Treatment of HBeAg-Negative Chronic Hepatitis B with Nucleoside/Nucleotide Analogs

Stephanos J. Hadziyannis

INTRODUCTION AND HISTORICAL VIEW

Hepatitis Be antigen-negative/hepatitis Be antigen, antibody-positive (HBeAg-negative/anti-HBe-positive) chronic hepatitis B (CHB) was first identified in the early 1980s [1-3]. At that time, interferon-alpha was not yet available, while several other drugs were tried in CHB and proved to have little if any therapeutic benefit. In the mid 1980s, when conventional interferon-alpha became available for HBeAg-positive CHB, its efficacy was also evaluated in HBeAg-negative liver disease [4]. Four small randomized controlled trials (RCTs) of a short duration were performed in Greece and Italy, where this form of CHB prevails [3-7]. Although high end-of-treatment (EOT) response rates were achieved in these early studies, most patients who responded during therapy, relapsed soon after interferon was stopped. Thus the overall sustained response (SR) rate was not promising [3, 4]. These early "negative" treatment results in a disease that was generally viewed as atypical and rare [5, 6], discouraged pharmaceutical companies and most - but not all - clinical investigators from further studies on interferon-based therapies in HBeAg-negative CHB [8]. At the same time molecular biology made an impressive entry in the field of viral hepatitis and in 1989 replication competent hepatitis B virus (HBV)

mutants harboring a novel pre-core stop-codon which abolishes HBeAg production were discovered in patients with HBeAg-negative CHB [9-11] and explained, on a molecular basis, how HBeAg (a marker of HBV replication) can be negative despite ongoing HBV replication. These observations stimulated further research on several aspects of HBeAg-negative CHB, including epidemiology and treatment [12-15]. At present its frequency appears to be increasing worldwide [12-14] and in the Mediterranean area of Europe as well as in France and Germany HBeAg-negative type CHB represents 65-95% of newly diagnosed cases of CHB [12, 14, Hadziyannis. (in press), M. Manns and P. Marcellin, personal communication].

Treatment of HBeAg-negative CHB with conventional interferon alpha has continued in the 1990s in certain centers in Greece and Italy and longer treatment periods and higher interferon doses have resulted in an increase in the SR rate, in HBsAg loss in a number of patients, and improvement in the life threatening complications of the disease over the years [16-18]. Thus interferon-alpha was recently recommended as a first line therapy for HBeAg-negative CHB by the European Association for the Study of the Liver (EASL) and Asian Pacific Association for the study of the Liver (APASL). Pegasys, a more potent pegylated compound of interferon-alpha-2a [19], is now under evaluation for efficacy, safety and tolerability in HBeAg-negative CHB in a large, multicenter, one year phase III trial (see chapters in this volume by P. Marcellin and F. Bonino). However, interferon-based therapies may be only applicable in clinical practice in patients with compensated liver disease and, even with the best results, a high SR is not expected [8]. On the other hand the practicing physician now has access to oral nucleos(t)ide analogs, a new class of potent antiviral agents, [6, 8]. These agents have already been given to most subsets of patients with CHB, saving lives and revolutionizing the treatment of many individuals particularly with severe and advanced forms of viral B liver disease both in the pre- and post-transplant setting.

The nucleoside analog era in HBeAg-negative CHB started with lamivudine in 1999 [20], adefovir dipivoxil was introduced in 2003 [21], now includes entecavir [22], telbivudine [23-24] and other compounds (see chapter by R. Esteban). The first two nucleos(t)ides have already been approved for the treatment of CHB in the USA, Europe and in most other parts of the world; entecavir has completed Phase III registration trials and telbivudine is currently under

evaluation in phase III pre-registration trials. The aim of all analogs is the suppression of HBV replication by treatment courses of finite duration (6 months to 2 years). However short-term treatment courses with nucleoside analogs in HBeAg-negative CHB have been extended to maintain effective HBV suppression by long-term and possibly indefinite administration without the development of viral resistance [26-27].

In this article the efficacy, safety, tolerance and other aspects of short- and long-term anti-viral therapy with the two approved (lamivudine and adefovir dipivoxil) and other non-yet approved nucleos(t)ide analogs in the various subsets of patients with HBeAg-negative chronic viral B liver disease will be critically reviewed. Some evidence-based recommendations for therapeutic decision making in clinical practice and of treatment strategies in the various settings of HBeAg-negative chronic HBV infection and/or of liver disease are also mentioned.

GENERAL REMARKS ON NUCLEOS(T)IDE ANALOGS IN CHRONIC HEPATITIS B AND THEIR MODE OF ACTION

In the past few years several agents mimicking the structure of natural nucleosides have been synthesized chemically. They are generally referred to as nucleoside analogs but some are already phosphorylated to their triphosphate counterparts and are therefore now specifically defined as nucleotide analogs. Nucleoside analogs can be produced in their natural D- but also in the unnatural L-configuration e.g. L-deoxythymidine (LDT), and therefore they are also referred to as enantiomers [6].

When nucleos(t)ides are incorporated into newly synthesized DNA of HBV, they cause chain termination, thus inhibiting viral replication. Some nucleoside analogs competitively inhibit the polymerase of HBV either in its reverse transcriptase activity (synthesis of HBV DNA from the pregenomic HBV RNA transcript) or in its DNA-dependent DNA-directed activity (synthesis of the positive DNA strand within core particles). Theoretically, nucleoside analogs could also inhibit the amplification and replenishment of the pool of covalently closed circular DNA (cccDNA) in the nuclei of already infected hepatocytes as well as its formation in newly infected cells. This activity has recently been well documented in congenitally infected ducks treated by a combination of lamivudine and a

deoxyguanosine pro-drug and has been found to be dependent on the cell cycle phase [6, 25].

Despite the potency of several oral nucleotide analogs against HBV and the supposedly low rate of viral resistance even after long-term administration, none has been found to posses the properties and clinical characteristics of an ideal anti-HBV agent. In fact few, if any, HBeAg-negative CHB patients treated with finite courses of nucleoside achieve sustained virologic and biochemical remission [12, 27]. Thus, the present goal of treatment of HBeAg-negative chronic viral B liver disease with already approved and newer nucleoside analogs is effective and continuous suppression of HBV replication without the development of viral resistance based on various long lasting or even indefinite regimens [12, 26-28].

PREREQUISITES FOR INITIATING TREATMENT IN HBEAG-NEGATIVE CHRONIC HEPATITIS B

A number of criteria must be met concerning the diagnosis, differential diagnosis and severity of liver disease before initiating treatment of HBeAg-negative chronic viral B liver disease.

Safe diagnosis

For a diagnosis of HBeAg-negative CHB to be made, patients must have chronic HBV infection with documented HBsAg seropositivity and HBeAg negativity (usually positivity of anti-HBe as well) for at least 6 months, preferably one year; persistent or intermittent elevations in alanine aminotransferase/aspartate aminotransferase (ALT/AST) activity, detectable serum HBV DNA exceeding 105 copies/mL at least once in the last 3 month period and ≥grade 3 necroinflammation on liver biopsy [3, 4, 12, 13, 29]. Other concomitant or superimposed causes of liver disease should also be absent.

Differentiation from the inactive HBsAg carrier state

The serological profile of HBeAg-negative CHB i.e. **"HBsAg(+)/HBeAg(-)/anti-HBe(+)"** is identical to that of the inactive HBsAg carrier state. Moreover, several patients with HBeAg-negative CHB exhibit an intermittent rather than a continuous pattern of biochemical activity, sometimes with quite long-lasting intervening

periods of biochemical and even virologic remission [30, 31] mimicking the inactive HBsAg carrier state. Therefore, differentiation between these two conditions may require follow–up [30-32]. In studies with frequent assays of ALT/AST and of serum HBV DNA levels, major fluctuations in viremia and serum aminotransferase levels have been observed in more than 40% of untreated HBeAg-negative CHB cases [10, 30-31]. In this context an arbitrary serum HBV DNA level of 10^5 cP/mL has been suggested as a cut-off point for differentiation between patients with HBeAg-negative CHB and inactive HBsAg carriers [7]. However, several patients with HBeAg-negative CHB may have serum HBV DNA levels below 10^5 cP/mL at certain moments [32, 33]. According to our data, 20-30% of patients with histologically documented HBeAg-negative CHB, first present with normal ALT levels and low HBV DNA values below the cut-off level of 10^5 and even of 10^4 copies/mL, and may initially be misclassified as inactive HBsAg carriers [29, 30]. Thus, HBsAg(+)/HBeAg(-) individuals should be followed-up clinically, have frequent ALT assays and in case of increasing ALT levels be further tested for serum HBV DNA levels and considered as possible candidates for anti-viral therapy [26].

Severity of liver disease

Untreated HBeAg-negative CHB often runs a progressive course frequently terminating in cirrhosis and portal hypertension, liver failure and/or hepatocellular carcinoma [3, 34, 35]. On the other hand, patients with histologically minimal or mild HBeAg-negative liver disease and usually minimal ALT elevations may run a non-deteriorating or a very slowly progressive course, never reaching cirrhosis. Thus, considering the cost of long-term nucleos(t)ide analog therapy and the frequent development of viral resistance, at least with lamivudine treatment [21, 29, 36-38], the decision to treat or not to treat patients with HBeAg-negative CHB should be based on liver histology [26, 27, 29, 39]. Thus, in patients with minimal or mild liver disease, initiation of treatment is not usually recommended unless serum chemistries and liver necroinflammation/fibrosis deteriorate.

Previous treatment with interferon

In the absence of overt cirrhosis, patients with HBeAg-negative CHB may first be treated with an interferon course of a finite duration [7, 8,

31], probably pegylated interferon-alpha-2a for one year [41], thus providing a chance for sustained remission. If patients fail to respond to a first or second course of interferon treatment, if they are reluctant to be treated or retreated by interferon, if their liver disease has already advanced to de-compensated or clinically overt cirrhosis or if interferon is contraindicated for any reason, then long-term therapy with nucleoside analogs unequivocally becomes the treatment of choice.

GOALS AND END-POINTS OF THERAPY IN HBEAG-NEGATIVE CHRONIC HEPATITIS B

In HBeAg-positive CHB, sustained seroconversion of HBeAg to anti-HBe is considered to be a reliable end-point [7]. However, loss of HBeAg and seroconversion to anti-HBe are not applicable to HBeAg-negative/anti-HBe-positive CHB. Biochemical and virologic remission during a course of therapy that is sustained after stopping treatment, is considered to be a viable goal in approximately 25% of HBeAg-negative patients treated by interferon [16-18]. In several such responders, loss of HBsAg may also follow [16, 31]. Moreover, in observational studies with long-term follow-up, a reduced risk of the development of liver de-compensation and/or HCC, as well as improved survival have been reported [17, 35]. However, the goal of a sustained response is rarely if ever achieved with finite courses of nucleoside analog therapy lasting for 1, 2 or even 3 years [13, 38]. Thus, very long-term or even indefinite nucleoside analog treatment to maintain effective HBV suppression without viral resistance appears to be the next therapeutic alternative in HBeAg-negative CHB [16, 26-28]. It remains to be seen whether or not, at what frequency and with which regimens and compounds, sustained virologic responses can be achieved after stopping successful very long-term (for 5 and more years) nucleoside analog therapy.

A NOTE ON THE DEFINITIONS AND VOCABULARY OF RESPONSE TO TREATMENT

In both HBeAg-positive and HBeAg-negative CHB, virologic and biochemical responses can be evaluated during therapy (on-therapy responses) or after discontinuation of therapy (off-therapy or sustained responses) [13, 42]. In particular, on-therapy responses may be

subdivided into initial (achieved within the first months of therapy), maintained (persisting throughout the course of therapy), and end-of-therapy (evaluated at the end of a course of therapy with defined duration) [42]. Biochemical responses are defined by decreases in ALT/AST to the normal range, while virologic responses, preferably evaluated by qualitative polymerase chain reaction (PCR) assays, are arbitrarily considered to be achieved when serum HBV DNA levels fall below 10^5 cP/mL [5, 7, 13]. In particular in HBeAg-positive CHB, a virologic response also requires loss of HBeAg and seroconversion to anti-HBe. Although serum HBV DNA levels of 10^5cP/mL are currently used in many definitions of a virologic response [7], effective HBV suppression is probably achieved when serum HBV DNA levels drop below 400cP/mL or even 200cP/mL which are the cut-off levels of the Roche Monitor assay or of most in-house real time PCR assays [43]. A response is defined as complete when a biochemical and virologic response is accompanied by loss of HBsAg [16, 42]. When virologic and biochemical responses are maintained for several months, histologic improvement can also be demonstrated and is usually being defined as a reduction of the necroinflammatory Histology Activity Index (HAI/Knodell) score by ≥2 points without worsening in fibrosis [7, 13].

TREATMENT OF HBEAG-NEGATIVE CHRONIC HEPATITIS B WITH LAMIVUDINE (LAM)

Lamivudine (3TC or (-)-2´,3´-dideoxy-3´-thiacytidine), which was begun for the treatment of CHB in the late nineties, is a safe drug with rare and generally mild side-effects [44].

In patients with HBeAg-negative CHB, a 12-month course of LAM at a daily dose of 100-150mg has been shown to provide initial biochemical and virologic responses even by sensitive PCR assays in more than two thirds of patients [20, 37]. Unfortunately, biochemical and virologic relapses are observed in the vast majority of patients after stopping a 12-24 month lamivudine course [45, 46]. Given its excellent tolerability and safety profile, long-term treatment with lamivudine could be an acceptable, beneficial maintenance therapy in HBeAg-negative CHB. Unfortunately in clinical practice only 30-40% of patients remain in remission after the third year of lamivudine monotherapy without developing viral resistance [37, 38, 42].

Virologic breakthroughs under LAM monotherapy in CHB usually develop after the first 6 months of treatment [45, 47] and if therapy is extended their rate increases progressively, reaching approximately 60% at the end of the 3rd year in both HBeAg–positive and HBeAg–negative CHB [38, 45, 47]. Although several questions were initially raised about the clinical significance of LAM resistance, it is now quite clear that in patients with HBeAg-negative CHB its development is almost invariably followed by increasing viremia levels ending in biochemical breakthroughs [37], which ultimately have an adverse effect on liver histology [47]. Breakthroughs may be quite severe and this becomes of particular concern in patients with advanced liver disease and cirrhosis since it may lead to liver decompensation and death regardless of HBeAg status [38]. Finally it should be noted that the LAM resistant mutation M204I, which develops quite frequently in HBeAg-negative CHB patients of genotype D [37, 47], also appears to be resistant to treatment with some newer nucleoside analogs that are active against other LAM resistant HBV strains [6, 22-24]; but fortunately it remains sensitive to ADV (Hadziyannis S., unpublished).

Another disadvantage of lamivudine therapy for HBeAg-negative CHB is that, as previously mentioned, no course of finite duration has been shown to achieve sustained off-therapy responses in a sizeable proportion of patients and that the optimal duration of therapy remains currently unknown [38]. For example, it is noteworthy that most Greek patients with HBeAg-negative CHB, who have discontinued effective LAM therapy after 3-5 years duration have virologic and biochemical relapses [SJ Hadziyannis. unpublished].

TREATMENT OF HBEAG–NEGATIVE CHRONIC HEPATITIS B WITH ADEFOVIR DIPIVOXIL

Adefovir is an acyclic nucleotide analog with strong inhibitory activity for HBV replication and other viruses [6, 48]. It is administered orally in the form of its prodrug adefovir dipivoxil (ADV) and is commercially available under the trade name Hepsera. ADV is adefovir esterified with two pivalic acid molecules and has good oral availability [49]. It is rapidly converted to adefovir in plasma and tissues, has a plasma half-life of 5-7 hours and 90% of the drug is excreted in urine within 24 hours [6]. After being transported intracellularly by a receptor-based mechanism, adefovir is

phosphorylated to its diphosphate form, which is an analog of deoxyadenosine-5'-triphosphate but without a 3'-hydroxylic root. Therefore, it results in competitive inhibition of DNA synthesis by DNA polymerases and reverse transcriptases [50-51]. ADV has been shown to have no significant interaction with other drugs [6, 51] and the 10mg/day dose is very well tolerated with a safety profile similar to placebo [21, 52]. A higher daily dose of 30mg was found to be associated with an increased risk of renal damage without a significant increase in efficacy [52]. Thus, the approved ADV dose for CHB is 10mg/day, which can be safely administered even in patients with hepatic or mild renal impairment. However, dosing interval adjustments are recommended for patients with creatinine clearance of <50mL/min and in patients requiring hemodialysis [53-54].

ADV therapy was approved in 2002 for the treatment of all subgroups of chronically HBV infected patients with active viral replication and evidence of liver damage, who require therapeutic intervention: patients with CHB or decompensated HBV cirrhosis or HBV transplant patients, irrespective of HBeAg status and of the development of LAM resistance.

Efficacy, safety and tolerance

ADV monotherapy, at a dose of 10mg once a day, has been evaluated in a multicenter, randomized, double-blind, placebo-controlled trial including 185 patients with HBeAg-negative [21]. ADV was significantly more effective than placebo for all end-points. At 48 weeks, histologic improvement occurred in 64% of patients treated with ADV compared to 33% of those treated with placebo ($P<0.001$). ADV was also found to effectively suppress virologic and biochemical activity, reducing HBV DNA levels by approximately 4 logs and normalizing ALT in 72% of patients during the first 48 weeks of therapy [21].

Long-term extension of this trial has provided further information on the safety and efficacy of ADV therapy in HBeAg-negative CHB [28, 39]. During the second and third year on-therapy biochemical and virologic responses were maintained without any significant toxicity [28, 39] and with infrequent and late development of viral resistance. At the end of the second and third years of therapy, serum HBV DNA levels were undetectable by PCR in 71% and 79% of ADV treated patients respectively and ALT remained normal in 73% and 69% [28,

277

39]. Liver biopsies performed at week 96 in a subset of the overall cohort of this study suggested that an additional histological benefit is obtained if the duration of ADV treatment is extended [28].

This ongoing trial on the course of HBeAg-negative CHB also provided important information concerning results after stopping ADV treatment. Approximately one fourth of the total patient population in the trial (one third of the patients initially treated with ADV) switched to placebo after the first year of therapy. Most of them experienced biochemical and virologic relapses as well as reversal of the histologic improvement obtained during the first year of active treatment. Moreover, post-treatment flares in serum ALT levels were seen in some patients after stopping ADV [28]. Although these events were probably self-limiting and not associated with hepatic decompensation, they nevertheless stress the need for careful monitoring of patients who stop ADV therapy.

HBV resistance

There are two recognized ADV resistant HBV mutants, the rtN236T and the rtA181V [40, 56]. The rtN236T mutation is the most frequent and is associated with a selection of a novel asparagine to threonine substitution at residue rt236 in domain D of the HBV polymerase. It should be noted, however, that the incidence of ADV resistant mutations is delayed and infrequent, with a cumulative probability of 0% after 48 weeks, 2% after 96 weeks and 3.9% after 144 weeks of ADV therapy [39-40].

Patients developing the rtN236T mutation experience rebound in HBV DNA of >1 $\log_{10}$ from nadir with an increase in ALT activity [40]. The model structure of the reverse transcriptase of HBV suggests that the side chain of the rtN236T mutant may have a more favourable interaction with the gamma-phosphate of dATP compared to adefovir diphosphate, thus providing selectivity against adefovir diphosphate versus the natural substrate. The rtN236T mutation results in >60% reduction in the replicative capacity of HBV, but it is susceptible to L-deoxythymidine (LdT) and entecavir *in vitro* and fully susceptible to lamivudine both *in vitro* and *in vivo*. The significance of the rtA181V mutation is not clear and needs to be further characterized.

Efficacy of ADV in patients with LAM resistance

ADV monotherapy is effective in all subsets of patients with resistance to LAM [42, 57, 58] with similar antiviral efficacy against all LAM resistant HBV mutants, as well as in cirrhotic and transplant patients irrespective of their HBeAg status [59-63]. In 95 LAM resistant CHB patients who continued to take this drug, virologic responses at 48 weeks (defined as reduction in serum HBV DNA levels to $<10^5$cP/mL or >2 $\log_{10}$) were observed in 85% (39/46) of those who received additional ADV therapy and in only 11% of those who received placebo ($P<0.001$). Normalization of ALT was achieved in 31% and 6% of the cases respectively ($P=0.002$) [62]. In a large study including 324 HBV decompensated cirrhotics (n=128) or transplant patients (n=196) with resistance to LAM, the addition of ADV resulted in a significant reduction in serum HBV DNA levels, frequent normalization of liver function tests and improvement in Child-Pugh score (>90% of patients in both cohorts) [61]. Similar findings have also been reported in studies including fewer patients [59, 60, 62, 63]. These observations have been further analysed and commented on in relation to combination therapy both as an initial and rescue treatment to optimize efficacy and avoid the problem of multiple drug resistance [64-69].

The question of whether CHB patients with resistance to lamivudine should be switched to ADV monotherapy (immediately or after a period of concurrent LAM therapy) or receive long-term combination therapy with ADV and LAM is still debatable, primarily because of the high cost of combination therapy with oral nucleoside analogs. However, in patients with severe liver disease the financial cost should not be a criterion since the benefit can mean saving a life [69].

TREATMENT OF HBEAG-NEGATIVE CHRONIC HEPATITIS B WITH NEWER NUCLEOSIDE ANALOGS

Several nucleoside analogs are currently under evaluation for efficacy, safety and tolerance in phase II and III trials either alone or in combination in various types of chronic HBV infection including HBeAg-negative CHB. Those in advanced pre-registration stages and/or the most promising drugs appear to be entecavir, telbivudine, emtricitabine, clevudine, and tenofovir [43]. Advantages of these

newer compounds over LAM and ADV in the short- and long-term treatment of CHB have been claimed on the basis of more profound and more rapid [6, 55] suppression of HBV replication. However, the actual frequency of HBV resistance during long-term administration of the newer nucleoside analogs is still unknown; but a resistance threshold as high as that of adefovir cannot be really expected. Moreover the resistance profile of most new nucleoside analogs does not appear to be much different from that of LAM, and, therefore, cross-resistance in the treatment of lamivudune failures should be anticipated. Finally the antiviral efficacy of entecavir in LAM resistant mutants has been reported to be associated with baseline YMDD mutations [22] while telbivudine has been found to be inactive against rt M204I which is the predominant LAM mutant in HBeAg-negative CHB genotype D [47].

CONCLUDING REMARKS

The combination of a high rate of HBV replication with a slow death rate of HBV infected hepatocytes suggests that in chronic HBV infection, long-term treatment with potent antiviral agents will usually be required. This seems to be particularly true for HBeAg-negative chronic hepatitis B where the success of finite courses of interferon-alpha therapy has been limited [12, 13, 55] Treatment with the currently available nucleoside analogs has clearly confirmed that this is indeed the case. However, long-term nucleoside analog treatment, though initially quite effective in most HBeAg-negative patients, now faces the problem of a progressive decrease in efficacy because of the development of high rates of HBV resistance. In HBeAg-negative CHB patients, long-term monotherapy with lamivudine the first oral nucleoside analog approved for the treatment of HBV infection, only remains effective for more than 3 years in 30-40% of treated patients. Virologic and biochemical breakthroughs during long-term LAM treatment in HBeAg-negative CHB, destroy the benefit gained during the earlier periods of treatment. Moreover, such breakthroughs and relapses can be quite severe and even life threatening, particularly in patients with advanced liver disease and overt cirrhosis, thus raising major concerns on the suitability of long-term LAM therapy in such clinical settings. Long-term monotherapy with adefovir dipivoxil (ADV, Hepsera), the second nucleoside analog approved for the treatment of hepatitis B, is effective in more than two thirds of

HBeAg-negative patients with CHB at least for the first 3-4 years with rare and delayed appearance of HBV resistance . Moreover it has been shown to be effective against both wild type and LAM-resistant HBV strains as well as against all HBV genotypes either with a positive or negative HBeAg phenotype [65]. Currently the high cost of ADV remains its major drawback, otherwise it would already have completely replaced the use of LAM in most parts of the world and in all settings of HBeAg-negative chronic viral B liver disease. However, based on the lessons learnt for the treatment of HIV infection, practicing clinicians know that the use of "monotherapy" with any nucleoside analog is not the best way for a long-term/indefinite suppression of HBV replication. Combinations of drugs with complementary mechanisms of antiviral activity and different HBV resistance profiles [66, 67] (such as adefovir or tenofovir in combination with telbivudine or entecavir or lamivudine or emtricitabine) may soon prevent the development of HBV resistance and/or induce sustained virologic responses.

REFERENCES

1. Hadziyannis SJ, Lieberman HM, Karvountzis GG, Shafritz DA. Analysis of liver disease, nuclear HBcAg, viral replication, and hepatitis B virus DNA in liver and serum of HBeAg vs. anti-HBe positive carriers of hepatitis B virus. Hepatology 1983;3:656-662.

2. Bonino F, Rosina F, Rizzetto M, Rizzi R, Chiaberge E, Tardanico R, Callea F, Verme G. Chronic hepatitis in HBsAg carriers with serum HBV-DNA and anti-HBe. Gastroenterology 1986;90:1268-1273.

3. Hadziyannis SJ. Hepatitis Be antigen negative chronic hepatitis B. From clinical recognition to pathogenesis and treatment. Vir Hep Rev 1995;1:7-36.

4. Brunetto MR, Oliveri F, Colombatto P, Capalbo M, Barbera C, Bonino F. Treatment of chronic anti-HBe-positive hepatitis B with interferon-alpha. J Hepatol 1995;22:S42-S44.

5. Hoofnagle JH, di Bisceglie AM. The treatment of chronic viral hepatitis. N Engl J Med 1997;336:347-356.

6. Karayiannis P. Hepatitis B virus: old, new and future approaches to antiviral treatment. J Antimicrob Chemother 2003;51:761-785.

7. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000-summary of a workshop. Gastroenterology 2001;120:1828-1853.

8. Hadziyannis SJ. Interferon alpha therapy in HBeAg-negative chronic hepatitis B: new data in support of long-term efficacy. J Hepatol 2002;36:280-282.

9. Carman WF, Jacyna MR, Hadziyannis S, Karayiannis P, McGarvey MJ, Makris A, Thomas HC. Mutation preventing formation of hepatitis Be antigen in patients with chronic hepatitis B infection. Lancet 1989;2:588-591.

10. Brunetto MR, Giarin MM, Oliveri F, Chiaberge E, Baldi M, Alfarano A, Serra A, Saracco G, Verme G, Will H. Wild-type and e antigen-minus hepatitis B viruses and course of chronic hepatitis. Proc Natl Acad Sci USA 1991;88:4186-4190.

11. Okamoto H, Tsuda F, Akahane Y, Sugai Y, Yoshiba M, Moriyama K, Tanaka T, Miyakawa Y, Mayumi M. Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigen. J Virol 1994;68:8102-8110.

12. Hadziyannis SJ, Vassilopoulos D. Hepatitis Be antigen-negative chronic hepatitis B. Hepatology 2001;34:617-624.

13. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2001;34:1225-1241.

14. Funk ML, Rosenberg DM, Lok AQS. World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants. J Viral Hepat 2002;9:52-61.

15. Lindh M, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus-large-scale analysis using a new genotyping method. J Infect Dis 1997;175:1285-1293.

16. Manesis EK, Hadziyannis SJ. Interferon alpha treatment and retreatment of hepatitis Be antigen-negative chronic hepatitis B. Gastroenterology 2001; 121:101-109.

17. Brunetto MR, Oliveri F, Coco B, Leandro G, Colombatto P, Gorin JM, Bonino F. Outcome of anti-HBe positive chronic hepatitis B in alpha-interferon treated and untreated patients: a long-term cohort study. J Hepatol 2002;36:263-270.

18. Lampertico P, Del Ninno E, Vigano M, Romeo R, Donato MF, Sablon E, Morabito A, Colombo M. Long-term suppression of hepatitis Be antigen-negative chronic hepatitis B by 24-month interferon therapy. Hepatology 2003;37:756-763.

19. Cooksley WG, Piratvisuth T, Lee SD, Mahachai V, Chao YC, Tanwandee T, Chutaputti A, Chang WY, Zahm FE, Pluck N. Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis Be antigen-positive chronic hepatitis B. J Viral Hepat 2003;10:298-305.

20. Tassopoulos NC, Volpes R, Pastore G, Heathcote J, Buti M, Goldin RD, Hawley S, Barber J, Condreay L, Gray DF. Efficacy of lamivudine in patients with hepatitis Be antigen-negative/hepatitis B virus DNA-positive (precore mutant) chronic hepatitis B. Lamivudine Precore Mutant Study Group. Hepatology 1999;29:889-896.

21. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Wulfsohn MS, Xiong S, Fry J, Brosgart CL; Adefovir Dipivoxil 438 Study Group. Adefovir dipivoxil for the treatment of hepatitis Be antigen-negative chronic hepatitis B. N Engl J Med 2003;348:800-807.

22. Chang TT, Hadziyannis S, Cianciara J, Rizzetto M, Schiff E, Pastore G, Klesczenski K, Danisky G, DeHertogh D, Hindes R. Sustained viral load and ALT reduction following 48 weeks of entecavir treatment in subjects with chronic hepatitis B who have failed lamivudine. Hepatology 2002;36:300A.

23. Lai CL, Leung NWY, Teo EK, Tong M, Wongt F, Hann HW, Han S, Poynard T, Myers M, Chao G, Lloyd D, Brown N. Results of a one-year international phase IIb comparative trial of telbivudine, lamivudine and the combination in patients with chronic hepatitis B. Hepatology 2003;38:262A.

24. Bryant ML, Bridges EG, Placidi L, Faraj A, Loi AG, Pierra C, Dukhan D, Gosselin G, Imbach JL, Hernandez B, Juodawlkis A, Tennant B, Korba B, Cote P, Marion P, Cretton-Scott E, Schinazi RF, Sommadossi JP. Antiviral L-

nucleosides specific for hepatitis B virus infection. Antimicrob Agents Chemother 2001;45:229-235.

25. Addison WR, Walters KA, Wong WW, Wilson JS, Madej D, Jewell LD, Tyrrell DL. Half-life of the duck hepatitis B virus covalently closed circular DNA pool in vivo following inhibition of viral replication. J Virol 2002;76:6356-6363.

26. Papatheodoridis GV, Hadziyannis SJ. Review article: current management of chronic hepatitis B. Aliment Pharmacol Ther 2004;19:25-37.

27. Keeffe EB, Dieterich DT, Han SH, Jacobson IM, Martin P, Schiff ER, Tobias H, Wright TL. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States. Clin Gastroenterol Hepatol 2004;2:87-106.

28. Hadziyannis S, Tassopoulos N, Heathcote E, Chang TT, Kittis G, Rizzetto M, Marcellin P, Lim SG, Chen SS, Wulfsohn M, Wollman M, Frei J, Brosgart C. Two year results from a double-blind, randomized, placebo-controlled study of adefovir dipivoxil (ADV) for presumed precore mutant chronic hepatitis B. J Hepatol 2003;38:143A.

29. Conjeevaram HS, Lok AS. Management of chronic hepatitis B. J Hepatol 2003;38:S90-S103.

30. Hadziyannis SJ, Bramou T, Alexopoulou A, Makris A. Immunopathogenesis and natural course of anti-HBe positive chronic hepatitis with replicating B virus. In: Hollinger FB, Lemon SM, Margolis HS, editors. Viral hepatitis and liver disease. Baltimore, MD: Williams & Wilkins 1991:673-676.

31. Brunetto MR, Oliveri F, Colombatto P, Coco B, Ciccorossi P, Bonino F. Treatment of HBeAg-negative chronic hepatitis B with interferon or pegylated interferon. J Hepatol 2003;39:S164-S167.

32. Manesis EK, Papatheodoridis GV, Sevastianos V, Cholongitas E, Papaioannou C, Hadziyannis SJ. Significance of hepatitis B viremia levels determined by a quantitative polymerase chain reaction assay in patients with hepatitis Be antigen-negative chronic hepatitis B virus infection. Am J Gastroenterol 2003;98:2261-2267.

33. Chu CJ, Hussain M, Lok AS. Quantitative serum HBV DNA levels during different stages of chronic hepatitis B infection. Hepatology 2002;36:1408-1415.

34. Naoumov NV, Schneider R, Grotzinger T, Jung MC, Miska S, Pape GR, Will H. Precore mutant hepatitis B virus infection and liver disease. Gastroenterology 1992;102:538-543.

35. Papatheodoridis GV, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-alpha treated and untreated patients with HBeAg-negative chronic hepatitis B. J Hepatol 2001;34:306-313.

36. Santantonio T, Mazzola M, Iacovazzi T, Miglietta A, Guastadisegni A, Pastore G. Long-term follow-up of patients with anti-HBe/HBV DNA-

positive chronic hepatitis B treated for 12 months with lamivudine. J Hepatol 2000;32:300-306.

37.   Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C. Efficacy of long-term lamivudine monotherapy in patients with hepatitis Be antigen-negative chronic hepatitis B. Hepatology 2000;32:847-851.

38.   Rizzetto M, Marzano A, Lagget M. Treatment of hepatitis Be antigen-negative chronic hepatitis B with lamivudine. J Hepatol 2003;39:S168-S171.

39.   Hadziyannis S, Tassopoulos N, Chang TT, Heathcote J, Kittis G, Rizzetto M, Marcellin P, Lim SG, Chen SS, Arterburn S, Ma J, Xiong S, Wollman M, James C, Currie G, Brosgart CL. Three year study of adefovir dipivoxil (ADV) demonstrates sustained efficacy in presumed precore mutant chronic hepatitis B (CHB) patients in a long-term safety and efficacy study (LTSES). J Hepatol 2004;40:17.

40.   Qi X, Snow A, Thibault V, Zhu Y, Curtis M, Hadziyannis SJ, Brosgart C, Currie G, Arterburn S, Gibbs CS, Miller MD, Xiong S. Long-term incidence of adefovir dipivoxil (ADV) resistance in chronic hepatitis B (CHB) patients after 144 weeks of therapy. J Hepatol 2004;40:20.

41.   Marcellin P, Lau GKK, Bonino F, Farci P, Hadziyannis S, Jin R Piratvisuth T, Germanidis G, Pluck N. Peginterferon alfa-2A (40KD) (PEGASYS®) monotherapy is more effective than lamivudine monotherapy in the treatment of HBeAg-negative chronic hepatitis B: 72-week results from a phase III, partially double-blind study of PEGASYS® alone vs. PEGASYS® plus lamivudine vs. lamivudine. J Hepatol 2004;40:34.

42.   Hadziyannis SJ, Papatheodoridis GV, Vassilopoulos D. Treatment of HBeAg-negative chronic hepatitis B. Semin Liver Dis 2003;121:101-109.

43.   Pawlotsky JM. Molecular diagnosis of viral hepatitis. Gastroenterology 2002;122:1554-1568.

44.   Jarvis B, Faulds D. Lamivudine: A review of its therapeutic poential in chronic hepatitis B. Drugs 1999;58:101-141.

45.   Tassopoulos N, Volpes R, Pastore G, Heathcote J, Buti M, Gray DF, Barber J, Hawley S. Post lamivudine treatment follow-up of patients with HBeAg-negative chronic hepatitis. Br J Hepatol 1999;30:117.

46.   Santantonio T, Mazzola M, Iacovazzi T, Miglietta A, Guastadisegni A, Pastore G. Long-term follow-up of patients with anti-HBe/HBV DNA -positive chronic hepatitis B treated for 12 months with lamivudine. J Hepatol 2000;32:300-306

47.   Papatheodoridis GV, Dimou E, Laras A, Papadimitropoulos V, Hadziyannis SJ. Course of virologic breakthroughs under long-term lamivudine in HBeAg-negative HBV liver disease. Hepatology 2002;36:219-126

48.   Nicoll AJ, Colledge DL, Toole JJ, Angus PW, Smallwood RA, Locarnini SA. Inhibition of duck hepatitis B virus replication by 9-(2-

phosphonylmethoxyethyl) adenine, an acyclic phosphonate nucleoside analogue. Antimicrobial Agents and Chemotherapy 1998;42:3130-3135.

49.   Cullen JM, Li DH, Brown C, Eisenberg EJ, Cundy KC, Wolfe J, Toole J, Gibbs C. Antiviral efficacy and pharmacokinetics of oral adefovir dipivoxil in chronically woodchuck hepatitis virus-infected woodchucks. Antimicrobial Agents and Chemotherapy 2001;45:2740-2745.

50.   Yu AS, Keeffe EB. Nucleoside analogues and other antivirals for treatment of hepatitis B in the peritransplant period. Clin Liver Dis 2003;7:551-572.

51.   Holy A, Votruba I, Metra A, Cerny J, Vesely J, Vlach J, Sediva P, Rosenberg I, Otmar M, Hrebabecky H. Acyclic nucleotide analogues: synthesis, antiviral activity and inhibitory effects on some cellular and virus-encoded enzymes in vitro. Antiviral Res 1990;23:295-311.

52.   Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, Jeffers L, Goodman Z, Wulfsohn MS, Xiong S, Fry J, Brosgart CL; Adefovir Dipivoxil 437 Study Group. Adefovir dipivoxil for the treatment of hepatitis B antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808-816.

53.   Mailliard ME, Gollan JL. Suppressing hepatitis B without resistance - so far, so good. New Engl J Med 2003;348:848-850.

54.   Knight W, Hayashi S, Benhamou Y, Currie G, Ebrahimi R, Gill S, Fry J, Kearney B, Brosgart C. Dosing guidelines for adefovir dipivoxil in the treatment of HBV infected patients with renal or hepatic impairment. J Hepatol 2002;36:136.

55.   Dusheiko G. Adefovir dipivoxil for the treatment of HBeAg-positive chronic hepatitis B: a review of the major clinical studies. J Hepatol 2003;39:S116-S123.

56.   Xiong S, Yang H, Westland CE, Delaney WE, Colledge D, Bartholomeusz A, Thibault V, Benhamou Y, Anguo P, Wulfsohn M, Gibbs CS, Fry J, Brosgart C, Locarnini S. Resistance surveillance of HBeAg- chronic hepatitis B (CHB) patients treated for two years with adefovir dipivoxil (ADV). J Hepatol 2003;38:182.

57.   Zoulim F, Trepo C, Poynard T, Pol S, Zarski JP, Marcellin P, Beaugrand M, Stitruk V, Trylesinski A, Kleber K, Van Doren S, Brosgart C. Adefovir dipivoxil (ADV) for the treatment of patients with chronic hepatitis B (CHB) failing lamivudine (LAM) therapy. J Hepatol 2003;38:184.

58.   Westland CE, Yang H, Namimi H, Lama N, Gibbs CS, Miller MD, Fry J, Brosgart C, Xiong S. Comparison of anti-HBV activity of adefovir against different lamivudine-resistant HBV strains in vitro and in liver transplant patients. Hepatology 2001;34:446A.

59.   Perrillo R, Schiff E, Yoshida E, Statler A, Hirsch K, Wright T, Gutfreund K, Lamy P, Murray A. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. Hepatology 2000;32:129-134.

60.   Ahmad J, Dodson SF, Balan V, Vargas HE, Fung JJ, Rakela J, Shakil AD. Adefovir dipivoxil suppresses lamivudine resistant hepatitis B virus in liver transplant recipients. Hepatology 2000;32:292A.

61.   Schiff ER, Lai CL, Hadziyannis S, Neuhaus P, Terrault N, Colombo M, Tillmann HL, Samuel D, Zeuzem S, Lilly L, Rendina M, Villeneuve JP, Lama N, James C, Wulfsohn MS, Namini H, Westland C, Xiong S, Choy GS, Van Doren S, Fry J, Brosgart CL; Behalf of the Adefovir Dipovoxil Study 435 International Investigators Group. Adefovir dipivoxil therapy for lamivudine-resistant hepatitis B in pre- and post-liver transplantation patients. Hepatology 38:1419-1427.

62.   Perrillo R, Hann HW, Mutimer D, Willems B, Leung N, Lee WM, Moorat A, Gardner S, Woessner M, Bourne E, Brosgart CL, Schiff E. Adefovir dipivoxil added to ongoing lamivudine in chronic hepatitis B with YMDD mutant hepatitis B virus. Gastroenterology 2004;126:81-90.

63.   Kuwahara R, Kumashiro R, Inoue H, Tanabe R, Tanaka E, Hino T, Ide T, Koga Y, Sata M. Adefovir dipivoxil as a treatment for hepatic failure caused by lamivudine-resistant HBV strains. Dig Dis Sci 2004;49:300-303.

64.   Peters MG, Hann HH, Martin P, Heathcote EJ, Buggisch P, Rubin R, Bourliere M, Kleber K, Ebrahimi R, Xiong S, Brosgart C. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology 2004;126:91-101.

65.   Westland C, Delaney W 4th, Yang H, Chen SS, Marcellin P, Hadziyannis S, Gish R, Fry J, Brosgart C, Gibbs C, Miller M, Xiong S. Hepatitis B virus genotypes and virologic response in 694 patients in phase III studies of adefovir dipivoxil1. Gastroenterology 2003;125:107-116.

66.   Shaw T, Locarnini S. Combination chemotherapy for hepatitis B virus: the final solution? Hepatology 2000;32:430-432.

67.   Shaw T, Bowden S, Locarnini S. Rescue therapy for drug resistant hepatitis B: another argument for combination chemotherapy? Gastroenterology 2004;126:343-347.

68.   EASL International consensus conference on hepatitis B. J Hepatol 2003;39:S3-S25.

69.   Zoulim F. Treatment of pre- and post-liver transplantation HBV infection: Should we aim at combination therapy? Hepatology 2003;38:1353-1355.