Antiviral Research 96 (2012) 100–104

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Comparison of rescue strategies in lamivudine-resistant patients with chronic hepatitis B

Pan Zhao [a,*], Chunya Wang [b], LiLi Huang [a], Dongping Xu [a], Tanshi Li [b]

[a] Liver Failure Therapy and Research Center, Beijing 302 Hospital, Beijing 100039, China
[b] Emergency Department, Beijing 301 Hospital (General Hospital of PLA), Beijing 100853, China

ARTICLE INFO

Article history:
Received 17 May 2012
Revised 21 August 2012
Accepted 23 August 2012
Available online 31 August 2012

Keywords:
Chronic hepatitis B
Lamivudine
Resistance
Adefovir dipivoxil
Entecavir

ABSTRACT

Lamivudine (LAM) resistance now poses a major problem in the management of patients with chronic hepatitis B virus (HBV) infection. We retrospectively collected clinical data on chronic HBV-infected patients who had developed LAM resistance under de novo LAM monotherapy and subsequently took nucleos(t)ide analogs as rescue strategy in our hospital. From initiation of rescue therapies to January 2012, incidence of antiviral drug resistance was 23.67%, 18%, 6.94% and 0% ($P = 0.007$) in the group of switching to adefovir dipivoxil (ADV) monotherapy, switching to entecavir (ETV) monotherapy, adding on ADV and switching to combination of ADV and ETV. At month 12, the median levels of serum HBV DNA were respectively 9300 IU/mL, 4648 IU/mL, 2054 IU/mL and 100 IU/mL ($P < 0.001$), and the cumulative rates of serum ALT normalization were respectively 75%, 84%, 93% and 100% ($P = 0.003$). Additionally, the strategy of switching to ADV monotherapy induced more single rtA181T mutations. In conclusion, switching to ADV monotherapy has been widely used in real-world clinical practice in China, however, due to the high incidence of drug resistance, switching to neither ADV nor ETV monotherapy is optimal when LAM resistance occurs; combination of ADV and ETV is most effective, whereas the strategy of adding on ADV is rational for most of LAM-resistant Chinese patients with chronic hepatitis B.

1. Introduction

Hepatitis B virus (HBV) infection frequently leads to liver cirrhosis and hepatocellular carcinoma. The development of oral nucleos(t)ide analogs (NA) has substantial impact in the anti-HBV treatment. Nevertheless, the emergence of drug-resistant mutations within the viral reverse-transcriptase (RT) gene is the major drawback of NA treatment (Locarnini and Bowden, 2010). Because viral drug resistance is associated with rebound in viral load levels and the subsequent worsening of liver disease (Billioud et al., 2011), it is becoming clinically important to adjust antiviral therapy when viral drug resistance occurs.

Lamivudine (LAM) has been widely used as a first-line therapy for chronic hepatitis B (CHB). However, a major shortcoming of LAM is the frequent emergence of drug resistance mutations and it was reported that the cumulative incidence of LAM resistance after 1 year and 4 years respectively arrived at 24% and 66% (Lai et al., 2003; Leung et al., 2001). As rescue therapies, switching to adefovir dipivoxil (ADV) or entecavir (ETV) monotherapy and adding on ADV were once suggested against LAM-resistant HBV (Lee et al., 2012), but studies about the efficacy of switching to combination of ADV and ETV were few. Additionally, incidence of drug resistance after initiation of rescue therapies varied markedly between studies (Perrillo et al., 2011; Lee et al., 2009; Dai et al., 2007). The aim of this article is to investigate various rescue strategies in LAM-resistant patients with CHB in real-world clinical practice in China and compare the incidence of drug resistance among them.

2. Patients and methods

2.1. Patients

We retrospectively collected clinical data between September 2007 and January 2012 on chronic HBV-infected patients who had developed antiviral drug resistance under de novo LAM monotherapy and subsequently took nucleos(t)ide analogs as rescue strategy for at least 12 months in our hospital. Individual rescue therapy regimens were chosen based on attending physicians' own discretion. The observational endpoint for every patient was the emergence of antiviral drug resistance for the administered

Abbreviations: HBV, hepatitis B virus; LAM, lamivudine; ADV, adefovir dipivoxil; ETV, entecavir; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase.

* Corresponding author. Address: Liver Failure Therapy and Research Center, Beijing 302 Hospital, No. 100 of West Fourth Ring Middle Road, Beijing 100039, China. Tel.: +86 10 66933392; fax: +86 10 66933020.
E-mail addresses: doczhaopan@126.com, zhaopan302@sina.com (P. Zhao).

0166-3542/$ - see front matter Crown Copyright © 2012 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.antiviral.2012.08.008

Table 1
Primers used for HBV reverse-transcriptase gene amplification.

Polarity	Sequences (5′–3′)	Position	Round
Sense	AGT CAG GAA GAC AGC CTAC TCC	3146–3167	First
Antisense	AGG TGA AGC GAA GTG CAC AC	1577–1596	
Sense	TTC CTG CTG GTG GCT CCA GTT C	54–75	Second
Antisense	TTC CGC AGT ATG GAT CGG CAG	1258–1278	

medications to January 2012. Antiviral drug resistance was defined as virological breakthrough (>1 log10 increase in serum HBV DNA level from the nadir in a patient who had an initial virological response, Lok and McMahon (2007)) accompanied with documented genotypic resistance. Patients with hepatic decompensation, past or current hepatocellular carcinomas or liver transplantation were excluded. Other criteria for exclusion were infection with hepatitis A, C, D, E or HIV, or the presence of other forms of liver diseases such as autoimmune or alcoholic liver disease, drug hepatitis or Wilson's disease. Informed written consent for the analysis was obtained from each patient. The study was approved by the ethics committee of Beijing 302 Hospital.

2.2. Biochemical and serological markers and quantification of HBV DNA

Serum HBV DNA, ALT, HBeAg were routinely detected in the Central Clinical Laboratory of Beijing 302 Hospital. Among these, HBV DNA level was determined by a popular real-time quantitative PCR (qPCR) kit (Fosun Pharmaceutical Co., Ltd., Shanghai, China) with a lower detection limit of 100 IU/mL. The normalized level of serum ALT was $\leqslant$40 U/L.

2.3. HBV RT gene amplification and sequencing

HBV gene fragment (nucleotides (nt) 54–1278) encompassing the complete RT gene (nt 130–1161) was amplified by nested PCR. The sense and antisense primers for first-round PCR and second-round PCR were described in Table 1. The first-round PCR consisted of 10 cycles of 94 °C for 35 s, 59 °C for 35 s (decreasing by 2 °C every other cycle), 72 °C for 70 s; and 30 cycles of 94 °C for 35 s, 56 °C for 35 s, 72 °C for 70 s. The second-round PCR (conducted in the same tube) consisted of 35 cycles of 94 °C for 25 s, 56 °C for 25 s, and 72 °C for 50 s. PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Sequencing was performed using an ABI 3730xl DNA Analyzers (Applied Biosystems, Foster City, CA). Analysis and assembly of sequencing data were performed with the Vector NTI Suite software package (Informax, Frederick, MD, USA).

2.4. HBV genotype analysis

The genotyping was based on S-gene sequences encompassing the RT domain of HBV, which was amplified as the above. HBV genotype was determined by molecular evolutionary analysis of the viral sequences using the MEGA4 software. Phylogenetic trees were constructed using neighbor-joining analysis with bootstrap test confirmation performed on 1000 resamplings. Standard reference sequences were acquired from the online hepatitis virus database (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi) as previously reported (Fang et al., 2009).

2.5. Statistical analysis

Data analyses were performed using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA). Continuous data were expressed as median (quartile range). Categorical data were expressed as the number of subjects. Group comparisons were performed using the Kruskal–Wallis test for continuous variables, and the Fisher's exact test for categorical variables. A probability value of less than 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

338 patients developing rtM204V/I and/or rtL80I/M, rtL180M, rtV173L mutations (Patients developing rt181 site mutation were not included) were finally included, and four rescue strategies were documented to treat patients with LAM resistance. Of these 338 patients, 207 switched to ADV monotherapy, accounting for 61.24%; 50 switched to ETV monotherapy, accounting for 14.79%; 72 added on ADV, accounting for 21.30%; 9 switched to combination of ADV and ETV, accounting for 2.66%. The baseline characteristics of these patients were summarized in Table 2. There were no statistically significant differences in sex composition, median age, cirrhosis composition, HBeAg status, genotypes, median serum HBV DNA level or median ALT level among the four groups. The median duration time after initiation of the above four rescue therapies was respectively 18 months, 17 months, 23 months and 18 months, and no statistical significance existed among the four groups ($P = 0.070$) (Fig. 1).

3.2. Drug resistance analysis

During the rescue treatment period, virological breakthrough and genotypic resistance were detected in 63 patients, including 49 of 207 patients (23.67%) who switched to ADV monotherapy,

Table 2
Baseline characteristics of LAM-resistant patients with CHB before receiving different rescue strategies.

Variables	Switching to ADV monotherapy	Switching to ETV monotherapy	Adding on ADV	Switching to ADV + ETV	*P* value
Sex (male/female)	185/22	43/7	65/7	6/3	0.183
Age (years)					
Median (quartile range)	43 (14)	43 (12)	39 (14.5)	45 (11)	0.310
Cirrhosis/no cirrhosis	36/171	8/42	20/52	2/7	0.233
HBeAg+/HBeAg-	187/20	42/8	61/11	8/1	0.365
Genotype (C/B)	182/25	40/10	57/15	7/2	0.150
HBV DNA (IU/mL)					
Median (quartile range)	890,000 (903,200)	772,900 (6,758,300)	830,000 (5,418,469)	899,600 (5,176,820)	0.230
ALT (U/L)					
Median (quartile range)	59 (86)	53 (68)	62 (92)	60 (82)	0.260
LAM-resistant mutations	68 (32.85%)	17 (34.00%)	29 (40.28%)	3 (33.33%)	
rtM204I/V					
rtM204I/V + (rtL80I/M and/or rtL180 M and/or rtV173L)	139 (67.15%)	33 (66.00%)	43 (59.72%)	6 (66.67%)	

LAM, lamivudine; ADV, adefovir; ETV, entecavir; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase.

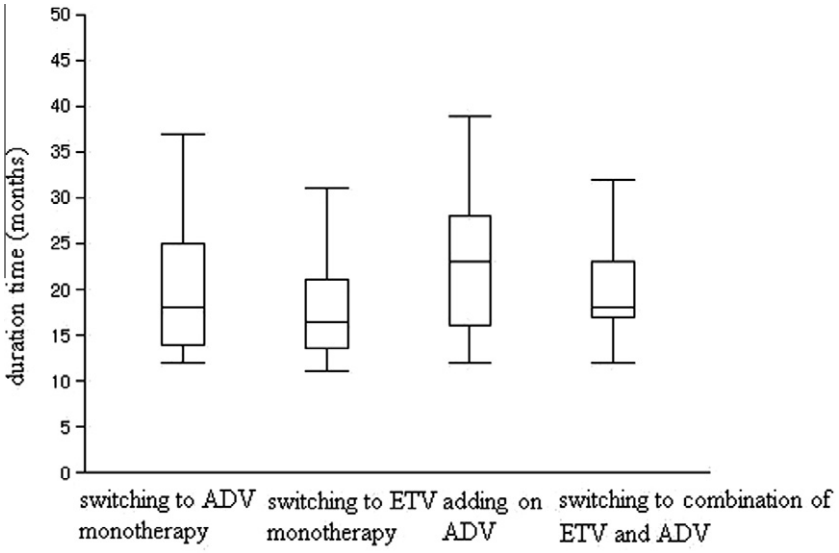

Fig. 1. The median duration time after initiation of rescue therapies for LAM-resistant patients with CHB. No statistically significant differences exit among the four groups ($P = 0.070$) (LAM, lamivudine; ADV, adefovir; ETV, entecavir; CHB, chronic hepatitis B).

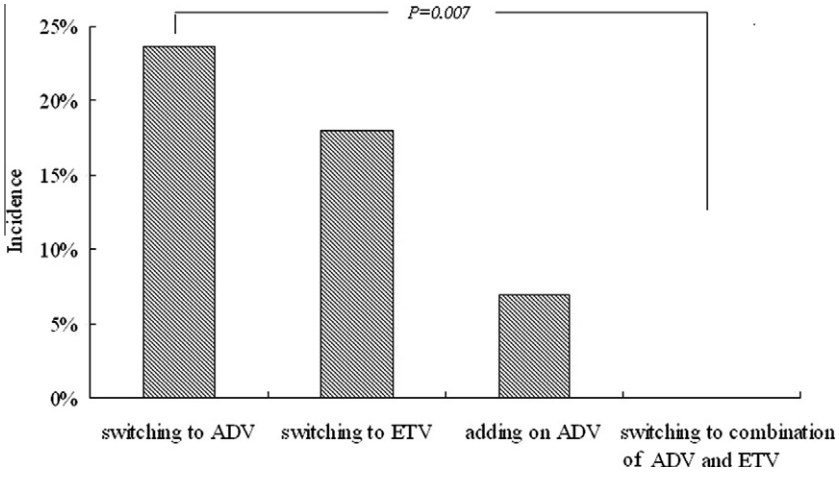

Fig. 2. Incidence of drug resistance after initiation of rescue therapies for LAM-resistant patients (LAM, lamivudine; ADV, adefovir; ETV, entecavir).

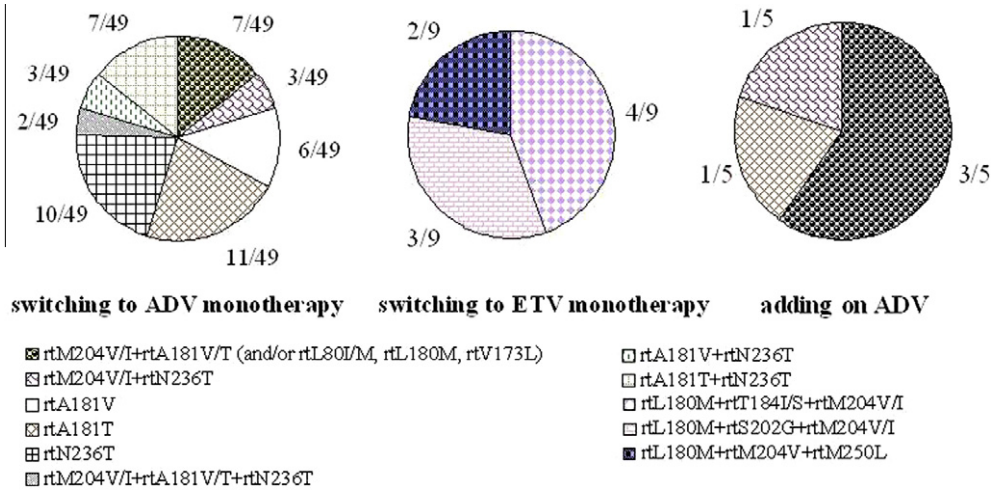

Fig. 3. Mutational patterns of genotypic resistance in LAM-resistant patients after initiation of rescue therapies (LAM, lamivudine; ADV, adefovir; ETV, entecavir).

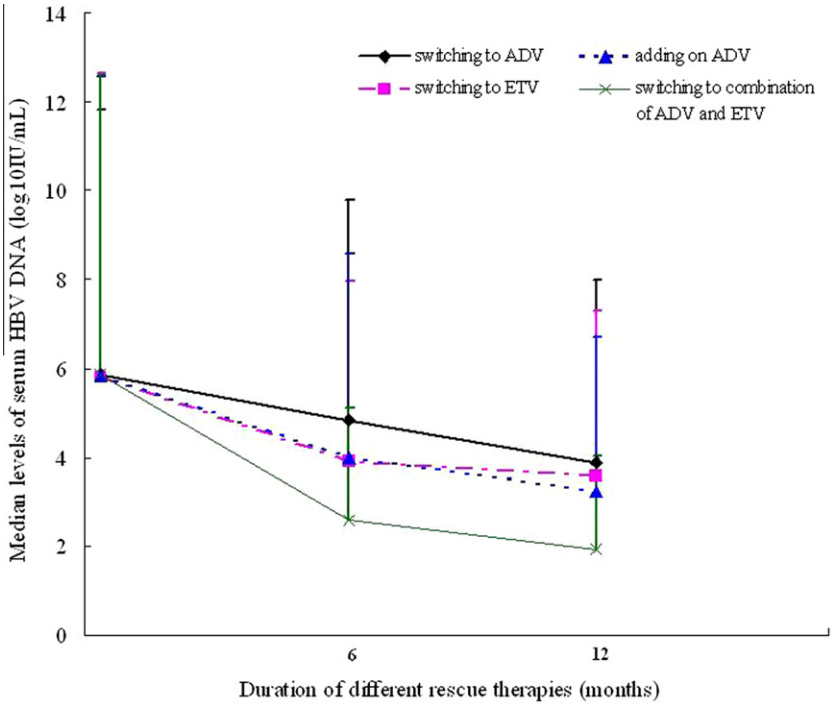

Fig. 4. Median log change in serum HBV DNA levels after initiation of rescue therapies (*P* values among the four groups at month 6 and month 12 are both less than 0.001.). Error bars indicate the quartile range (LAM, lamivudine; ADV, adefovir; ETV, entecavir).

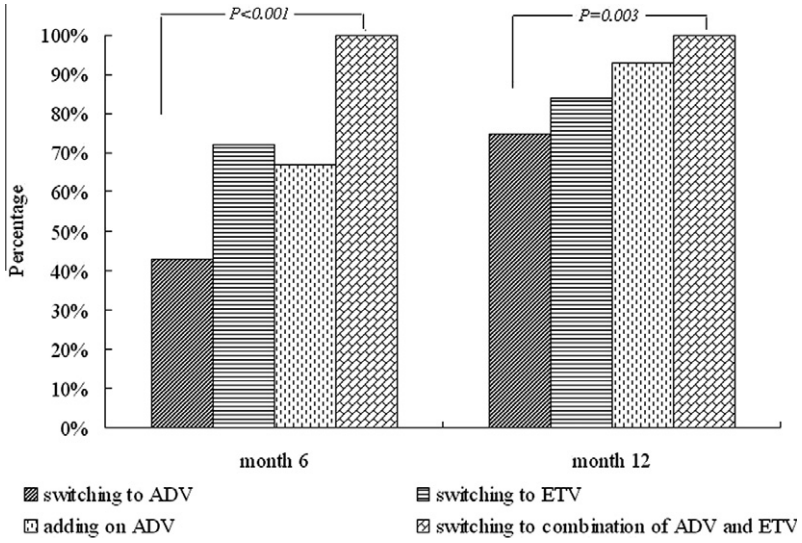

Fig. 5. Cumulative rates of achieving normalization of serum ALT after initiation of rescue therapies at month 6 and month 12.

9 of 50 patients (18%) who switched to ETV monotherapy and 5 of 72 patients (6.94%) who added on ADV. No resistance occurred in patients who switched to combination of ADV and ETV. A statistically significant difference ($P = 0.007$) existed among the four groups (Fig. 2).

Mutational patterns of genotypic resistance after initiation of rescue therapies were as follows. Single rtA181T, single rtA181V, single rtN236T, rtA181T + rtN236T, rtA181V + rtN236T, rtM204V/I + rtA181V/T (and/or rtL80I/M, rtL180M, rtV173L), rtM204V/I + rtN236T, rtM204V/I + rtA181V/T + rtN236T were developed respectively in 11, 6, 10, 7, 3, 7, 3 and 2 patients who switched to ADV monotherapy; rtM204V/I + rtA181V/T (and/or rtL180M, rtV173L), single rtA181T, rtM204V/I + rtN236T were developed respectively in 3, 1 and 1 patients who added on ADV; rtL180M + rtT184I/S + rtM204V/I, rtL180M + rtS202G + rtM204V/I, rtL180M + rtM204V + rtM250L were developed respectively in 4, 3 and 2 patients who switched to ETV monotherapy. Details were described in Fig. 3.

Fig. 4 showed the median log change in serum HBV DNA levels after initiation of rescue therapies. At month 6, the median levels of serum HBV DNA were respectively 81,138 IU/mL (94,900 IU/mL), 9739 IU/mL (10,999 IU/mL), 11,567 IU/mL (38,127 IU/mL), 485 IU/mL (332 IU/mL) in the group of switching to ADV monotherapy, switching to ETV monotherapy, adding on ADV

and switching to combination of ADV and ETV, with statistical significance ($P < 0.001$) among the four groups, and at month 12, the median levels of serum HBV DNA were respectively 9300 IU/mL (12,030 IU/mL), 4648 IU/mL (5109 IU/mL), 2054 IU/mL (2881 IU/mL), 100 IU/mL (128 IU/mL), with statistical significance ($P < 0.001$) among the four groups.

Fig. 5 showed the cumulative rates of achieving normalization of serum ALT after initiation of rescue therapies at month 6 and month 12. The cumulative rates of achieving normalization of serum ALT in the group of switching to ADV monotherapy, switching to ETV monotherapy, adding on ADV and switching to combination of ADV and ETV at month 6 were respectively 43% (89/207), 72% (36/50), 67% (48/72) and 100% (9/9), with statistical significance ($P < 0.001$) among the four groups, and the cumulative rates at month 12 were respectively 75% (156/207), 84% (42/50), 93% (67/72) and 100% (9/9), with statistical significance ($P = 0.003$) among the four groups.

4. Discussion

HBV infection is a highly endemic disease in China (Luo et al., 2011). Related to its low cost and safety profile, LAM has been extensively applied for patients with CHB during the past decade. However, most patients with CHB require long-term LAM treatment, which results in the emergence of LAM-resistant mutants. Thus, LAM resistance now poses a major problem and becomes a challenge in the management of patients with CHB in China.

As salvage for LAM resistance, the strategy of switching to ADV monotherapy was widely adopted in China. ETV, a guanosine analog, was proved to be potent against LAM-resistant HBV not only in vivo (Tenney et al., 2007) but also in vitro (Levine et al., 2002). However, monotherapy with either ADV or ETV was reported to have a higher risk for developing subsequent genotypic resistance to ADV or ETV and even to induce more multi-drug resistant mutants compared with adding-on ADV therapy (Rapti et al., 2007; Chung et al., 2011; Kim et al., 2010). In our study, we found that incidence of drug resistance in the group of switching to ADV or ETV monotherapy was much higher (respectively 23.67% and 18%) than that in the group of adding on ADV (6.94%) or switching to combination of ADV and ETV (0%). Additionally, the group of switching to combination of ADV and ETV showed the lowest median level of serum HBV DNA and highest cumulative rates of serum ALT normalization among the four groups. Nevertheless, the combination therapy of ADV and ETV was not commonly used in China (only nine patients adopting this regimen in this study), the reason of which might be that its high cost restricted its wide application.

Previous studies showed that rtA181T mutation was frequently developed in lamivudine-resistant or lamivudine-to-adefovir dipivoxil sequential patients (Yatsuji et al., 2006; Yeon et al., 2006) and it could lead to cross resistance to lamivudine and adefovir dipivoxil (Villet et al., 2008). Our study also showed that incidence of single rtA181T mutation was relatively high (11/49) in patients switching to ADV monotherapy. It seemed that the effect of rtA181T mutant was equal to that of multi-drug resistant mutant, and moreover, this mutation might be more harmful because of its potential association with the development of hepatocellular carcinoma (Hosaka et al., 2010; Yeh et al., 2011).

In summary, switching to ADV monotherapy has been widely used in real-world clinical practice in China, however, due to the high incidence of drug resistance, switching to neither ADV nor ETV monotherapy is optimal when LAM resistance occurs; combination of ADV and ETV is most effective, whereas the strategy of adding on ADV is rational for most of LAM-resistant Chinese patients with chronic hepatitis B.

References

Billioud, G., Pichoud, C., Puerstinger, G., Neyts, J., Zoulim, F., 2011. The main hepatitis B virus (HBV) mutants resistant to nucleoside analogs are susceptible in vitro to non-nucleoside inhibitors of HBV replication. Antiviral Res. 92 (2), 271–276.

Chung, G.E., Kim, W., Lee, K.L., Hwang, S.Y., Lee, J.H., Kim, H.Y., Jung, Y.J., Kim, D., Jeong, J.B., Kim, B.G., Kim, Y.J., Yoon, J.H., Lee, H.S., 2011. Add-on adefovir is superior to a switch to entecavir as rescue therapy for lamivudine-resistant chronic hepatitis B. Dig. Dis. Sci. 56 (7), 2130–2136.

Dai, C.Y., Chuang, W.L., Hsieh, M.Y., Lee, L.P., Huang, J.F., Hou, N.J., Lin, Z.Y., Chen, S.C., Hsieh, M.Y., Wang, L.Y., Tsai, J.F., Chang, W.Y., Yu, M.L., 2007. Adefovir dipivoxil treatment of lamivudine-resistant chronic hepatitis B. Antiviral Res. 75 (2), 146–151.

Fang, Z.L., Sabin, C.A., Dong, B.Q., Wei, S.C., Chen, Q.Y., Fang, K.X., Yang, J.Y., Wang, X.Y., Harrison, T.J., 2009. The association of HBV core promoter double mutations (A1762T and G1764A) with viral load differs between HBeAg positive and anti-HBe positive individuals: a longitudinal analysis. J. Hepatol. 50, 273–280.

Hosaka, T., Suzuki, F., Kobayashi, M., Hirakawa, M., Kawamura, Y., Yastuji, H., Sezaki, H., Akuta, N., Suzuki, Y., Saitoh, S., Arase, Y., Ikeda, K., Miyakawa, Y., Kumada, H., 2010. Development of HCC in patients receiving adefovir dipivoxil for lamivudine-resistant hepatitis B virus mutants. Hepatol. Res. 40, 145–152.

Kim, H.J., Park, J.H., Park, D.I., Cho, Y.K., Sohn, C.I., Jeon, W.K., Kim, B.I., 2010. Rescue therapy for lamivudine-resistant chronic hepatitis B: comparison between entecavir 1.0 mg monotherapy, adefovir monotherapy and adefovir add-on lamivudine combination therapy. J. Gastroenterol. Hepatol. 25 (8), 1374–1380.

Lai, C.L., Dienstag, J., Schiff, E., Leung, N.W., Atkins, M., Hunt, C., Brown, N., Woessner, M., Boehme, R., Condreay, L., 2003. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin. Infect. Dis. 36 (6), 687–696.

Lee, J.M., Kim, H.J., Park, J.Y., Lee, C.K., Kim do, Y., Kim, J.K., Lee, H.W., Paik, Y.H., Lee, K.S., Han, K.H., Chon, C.Y., Hong, S.P., Nguyen, T., Ahn, S.H., 2009. Rescue monotherapy in lamivudine-resistant hepatitis B e antigen-positive chronic hepatitis B: adefovir versus entecavir. Antiviral Ther. 14(5), 705–712.

Lee, J.H., Yoon, J.H., Cho, E.J., Yang, H.J., Jang, E.S., Kwak, M.S., Hwang, S.Y., Yu, S.J., Lee, C.H., Kim, Y.J., Kim, C.Y., Lee, H.S., 2012. Simple scoring system predicting genotypic resistance during rescue therapy for lamivudine-resistant chronic hepatitis B. J. Clin. Gastroenterol. 46 (3), 243–250.

Leung, N.W., Lai, C.L., Chang, T.T., Guan, R., Lee, C.M., Ng, K.Y., Lim, S.G., Wu, P.C., Dent, J.C., Edmundson, S., Condreay, L.D., Chien, R.N., Asia Hepatitis Lamivudine Study Group, 2001. Extended lamivudine treatment in patients with chronic hepatitis B enchances hepatitis B e antigen seroconversion rates: results after three years of therapy. Hepatology 33, 1527–1532.

Levine, S., Hernandez, D., Yamanaka, G., Zhang, S., Rose, R., Weinheimer, S., Colonno, R.J., 2002. Efficacies of entecavir against lamivudine-resistant hepatitis B virus replication and recombinant polymerases in vitro. Antimicrob. Agents Chemother. 46 (8), 2525–2532.

Locarnini, S., Bowden, S., 2010. Drug resistance in antiviral therapy. Clin. Liver Dis. 14 (3), 439–459.

Lok, A.S., McMahon, B.J., 2007. Chronic hepatitis B. Hepatology 45, 507–539.

Luo, Z., Xie, Y., Deng, M., Zhou, X., Ruan, B., 2011. Prevalence of hepatitis B in the southeast of China: a population-based study with a large sample size. Eur. J. Gastroenterol. Hepatol. 23 (8), 695–700.

Perrillo, R.P., Hann, H.W., Schiff, E., Mutimer, D., Willems, B., Leung, N., Lee, W.M., Dixon, S., Woessner, M., Brosgart, C.L., Condreay, L.D., Gardner, S.D., 2011. Extended treatment with lamivudine and adefovir dipivoxil in chronic hepatitis B patients with lamivudine resistance. Hepatol. Int. 5 (2), 654–663.

Rapti, I., Dimou, E., Mitsoula, P., Hadziyannis, S.J., 2007. Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. Hepatology 45 (2), 307–313.

Tenney, D.J., Rose, R.E., Baldick, C.J., Levine, S.M., Pokornowski, K.A., Walsh, A.W., Fang, J., Yu, C.F., Zhang, S., Mazzucco, C.E., Eggers, B., Hsu, M., Plym, M.J., Poundstone, P., Yang, J., Colonno, R.J., 2007. Two-year assessment of entecavir resistance in lamivudine-refractory hepatitis B virus patients reveals different clinical outcomes depending on the resistance substitutions present. Antimicrob. Agents Chemother. 51 (3), 902–911.

Villet, S., Pichoud, C., Billioud, G., Barraud, L., Durantel, S., Trépo, C., Zoulim, F., 2008. Impact of hepatitis B virus rtA181V/T mutants on hepatitis B treatment failure. J. Hepatol. 48, 747–755.

Yatsuji, H., Noguchi, C., Hiraga, N., Mori, N., Tsuge, M., Imamura, M., Takahashi, S., Iwao, E., Fujimoto, Y., Ochi, H., Abe, H., Maekawa, T., Tateno, C., Yoshizato, K., Suzuki, F., Kumada, H., Chayama, K., 2006. Emergence of a novel lamivudine-resistant hepatitis B virus variant with a substitution outside the YMDD motif. Antimicrob. Agents Chemother. 50, 3867–3874.

Yeh, C.T., Chen, T., Hsu, C.W., Chen, Y.C., Lai, M.W., Liang, K.H., Chen, T.C., 2011. Emergence of the rtA181T/sW172* mutant increased the risk of hepatoma occurrence in patients with lamivudine-resistant chronic hepatitis B. BMC Cancer 11, 398.

Yeon, J.E., Yoo, W., Hong, S.P., Chang, Y.J., Yu, S.K., Kim, J.H., Seo, Y.S., Chung, H.J., Moon, M.S., Kim, S.O., Byun, K.S., Lee, C.H., 2006. Resistance to adefovir dipivoxil in lamivudine resistant chronic hepatitis B patients treated with adefovir dipivoxil. Gut 55, 1488–1495.