SMGroup

SM Virology

Review Article

Effects of Antiviral Treatment on Chronic Hepatitis B-Related Hepatocellular Carcinoma and Recurrence after Surgical Treatment

Xiaomei Hou[1], Jue Wang[2] and Yan Du[2]*

[1]*Department of Epidemiology, Second Military Medical University, Shanghai 200433, PR China*
[2]*Obstetrics and Gynecology Hospital of Fudan University, Shanghai 200011, PR China*

Article Information

Received date: Jan 01, 2016
Accepted date: Feb 25, 2016
Published date: Mar 01, 2016

***Corresponding author**

Yan Du, Obstetrics and Gynecology Hospital of Fudan University, Shanghai 200011, PR China,
Tel: +86-21-56096012;
Fax: +86-21-56096012;
Email: sophiedu_61@163.com

Keywords Hepatitis B Virus; Hepatocellular Carcinoma; Recurrence; Antiviral Therapy; Interferon; Nucleos(T) Ide Analogues

Abbreviations AASLD: American Association for the Study of Liver Disease; ADV: adefovir dipivoxil; ALT: alanine aminotransferase; APASL: Asian Pacific Association for the Study of the Liver; AST: aspartate transaminase; cccDNA: covalently closed circular DNA; EASL: European Association for the Study of the Liver; EnhII/BCP/PC: enhancer II/basal core promoter/precore; ETV: entecavir; HBcrAg: hepatitis B core-related antigen; HBeAg: hepatitis B e antigen; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; IFN: interferon; LAM: lamivudine; LC: liver cirrhosis; NA: nucleos(t)ide analogue; PEG-IFN: pegylated interferon; TBV: telbivudine; TDF: tenofovir disoproxil fumarate; UD: undetectable; ULN: upper limit of normal; VEGF: vascular endothelial growth factor; YMDD: tyrosine–methionine–aspartate–aspartate

Abstract

Hepatocellular Carcinoma (HCC) is one of the most common and aggressive malignancies, and the high rate of recurrence is a major obstacle to improving prognosis. Chronic Hepatitis B Virus (HBV) is one of the major causes of HCC, and high viral replication rate and related hepatic inflammation are major risk factors of HCC recurrence after surgical resection. Current approved antiviral medications for the treatment of chronic hepatitis B are interferon-α (IFNα) and nucleos (t) ide analogues (NAs), including lamivudine, adefovir dipivoxil, telbivudine, entecavir, and tenofovir disoproxil fumarate. IFNα treatment significantly reduces HBV-related HCC in sustained responders, but its usage is limited by adverse effects. NAs treatment significantly reduces disease progression into cirrhosis and thus HCC incidence, especially in HBV e antigen-positive patients. However, the long-term continuous treatment of NAs may result in drug resistance due to viral mutations. The effect of IFNα treatment on HCC recurrence remains controversial, while evidence has suggested that postoperative NAs therapy can improve both recurrence-free survival and overall survival in patients with HBV-related HCC. There is a great need to develop more effective and affordable new agents with a better safety record. More high-quality prospective trials are needed to quantitatively estimate treatment efficacy and identify predictive factors of HCC development and progression.

Introduction

Hepatocellular Carcinoma (HCC) is one of the most common and fatal malignancies worldwide [1]. So far, the main treatment for HCC is surgical resection. However, it is only limited to a proportion of the patients, and is dampened by a high recurrence rate of about 70% within 5 years after surgery [2]. Chronic infection with Hepatitis B Virus (HBV) or Hepatitis C Virus (HCV) is the most common underlying cause of HCC. There are 8 genotypes (A-H) of HBV according to a sequence divergence greater than 8% in the entire HBV genome. HBV genotypes have distinct geographical distributions, and have been shown to differ with regard to hepatitis B e antigen (HBeAg) seroconversion, clinical outcomes, prognosis, and antiviral responses [3]. In HBV endemic areas such as Asia and Africa, HBV genotype C (*vs.* genotype B), HBeAg expression, high viral load ($>1\times10^4$ copies/mL), and viral mutations in the enhancer II/basal core promoter/precore (EnhII/BCP/PC) and the preS regions of the HBV genome are associated with increased risks of Liver Cirrhosis (LC) and/or HCC in chronic HBV-infected patients [4]. The dynamic process of the interplay between the hepatitis viruses and host inflammatory factors creates a tumor-friendly microenvironment that is essential for carcinogenesis and metastasis. For chronic HBV infected individuals, antiviral therapy is proven to be effective for HCC prevention [5]. In addition, antiviral therapy before and after surgical resection may prevent HCC recurrence and improve survival [6,7]. There are currently two main categories of medications, Interferon-α (IFNα) and Nucleos (t)ide Analogue (NA), approved for the treatment of chronic hepatitis B. The conventional IFNα was introduced in 1992, later in 2005 the pegylated, long-acting formulation (PEG-IFN) was introduced to solve the tolerability issue. For NAs, there are currently 5 approved drugs for HBV treatment: lamivudine (LAM), adefovir dipivoxil (ADV), telbivudine (TBV), entecavir (ETV), and tenofovir disoproxil fumarate (TDF), and each with pros and cons [8]. However, current treatments do not eradicate HBV, and not all patients with chronic HBV infection will develop cirrhosis, HCC or other liver complications. In addition, host immune response can result in spontaneous remission which can be long-lasting. Furthermore, long-term treatment is associated with risks of drug resistance, very high costs, potential adverse events, and non-adherence. Therefore, it is important to identify those patients who are most beneficial to receive antiviral therapy.

Antiviral Therapy and Prevention of HBV-Related HCC

There are four phases of chronic HBV infection, namely immune tolerant, immune clearance, inactive (carrier), and reactivation phase. Each phase has different characteristics on HBeAg

positivity, serum Alanine Amino Transferees (ALT) levels, and HBV DNA levels [8]. Patients may go through various phases, which are influenced by the HBV genotype and host immune status [9-11]. The goal of antiviral treatment is to achieve maximum clinical benefits including reducing complications such as LC and HCC. Decision for the treatment initiation is mainly based on the stage of liver diseases, initial virus replication status, liver inflammation and/or fibrosis status. Different associations, including the American Association for the Study of Liver Diseases (AASLD), the Asian Pacific Association for the Study of the Liver (APASL), and the European Association for the Study of the Liver (EASL) all have provided guidelines for the initiation of antiviral treatment, choice of antiviral drugs, and the stop signs of the treatment (Table 1) [12-14]. In addition, it should also consider patient's age, family history of HCC, occupation, adverse effects, drug resistance, and costs of the treatment. Moreover, patients should be closely monitored for their treatment responses so that timely modifications could be made according to their responses. Intermediate outcomes, including a decrease in levels of serum HBV DNA, HBeAg seroconversion, loss of Hepatitis B Surface Antigen (HBsAg), normalization of ALT levels, and a decrease in hepatic inflammation, are usually used as clinical assessment of the treatment response. HBV demonstrates "mutation-selection-adaptation", a viral evolutionary process involved in hepatocarcinogenesis. Non-resolving inflammation caused by HBV infection contributes to the generation of HBV mutations, and further facilitates the selection of HCC-risk HBV mutations possibly through inducing the expression of cytidine deaminases [15]. Previous studies have shown that C1653T, T1753V, A1762T/G1764A, T1674C/G and C1766T/T1768A in the EnhII/BCP regions; G1899A, C2002T, A2159G, A2189C, and G2203A/T in the precore/core region; as well as T53C, preS2 start codon mutation, preS1 deletion, C2964A, A2962G, C3116T, and C7A in the preS region are significantly associated with an increased risk of HCC occurrence [16 -21]. Some evidences have shown that HCC-risk HBV mutations may affect the outcomes of antiviral treatment [22]. PreS deletion, the most common mutation in the preS region, is usually generated during the progression of chronic hepatitis B, especially in IFN-treated patients [23]. In addition, drug-resistant viral mutations limit NA therapeutic effect and may also promote hepatocarcinogenesis [2].

Effects of IFNα on HBV-Related HCC

IFNα has an immunomodulatory activity. The main advantages of IFNα-based treatment are short administration course, a higher rate of HBeAg seroconversion and HBsAg loss, and a sustainable off-treatment response once achieved. In addition, the introduction of PEG-IFN allows for weekly injections while maintaining antiviral efficacy [24]. Factors associated with response to PEG-IFN treatment in patients with HBeAg-positive hepatitis include older age, female sex, high ALT, low HBV DNA, and absence of previous IFN therapy. Patients with genotype A and high ALT or low HBV DNA, and those with genotypes B or C and both high ALT and low HBV DNA had better outcomes [25]. Previous studies have shown that overall, IFNα-based treatment facilitated HBsAg clearance and HBeAg seroconversion, decreased incidence of liver complications including cirrhosis and HCC, and improved overall survival [26-29]. These data indicated that IFN is most beneficial for young patients, particularly among HBeAg-positive patients who have a genotype A infection. IFNα may be used in patients with compensated cirrhosis with careful monitoring. However, it should not be used in patients with decompensate cirrhosis, in patients with severe exacerbations of chronic hepatitis B or acute liver failure, and in those undergoing immunosuppressive or cancer chemotherapy. IFNα-based treatment has adverse effects, including initial flu-like illness, fatigue, bone marrow suppression, and unmasking or exacerbation of autoimmune illnesses, which are tolerable but should be closely monitored. In addition, low probability of achieving a response and high costs as well as side-effects may limit its long-time clinical use.

Effects of NAs on HBV-Related HCC

An oral sequential therapy with NAs is preferable for patients who do not respond to IFNα.The efficacy of NAs treatment is predictable and the side effects are minimal. However, the long-term continuous usage of NAs will cause antiviral drug resistance [30]. The pros and cons of each NA for the treatment of chronic hepatitis B infection have been previously summarized [8]. Longitudinal studies as well as meta-analyses have shown that HCC incidence is significantly lower in patients receiving NAs treatment compared to untreated patients [31-34]. Nevertheless, the risk of HCC does not reduce to zero for patients who receive NAs treatment, especially in those with pre-existing cirrhosis conditions. Therefore, patients with chronic hepatitis B should be closely monitored during their course of antiviral therapy [33,35]. Rather than directly inhibiting Covalently Closed Circular DNA (cccDNA), NAs primarily inhibit the reverse transcription of the pregenomic HBV RNA to the first strand of HBV DNA. As a result, it is common to see hepatitis relapses on drug withdrawal. Furthermore, patients with inadequate or slow decline of serum HBV DNA levels at the first 12-24 weeks of treatment are prone to antiviral drug resistance during continued therapy. Additional therapy should be given to those patients who receive NAs with a low genetic barrier (LAM and TBV) and could not achieve adequate initial viral decline. Patients may stay on the same drug if they receive NAs with a high genetic barrier to resistance (ETV and TDF), and attain continuously declined serum HBV DNA levels [36,37].NAs treatment should be chosen based on patient's age, initial HBV-DNA level, HBV genotype/subgenotype, and any contraindication; and should be initiated with high genetic barrier NAs to lower the chance of developing drug resistance. Candidates for NAs treatment are those patients with decompensated liver diseases or poor response to INFα, and committed to long-term treatment duration.

Drug-Resistant Viral Mutations Limit the Effects of NAs and May Promote HBV-Related HCC

Emergence of drug resistance drastically reduces the effectiveness of NAs treatment. LAM is the first approved NAs to treat chronic hepatitis B and can significantly reduce the risk of HCC [34]. LAM resistance develops in 14-23% of cases after one year and approximately 70% after five years [38]. The most frequently encountered LAM-resistant mutant is rtM204V/I, which harbors a mutation located at the catalytic tyrosine–methionine–aspartate–aspartate (YMDD) motif [39] .The rtL180M mutation usually concurrently occurs with the rtM204V mutation [39]. A substantial proportion of LAM-resistant patients carry the rtA181T mutation, which increases HCC risk during the subsequent course of antiviral therapy [40,41]. Due to the overlap between S and polymerase genes, the sW172* nonsense mutation also exists in a great proportion of patients carrying the rtA181T mutation. The emergence of both mutations

Table 1: Guidelines for the treatment of chronic hepatitis B infection.

	AASLD 2015[12]	APASL 2015[13]	EASL 2012[14]
Choice of antiviral drugs			
IFN	PEG-IFN (not for de-compensated cirrhosis)	IFN (conventional or PEG) PEG-IFN is preferred in young patients	PEG-IFN (not for de-compensated cirrhosis) for patients with high ALT and low HBV DNA
NAs	ETV or TDF	ETV or TDF	ETV or TDF
Recommendations regarding when to stop NAs			
HBeAg+	HBeAg seroconversion and UD HBV DNA+ ≥6 month consolidation	HBeAg seroconversion and UD HBV DNA+ ≥12 months	HBeAg seroconversion+ 12 month consolidation
HBeAg-	HBsAg loss	(1) HBsAg loss following either anti-HBs seroconversion or ≥12 months of a post-HBsAg clearance consolidation period (2) Treatment ≥2 year with UD HBV DNA ≥3 occasions 6 month apart	HBsAg loss?
Cirrhosis	Compensated: HBsAg loss De-compensated: DO NOT STOP	With a careful off-therapy monitoring plan	DO NOT STOP
Indications for Treatment of Non-Cirrhotic HBeAg+ Patients			
Treat HBV DNA (IU/mL) ALT (ULN)	>20,000 and >2x	>20,000 and >2x	>20,000 and/or >1x and Biopsy or non-invasive assessment ≥A2 or ≥F2
HBV DNA (IU/mL) ALT (ULN) Age	>20,000 and 1-2 or >40 or Family history of HCC or Previous treatment history	>20,000 and any >35 or Family history of HCC or cirrhosis	
Treat if modest/severe inflammation or fibrosis	Biopsy	Biopsy or non-invasive assessment	Biopsy or non-invasive assessment
Indications for Treatment of Patients with Compensated Cirrhosis			
HBV DNA (IU/mL) ALT (ULN) HBeAg	>20,000 and Any + or -	>2,000 and Any + or -	detected Any + or -
HBV DNA (IU/mL) ALT (ULN)	<2,000 and Any		
Indications for Treatment of Patients with De-compensated Cirrhosis			
HBV DNA (IU/mL) ALT (ULN) HBeAg	Any Any + or -	Any Any + or -	Any Any + or -
Refer for liver transplant	Yes	Yes	Yes

x, time(s);? Not conclusive

Adapted and summarized from references [12-14], does not include special patient groups such as children and pregnant women.

causes truncation of the pre-S/S reading frames, which is partially responsible for the development of HCC in patients not responding to NAs treatment [8].The rate of ADV resistance is 2-3% after two years and 28-29% after five years of monotherapy in treatment naïve patients [39]. Furthermore, rtN236T and rtA181T/V, two major ADV-resistant mutations, emerge more frequently in LAM-resistant patients than in treatment naïve patients [42-44]. Resistance to ETV is rare in treatment naïve patients even with long-term therapy (1.5% by the fifth year) [36]. However, the cumulative probability of genotypic ETV resistance increases with a combination of substitutions I169T and M250V, or T184G and S202I in LAM-resistant patients [45]. TBV resistance develops in 2-3% and 21% treatment-naïve HBeAg-positive patients after 1 and 2 years of therapy, respectively [46]. TBV resistance is associated with a signature M204I mutation in viral polymerase [46]. Tenofovir disoproxil has been reported to be safe and effective in suppressing HBV replication with a low risk of drug resistance, and effective against various NAs resistant or cross-resistant mutants [47,48].

Antiviral Therapy and Prevention of HBV-Related HCC Recurrence

The high rate of recurrence after curative resection is a major obstacle to improve HCC prognosis [49]. Early recurrence (within 2 years) is related to metastaisis and dissemination of primary tumor, whereas late recurrence (after ≥2 years) mainly results from *de novo* tumors because of the "field effect" in the diseased liver and is closely associated with high viral load and hepatic inflammatory activity [50].The expression of HBeAg either before or after curative treatment is significantly associated with HCC early recurrence and poor survival [51]. High serum Hepatitis B Core-Related Antigen (HBcrAg) is associated with HCC recurrence [52]. A high level of HBV DNA in peritumoral liver tissues is an independent predictor of poor disease free survival and overall survival after surgical resection [53].In addition, high HBV viral load is one of the prognostic factors for local recurrence after complete radiofrequency ablation of small HBV-related HCC [51].All these data support that high and persistent viral replication is associated with a high risk of HCC recurrence. Therefore, antiviral therapy both before and after curative treatment may be crucial in preventing HCC recurrence and improving survival.

Effects of IFNα on HBV-Related HCC Survival and Recurrence

IFNα has anticancer effects, possibly through suppression of HBV replication, inhibition of inflammatory signaling, and tumoricidal effect [54,55]. IFNα mediates the expression of Vascular Endothelial Growth Factor (VEGF)[54], and targets Wnt signaling via inducing nuclear export of β-catenin [56]. Several studies have shown that IFNα significantly improved survival[6,57,58] and reduced recurrence rate of HBV-related HCC [58-61]. A meta-regression study including nine randomized trials and four cohort studies showed that overall IFNα improved the 1-year, 2-year, and 3-year recurrence-free survival [62]. However, a recent systematic review and meta-analysis pooled data from both randomized controlled trials and non-randomized studies, and observed little evidence indicating that adjuvant interferon therapy improved recurrence-free survival and overall survival among HBV-related HCC patients[63].Therefore, the use of IFNα treatment to improve survival and prevent recurrence should be further investigated among HBV-related HCC patients.

Effects of NAs on HBV-Related HCC Survival and Recurrence

The aims of NAs treatment are to improve the liver function of HBV-related HCC patients as confirmed by several studies [64,65]. HBV DNA level is a risk factor of HCC and NAs target HBV DNA polymerase. NAs treatment can inhibit HBV DNA replication, improve serum albumin, normalize Aspartate Transaminase (AST) and ALT levels, reduce Child-Pugh scores, slow down disease progression to cirrhosis, and subsequently reduce the risk of *de novo* tumors after curative treatment [66,67]. Evidence from cohort study [68] and randomized trials [69,70] has further shown that NAs therapy can provide benefits for patients with HBV-related HCC after curative treatment. Meta-analyses have also supported the notion that NAs therapy could improve survival and reduce early recurrence of patients with HBV-related HCC after curative treatment [71,72]. However, long-term usage of NAs is required to achieve a potential beneficial effect in preventing HCC recurrence and improving survival, leading to the development of drug resistant strains [73].

Conclusions

HBV-related HCC is a huge public health burden, especially in HBV endemic areas such as Asia and Sub-Saharan Africa. HBV is one of the most important risk factors for HCC development, and antiviral treatment of chronic hepatitis B is so far the only option to prevent HCC. IFN and NAs are currently the major antiviral drugs in clinical application. IFNα treatment of chronic hepatitis B may significantly reduce the risk of HCC development; however, the adverse effects may limit its long-term use. Oral administration of NAs can reduce HCC incidence, especially in HBeAg-positive patients, while long-term continuous treatment of NAs may result in drug resistance due to viral mutations. Patients should be closely monitored during their course of antiviral treatment so timely adjustment can be given. There is a great need to develop more effective and affordable new agents with a better safety record, especially for patients with hepatic decomposition. A high viral load has been associated with increased risk of HCC development, as well as HCC recurrence after curative treatment. Therefore, it is reasonable to speculate that antiviral treatment can also reduce the risk of HCC recurrence after surgical resection and improve survival. While IFNα treatment is effective against primary HCC, the question of whether it can also prevent HCC recurrence remains controversial. For NAs treatment, a significant body of evidence suggests that postoperative NAs therapy improves both recurrence-free survival and overall survival in patients with HBV-related HCC. However, it is important to note that antiviral therapy is not the only factor affecting outcomes. HCC development and recurrence are determined by complex interactions among viral factors, host immunity, and environmental determinants. While the underlying mechanisms are still being investigated, more high-quality prospective trials are expected to quantitatively estimate treatment efficacy and identify predictive factors of HCC development and progression.

Acknowledgement

Supported by: National Natural Science Foundation of China (No. 81302492 to Yan Du).

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D, et al. Global cancer statistics. CA Cancer J Clin. 2011; 61: 69-90.
2. Du Y, Su T, Ding Y, Cao G. Effects of antiviral therapy on the recurrence of Hepatocellular carcinoma after curative resection or liver transplantation. Hepat Mon. 2012; 12: e6031.
3. Zhang Q, Cao G. Genotypes, mutations, and viral load of hepatitis B virus and the risk of Hepatocellular carcinoma: HBV properties and hepatocarcinogenesis. Hepat Mon. 2011; 11: 86-91.
4. Xie J, Zhang Y, Zhang Q, Han Y, Yin J, Pu R, et al. Interaction of signal transducer and activator of transcription 3 polymorphisms with hepatitis B virus mutations in Hepatocellular carcinoma. Hepatology. 2013; 57: 2369-2377.
5. Lok AS. Does antiviral therapy for hepatitis B and C prevent Hepatocellular carcinoma? J Gastroenterol Hepatol. 2011; 26: 221-227.
6. Chan AC, Chok KS, Yuen WK, Chan SC, Poon RT, Lo CM, et al. Impact of antiviral therapy on the survival of patients after major hepatectomy for hepatitis B virus-related hepatocellular carcinoma. Arch Surg. 2011; 146: 675-681.
7. Miao RY, Zhao HT, Yang HY, Mao YL, Lu X, Zhao Y, et al. Postoperative

adjuvant antiviral therapy for hepatitis B/C virus-related Hepatocellular carcinoma: a meta-analysis. World J Gastroenterol. 2010; 16: 2931-2942.

8. Chen LP, Zhao J, Du Y, Han YF, Su T, Hong-Wei Zhang, et al. Antiviral treatment to prevent chronic hepatitis B or C-related hepatocellular carcinoma. World J Virol. 2012 1: 174-183.

9. Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med. 2002; 347: 168-174.

10. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006; 295: 65-73.

11. Wu CF, Yu MW, Lin CL, Liu CJ, Shih WL, Tsai KS, et al. Long-term tracking of hepatitis B viral load and the relationship with risk for Hepatocellular carcinoma in men. Carcinogenesis.2008; 29: 106-112.

12. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH, et al. AASLD guidelines for treatment of chronic hepatitis B. Hepatology. 2016; 63: 261-283.

13. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016; 10: 1-98.

14. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol. 2012; 57: 167-185.

15. Deng Y, Du Y, Zhang Q, Han X, Cao G. Human cytidine deaminases facilitate hepatitis B virus evolution and link inflammation and Hepatocellular carcinoma. Cancer Lett. 2014; 343: 161-171.

16. Yin J, Xie J, Liu S, Zhang H, Han L, Lu W, et al. Association between the various mutations in viral core promoter region to different stages of hepatitis B, ranging of asymptomatic carrier state to Hepatocellular carcinoma. Am J Gastroenterol. 2011; 106: 81-92.

17. Liu S, Zhang H, Gu C, Yin J, He Y, Xie J, et al. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. J Natl Cancer Inst. 2009 101: 1066-1082.

18. Yin J, Xie J, Zhang H, Shen Q, Han L, Lu Wet, al. Significant association of different preS mutations with hepatitis B-related cirrhosis or hepatocellular carcinoma. J Gastroenterol. 2010 45: 1063-1071.

19. Zhu Y, Jin Y, Guo X, Bai X, Chen T, Wang J, et al. Comparison study on the complete sequence of hepatitis B virus identifies new mutations in core gene associated with hepatocellular carcinoma. Cancer Epidemiol Biomarkers Prev. 2010; 19: 2623-2630.

20. Xie JX, Zhao J, Yin JH, Zhang Q, Pu R, Lu WY, et al. Association of novel mutations and haplotypes in the preS region of hepatitis B virus with hepatocellular carcinoma. Front Med China. 2010; 4: 419-429.

21. Liu S, Xie J, Yin J, Zhang H, Zhang Q, Pu R, et al. A matched case-control study of hepatitis B virus mutations in the preS and core promoter regions associated independently with hepatocellular carcinoma. J Med Virol. 2011; 83: 45-53.

22. Yin J, Wang J, Pu R, Xin H, Li Z, Han X, et al. Hepatitis B Virus Combo Mutations Improve the Prediction and Active Prophylaxis of Hepatocellular Carcinoma: A Clinic-Based Cohort Study. Cancer Prev Res (Phila). 2015; 8: 978-988.

23. Zhang D, Dong P, Zhang K, Deng L, Bach C, Chen W et al. Whole genome HBV deletion profiles and the accumulation of preS deletion mutant during antiviral treatment. BMC Microbiol. 2012; 12: 307.

24. van Zonneveld M, Honkoop P, Hansen BE, Niesters HG, Darwish Murad S, de Man RA, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology. 2004; 39: 804-810.

25. Buster EH, Hansen BE, Lau GK, Piratvisuth T, Zeuzem S, Steyerberg EW et al. Factors that predict response of patients with hepatitis B e antigen-positive chronic hepatitis B to peginterferon-alfa. Gastroenterology. 2009; 137: 2002-2009.

26. Fontana RJ, Hann HW, Perrillo RP, Vierling JM, Wright T, Rakela J, et al. Determinants of early mortality in patients with decompensated chronic hepatitis B treated with antiviral therapy. Gastroenterology. 2002; 123: 719-727.

27. Lin SM, Yu ML, Lee CM, Chien RN, Sheen IS, Chu CM, et al. Interferon therapy in HBeAg positive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. J Hepatol. 2007; 46: 45-52.

28. Li WC, Wang MR, Kong LB, Ren WG, Zhang YG, Nan YM. et al. Peg interferon alpha-based therapy for chronic hepatitis B focusing on HBsAg clearance or seroconversion: a meta-analysis of controlled clinical trials. BMC Infect Dis. 2011; 11: 165.

29. Hansen BE, Rijckborst V, Ter Borg MJ, Janssen HL. HBV DNA suppression in HBeAg-positive chronic hepatitis B patients treated with peg interferon or placebo. J Med Virol. 2011; 83: 1917-1923.

30. Sonneveld MJ, Janssen HL. Chronic hepatitis B: peginterferon or nucleos(t) ide analogues? Liver Int 31 Suppl. 2011; 1: 78-84.

31. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. Hepatology. 2013; 58: 98-107.

32. Sung JJ, Tsoi KK, Wong VW, Li KC, Chan HL Meta-analysis. Treatment of hepatitis B infection reduces risk of hepatocellular carcinoma. Aliment Pharmacol Ther. 2008; 28: 1067-1077.

33. Papatheodoridis GV, Lampertico P, Manolakopoulos S, Lok A. Incidence of hepatocellular carcinoma in chronic hepatitis B patients receiving nucleos(t) ide therapy: a systematic review. J Hepatol. 2010; 53: 348-356.

34. 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; 351: 1521-1531.

35. Thursz M, Brown A. Can antiviral therapy of chronic hepatitis B prevent the development of hepatocellular carcinoma? Gut. 2011; 60: 1025-1026.

36. 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; 49: 1503-1514.

37. Snow-Lampart A, Chappell B, Curtis M, Zhu Y, Myrick F, 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: 763-773.

38. Lok AS, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, et al. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. Gastroenterology. 2003; 125: 1714-1722.

39. Yeh CT, Chen T, Hsu CW, Chen YC, Lai MW, Liang KH et al. Emergence of the rtA181T/sW172* mutant increased the risk of hepatoma occurrence in patients with lamivudine-resistant chronic hepatitis B. BMC Cancer. 2011; 11: 398.

40. Papatheodoridis GV, Deutsch M. Resistance issues in treating chronic hepatitis B. Future Microbiol. 2008; 3: 525-538.

41. EASL Clinical Practice Guidelines: management of chronic hepatitis B. J Hepatol. 2009; 50: 227-242.

42. Yeon JE, Yoo W, Hong SP, Chang YJ, Yu SK, Kim JH et al. Resistance to adefovir dipivoxil in lamivudine resistant chronic hepatitis B patients treated with adefovir dipivoxil. Gut. 2006; 55: 1488-1495.

43. Schildgen O, Sirma H, Funk A, Olotu C, Wend UC, Hartmann H, et al. Variant of hepatitis B virus with primary resistance to adefovir. N Engl J Med. 2006; 354: 1807-1812.

44. 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; 43: 1385-1391.

45. Sherman M, Yurdaydin C, Sollano J, Silva M, Liaw YF, Cianciara J et al. Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. Gastroenterology. 2006; 130: 2039-2049.

46. Tillmann HL, McHutchison JG .Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 358: 1517; author reply. 2008; 1517-1518.

47. 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: 247-254.

48. Van Bommel F, de Man RA, Wedemeyer H, Deterding K, Petersen J, 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.

49. Llovet JM, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. Semin Liver Dis. 2005; 25: 181-200.

50. Wu JC, Huang YH, Chau GY, Su CW, Lai CR, Lee PC et al. Risk factors for early and late recurrence in hepatitis B-related hepatocellular carcinoma. J Hepatol. 2009; 51: 890-897.

51. Xia F, Lai EC, Lau WY, Ma K, Li X, Bie P, et al. High serum hyaluronic acid and HBV viral load are main prognostic factors of local recurrence after complete radiofrequency ablation of hepatitis B-related small hepatocellular carcinoma. Ann Surg Oncol. 2012; 19: 1284-1291.

52. Hosaka T, Suzuki F, Kobayashi M, Hirakawa M, Kawamura Y, Yatsuji H, et al. HBcrAg is a predictor of post-treatment recurrence of hepatocellular carcinoma during antiviral therapy. Liver Int. 2010; 30: 1461-1470.

53. Yeh CT, So M, Ng J, Yang HW, Chang ML, Lai MW, et al. Hepatitis B virus-DNA level and basal core promoter A1762T/G1764A mutation in liver tissue independently predict postoperative survival in hepatocellular carcinoma. Hepatology. 2010; 52: 1922-1933.

54. von Marschall Z, Scholz A, Cramer T, Schafer G, Schirner M, Oberg K, et al. Effects of interferon alpha on vascular endothelial growth factor gene transcription and tumor angiogenesis. J Natl Cancer Inst. 2003; 95: 437-448.

55. Wang L, Tang ZY, Qin LX, Wu XF, Sun HC, Xue Q et al. High-dose and long-term therapy with interferon-alfa inhibits tumor growth and recurrence in nude mice bearing human hepatocellular carcinoma xenografts with high metastatic potential. Hepatology. 2000; 32: 43-48.

56. Thompson MD, Dar MJ, Monga SP. Pegylated interferon alpha targets Wnt signaling by inducing nuclear export of beta-catenin. J Hepatol. 2011; 54: 506-512.

57. Sun HC, Tang ZY, Wang L, Qin LX, Ma ZC, Ye QH, et al. Postoperative interferon alpha treatment postponed recurrence and improved overall survival in patients after curative resection of HBV-related hepatocellular carcinoma: a randomized clinical trial. J Cancer Res Clin Oncol. 2006; 132: 458-465.

58. Qu LS, Jin F, Huang XW, Shen XZ. Interferon-alpha therapy after curative resection prevents early recurrence and improves survival in patients with hepatitis B virus-related hepatocellular carcinoma. J Surg Oncol. 2010; 102: 796-801.

59. Lin SM, Lin CJ, Hsu CW, Tai DI, Sheen IS, Lin DY, et al. Prospective randomized controlled study of interferon-alpha in preventing hepatocellular carcinoma recurrence after medical ablation therapy for primary tumors. Cancer. 2004; 100: 376-382.

60. Someya T, Ikeda K, Saitoh S, Kobayashi M, Hosaka T, Sezaki Het, al. Interferon lowers tumor recurrence rate after surgical resection or ablation of hepatocellular carcinoma: a pilot study of patients with hepatitis B virus-related cirrhosis. J Gastroenterol. 2006; 41: 1206-1213.

61. Hsu YC, Ho HJ, Wu MS, Lin JT, Wu CY. Postoperative peg-interferon plus ribavirin is associated with reduced recurrence of hepatitis C virus-related hepatocellular carcinoma. Hepatology. 2013; 58: 150-157.

62. Shen YC, Hsu C, Chen LT, Cheng CC, Hu FC, Hu FC et al. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. J Hepatol. 2010; 52: 889-894.

63. Huang TS, Shyu YC, Chen HY, Yuan SS, Shih JN, Shih JN et al. A systematic review and meta-analysis of adjuvant interferon therapy after curative treatment for patients with viral hepatitis-related hepatocellular carcinoma. J Viral Hepat. 2013; 20: 729-743.

64. Kuzuya T, Katano Y, Kumada T, Toyoda H, Nakano I, Hirooka Y, et al. Efficacy of antiviral therapy with lamivudine after initial treatment for hepatitis B virus-related hepatocellular carcinoma. J Gastroenterol Hepatol. 2007; 22: 1929-1935.

65. Thia TJ, Lui HF, Ooi LL, Chung YF, Chow PK, Cheow PC et al. A study into the risk of exacerbation of chronic hepatitis B after liver resection for hepatocellular carcinoma. J Gastrointest Surg. 2007; 11: 612-618.

66. Koda M, Nagahara T, Matono T, Sugihara T, Mandai M, Ueki M, et al. Nucleotide analogs for patients with HBV-related hepatocellular carcinoma increase the survival rate through improved liver function. Intern Med. 2009; 48: 11-17.

67. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med. 1999; 341: 1256-1263.

68. Wu CY, Chen YJ, Ho HJ, Hsu YC, Kuo KN, Wu MS et al. Association between nucleoside analogues and risk of hepatitis B virus-related hepatocellular carcinoma recurrence following liver resection. JAMA. 2012; 308: 1906-1914.

69. Yin J, Li N, Han Y, Xue J, Deng Y, Shi J et al .Effect of antiviral treatment with nucleotide/nucleoside analogs on postoperative prognosis of hepatitis B virus-related hepatocellular carcinoma: a two-stage longitudinal clinical study. J Clin Oncol. 2013; 31: 3647-3655.

70. Huang G, Lau WY, Wang ZG, Pan ZY, Yuan SX, Shen F, et al. Antiviral therapy improves postoperative survival in patients with hepatocellular carcinoma: a randomized controlled trial. Ann Surg. 2015; 261: 56-66.

71. Ke Y, Wang L, Li LQ, Zhong JH. Nucleos (t) ide analogues to treat hepatitis B virus-related hepatocellular carcinoma after radical resection. World J Hepatol. 2014; 6: 652-659.

72. Xia BW, Zhang YC, Wang J, Ding FH, He XD. Efficacy of antiviral therapy with nucleotide/nucleoside analogs after curative treatment for patients with hepatitis B virus-related hepatocellular carcinoma: A systematic review and meta-analysis. Clin Res Hepatol Gastroenterol. 2015; 39: 458-468.