J Gastroenterol (2013) 48:999–1005
DOI 10.1007/s00535-012-0742-5

REVIEW

Combination therapy with a nucleos(t)ide analogue and interferon for chronic hepatitis B: simultaneous or sequential

Masaru Enomoto · Akihiro Tamori · Shuhei Nishiguchi · Norifumi Kawada

Received: 2 October 2012 / Accepted: 11 December 2012 / Published online: 22 January 2013

Abstract Currently available antiviral treatment for chronic hepatitis B virus infection can be divided into two classes of therapeutic agents: nucleos(t)ide analogues (NAs) and interferon (IFN). The major advantages of NAs are good tolerance and potent antiviral activity associated with high rates of on-treatment response to therapy; the advantages of IFN include a finite course of treatment, absence of drug resistance, and an opportunity to obtain a post-treatment durable response to therapy. The use of these two antiviral agents with different mechanisms of action in combination is theoretically an attractive approach for treatment. Here, we have reviewed previous reports of either simultaneous or sequential combination therapy with NA and IFN for chronic hepatitis B patients. In previous studies comparing the lamivudine/IFN combination and lamivudine monotherapy in a finite course, combination therapy was associated with higher rates of sustained post-treatment response and lower rates of drug resistance than lamivudine monotherapy. However, NAs such as lamivudine are generally administered indefinitely because of high rates of post-treatment relapse. In addition, concern for drug resistance has decreased significantly with newer, high-potency NAs even when administered alone. In previous studies comparing the lamivudine/IFN combination and IFN monotherapy, the combination therapy showed greater on-treatment viral suppression, but no difference was observed in the post-treatment sustained response. Thus, whether combination therapy confers an additional benefit compared to monotherapy for treating chronic hepatitis B remains unclear. The efficacy of IFN in combination with a more potent NA, such as entecavir or tenofovir, remains to be comprehensively evaluated.

Keywords Chronic hepatitis B · Lamivudine · Adefovir · Entecavir · Interferon · Nucleos(t)ide analogue

Abbreviations

ALT	Alanine aminotransferase
CccDNA	Covalently closed circular DNA
HBcrAg	Hepatitis B core-related antigen
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
IFN	Interferon
NA	Nucleos(t)ide analogue

Introduction

More than 350 million people worldwide are infected with hepatitis B virus (HBV) which is a leading cause of liver-related morbidity that accounts for 1 million deaths annually [1, 2]. Currently available antiviral treatment for chronic hepatitis B can be divided into two classes of therapeutic agents: nucleos(t)ide analogues (NAs) and interferon (IFN). Nucleosides include lamivudine [3, 4], telbivudine [5], and entecavir [6, 7]; nucleotides include adefovir [8, 9] and tenofovir [10]. The direct, potent antiviral effects of NAs induce an on-treatment response in

M. Enomoto (✉) · A. Tamori · N. Kawada
Department of Hepatology,
Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan
e-mail: enomoto-m@med.osaka-cu.ac.jp

S. Nishiguchi
Department of Internal Medicine,
Hyogo College of Medicine, Nishinomiya, Hyogo, Japan

most patients, but post-treatment relapse commonly occurs after treatment discontinuation. Long-term use of NAs triggers the emergence of drug-resistant variants possessing mutations in the hepatitis B virus (HBV) polymerase gene. Among the NAs currently available, entecavir or tenofovir is recommended as the first-line treatment because of the low rate of drug resistance. In contrast, IFN has both antiviral and immunomodulatory actions [11, 12]. The major advantages of IFN include a finite course of treatment, absence of drug resistance, and an opportunity to obtain a post-treatment durable response to therapy; however, a response to IFN is achieved in only a minority of patients with chronic hepatitis B.

In this article, we have reviewed previous reports on combination therapy with NA and IFN for chronic hepatitis B. Regimens of combination therapy can be classified into two main groups: (1) simultaneous therapy with the drugs in the combination and (2) sequential combination therapy in which treatment with one drug follows that of the previously administered one. To compare the results of previous trials, we noted that age/sex of the included subjects, HBV genotypes, and mode of vial transmission varies among the different studies. These differences may affect the results and their interpretations as older age, male sex, HBV genotypes C and D (vs. A and B), and vertical transmission are associated with a poor response to IFN therapy [13–15]. In particular, HBV genotypes have specific geographic distributions, with genotype A being prevalent in Northwest Europe, North America, and Central Africa, genotypes B and C being common in Southeast Asia, China, Japan, and Oceania, and genotype D being prevalent in Southern Europe, the Middle East, and India, although it has a nearly worldwide distribution.

Theoretical background

Nucleos(t)ide analogues directly inhibit viral replication by targeting at least one of the three replication steps: priming of HBV DNA polymerase, reverse transcription of negative-strand HBV DNA from pregenomic RNA, and synthesis of positive-strand HBV DNA. IFN also possesses antiviral activity but does not act directly on the virus or replication complex. Instead, it acts by inducing IFN-stimulated genes to establish a non-virus-specific antiviral state within the cell. In addition to their role as antivirals, IFNs are important immunomodulators that interact with the adaptive and innate immune responses. Combining NA and IFN, with their different mechanisms of action, in a therapeutic regimen is theoretically an attractive approach for treating chronic hepatitis B.

The action of NAs has little or no effect on the decrease in the intrahepatic HBV replicative intermediate, covalently closed circular DNA (cccDNA). In the experimental woodchuck hepatitis virus system, cccDNA persisted even when viral production was strongly reduced by NA treatment [16, 17]. To reduce the level of intrahepatic cccDNA, the immunomodulatory activity of IFN, which presumably induces cytotoxic T cell activity for immune clearance of infected cells, may be required. However, a high HBV DNA load is associated with an inefficient T cell response to HBV-related antigens, such as hepatitis B surface antigen (HBsAg) [18]. Several studies have shown that a decreased viral load induced by NA treatment can result in the subsequent restoration of CD4 followed by CD8 cellular immune response against HBV [19, 20]. The rationale for combination therapy is based on the concept that suppression of viral replication by NA can decrease viral protein synthesis on the surface of hepatocytes, which may restore the immune response and optimize the immunomodulatory effects of IFN for clearing infected cells.

Simultaneous combination with NA and IFN

Table 1 shows a summary of previous studies examining simultaneous combination therapy with NA and IFN for chronic hepatitis B. The first trial was reported by Mutimer et al. [21] in the UK. Since this study was designed to assess the safety and tolerability of combination treatment, the duration of treatment was only 16 weeks, and few patients showed sustained seroconversion from hepatitis B e antigen (HBeAg) to anti-HBe (antibody to HBe) by this short-term therapy with lamivudine and IFN-α. Barbaro et al. [22] reported the results of a randomized trial conducted in Italy where the 24-week combination with lamivudine and IFN-α increased the rate of sustained HBeAg seroconversion compared to the 52-week lamivudine monotherapy (33 vs. 15 %; $P = 0.014$). Tatulli et al. [23] in Italy found that the 52-week combination with lamivudine and IFN-α resulted in a sustained loss of serum HBV DNA, based on the results of a solution hybridization assay, and normalization of alanine aminotransferase (ALT) in only 14 % of HBeAg-negative patients, but drug-resistant mutation variants did not emerge in any patients. However, from these previous studies, it is still unclear whether combination therapy with lamivudine and IFN confers an additional benefit compared to IFN monotherapy.

Three randomized controlled trials (2 in HBeAg-positive patients [24, 25] and 1 in HBeAg-negative patients [26]) did not show that 1-year combination therapy with lamivudine and pegylated IFN-α was superior to monotherapy with pegylated IFN-α in terms of the rate of sustained response. The results of these globally conducted

Table 1 Simultaneous combination therapy with nucleos(t)ide analogues and interferon

Reference (first author)	HBeAg	*n* (genotype)	Age (years)	Male (%)	Regimens	Biochemical response (%)	Virologic response (%)
Mutimer [21]	+	20 (N.D.)	39 ± 11[a]	95	LAM + IFN for 12–16 weeks	0	5
Barbaro [22]	+	76 (N.D.)	42 (33–50)[b]	84	LAM + IFN for 24 weeks	37	33
Tatulli [23]	−	29 (N.D.)	44 (27–64)[b]	90	LAM + IFN for 52 weeks	14	14
Janssen [24]	+	130 (A43/B11/C18/D52)	34 ± 12[a]	75	LAM + PEG for 52 weeks	35	35
Lau [25]	+	271 (A18/B82/C156/D11)	32 ± 10[a]	77	LAM + PEG for 48 weeks	39	28
Marcellin [26]	−	179 (N.D.)	41 ± 11[a]	82	LAM + PEG for 48 weeks	60	44
Wursthorn [29]	±	26 (A8/B0/C1/D14)	34 (19–55)[b]	77	ADV + PEG for 48 weeks	N.D.	N.D.
Takkenberg [30]	±	40 (A20/B2/C2/D9)	40 ± 10[a]	88	ADV + PEG for 48 weeks	N.D.	50

HBeAg hepatitis B e antigen, *LAM* lamivudine, *ADV* adefovir dipivoxil, *IFN* interferon, *PEG* pegylated interferon, *N.D.* not described

[a] Mean (± standard deviation, SD)

[b] Median (range)

trials, which included many patients with various HBV genotypes, appear to be reliable. All studies found that the combination therapy had greater on-treatment viral suppression and higher rates of sustained post-treatment response than therapy with lamivudine alone, but no difference was observed in the sustained post-treatment virologic response compared to that with pegylated IFN-α alone. Janssen et al. [24], for example, found that more patients in the patient group receiving the 52-week pegylated IFN-α + lamivudine combination than in the group receiving the 52-week pegylated IFN-α monotherapy showed a response, as assessed by serum HBeAg loss at the end of treatment (44 vs. 29 %; $P = 0.01$). However, this difference was not sustained; 35 % of the combination-therapy group and 36 % of the monotherapy group showed a sustained HBeAg loss at the end of follow-up ($P = 0.91$). The trial also showed that pegylated IFN-α therapy improves liver histology, particularly in responders to therapy, but that the addition of lamivudine to therapy with pegylated IFN-α did not further improve histological outcome [27] and that genotypes C and D are associated with a lower rate of response to IFN than genotypes A and B.

To date, few studies have examined the combination of IFN and other NAs that are more potent than lamivudine. The telbivudine + IFN combination is prohibited because of the high risk of severe polyneuropathy [28]. Interestingly, Wursthorn et al. [29] from Germany found that 48-week combination therapy with adefovir dipivoxil and pegylated IFN-α led to marked decreases in cccDNA in the liver, which has been correlated with reduced HBsAg in serum. However, the rate of the post-treatment sustained response was not reported in this study. Another group from the Netherlands [30] showed that intrahepatic cccDNA levels at the end of 48-week treatment with adefovir dipivoxil and pegylated IFN-α were predictive of a sustained response defined as HBV DNA <2,000 IU/mL and normal ALT. The efficacy of combining IFN and other NAs, such as entecavir or tenofovir, remains to be elucidated.

Sequential combination starting with IFN followed by NA

Table 2 shows a summary of previous reports concerning sequential combination therapy starting with IFN followed by NAs for chronic hepatitis B. Hasan et al. reported that the rate of sustained HBeAg seroconversion was only 6.2 % in patients in Kuwait receiving IFN-α alone for 4 weeks, followed by the IFN-α + lamivudine combination for 12 weeks, and lastly by lamivudine alone for 36 weeks; this rate was similar to that observed in patients receiving lamivudine alone for 48 weeks [31].

In contrast, a randomized trial by Chan et al. in China [32] showed that the rate of sustained virologic response, defined as HBeAg seroconversion and a HBV DNA level of <500,000 copies/mL, was 36 % in patients receiving pegylated IFN-α alone for 8 weeks, followed by the pegylated IFN-α + lamivudine combination for 24 weeks, and lastly by lamivudine alone for 28 weeks; this rate was

Table 2 Sequential combination therapy starting with IFN followed by nucleos(t)ide analogues

Reference (first author)	HBeAg	*n* (genotype)	Age (years)	Male (%)	Regimens	Biochemical response (%)	Virologic response (%)
Hasan [31]	+	32 (N.D.)	32 (17–63)[b]	88	IFN for 4 weeks, IFN + LAM for 12 weeks, and then LAM for 36 weeks	9.3	6.2
Chan [32]	+	50 (A0/B18/C35/D0)	32 (19–57)[b]	62	PEG for 8 weeks, PEG + LAM for 24 weeks, and then LAM for 28 weeks	50	36

[a] Mean (range)

[b] Median (range)

significantly higher than that observed in patients receiving lamivudine alone for 52 weeks (14 %; $P = 0.011$). At the end of the treatment period, 21 % of patients in the sequential combination treatment group developed a lamivudine-resistant mutant, compared to 40 % of patients in the lamivudine monotherapy group. Follow-up of this study demonstrated that sequential combination with pegylated IFN-α followed by lamivudine maintained a higher long-term virologic response than lamivudine monotherapy for up to 3 years [33]. However, this study did not include a study arm of pegylated IFN-α alone.

Sequential combination starting with NA followed by IFN

Table 3 shows a summary of previous studies which examined sequential combination therapy starting with NAs followed by IFN for chronic hepatitis B. In a pilot study [34] by Serfaty et al. in France, sustained responses, defined as serum HBV DNA clearance based on the results of a branched DNA assay and ALT normalization, were achieved in 57 % of patients who received lamivudine alone for 20 weeks followed by the lamivudine + IFN-α combination for 4 weeks, and lastly by IFN-α alone for 24 weeks.

Some groups have studied similar protocols for sequential therapy, but the results have been conflicting. Consistent with the results reported by Serfaty et al. [34], Sarin et al. [35] in India reported that the addition of 4-week lamivudine before starting 24-week pegylated IFN-α therapy resulted in a significantly higher rate of sustained HBeAg clearance (39 %) than that with 24-week pegylated IFN-α monotherapy (14 %; $P = 0.05$). In contrast, Manesis et al. [36] found that in HBeAg-negative patients in Greece, where genotype D is predominant, the rate of sustained response to sequential therapy, defined as HBV DNA of ≤30,000 copies/mL and normal ALT, was only 22 %, which did not differ from that obtained in age/sex-matched historical controls treated with IFN-α alone for 12 months (14 %; $P = 0.36$). In another report in Greece [37], sequential therapy did not raise the rate of sustained virologic response, defined as HBV DNA levels of <400 copies/mL, in HBeAg-negative patients compared to lamivudine monotherapy for a median duration of 25 months (33 vs. 17 %; $P = 0.40$), although no patients in the sequential therapy group showed emerging resistance to lamivudine. A group in China, where genotype B or C is predominant, reported very similar results [38].

In Japan and other East Asian countries, genotype C is the most prevalent HBV type [39, 40], and most patients with chronic hepatitis B acquire the virus perinatally [13]. Thus, response rates to IFN-based therapy in these countries are lower than those reported in Europe and the USA. In our previous study [41] using sequential therapy with lamivudine alone for 16–32 weeks, followed by the lamivudine + IFN combination for 4 weeks and lastly by IFN alone for 20 weeks, the rate of sustained loss of HBeAg was only 29 %. The rate of HBeAg loss during lamivudine treatment was higher among sustained responders than that among non-responders. In a multicenter trial, Minami et al. [42] found that patients who lost HBeAg during lamivudine treatment were more likely to show a sustained response to sequential therapy. Okuse et al. [43] reported that sequential therapy was effective for patients with acute exacerbations of chronic hepatitis B, particularly those in whom HBeAg had become negative during lamivudine treatment.

To date, a small study by Moucari et al. [44] from France has been the only one to evaluate the efficacy of sequential therapy with adefovir dipivoxil followed by IFN-α. Sustained virologic response, defined as serum HBV DNA of <10,000 copies/mL, was achieved in 50 % of patients, but only 20 HBeAg-negative patients were included in this study.

We recently reported the outcomes of sequential therapy with entecavir followed by IFN-α [45]. Among the 24 patients receiving entecavir alone for 36–52 weeks, followed by the entecavir + IFN-α combination for 4 weeks, and lastly by IFN-α alone for 20 weeks, the rate of sustained response, defined by HBeAg loss, HBV DNA of <10,000 copies/mL, and normal ALT, was 21 %; this was not higher than the rate found in our previous study using lamivudine [41]. In the study carried out in China, Chen

Table 3 Sequential combination therapy starting with a nucleos(t)ide analogue followed by IFN

Reference (first author)	HBeAg	*n* (genotype)	Age (years)	Male (%)	Regimens	Biochemical response (%)	Virologic response (%)
Serfaty [34]	±	14 (A6/B0/C1/D4)	40 (30–57)[a]	100	LAM for 20 weeks, followed by LAM + IFN for 4 weeks, and then IFN for 24 weeks	57	57
Sarin [35]	+	36 (N.D.)	33 ± 11[b]	93	LAM for 4 weeks, followed by PEG for 24 weeks	36	39
Manesis [36]	−	36 (N.D.)	55 (46–66)[a]	69	LAM for 6 months, followed by LAM + IFN for 6 months, and then IFN for 6 months	39	22
Vassiliadis [37]	−	18 (N.D.)	42 (19–63)[a]	83	LAM for 3 months, followed by LAM + PEG for 3 months, and then by PEG for 9 months	72	33
Shi [38]	−	64 (N.D.)	35 (21–56)[a]	60	LAM for 20 weeks, followed by LAM + IFN for 4 weeks, and then IFN for 24 weeks	53	14
Enomoto [41]	+	24 (C)	37 ± 11[b]	88	LAM for 16–32 weeks, followed by LAM + IFN for 4 weeks, and then IFN for 20 weeks	46	29
Minami [42]	±	37 (N.D.)	N.D.	N.D.	LAM for 20 weeks, followed by LAM + IFN for 4 weeks, and then IFN for 20 weeks	46	35
Okuse [43]	±	12 (C)	32 ± 8[b]	83	LAM for 20 weeks, followed by LAM + IFN for 4 weeks, and then IFN for 20 weeks	N.D.	58
Moucari [44]	−	20 (A5/B3/C1/D9)	44 (41–52)[a]	85	ADV for 20 weeks, followed by ADV + PEG for 4 weeks, and then PEG for 44 weeks	50	50
Enomoto [45]	+	24 (A1/B0/C23/D0)	39 ± 7[b]	96	ETV for 36–52 weeks, followed by ETV + IFN for 4 weeks, and then IFN for 20 weeks	29	21
Chen [46]	±	32 (A0/B23/C9/D0)	35 ± 5[b]	72	ETV for 12–26 days, followed by ETV + PEG for 2 weeks, and then PEG for 22–46 weeks	61	74

[a] Median (range)

[b] Mean (± SD)

ETV Entecavir

et al. [46] included only patients with acute exacerbation (ALT >10-fold the upper limit of normal) who were treated with entecavir alone for 12–26 days before the ALT had declined to five- to ten-fold the upper limit of normal, followed by the entecavir + pegylated IFN-α combination for 2 weeks, and then by pegylated IFN-α alone for 22–46 weeks. Sustained virologic response, defined as HBV DNA of <10,000 copies/mL, was obtained in 69 % of HBeAg-positive and in 80 % of HBeAg-negative patients with acute exacerbation of chronic hepatitis B.

One objective of sequential therapy starting with NA is to lower the viral load before IFN therapy is initiated, thereby restoring treatment sensitivity as low HBV DNA levels are associated with a favorable response to IFN. Another objective of sequential therapy is to prevent the relapse of hepatitis following the discontinuation of NA therapy through the use of IFN. The high risk of viral relapse after treatment may be attributed to the persistence of cccDNA in the liver, which is correlated with HBV antigen levels in the serum. Using HBsAg and hepatitis B core-related antigen (HBcrAg) levels, Matsumoto et al. [47] proposed a model for predicting relapse after the discontinuation of NA therapy. In our study of sequential therapy using entecavir [45], few patients showed a decrease in HBsAg or HBcrAg to the level meeting the criteria of safe discontinuation of NA. Taken together with the fact that at least 21 % of our patients achieved a sustained response, we suggest that the switch to IFN-α contributes to the safe termination of NA therapy in some patients [48].

Combination with NA and IFN in the guidelines

Combination therapy with NA and IFN is not recommended in the guidelines proposed by the Asian–Pacific Association for the Study of the Liver (updated in 2008) [13] and the American Association for the Study of Liver Diseases (updated in 2009) [14] because there has been no large clinical trial to confirm the benefits of combination therapy over monotherapy in inducing a higher rate of sustained response. The most recently updated guidelines proposed by the European Association for the Study of the Liver (updated in July 2012) [15] also does not recommend combination therapy of IFN with lamivudine or telbivudine. However, the limited information currently available on the efficacy and safety of combining IFN with other NAs has raised an unresolved issue of assessing the safety and efficacy of combining IFN with a more potent NA, such as entecavir or tenofovir.

Guidelines proposed by the Japanese Study Group of the Standardization of Treatment of Viral Hepatitis [49] basically recommend IFN as the first-line treatment for chronic hepatitis B patients aged <35 years to attain a "drug-free state" and entecavir for patients aged ≥35 years to persistently suppress HBV DNA (as tenofovir disoproxil fumarate has not been licensed in Japan to date). In patients aged <35 years and harboring HBV DNA in titers of ≥7 log copies/mL, sequential treatment with entecavir followed by IFN is recommended as the first-line therapy if HBeAg is negative and as the second-line therapy (next to IFN monotherapy) if HBeAg is positive. In patients aged ≥35 years and harboring HBV DNA of ≥7 log copies/mL, sequential treatment is recommended as the second-line therapy (next to entecavir) if the HBeAg is positive.

Conclusions

It remains unclear whether combination therapy is superior to monotherapy for treating chronic hepatitis B. Consequently, controlled trials comparing combination and monotherapy are necessary. In previous studies comparing the lamivudine + IFN combination and lamivudine monotherapy in a finite course, combination therapy was associated with higher rates of sustained post-treatment response and lower rates of drug resistance than lamivudine monotherapy. However, NAs are generally administered indefinitely due to high rates of post-treatment relapse. Additionally, even when NAs are administered alone, concern for drug resistance has significantly decreased (≤1.2 % in 3–5 years [50, 51]) with the development of newer high-potency NAs, such as entecavir and tenofovir. In previous studies comparing the lamivudine + IFN combination and IFN monotherapy, combination therapy showed greater on-treatment viral suppression, but no difference in the post-treatment sustained response was observed when compared to therapy with IFN alone. The efficacy of combining IFN with a more potent NA remains to be evaluated. Further studies are needed to determine whether switching to IFN contributes to the safe discontinuation of therapy, particularly in patients with decreased HBsAg and/or HBcrAg levels during long-term NA treatment [52].

Conflict of interest Dr. S. Nishiguchi has received research grants from Bristol-Myers K.K., MSD K.K., and Chugai Pharmaceutical Co., Ltd. Dr. N. Kawada has received grants from Bristol-Myers K.K., MSD K.K., and Chugai Pharmaceutical Co., Ltd.

References

1. Dienstag JL. Hepatitis B virus infection. N Engl J Med. 2008;359:1486–500.
2. Liaw YF, Chu CM. Hepatitis B virus infection. Lancet. 2009;373:582–92.
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;339:61–8.
4. 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;341:1256–63.
5. 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;357:2576–88.
6. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med. 2006;354:1001–10.
7. 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;354:1011–20.
8. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med. 2003;348:808–16.
9. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. N Engl J Med. 2005;352:2673–81.
10. 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.
11. Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med. 1993;119:312–23.
12. Yang YF, Zhao W, Xia HM, Zhong YD, Huang P, Wen J. Long-term efficacy of interferon alpha therapy on hepatitis B viral replication in patients with chronic hepatitis B: a meta-analysis. Antiviral Res. 2010;85:361–5.
13. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int. 2008;2:263–83.
14. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology. 2009;50:661–2.
15. 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.
16. Moraleda G, Saputelli J, Aldrich CE, Averett D, Condreay L, Mason WS. Lack of effect of antiviral therapy in nondividing hepatocyte cultures on the closed circular DNA of woodchuck hepatitis virus. J Virol. 1997;71:9392–9.
17. Dandri M, Burda MR, Will H, Petersen J. Increased hepatocyte turnover and inhibition of woodchuck hepatitis B virus replication by adefovir in vitro do not lead to reduction of the closed circular DNA. Hepatology. 2000;32:139–46.
18. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol. 1995;13:29–60.
19. Boni C, Bertoletti A, Penna A, Cavalli A, Pilli M, Urbani S, et al. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. J Clin Invest. 1998;102:968–75.
20. Boni C, Penna A, Ogg GS, Bertoletti A, Pilli M, Cavallo C, et al. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. Hepatology. 2001;33:963–71.
21. Mutimer D, Naoumov N, Honkoop P, Marinos G, Ahmed M, de Man R, et al. Combination alpha-interferon and lamivudine therapy for alpha-interferon-resistant chronic hepatitis B infection: results of a pilot study. J Hepatol. 1998;28:923–9.

22. Barbaro G, Zechini F, Pellicelli AM, Francavilla R, Scotto G, Bacca D, et al. Long-term efficacy of interferon alpha-2b and lamivudine in combination compared to lamivudine monotherapy in patients with chronic hepatitis B. An Italian multicenter, randomized trial. J Hepatol. 2001;35:406–11.
23. Tatulli I, Francavilla R, Rizzo GL, Vinciguerra V, Ierardi E, Amoruso A, et al. Lamivudine and alpha-interferon in combination long term for precore mutant chronic hepatitis B. J Hepatol. 2001;35:805–10.
24. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet. 2005;365:123–9.
25. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med. 2005;352:2682–95.
26. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med. 2004;351:1206–17.
27. van Zonneveld M, Zondervan PE, Cakaloglu Y, Simon C, Akarca US, So TM, et al. Peg-interferon improves liver histology in patients with HBeAg-positive chronic hepatitis B: no additional benefit of combination with lamivudine. Liver Int. 2006;26:399–405.
28. Marcellin P, Avila C, Wursthorn K, Chuang WL, Lau GK, Peng CY, et al. Telbivudine (LDT) plus peg-interferon (PEGIFN) in HBeAg-positive chronic hepatitis B—very potent antiviral efficacy but risk of peripheral neuropathy (PN). J Hepatol. 2010;52:S6–7.
29. Wursthorn K, Lutgehetmann M, Dandri M, Volz T, Buggisch P, Zollner B, et al. Peginterferon alpha-2b plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. Hepatology. 2006;44:675–84.
30. Takkenberg B, Terpstra V, Zaaijer H, Weegink C, Dijkgraaf M, Jansen P, et al. Intrahepatic response markers in chronic hepatitis B patients treated with peginterferon alpha-2a and adefovir. J Gastroenterol Hepatol. 2011;26:1527–35.
31. Hasan F, al-Khaldi J, Asker H, Varghese R, Siddique I, al-Shammali M, et al. Treatment of chronic hepatitis B with the sequential administration of interferon and lamivudine. Hepatogastroenterology. 2003;50:2040–2.
32. Chan HL, Leung NW, Hui AY, Wong VW, Liew CT, Chim AM, et al. A randomized, controlled trial of combination therapy for chronic hepatitis B: comparing pegylated interferon-alpha2b and lamivudine with lamivudine alone. Ann Intern Med. 2005;142:240–50.
33. Chan HL, Hui AY, Wong VW, Chim AM, Wong ML, Sung JJ. Long-term follow-up of peginterferon and lamivudine combination treatment in HBeAg-positive chronic hepatitis B. Hepatology. 2005;41:1357–64.
34. Serfaty L, Thabut D, Zoulim F, Andreani T, Chazouillères O, Carbonell N, et al. Sequential treatment with lamivudine and interferon monotherapies in patients with chronic hepatitis B not responding to interferon alone: results of a pilot study. Hepatology. 2001;34:573–7.
35. Sarin SK, Sood A, Kumar M, Arora A, Amrapurkar D, Sharma BC, et al. Effect of lowering HBV DNA levels by initial antiviral therapy before adding immunomodulator on treatment of chronic hepatitis B. Am J Gastroenterol. 2007;102:96–104.
36. Manesis EK, Papatheodoridis GV, Hadziyannis SJ. A partially overlapping treatment course with lamivudine and interferon in hepatitis B e antigen-negative chronic hepatitis B. Aliment Pharmacol Ther. 2006;23:99–106.
37. Vassiliadis T, Tziomalos K, Patsiaoura K, Zagris T, Giouleme O, Soufleris K, et al. Lamivudine/pegylated interferon alfa-2b sequential combination therapy compared with lamivudine monotherapy in HBeAg-negative chronic hepatitis B. J Gastroenterol Hepatol. 2007;22:1582–8.
38. Shi M, Wang RS, Zhang H, Zhu YF, Han B, Zhang Y, et al. Sequential treatment with lamivudine and interferon-alpha monotherapies in hepatitis B e antigen-negative Chinese patients and its suppression of lamivudine-resistant mutations. J Antimicrob Chemother. 2006;58:1031–5.
39. Orito E, Ichida T, Sakugawa H, Sata M, Horiike N, Hino K, et al. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology. 2001;34:590–4.
40. Enomoto M, Tamori A, Nishiguchi S. Hepatitis B virus genotypes and response to antiviral therapy. Clin Lab. 2006;52:43–7.
41. Enomoto M, Tamori A, Kohmoto MT, Hayashi T, Jomura H, Habu D, et al. Lamivudine and IFN-beta sequential therapy in HBe antigen-positive patients with chronic hepatitis B virus genotype C infection. J Interferon Cytokine Res. 2007;27:201–7.
42. Minami M, Okanoue T. Management of HBV infection in Japan. Hepatol Res. 2007;37[s1]:S79–82.
43. Okuse C, Yotsuyanagi H, Yamada N, Okamoto M, Ikeda H, Kobayashi M, et al. Effect of nucleoside analog-interferon sequential therapy on patients with acute exacerbation of chronic hepatitis B. Hepatol Res. 2010;40:461–9.
44. Moucari R, Boyer N, Ripault MP, Castelnau C, Mackiewicz V, Dauvergne A, et al. Sequential therapy with adefovir dipivoxil and pegylated interferon alfa-2a for HBeAg-negative patients. J Viral Hepat. 2011;18:580–6.
45. Enomoto M, Nishiguchi S, Tamori A, Kobayashi S, Sakaguchi H, Shiomi S, et al. Entecavir and interferon-α sequential therapy in Japanese patients with hepatitis B e antigen-positive chronic hepatitis B. J Gastroenterol 2012. doi: 10.1007/s00535-012-0645-5.
46. Chen CC, Wang PC, Chang HW, Chen CF. Safety and efficacy of two-step peginterferon α-2a treatment in patients of chronic hepatitis B with acute exacerbation. J Viral Hepat. 2012;19:161–72.
47. Matsumoto A, Tanaka E, Suzuki Y, Kobayashi M, Tanaka Y, Shinkai N, et al. Combination of hepatitis B viral antigens and DNA for prediction of relapse after discontinuation of nucleos(t)ide analogs in patients with chronic hepatitis B. Hepatol Res. 2012;42:139–49.
48. Kozuka R, Enomoto M, Morikawa H, Tamori A, Kawada N. Anti-hepatitis B virus therapy: to stop, or not to stop: has the question been solved? Hepatol Res. 2012;42:946–7.
49. Kumada H, Okanoue T, Onji M, Moriwaki H, Izumi N, Tanaka E, et al. Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan. Hepatol Res. 2010;40:1–7.
50. 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-naïve patients is rare through 5 years of therapy. Hepatology. 2009;49:1503–14.
51. 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.
52. Kittner JM, Sprinzl MF, Grambihler A, Weinmann A, Schattenberg JM, Galle PR, et al. Adding pegylated interferon to a current nucleos(t)ide therapy leads to HBsAg seroconversion in a subgroup of patients with chronic hepatitis B. J Clin Virol. 2012;54:93–5.