especially when treated with low genetic barrier NAs. As a new antiviral drug for CHB, tenofovir disoproxil (TDF) has recently been recommended as the first line of anti-HBV NAs medication by domestic and international CHB prevention and treatment guidelines.[1-3] At present, little is known about the efficacy and safety of TDF for CHB patients with genotypic resistance in China.

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Therefore, our research aimed at investigating the clinical efficacy and safety of TDF monotherapy in Chinese CHB patients with genotypic resistance, which might provide a reference for our national antiviral therapy for CHB patients.

Methods

Study patients and data collection

From September 2012 to September 2015, data were collected from 33 patients diagnosed with CHB and genotypic resistance who were treated at the First Affiliated Hospital, College of Medicine, Zhejiang University. The research protocol was approved by the Human Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (No. 2016-486). In accordance with "The guideline for prevention and treatment of CHB in China in 2010",[4] enrollment and exclusion criteria were established, with the enrollment criteria including the following: (1) in accordance with the diagnostic criteria for CHB in China in 2010, including CHB hepatitis B e antigen [HBeAg]-positive and HBeAg-negative patients; (2) patients who were 18–65 years old; (3) patients who took non-TDF NAs antiviral therapy for more than 1 year and had occurrences of virological breakthrough and abnormal hepatic function; (4) patients who had genotypic resistance loci determined by the detection of HBV P-region sites; (5) patients who signed informed consent; and (6) patients who had previous therapies replaced with TDF monotherapy and received follow-up observation and monitoring of relevant indicators for every 3 months. The exclusion criteria included the following: (1) presence of hepatitis C virus infection; (2) presence of AIDS or other immunodeficiency diseases; (3) presence of autoimmune diseases; (4) alcoholism; (5) presence of renal diseases; and (6) having poor compliance to medication and outpatient follow-ups.

All the 33 patients were followed up for every 12 weeks (at day 0, the 12th, 24th, 36th, and 48th weeks) in hospital clinic of the First Affiliated Hospital, College of Medicine, Zhejiang University. The specific workers monitored and recorded these patients' data at every follow-up point (including age, sex, history of HBV therapy, HBeAg status, HBV DNA levels, alanine aminotransferase [ALT] levels, serum creatinine, urinary protein, genotypic assay, clinical symptoms, and liver color ultrasound examinations; these indicators were tested by the Department of Clinical Laboratory of the First Affiliated Hospital, College of Medicine, Zhejiang University).

Therapeutic method

All patients received TDF (Gilead Sciences Inc., USA) monotherapy that included an oral 300 mg, 1 time/day for at least 48 weeks.

Statistical analysis

Statistical analyses were carried out using rank sum test or rank correlation by SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were described by mean ± standard deviation or median (quartile range). Categorical variables were described by percentage. HBV DNA levels were logarithmically transformed for analysis (to analyze the data, we assigned the undetectable HBV DNA at a level of 2.00 $\log_{10}$U/ml). A $P < 0.05$ was considered statistically significant. When we compared the data of different follow-up points, we used Bonferroni correction method to adjust the probability ($P < 0.005$).

Results

Baseline characteristics

Among the 33 CHB patients, 21/33 (64%) were male, and the mean age was 45.1 ± 11.0 years. There were 25/33 (76%) HBeAg-positive patients and 8/33 (24%) HBeAg-negative patients. About 13/33 (39%) treatment-experienced patients had taken one kind of non-TDF NAs and 20/33 (61%) treatment-experienced patients had taken two or more kinds of non-TDF NAs. The median baseline serum ALT level was 56 (31–89) U/L and 21/33 (64%) patients had abnormal ALT levels. The median baseline HBV DNA level was 5.48 (4.49–6.57) $\log_{10}$ U/ml [Table 1].

Virological response

The HBV DNA levels of all the 33 CHB patients were significantly decreased after TDF monotherapy. Statistically significant differences were observed by comparing HBV DNA levels during 48 weeks ($P < 0.001$) [Figure 1]. There were significant differences by comparing HBV DNA levels at day 0, with the 12th, 24th, 36th and 48th weeks ($Z = 5.01$, $P < 0.001$). There were significant differences by comparing HBV DNA levels at the 12th week with the 24th, 36th, and 48th weeks ($Z = 4.02$, $P < 0.001$). However, there were no significant differences by comparing HBV DNA levels at the

Table 1: Baseline characteristics of patients with chronic hepatitis B and genotypic resistance (n = 33)

Variables	Results
Age (years)	45.1 ± 11.0
Gender (male/female)	21/12
Treatment-experienced drugs, *n* (%)	
LAM	5 (15)
ADV	0
LdT	6 (18)
ETV	2 (6)
Two or more kinds	20 (61)
Genotypic resistance loci, *n* (%)	
1	8 (24)
2	15 (46)
3	9 (27)
>3	1 (3)
HBeAg positive, *n* (%)	25 (76)
Baseline HBV DNA levels ($\log_{10}$ U/ml)	5.48 (4.49–6.57)
Baseline ALT levels (U/L)	56 (31–89)

Values are presented as the mean ± standard deviation, median (quartile range), or percentage. LAM: Lamivudine; ADV: Adefovir dipivoxil; LdT: Telbivudine; ETV: Entecavir; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; ALT: Alanine aminotransferase.

24th week with the 36th and 48th weeks ($Z = 1.83$, $P = 0.068$ and $Z = 1.83$, $P = 0.070$; respectively). Moreover, there was no significant difference by comparing HBV DNA levels at the 36th week with the 48th week ($Z = 1.00$, $P = 0.317$). During the follow-up period, the median time of HBV DNA reaching undetectability was 24 (12–24) weeks. There was a significant correlation between the time it took to achieve undetectable HBV DNA and the number of HBV genotypic resistance loci in the 33 CHB patients ($r_s = 0.36$, $P = 0.040$). If the patient had multiple genotypic resistance loci, it took more time to achieve undetectable HBV DNA.

The virological response time and the baseline HBV DNA levels in the 33 CHB patients were significantly related ($r_s = 0.39$, $P = 0.030$). Specifically, if the baseline HBV DNA levels were higher, the time it took to achieve undetectable HBV DNA was longer. On the contrary, the lower the baseline HBV DNA levels were, the earlier virological responses were reached [Figure 2].

The ratio of HBeAg negative conversion indicates the ratio of HBeAg transforms from positive to negative (including HBeAg seroclearance and seroconversion). During the follow-up period, HBeAg negative conversion was reached in 7 of the 25 (28%) patients. Among these patients, HBeAg seroclearance was reached in 4 of the 25 (16%) patients and HBeAg seroconversion was reached in 3 of the 25 (12%) patients. Among them, one patient's HBeAb changed from negative to positive.

Biochemical responses

During TDF monotherapy, the rate of ALT normalization gradually increased from 12% at day 0 to 91% at the 48th week. There were significant differences in the comparison of ALT levels at the 24th week with the 36th and 48th weeks ($Z = 3.69$, $P < 0.001$ and $Z = 4.48$, $P < 0.001$; respectively). However, there were no significant differences in the comparison of ALT levels at day 0 with the 12th and 24th weeks ($Z = 1.68$, $P = 0.092$ and $Z = 3.31$, $P = 0.010$; respectively). Moreover, there was no significant difference in the comparison of ALT levels at the 36th with the 48th week ($Z = 2.27$, $P = 0.023$). The results showed that ALT levels decreased rapidly from 24 to 36 weeks, but decreased slowly in the first 24 weeks and after the 36th weeks [Table 2].

Adverse events

All the 33 patients achieved virological response and had no occurrence of virological breakthrough at the end of the 48th week. All the 33 patients tolerated the TDF monotherapy well, and there were no clinically significant side effects such as exacerbation of symptoms or death occurred during the follow-up period; 31/33 (94%) patients showed a normal level of serum creatinine and were negative for urinary protein throughout the treatment period, 2/33 (6%) patients' serum creatinine levels increased; however, there were no significant changes in renal function confirmed by ultrasound examination.

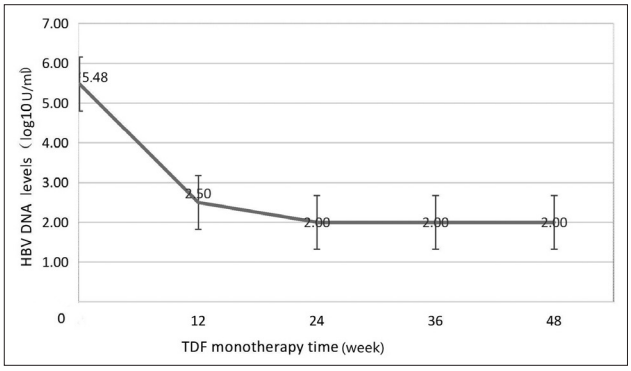

Figure 1: The characteristics between HBV DNA levels and TDF monotherapy time: all the 33 CHB patients' HBV DNA levels were significantly decreased after TDF monotherapy. TDF: Tenofovir disoproxil; HBV: Hepatitis B virus; CHB: Chronic hepatitis B.

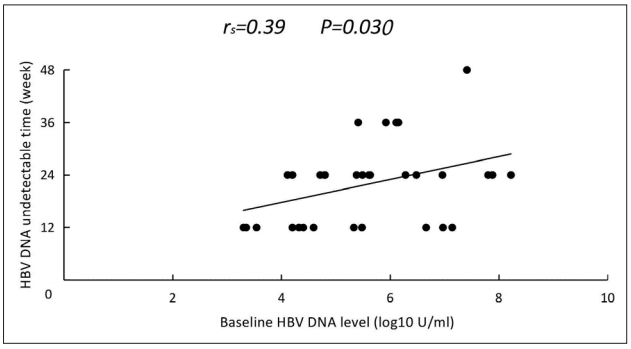

Figure 2: The correlation between baseline HBV DNA levels and the time to reach undetectable HBV DNA levels ($n = 33$). The baseline HBV DNA levels were high, the time of HBV DNA to reach undetectable level was longer. HBV: Hepatitis B virus.

Table 2: Treatment and response characteristics of patients' HBV DNA and ALT levels ($n = 33$)

Characteristics	Baseline	12th week	24th week	36th week	48th week
HBV DNA ($\log_{10}$ U/ml)					
Measured value	5.48 (4.49–6.57)	2.50 (2.00–3.35)	2.00 (2.00–2.00)	2.00 (2.00–2.00)	2.00 (2.00–2.00)
Cumulative decreased range	–	2.59 (2.06–3.55)	3.41 (2.49–4.57)	3.48 (2.59–4.57)	3.48 (2.59–4.57)
ALT					
Measured value (U/L)	56.00 (31.00–89.00)	45.00 (30.00–78.00)	36.00 (28.50–47.50)	33.00 (23.50–38.00)	26.00 (20.00–35.00)
Normal, *n* (%)	12 (36)	15 (45)	19 (58)	28 (85)	30 (91)
Abnormal, *n* (%)	21 (64)	18 (55)	14 (58)	5 (15)	3 (9)
Cumulative probability of recovering normal (%)	–	14	33	76	86

Values are presented as median (quartile range), or percentage. ALT: Alanine aminotransferase; HBV: Hepatitis B virus; –: Not applicable.

Discussion

In CHB antiviral therapy, TDF shows strong efficacy and fewer adverse reactions, and it is an ideal drug for the rescue treatment of CHB patients with genotypic resistance.[5] At present, there are many clinical studies of multicenter, large-scale samples of TDF here and abroad.[6-14] Among them, TDF *in vitro* experiments showed that it had a strong antiviral capacity against both hepatotropic viruses and retroviruses, and it can inhibit the activity of HBV DNA polymerase, which had a good inhibitory effect on HBV DNA replication in wild-type HBV. *In vitro* studies of TDF treatment for HBV infection with lamivudine (LAM) and adefovir dipivoxil (ADV) resistance also showed that TDF has a good inhibitory effect on viral replication. *In vivo* researches of TDF also showed strong inhibition of HBV DNA replication in treatment-naive or treatment-experienced patients having resistance to other kinds of NAs, and to some extent, TDF can prevent the progression of hepatic fibrosis and cirrhosis. However, these studies were carried out in American and European countries where the HBV genotype of human infections is A or D type, whereas Chinese people were mostly infected by B or C type HBV. Therefore, the efficacy and safety of TDF in antiviral treatment of CHB patients have not yet been confirmed by further clinical trials in China.[15,16] The research analyzed the efficacy and safety of the 33 Chinese CHB patients previously treated with non-TDF NAs (including LAM, telbivudine [LdT], entecavir [ETV], and ADV monotherapy or combined therapy) after 48 weeks of treatment. During the course of antiviral therapy, these patients experienced virological breakthrough and had clearly detected genotypic resistance loci and voluntarily switched to TDF monotherapy. The results showed that TDF still had an excellent antiviral effect and safety after 48 weeks for most Chinese CHB patients who were previously treated by NAs and had genotypic resistance.

In the study, we found that there was significant correlation between the duration of undetectable HBV DNA and the number of genotypic resistance loci. The results that the more genotypic resistance loci, the longer it took to reach a complete response to the virus were similar to the current studies abroad.[17] There was a positive correlation between the baseline HBV DNA levels and duration of undetectable HBV DNA. Therefore, CHB patients should detect serum HBV DNA levels regularly during antiviral therapy, and once virological breakthrough occurs, patients should have the HBV P-resistant gene region inspected as early as possible. At the same time, patients should switch to TDF rescue treatment as soon as possible to suppress HBV replication in time and achieve a virological response as early as possible. The research results show that HBV DNA levels were significantly decreased and reached undetectability at the end of the study. What is more, the therapeutic effect was achieved during 24 weeks. In our study, with the prolonged treatment duration, the range of HBV DNA levels decline was increased, and HBV DNA levels decline reached 3.48 (2.59–4.57) $\log_{10}$U/ml at the 48th week; The result was essentially similar to a study by Patterson *et al*.,[18] which reported that the mean range of decline of HBV DNA levels was (3.75±1.33) $\log_{10}$U/ml for CHB patients with LAM resistance who took TDF monotherapy for 48 weeks. In our study, the rate of HBV DNA undetectability rose from 37% at the 12th week to 100% at the 48th week, the result was similar to a study by van Bömmel *et al*.[19] In TDF rescue therapy, the cumulative probability of HBV DNA undetectability gradually increased, thus TDF showed an excellent antiviral efficacy.

HBeAg negative conversion shows the process of the clearance of HBV by the immune system, and it is also an important clinical reference of achieving virological response. The research showed that the rate of HBeAg seroconversion was 12% in TDF rescue therapy after 48 weeks. However, a study abroad[20] reported that the rate of HBeAg seroconversion was 21% in TDF treatment-naive CHB patients who were HBeAg positive. Our results were lower, and we consider that it may be related to our patients having been treated with NAs as well as the small size of our cohort. We further analyzed the baseline characteristics of three patients who exhibited HBeAg seroconversion, including one male and two females (34, 46, and 46 years old, respectively), with early symptoms that were less harsh. The baseline HBV DNA levels of these individuals were between 6.00 and 7.00 $\log_{10}$ U/ml and their genotypic resistance locus was 240I. The liver color Doppler ultrasound showed mild inflammation and their complete virological response times were 12, 24, and 36 weeks, respectively, with ALT being mildly increased. From the above analysis, we may speculate that the realization of HBeAg seroconversion may be related to the mild increase of ALT, being female, having nonhepatic cirrhosis, and having a low HBV DNA level. This is similar to the principles of optimization of interferon anti-HBV treatment. However, this still needs further analysis using large-scale sample data.

TDF shows not only potent inhibition of viral replication but also powerful ALT recovery capacity for CHB patients treated with NAs and having genotypic resistance. With prolonged treatment duration, the rate of ALT normalization gradually increased. The rate of ALT normalization reached 91% at the end of our study. The cumulative recovery rate of ALT in our study was 33% at the 24th week, which was significantly different from 77% at the 24th week in European TDF-naive treatment for CHB patients[21] and is similar to the 76% observed at the 36th week in our study. The reasons may be that our research patients were all experience-treated and exhibited genotypic resistance.

At present, many reports showed that TDF had no resistance in *in vivo* studies,[2,22-24] but some *in vitro* studies reported that the A181V/T+N236T mutation strains had decreased TDF sensitivity.[25,26] Koziel and Peters[27] found that the V214 and Q215 mutations decreased TDF susceptibility by analyzing

the related HBV resistance mutations in five kinds of NAs (including TDF). However, the patients with rtN236T and rtA181V double mutations were still sensitive to TDF in clinical practice. A number of studies[11-13,28] showed that no matter what is used, TDF monotherapy or combined therapy, TDF has shown an excellent clinical efficacy. Therefore, it is still unknown whether TDF resistance requires several genetic resistance loci at the same time. However, Suzuki *et al*.[29] recently reported the occurrence of virological breakthrough during the long-term follow-up period after TDF plus ETV treatment of patients, suggesting that TDF rescue therapy for drug-resistant patients still need to be closely followed up. The study results, by detecting gene loci, did not show the loci that decreased TDF susceptibility. Among them, there are three patients with the 181V resistance locus. We further analyzed complete virologic response durations and they all achieved HBV DNA undetectability at the 24th and 36th weeks, demonstrating that the efficacy of TDF is excellent. In our study, all the 33 CHB patients achieved completely virological response after 48 weeks and no virological breakthrough or drug resistance. Our findings are consistent with those reporting no drug resistance.

The common adverse effects of taking TDF are nausea, vomiting, diarrhea, and abdominal distension. Among these, the most attention is paid to the rare renal toxicity. There were reports of severe complications such as acute tubular necrosis and Fanconi syndrome.[29-33] However, most researches showed that TDF had excellent tolerability and safety in recent years. For treatment-naive CHB patients, studies have shown that the incidence of renal adverse events was <2.2% after treating men with TDF for 8 years.[34] For ADV treatment-experienced CHB patients who were treated with TDF monotherapy and FTC plus TDF treatment for 168 weeks, there were no patients diagnosed with renal adverse events in two groups.[11] Our study showed that the tolerability and safety of TDF were excellent for CHB patients after 48 weeks in China. However, as for its long-term safety, TDF still needs further study. During the follow-up period, most of the serum creatinine levels of patients were stable or were not significantly increased after taking TDF. There were no instances of renal injuries, which was consistent with researches in other countries.

In conclusion, TDF monotherapy is an effective and safe option for CHB patients with genotypic resistance to other NAs. However, this study had several limitations: it was a single-center study, the sample size was small, and the follow-up time was short that might cause few bias in the results. It is necessary to further study the long-term efficacy of TDF and to monitor adverse reactions.

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Conflicts of interest

There are no conflicts of interest.

References

1. Liaw YF, Kao JH, Piratvisuth T, Chan HL, Chien RN, Liu CJ, *et al.* Asian-Pacific consensus statement on the management of chronic hepatitis B: A 2012 update. Hepatol Int 2012;6:531-61. doi: 10.1007/s12072-012-9365-4.
2. European Association for the Study of the Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012;57:167-85. doi: 10.1016/j.jhep.2012.02.010.
3. Chinese Society of Hepatology, Chinese Medical Association. Guideline for prevention and treatment of chronic hepatitis B (2015 Edition) (in Chinese). J Chin Hepatol 2015;31:1941-60. doi: 10.3969/j.issn.1001-5256.2015.12.002.
4. Chinese Society of Hepatology, Chinese Medical Association. Guideline for prevention and treatment of chronic hepatitis B (2010 Edition) (in Chinese). Chin Hepatol 2011;16:2-16. doi: 10.14000/j.cnki.issn.1008-1704.2011.01.001.
5. 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 2011;53:763-73. doi: 10.1002/hep.24078.
6. Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, *et al.* Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: A 5-year open-label follow-up study. Lancet 2013;381:468-75. doi: 10.1016/S0140-6736(12)61425-1.
7. Seto WK, Liu K, Wong DK, Fung J, Huang FY, Hung IF, *et al.* Patterns of hepatitis B surface antigen decline and HBV DNA suppression in Asian treatment-experienced chronic hepatitis B patients after three years of tenofovir treatment. J Hepatol 2013;59:709-16. doi: 10.1016/j.jhep.2013.06.007.
8. Gerada J, Borg E, Formosa D, Magro R, Pocock J. Tenofovir as rescue therapy following clinical failure to lamivudine in severe acute hepatitis B. Mediterr J Hematol Infect Dis 2013;5:e2013035. doi: 10.4084/MJHID.2013.035.
9. Kuo A, Dienstag JL, Chung RT. Tenofovir disoproxil fumarate for the treatment of lamivudine-resistant hepatitis B. Clin Gastroenterol Hepatol 2004;2:266-72. doi: 10.1016/S1542-3565(04)00017-5.
10. Batirel A, Guclu E, Arslan F, Kocak F, Karabay O, Ozer S, *et al.* Comparable efficacy of tenofovir versus entecavir and predictors of response in treatment-naïve patients with chronic hepatitis B: A multicenter real-life study. Int J Infect Dis 2014;28:153-9. doi: 10.1016/j.ijid.2014.09.004.
11. Berg T, Zoulim F, Moeller B, Trinh H, Marcellin P, Chan S, *et al.* Long-term efficacy and safety of emtricitabine plus tenofovir DF vs. tenofovir DF monotherapy in adefovir-experienced chronic hepatitis B patients. J Hepatol 2014;60:715-22. doi: 10.1016/j.jhep.2013.11.024.
12. Lim YS, Byun KS, Yoo BC, Kwon SY, Kim YJ, An J, *et al.* Tenofovir monotherapy versus tenofovir and entecavir combination therapy in patients with entecavir-resistant chronic hepatitis B with multiple drug failure: Results of a randomised trial. Gut 2016;65:852-60. doi: 10.1136/gutjnl-2014-308353.
13. Kim HJ, Cho JY, Kim YJ, Gwak GY, Paik YH, Choi MS, *et al.* Long-term efficacy of tenofovir disoproxil fumarate therapy after multiple nucleos(t)ide analogue failure in chronic hepatitis B patients. Korean J Intern Med 2015;30:32-41. doi: 10.3904/kjim.2015.30.1.32.
14. van Hemert FJ, Berkhout B, Zaaijer HL. Differential binding of tenofovir and adefovir to reverse transcriptase of hepatitis B virus. PLoS One 2014;9:e106324. doi: 10.1371/journal.pone.0106324.
15. Ge Y, Li D, Fan Y, Yang H, Lyu W, Fan H, *et al*. Tenofovir rescue therapy for chronic hepatitis B patients after suboptimal response to treatment with lamivudine plus adefovir dipivoxil (in Chinese). Chin J Intern Med 2014;53:697-700. doi: 10.3760/cma.j.issn.0578-1426.2014.09.006.
16. Huang M, Li X, Wu Y, Tao L, Jie Y, Li X, *et al.* Efficacy of 48-week tenofovir disoproxil fumarate therapy in patients who were unresponsive to nucleoside-analogue treatments (in Chinese). Chin J Hepatol 2014;22:266-71. doi: 10.3760/cma.j.issn.1007-3418.2014.04.006.
17. Lim YS, Lee TH, Heo NY, Shim JH, Lee HC, Suh DJ. Entecavir plus adefovir combination treatment for chronic hepatitis B patients after failure of nucleoside/nucleotide analogues. Antivir Ther 2012;17:53-60. doi: 10.3851/IMP1914.

18. 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;60:247-54. doi: 10.1136/gut.2010.223206.
19. van Bömmel 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;51:73-80. doi: 10.1002/hep.23246.
20. 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;359:2442-55. doi: 10.1056/NEJMoa0802878.
21. Kitrinos KM, Corsa A, Liu Y, Flaherty J, Snow-Lampart A, Marcellin P, *et al.* No detectable resistance to tenofovir disoproxil fumarate after 6 years of therapy in patients with chronic hepatitis B. Hepatology 2014;59:434-42. doi: 10.1002/hep.26686.
22. Buti M, Tsai N, Petersen J, Flisiak R, Gurel S, Krastev Z, *et al*. Seven-year efficacy and safety of treatment with tenofovir disoproxil fumarate for chronic hepatitis B virus infection. Dig Dis Sci 2015;60:1457-64. doi: 10.1007/s10620-014-3486-7.
23. Gordon SC, Krastev Z, Horban A, Petersen J, Sperl J, Dinh P, *et al*. Efficacy of tenofovir disoproxil fumarate at 240 weeks in patients with chronic hepatitis B with high baseline viral load. Hepatology 2013; 58:505-13. doi: 10.1002/hep.26277.
24. Liu Y, Wang CM, Cheng J, Liang ZL, Zhong YW, Ren XQ, *et al*. Hepatitis B virus in tenofovir-naive Chinese patients with chronic hepatitis B contains no mutation of rtA194T conferring a reduced tenofovir susceptibility. Chin Med J 2009;122:1585-6. doi: 10.3760/cma.j.issn.0366-6999.2009.13.021.
25. Qi X, Xiong S, Yang H, Miller M, Delaney WE 4th. *In vitro* susceptibility of adefovir-associated hepatitis B virus polymerase mutations to other antiviral agents. Antivir Ther 2007;12:355-62.
26. Gish R, Jia JD, Locarnini S, Zoulim F. Selection of chronic hepatitis B therapy with high barrier to resistance. Lancet Infect Dis 2012;12:341-53. doi: 10.1016/S1473-3099(11)70314-0.
27. Koziel MJ, Peters MG. Viral hepatitis in HIV infection. N Engl J Med 2007;356:1445-54. doi: 10.1056/NEJMra065142.
28. Wu YS, Zhang WW, Ling XM, Yang L, Huang SB, Wang XC, *et al.* Efficacy and safety of tenofovir and lamivudine in combination with efavirenz in patients co-infected with human immunodeficiency virus and hepatitis B virus in China. Chin Med J 2016;129:304-8. doi: 10.4103/0366-6999.174509.
29. Suzuki F, Sezaki H, Akuta N, Suzuki Y, Kawamura Y, Hosaka T, *et al.* Virologic breakthrough in a patient with chronic hepatitis B by combination treatment with tenofovir disoproxil fumarate and entecavir. Drug Des Devel Ther 2014;8:869-73. doi: 10.2147/DDDT.S65349.
30. Viganò M, Brocchieri A, Spinetti A, Zaltron S, Mangia G, Facchetti F, *et al.* Tenofovir-induced Fanconi syndrome in chronic hepatitis B monoinfected patients that reverted after tenofovir withdrawal. J Clin Virol 2014;61:600-3. doi: 10.1016/j.jcv.2014.09.016.
31. Gracey DM, Snelling P, McKenzie P, Strasser SI. Tenofovir-associated Fanconi syndrome in patients with chronic hepatitis B monoinfection. Antivir Ther 2013;18:945-8. doi: 10.3851/IMP2649.
32. Samarkos M, Theofanis V, Eliadi I, Vlachogiannakos J, Polyzos A. Tenofovir-associated Fanconi syndrome in a patient with chronic hepatitis B. J Gastrointestin Liver Dis 2014;23:342. doi: 10.5604/16652681.1193725.
33. Hwang HS, Park CW, Song MJ. Tenofovir-associated Fanconi syndrome and nephrotic syndrome in a patient with chronic hepatitis B monoinfection. Hepatology 2015;62:1318-20. doi: 10.1002/hep.27730.
34. Marcellin P, Gane EJ, Flisiak R, Trinh HN, Petersen J, Gurel S, *et al*. Long Term Treatment with Tenofovir Disoproxil Fumarate for Chronic Hepatitis B Infection is Safe and Well Tolerated and Associated with Durable Virologic Response with no Detectable Resistance: 8 Year Results from Two Phase 3 Trials. Hepatology 2014;60(S1):313A.Abs.229. doi: 10.1002/hep.27486.