REVIEW

Treatment Options in Chronic Hepatitis B: Focus on Entecavir

Aarthi Chary[1,2] and Vivian Levy[1,3]

[1]Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA, USA.
[2]VA Palo Alto Health Care System, Palo Alto, CA, USA. [3]San Mateo County Health Systems, San Mateo, CA, USA.
Corresponding author email: charya@stanford.edu

Abstract: Chronic hepatitis B virus (HBV) infection is a major cause of liver-related morbidity and mortality worldwide and is endemic in many areas of Asia and Africa. Adequate treatment of hepatitis B with effective antiviral agents can improve disease burden. Entecavir is a potent, selective nucleoside analogue with activity against HBV. In recent years, studies have been done on the safety, efficacy, resistance profile and therapeutic use of entecavir. This review will discuss pharmacological and clinical aspects of oral entecavir for use in adults with chronic hepatitis B virus infection.

Keywords: hepatitis B virus, entecavir, antiviral agents/therapeutic use, antiviral agents/pharmacology, adults

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Introduction

Hepatitis B virus (HBV) is an enveloped DNA virus of the *Hepadnaviridae* family, and is a leading cause of liver-related morbidity and mortality worldwide. In contrast to low-prevalence areas—such as Western countries—in which HBV infection typically occurs in immunocompetent adolescents or adults and results in acute infection with subsequent clearance of infection, perinatal and horizontal (childhood) transmission is frequent in countries where HBV is endemic. In these regions, such as Asia and Africa, over 90% of those infected develop chronic infection. In the US, routine screening for hepatitis B surface antigen (HBsAg) is recommended for newly-arrived immigrants from countries where HBV seroprevalence is greater than 2%, as well as for men who have sex with men and injection drug users.[1] Over 300 million people worldwide are affected by chronic HBV. An estimated 600,000 deaths annually are attributed to HBV-related disease.[2,3] Chronic HBV infection is associated with increased risk of hepatocellular carcinoma (HCC), cirrhosis, end-stage liver disease (ESLD), and death. Chronic HBV-infected persons with persistent hepatitis B e antigen (HBeAg) have an estimated incidence of cirrhosis of 3.5% per year.[4] In those with cirrhosis, HCC develops at an incidence of 3%–6% per year.[5]

The primary treatment aim for chronic HBV is permanent viral suppression. Predictors of cirrhosis or HCC in persons chronically infected with HBV include persistently elevated HBV DNA and alanine aminotransferase (ALT) levels, genotype C HBV strain, presence of HBeAg, male sex, older age at infection, and co-infection with HIV, HCV or hepatitis D virus.[6] Though all of these factors may impact outcome of HBV-related disease, high serum HBV DNA and/or seropositivity for HBeAg are among the most significant risk factors for cirrhosis and HCC development.[7,8]

Thus, antivirals that suppress HBV DNA and lead to seroconversion (loss of HBeAg and development of anti-HBe antibody) may decrease the risk of hepatocellular carcinoma (HCC) and advanced liver disease.[9] In addition to interferon, various oral nucleoside and nucleotide analogs, known as nucleo(s) tide reverse transcriptase inhibitors (NRTIs), studied in recent years have been shown to suppress HBV viremia. Entecavir, approved in the U.S. in 2005, has emerged as one of the first-line agents in the treatment of chronic HBV due to its favorable tolerability profile, high potency against HBV, and high genetic barrier to resistance.

Natural History and Treatment Indications

Phases of HBV infection due to viral-host interactions are relevant when considering treatment. The early, *immune tolerant* phase is often seen in young individuals infected perinatally or horizontally, and is characterized by hepatitis B surface antigen (HBsAg) positivity, HBeAg positivity and high levels of serum HBV DNA ($>2 \times 10^6$ to 2×10^7 IU/ml where 1 IU/ml = 5 copies/ml), little immune response against the virus with normal or mildly elevated aminotransferases, and mild or no liver inflammation on histology. These persons have minimal disease progression at five year follow up, low rate of HBeAg loss, and can be clinically monitored without antiviral treatment unless there is evidence of progressive liver disease.[10] After perinatal infection, this phase may last for decades, during which there is a very low rate of spontaneous HBeAg seroconversion. The cumulative rate of spontaneous HBeAg clearance is estimated at 2% for the first 3 years of life and only 15% after two decades in perinatally-acquired infection.[11] Among immunocompetent adults, this phase is typically present only during the incubation period. During the second phase (*immune active*) one sees increased or fluctuating levels of aminotransferases precipitated by the immune response, moderate to severe liver inflammation on histology and associated decreasing HBV DNA. This phase may occur after several years of immune tolerance and is seen more frequently in subjects infected during adulthood. Ideally, the host immune response to HBV leads to a third phase, in which HBeAg seroconversion occurs, often with coinciding reduction in HBV DNA replication.

In patients who are in the *inactive HBV carrier* phase (low or undetectable serum HBV DNA levels and normal ALT with HBsAg and anti-HBe), antiviral treatment is not indicated. The estimated annual incidence of spontaneous HBeAg seroconversion is 2%–15% depending on factors such as age, ALT levels, and HBV genotype.[12] In perinatally-acquired infection, this period usually occurs in the second to third decade of life, when annual seroconversion rates

may approach 10%–20%. Similar rates are observed in adults who develop chronic infection.[13,14]

However, some patients can enter into an *HBeAg-negative chronic hepatitis B infection* phase during the course of disease, caused by nucleotide substitutions in the precore and/or basal core-promoter regions of the HBV genome. Though HBV strains in these patients may not express detectable levels of HBeAg, this phase of disease is often associated with progressive liver disease, fluctuating ALT activity, and elevated—though lower than in HBeAg-positive disease—serum HBV DNA levels.[15] Patients in this phase may present clinically as a new diagnosis of chronic hepatitis B or as HBV reactivation. The prevalence of the HBeAg-negative chronic hepatitis B has been increasingly recognized, and is related to duration of infection, as suggested by the older age at presentation; thus, it appears more prevalent in geographic areas where perinatal/horizontal transmission predominates.[16]

Treatment is recommended for HBeAg-positive and HBeAg-negative chronic hepatitis B if HBV DNA is >2,000 IU/mL and/or the serum ALT is above the upper limit of normal (ULN) and liver biopsy shows necroinflammation and/or fibrosis (i.e. at least grade 2 or stage 2 by META-VIR scoring or Ishak/Knodell score of at least 3). Patients with HBV DNA levels >20,000 IU/mL and elevated ALT levels above the ULN should be treated, regardless of whether a liver biopsy is performed.[17] In newly-diagnosed HBeAg-positive patients with compensated liver disease, however, antiviral treatment should be delayed 3–6 months to determine whether spontaneous HBeAg seroconversion will occur. Patients with compensated cirrhosis and detectable HBV DNA should be considered for treatment regardless of ALT and DNA levels. Patients with decompensated cirrhosis, rapid deterioration of liver function, cirrhosis or advanced fibrosis and detectable serum HBV DNA, or reactivation of chronic HBV after chemotherapy or immunosuppression need urgent antiviral treatment.[3,17,18] In addition, pre-emptive or prophylactic HBV therapy should be considered in individuals at high risk of hepatitis B reactivation, particularly HBsAg-positive carriers, prior to initiation of chemotherapy or immunosuppressive drugs.

The goal of antiviral treatment for HBV is to prevent progression to cirrhosis, ESLD, and HCC. This is achieved through sustained viral suppression to below the lower limits of detection of current real-time PCR assays (10 IU/ml) and loss of HBeAg. Sustained viral suppression also reduces the risk of resistance to nucleos(t)ide analogs. Currently, HBV infection cannot be completely eradicated with antiviral therapy due to the persistence of a reservoir of viral covalently closed circular DNA (cccDNA) in the nucleus of infected hepatocytes.[19,20,21] In patients treated with antivirals, HBV DNA should be checked every 12 weeks. Nucleos(t)ide therapy can be stopped 24 to 48 weeks after HBeAg seroconversion with undetectable HBV DNA documented on two separate occasions at least 6 months apart. Treatment duration is less clear in HBeAg negative patients, but consideration should be given to stopping antivirals in patients with undetectable HBV DNA on 3 separate occasions at least 6 months apart.[3]

Entecavir: Mechanisms of Action, Metabolism and Pharmacokinetic Profile
Mechanism of action
Entecavir has been approved in the U.S., European Union, and many other countries for the treatment of chronic HBV in adults with active HBV replication and evidence of active liver disease, as demonstrated either by elevated transaminases (AST or ALT) or liver tissue histology.

Entecavir (ETV, formerly called BMS 200475 and SQ 45676) is a cyclopentyl deoxyguanosine (nucleoside) analogue with the chemical name 2-amino-1,9-dihydro-9-[(1S,3R,4S)-4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6H-purin-6-one (Fig. 1).[22] Initially developed as a potential antiherpetic drug, entecavir was found to be a potent and specific inhibitor of HBV replication with minimal activity against other DNA and RNA viruses, such as herpes simplex virus, HIV, and influenza.[23] Like other nucleos(t)ide analogues, the monohydrate form of entecavir is phosphorylated intracellularly to its active triphosphate moiety (ETV-TP). ETV-TP competitively inhibits HBV polymerase by competing with its natural substrate, deoxyguanosine triphosphate.[23,24] This selective inhibition of HBV polymerase effectively blocks HBV replication at multiple steps of synthesis: protein-linked priming, RNA-directed first-strand synthesis or reverse transcription, and second
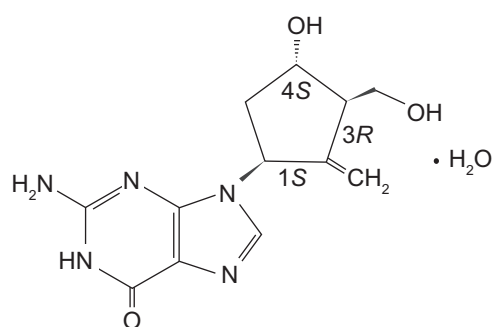
Figure 1. Entecavir structure.

strand DNA-dependent DNA synthesis.[25,26] ETV-TP is only a weak inhibitor of cellular DNA polymerases α, β, and δ and mitochondrial DNA polymerase γ (K_i values ranging from 18 to >160 μM).[22]

Clinical pharmacology

Early enzymatic and cell culture studies *in vitro* demonstrated entecavir had greater antiviral potency than other NRTIs against wild-type HBV and showed efficacy against lamivudine(LAM)-resistant HBV strains, albeit at higher concentrations.[23,27,28] In wild-type HBV-transfected HepG2 2.2.15 cells, the effective concentration of the drug required to reduce HBV replication by 50% (EC_{50}) was significantly lower for entecavir (0.00375 μM) than for lamivudine (0.116 μM) or other nucleoside analogs evaluated (range 128 nM to >100 μM).[23] In contrast, entecavir demonstrates much lower potency against HIV-1 virus, with EC_{50} ranging from 0.026 to >10 μM.[29] However, this antiviral efficacy is enhanced with reduced viral inoculum and incubation, and both *in vivo* and *in vitro* studies have demonstrated that entecavir may have some partial antiviral activity on HIV-1 and exert inhibitory pressure, with selection of the HIV reverse transcriptase M184V mutation particularly at high entecavir concentrations.[29–31]

Pharmacokinetics

Entecavir reaches peak plasma concentrations in healthy subjects in 0.5 to 1.5 hours following oral administration. Steady state is reached after 6–10 days of once-daily administration and both C_{max} and AUC (area under the plasma concentration versus time curve) are linearly related to dose. The C_{max} occurring after the standard 0.5-mg and 1-mg oral doses are 4.2 ng/mL and 8.2 ng/mL, respectively.[22]

The current recommended dosage of entecavir is 0.5 mg once daily for nucleoside-naive patients and 1.0 mg once daily for patients (age ≥ 16 years) with a history of LAM-refractory viremia or lamivudine or telbivudine resistance mutations. The drug is available in both tablet and solution, which have equivalent bioavailability; bioavailability of entecavir is estimated to be at least 70%, based on urinary excretion.[32] Administration on an empty stomach is recommended, at least 2 hours after a meal and 2 hours before the next meal, as high-fat or light meals have been shown to delay absorption and lead to lower observed C_{max}, by 44%–46%, and AUC, by 18%–20%.[22] Entecavir is approximately 13% protein-bound in human serum, and has large estimated volume of distribution.[22,32] Entecavir is metabolized in the liver and is not a substrate for, nor inhibitor/inducer of, the cytochrome P450 system. Clearance is primarily renal, by glomerular filtration and tubular secretion, and ranges from 360–471 mL/min regardless of dose.[33] Terminal half-life is 128–149 hours; as the drug has a 2-fold accumulation over the steady-state period following recommended dosages, the effective accumulation half-life is approximately 24 hours.[33]

While no significant gender or race differences in entecavir pharmacokinetics have been observed, all nucleos(t)ides are cleared renally and doses need to be adjusted for decreased creatinine clearance. Studies in healthy subjects demonstrated a 29.3% greater entecavir AUC in elderly (≥65 years), compared to young (18–40 years), subjects following a 1-mg dose.[22,32] An increase in AUC and C_{max} and decrease in clearance was also noted in non-HBV-infected subjects with varying degrees of renal impairment after a single 1-mg dose.[20] Only 13% of entecavir was removed over 4 hours with hemodialysis in subjects with end-stage renal disease.[20] Thus, dose adjustments are recommended in patients with creatinine clearance <50 mL/min, including those on hemodialysis or peritoneal dialysis. Dose-adjusted entecavir may be a better treatment option than adefovir or tenofovir in patients with renal insufficiency as entecavir has not been reported to cause renal insufficiency. While further studies are being done on the safety and efficacy of entecavir in subjects with moderate-to-severe hepatic impairment and liver transplant patients, there do not appear to be altered pharmacokinetics, independent

of renal function, and no dose adjustment for hepatic impairment is currently recommended.[22]

Clinical Studies and Experience
Preclinical and phase 1 and 2 trials
Preclinical studies of entecavir demonstrated an >100-fold increase in potency over other NRTIs in HBV cell culture, per EC_{50} results.[26] Hepadnavirus animal models demonstrated significant reductions in the woodchuck hepatitis virus DNA in serum of chronically-infected woodchucks given 0.2–0.5 mg/kg per day of oral entecavir.[34] Similarly, entecavir led to potent suppression of duck HBV in infected duck hepatocytes ($EC_{50} = 0.13$ nM, more than 1000-fold more potent than lamivudine, $EC_{50} = 138$ nM).[35] Sustained antiviral activity of entecavir, significant reduction of cccDNA levels and viral antigen levels, and improved survival was also demonstrated in woodchucks receiving long-term (>2 years) entecavir.[36]

A phase 2 randomized, double-blind multicenter clinical trial in 169 HBeAg-positive and negative patients compared the safety and efficacy of entecavir (0.01 mg/day, 0.1 mg/day, and 0.5 mg/day orally) to lamivudine 100 mg/day; patients were not allowed to have more than 12 weeks of prior lamivudine, and no HBV therapy within the 24 weeks prior to enrollment. Entecavir was found to be superior to lamivudine in reducing HBV DNA, with a dose-response relationship: after 24 weeks of therapy, 83.7% of patients treated with entecavir 0.5 mg had an HBV DNA level below the lower limit of detection (LLOD) compared to 57.5% treated with lamivudine, $P = 0.008$). Entecavir was well-tolerated with adverse events occurring at equivalent rates in both study arms.[37] A similarly-designed phase II study evaluated switching LAM-refractory (defined as continued viremia despite lamivudine treatment for >24 weeks or documented genotypic lamivudine resistance) patients to entecavir (0.1 mg/day, 0.5 mg/day, or 1.0 mg/day) versus continued lamivudine;[38] the 1.0 mg dose was based on *in vitro* studies demonstrating higher median EC_{50} were required in the presence of lamivudine resistance mutations at rtM204 and rtL180, compared to wild-type HBV strains (0.026 μM, versus 0.004 μM).[39] Patients treated with all doses of entecavir had significantly greater reductions in HBV DNA compared to lamivudine (2.85–5.06 log_{10} copies/mL mean reduction vs. 1.37 log_{10} copies/mL), and more patients normalized ALT (47%–68% vs. 47%).[38] All doses were well-tolerated and a dose-response relationship was noted, suggesting an optimal dose of 1.0 mg/day in LAM-refractory patients.

Phase 3 clinical trials
Entecavir was approved for the treatment of chronic hepatitis B infection in adults based on the results of three large prospective, multicenter, randomized double-blind phase 3 trials comparing entecavir to lamivudine, designed by the manufacturer and the Benefits of Entecavir for Hepatitis B Liver Disease (BEHoLD) Study Group. These studies were comprised of 1633 nucleoside-naïve and LAM-refractory patients (age ≥16 years) with chronic HBV infection based on HBsAg, chronic hepatitis on liver biopsy, HBV DNA level, and elevated ALT, with compensated liver function. Two of these phase 3 trials were conducted in HBeAg-positive (ETV-022)[40] and HBeAg-negative (ETV-027)[41] nucleoside-naïve patients, and the third in LAM-refractory HBeAg-positive patients (ETV-026).[42] Studies in nucleoside-naïve patients limited prior lamivudine use to a maximum of 12 weeks total, and patients could not have received any antiviral therapy within the preceding 24 weeks. The primary efficacy end point at 48 weeks was the proportion of patients with histologic improvement (decrease by at least 2 points in Knodell fibrosis score) compared to baseline; secondary endpoints were reduction in HBV DNA from baseline, proportion of patients with undetectable HBV DNA (lower limit of quantification 300 copies/mL), decrease in Ishak fibrosis score, HBeAg loss and HBeAg seroconversion (in HBeAg-positive patients only), and normalization of ALT. In addition to these, a fourth study evaluated entecavir in comparison to lamivudine in a large, mixed (HBeAg positive and negative) nucleoside-naïve population in China (ETV-023).[43] Primary endpoints and results of these trials are summarized in Table 1.

Nucleoside-naïve HBeAg-positive or HBeAg-negative patients
Both phase 3 studies in nucleoside-naïve subjects demonstrated superiority of entecavir over lamivudine with regards to the occurrence of histologic

Table 1. Efficacy of entecavir (ETV) for chronic hepatitis B (CHB): summary of phase 2 and phase 3 dose-ranging and lamivudine (LAM)-comparison arm clinical trials.[a]

Study population	Trial number [ref]	Study arms[b]	Composite endpoint[c] (% of pts)	Histologic improvement[d] (% of pts)	HBeAg seroconversion (% of pts)	Undetectable HBV DNA[e] (% of pts)	Mean decline in HBV DNA, from baseline (log copies/mL)	ALT normalization[f] (% of pts)
Nucleoside-naïve patients								
HBeAg+	ETV-022[40]	ETV 0.5 mg	–	72%*	21%	67%*	6.9*	68%
		LAM 100 mg	–	62%	18%	36%	5.4	60%
HBeAg–	ETV-027[41]	ETV 0.5 mg	85%*	70%*	–	90%*	5.0*	78%*
		LAM 100 mg	78%	61%	–	72%	4.5	71%
Mixed population (HBeAg+/HBeAg–)	ETV-023[43]	ETV 0.5 mg	90%*	–	15%	76%*	5.9*	90%*
		LAM 100 mg	67%	–	18%	43%	4.33	78%
Lamivudine-refractory patients								
HBeAg+	ETV-026[42]	ETV 1.0 mg	55%*	55%*	8%	19%*	5.11*	61%*
		LAM 100 mg	4%	28%	3%	1%	0.48	15%
Mixed population (HBeAg+/HBeAg–)	ETV-014[38,g]	ETV 1.0 mg	29%*	–	4%	26%*	5.06*	68%*
		ETV 0.5 mg	19%*	–	3%	26%*	4.46*	59%*
		LAM 100 mg	4%	–	6%	4%	1.37	6%
Mixed population (HBeAg+/HBeAg–)	ETV-052[63]	ETV 0.5 mg	15%	52%	17%	33%	3.58	17%
		ETV 1.0 mg	14%	60%	15%	33%	3.75	15%

aFrom week 48–52 week data.

bETV, entecavir; LAM, lamivudine.

cComposite endpoint, when reported, was defined by protocol as HBV DNA <0.7 MEq/mL by bDNA assay and alanine aminotransferase <1.25 times the upper limit of normal; for ETV-014, HBeAg loss was also included in definition.

dHistologic improvement defined as at least a two-point reduction in the Knodell necroinflammatory score and no worsening in the Knodell fibrosis score.

eHBV DNA was measured by PCR assay, with undetectable HBV DNA defined as <300 copies/mL for all studies except for ETV-014, in which lower limit of detection was 400 copies/mL.

fALT, alanine aminotransferase. Normalization of ALT was defined in referenced trials as <1–1.25 times the upper limit of normal.

gData from the ETV 0.1 mg are included; the 1.0 mg ETV dose has subsequently been chosen for the treatment of LAM-refractory CHB.

*Denotes values significantly different ($P < 0.05$) from comparatory LAM with the exception of trial ETV-052, in which ETV 0.5 mg and ETV 1.0 arms were compared.

improvement at week 48, as well as secondary endpoints of reduction in HBV viremia and ALT normalization. In both studies, patients were a mean age of 35–44 years, primarily male, predominantly Asian (39%–57%) and Caucasian (40%–58%), with mean baseline Knodell necroinflammatory scores of 7.8;13% of patients in both groups had previously received interferon.[40,41]

Chang et al (ETV-022)[40] evaluated 709 HBeAg-positive nucleoside-naïve patients randomized to entecavir 0.5 mg daily or lamivudine 100 mg daily for 52 weeks. Significantly more patients on entecavir achieved the primary endpoint of histologic improvement (72% vs. 62% on lamivudine, $P = 0.009$). More patients on entecavir had normalization of serum ALT compared to lamivudine (68% vs. 60%, $P = 0.02$). Significantly more patients on entecavir reached all virologic end points as well, with more patients achieving undetectable HBV DNA (67% vs. 36%) and greater mean reductions in HBV DNA (6.9 vs. 5.4 $\log_{10}$ copies/mL). In both groups, approximately one-fifth of patients had a loss in HBeAg with or without seroconversion, and $\leq 2\%$ had loss of HBsAg. Follow-up analysis at 96 weeks and 5 years demonstrated continued benefits of entecavir treatment in this nucleoside-naïve HBeAg-positive group at 96 weeks, with 74% of entecavir-treated patients (vs. 37% of lamivudine-treated) achieving undetectable HBV DNA levels.[44,45] Cumulatively, there was a trend towards greater rates of HBeAg seroconversion in entecavir patients (31% of patients vs. 25% of patients on lamivudine).[44] Patients who were continued on 1.0 mg entecavir daily in a rollover study thereafter were found to have superior outcomes at year 5 compared with lamivudine, with 94% (88/94) achieving undetectable HBV DNA (<300 copies/mL), 80% with normal ALT levels, and 23% (33/141) of additional patients achieving HBeAg seroconversion on-treatment.[45]

Lai et al (ETV-027)[41] followed a study protocol similar to that followed in ETV-022, but in 683 patients with HBeAg-negative chronic HBV. Compared to lamivudine, significantly more patients on entecavir achieved histologic improvement (70% vs. 61%, $P = 0.01$) and normalization of ALT (78% vs. 71%, $P = 0.045$). Similar to the findings of Chang et al the proportion of patients with undetectable HBV DNA levels at 48 weeks (90%) and the mean

reduction in serum HBV DNA (5.0 $\log_{10}$ copies/mL) were significantly greater in the entecavir group compared to lamivudine group (72% and 4.5 $\log_{10}$ copies/mL, respectively).[41]

Another similarly-designed study (ETV-023) reported data from a mixed HBeAg-positive and HBeAg-negative population, and also found significantly greater percentages of patients achieved their primary endpoint (reduction in branched-chain HBV DNA to <0.7 MEq/ml with ALT normalization) as well as reached an undetectable HBV DNA with entecavir compared to lamivudine.[43] Histologic data is not available for this study.

Lamivudine-refractory patients
The third large, multi-center, randomized, double-blind phase 3 study evaluating the efficacy of entecavir versus lamivudine was conducted in HBeAg-positive patients refractory to lamivudine (ETV-026).[42] This was defined as persistently detectable HBV bDNA after ≥ 36 weeks of lamivudine, repeated recurrence of detectable bDNA after a prior undetectable bDNA on lamivudine, evidence of HBV replication after discontinuation of lamivudine, and/or viremia with documented substitution of the methionine in the tyrosine-methionine-aspartate-aspartate (YMDD) nucleotide binding site of HBV DNA polymerase (codon 204). This study enrolled 286 patients to continue lamivudine or switch to/begin entecavir 1.0 mg for ≥ 52 weeks. Entecavir was associated with improved histology in 55% of patients (vs. 28% with lamivudine, $P < 0.0001$) and with undetectable HBV DNA in 19% of patients (vs. 1% with lamivudine, $P < 0.0001$), as well as superiority over lamivudine in improvement of fibrosis scores (34% vs. 6%) and percentage of patients achieving ALT normalization (61% vs. 15%). Subsequent analysis of patients at 96 weeks of treatment demonstrated continued improvements in proportions of patients achieving undetectable HBV DNA (to 40%) and in ALT normalization. There was a minimal increase in patients achieving HBeAg seroconversion between the end of years one and two (8% to 10%).[46]

HIV/HBV co-infected patients
Entecavir has anti-HIV activity, particularly at higher concentrations, and should not be used as monotherapy in patients with HBV-HIV coinfection who need

HBV treatment.[47] One small prospective study of HIV co-infected patients has been published, evaluating the addition of entecavir 1.0 mg daily in addition to patients' continued lamivudine-containing highly active antiretroviral therapy (HAART) in patients who experienced HBV breakthrough viremia.[48] The study was randomized, placebo-controlled and double-blind for 24 weeks then open-label; patients on tenofovir and those with lamivudine resistance mutations were not included, and all patients had virologic suppression of HIV. Of the 68 patients, the mean reduction in HBV DNA was significantly greater for entecavir than placebo (-3.65 $\log_{10}$ copies/mL vs. $+0.11$ $\log_{10}$ copies/mL), and significantly more patients on entecavir normalized ALT (34% vs. 8%). No severe adverse events were felt to be associated with entecavir, and suppression of HIV viremia was not affected through week 48.[48] However, at the time of this study, tenofovir had not yet been approved in treating chronic hepatitis B. Due to concerns for emergence of resistance, current recommendations are that HIV-HBV co-infected patients should almost always be treated simultaneously for both viruses; tenofovir—not entecavir—in combination with either lamivudine or emtricitabine is recommended as first-line HBV therapy in this clinical setting. If tenofovir cannot be used, another agent with anti-HBV activity may be used with lamivudine or emtricitabine for HBV treatment, in conjunction with a maximally-suppressive antiretroviral HIV treatment regimen. If antiretroviral therapy for HIV is not initiated, HBV therapy should consist only of agents with the least potential of selecting for HIV resistance mutations, such as pegylated interferon or telbivudine.[17,18,49]

Decompensated cirrhosis and liver transplant

Although entecavir is not currently approved for use in patients with decompensated cirrhosis, a recent nonrandomized prospective study demonstrated promising preliminary data on this population. Fifty-five patients with HBV-related decompensated cirrhosis and 144 patients with chronic hepatitis B or compensated cirrhosis, all of whom were naive to prior nucleos(t)ide analog treatment or other antiviral HBV therapy, were treated with at least 12 months of entecavir 0.5 mg daily monotherapy.[50] Biochemical and virologic outcomes were not significantly different between the same groups, with 92.3% HBV DNA negativity, 54% HBeAg loss, and 87% transplantation-free survival at 1 year in the group cumulatively. Furthermore, over 1 year, entecavir improved underlying liver function—as measured by Child-Turcotte-Pugh and Model for End-Stage Liver Disease (MELD) scores—in patients with advanced, decompensated disease.[50]

Safety/tolerability

Entecavir is generally well-tolerated with relatively low incidence of serious side effects. Given the low EC_{50} of entecavir against HBV, a very low dose of entecavir is required to treat HBV infection; this contributes to the favorable side effect profile of entecavir in comparison to other HBV antivirals. Based on aggregate results of clinical trials—including the three major phase 3 trials in nucleoside-naive (studies 022 and 027) and LAM-refractory (study 026) patients and the 1.0 mg entecavir and lamivudine arms of a phase 2 clinical trial in LAM-refractory patients (study 014), the most common adverse effects (incidence $\geq$3%) with a possible association with entecavir in study subjects were headache, dizziness, nausea and fatigue.[38,40–42] Clinical adverse events occurred with equivalent or lower frequency with entecavir compared to lamivudine; moderate-severe adverse reactions (grade 2–4) occurred in 15% (vs. 18% with lamivudine) of nucleoside-naive patients, and 22% (vs. 23%) in LAM-refractory patients.[22] The most frequent laboratory abnormalities were ALT elevations (11%–12%), hematuria (9%), and elevations in lipase of $\geq$2.1 times the upper limit of normal (ULN) (7%).[22] Elevations in ALT of $>$10 times the ULN or $>$2 times of baseline—which occurred in 2% both nucleoside-naive and LAM-refractory entecavir-treated patients, versus 4% and 11% of lamivudine-treated patients, respectively—were frequently associated with a preceding or concurrent $\geq$2 $\log_{10}$/mL reduction in HBV viral load, and generally resolved with continued treatment. Only one study, of HBeAg-positive nucleoside-naive patients, demonstrated a significant difference in the percentage of patients discontinuing treatment due to adverse events (i.e. more discontinuations in the lamivudine arm);[40] the aggregate discontinuation rate from these studies was 1% in entecavir arms and 4% in lamivudine arms.[22]

Entecavir carries a black box warning stating that severe acute exacerbations of hepatitis B have been

reported upon discontinuation of anti-hepatitis B therapy, including entecavir, so hepatic function should be monitored closely for at least several months after treatment discontinuation. Among patients in phase 2 trials for whom information was available, exacerbations of hepatitis—defined as ALT flare of >10 times ULN and >2 times baseline—occurred in 2% of HBeAg-positive, 8% of HBeAg-negative, and 12% of LAM-refractory patients treated with entecavir.[22] In addition, nucleoside analog drugs have been associated with lactic acidosis and severe hepatomegaly with steatosis; thus, it is recommended that entecavir be discontinued if lactic acidosis or significant hepatotoxicity develop.

There is insufficient data regarding the safety of entecavir in pregnant women (U.S. Food and Drug Administration Category C), breastfeeding women, and children under the age of 16 years.

Resistance

Entecavir is felt to have a high barrier to the emergence of resistance. As nucleoside analogs require recognition of their triphosphate form by the HBV polymerase, resistance may develop when amino acid substitutions occur in HBV Pol. Primary resistance to lamivudine results when substitutions occur in the highly-conserved YMDD motif of HBV Pol reverse transcriptase; mutations documented to confer resistance to LAM include primary resistance mutations rtM204V/I and the secondary rtL180M mutation.[51] Emergence of resistance has become a significant concern with nucleoside analogs; one-year genotypic resistance rates to lamivudine are 6%–32%, with frequency of both genotypic and virologic breakthrough occurring in 6%–15% of patients.[52] Telbivudine resistance is also associated with mutations at the rtM204 position.[53]

In contrast, resistance to entecavir requires multiple (3–4) reverse transcriptase substitutions, including two associated with primary lamivudine resistance (M204V with or without L180M) in addition to one or more specific entecavir resistance mutations at position rtT184, rtS202, and/or rtM250.[26,51,54,55] Resistance to entecavir appears to be enhanced by pre-existing lamivudine resistance substitutions, and HBV strains with lamivudine resistance mutations are 8-fold less susceptible to entecavir than wild-type strains;[54,55,56] this suggests a more

important role for entecavir in primary treatment than in lamivudine-exposed patients. When clones containing all possible amino acid substitutions at the three primary entecavir resistance positions in LAM-resistant HBV strains were tested, widely varying reductions in susceptibility were found, from 8-fold to >400-fold above wild-type.[55]

Results from phase 3 clinical trials (ETV-022 and ETV-027) demonstrated low rates of emergence of resistance in nucleoside-naive patients placed on entecavir. In HBeAg-positive subjects, several amino acid substitutions were seen within the HBV reverse transcriptase with low frequency; these were not associated with phenotypic reduced entecavir susceptibility. Virologic rebound occurred in 2% of those receiving entecavir, vs. 18% of lamivudine-treated patients.[40] In HBeAg-negative subjects, 2% of patients receiving entecavir had virologic rebound, vs. 8% on lamivudine.[41] In both studies, 48-week isolates from patients with virologic rebound on entecavir retained full susceptibility to entecavir and demonstrated no emergent substitutions from baseline, while the majority (71%–80%) of isolates from lamivudine patients with rebound revealed YMDD mutations.[40,41]

Five-year follow-up of 146 of these HBeAg-positive patients identified one patient, who had received lamivudine with entecavir prior to entecavir alone, developed entecavir resistance, with simultaneous emergence of rtM204V, rtL180M, and rtS202G in year 3 with subsequent virologic breakthrough.[45] Cumulative results from long-term genotypic data in six phase 2 and phase 3 entecavir studies showed low rates of both genotypic resistance and genotypic resistance accompanied by virologic breakthrough in nucleoside-naive patients, with 5-year rates of 1.2% and 0.8%, respectively;[57] entecavir resistance remained stable based on preliminary 6-year data.[58] While emergence of entecavir resistance in nucleoside-naive patients is uncommon, case reports of genotypic resistance with virologic breakthrough have been documented.[59]

In phase 3 clinical trials of LAM-refractory patients (ETV-026, n = 286), most patients (85%) had lamivudine resistance mutations rtM204I/V +/− rtL180M at baseline, and 6% (including 7 of 141 patients who subsequently were randomized to entecavir) had mutations associated with entecavir

Table 2. Selected features of approved agents for the treatment of chronic hepatitis B.

Drug, abbreviation [references]	Drug class[b]	Clinical indication[c]	Adult treatment dose and duration studied[d]	HBeAg seroconversion (% of pts)	HBV DNA undetectable[e] (% of pts)
Peginterferon alfa-2a[i] *PegIFN-α*[64,65]	Interferon, immunologic agent	CHB, HDV co-infection, HIV co-infection not on HAART (*age ≥18 years*)	180 mg/ week SC, 48 weeks	27%	25%
				(32% at 24 weeks after treatment)	63%
Entecavir *ETV*[40,41]	Nucleoside (Guanosine) analog RTI	CHB, some effect in LAM-refractory CHB (*age ≥16 yrs*)	0.5 mg/day PO, 48 weeks; LAM refractory: 1.0 mg/day, 48 weeks	21%	67% 90% 19%
Lamiduvine *3TC, LAM*[40,41,64–68]	Nucleoside (Cytidine) analog RTI	CHB, decompensated cirrhosis (*age >2 yrs*)	100 mg/day PO, 48–52 weeks	16%–21%[k]	36%–40%[k] 71%–73%[k]
Telbivudine *TBV, LdT*[68]	Nucleoside (Thymidine) analog RTI	CHB (*age ≥16 yrs*)	600 mg/day PO, 52 weeks	22%	60% 88%
Adefovir dipovoxil *ADV*[69,70]	Nucleotide (Adenosine) analog RTI	CHB, decompensated cirrhosis, LAM-refractory CHB (*age ≥12 yrs*)	10 mg/day PO, 48 weeks; LAM-refractory: 10 mg/day with 2nd agent, 48 weeks	12%	21% 51%
Tenofovir disoproxil fumarate *TDF*[71]	Nucleotide (Adenosine) analog RTI	CHB, LAM-refractory CHB (*age ≥18 yrs*)	300 mg/day PO, 48 weeks	21%	76% 93%

(*Continued*)

Table 2. (Continued)

Histologic improvement[f] (% of pts)	Viral resistance[g]	Side effects	Pregnancy FDA class[h]	Estimated cost per dose (U.S. dollars)[72–74]
38%[j]	none	Influenza-like symptoms, fatigue, weight loss, hair/skin changes, headache, insomnia, depression, anxiety, cytopenias (anemia, neutropenia, thrombocytopenia)	C/I	$385.00
48%				
72%	none	Nausea, dizziness, headache, fatigue, hepatitis, hepatomegaly, steatosis, lactic acidosis	C	$23.82
70%				
55%				
56%–62%[k]	15%–30%	Nausea/vomiting, headache, fatigue, anorexia, hepatomegaly, pancreatitis, lactic acidosis	C	$6.80
61%–66%[k]				
65%	2.2%–5%	Elevated creatine kinase, headache, cough, fatigue, influenza-like symptoms, elevated liver enzymes, steatosis, lactic acidosis	B	$16.23
67%				
53%	none	Asthenia, diarrhea, abdominal pain, anorexia, rash, nephrotoxicity, elevated liver enzymes/hepatitis, hepatomegaly, pancreatitis, lactic acidosis	C	$18.11
64%				
74%	<1%	Rash, diarrhea, nausea/vomiting, asthenia, Fanconi-like syndrome, diabetes insipidis, acute renal failure/tubular necrosis, hepatomegaly, steatosis, osteopenia, lactic acidosis	B	$15.92
72%				

[a]Data from 1-year results of trials comparing PegIFN-a to LAM, ETV to LAM, LAM to placebo (and as control), TBV to LAM, and TDF to ADV; all data from 48–52 week data of clinical trials, unless otherwise specified.
[b]RTI, reverse transcriptase inhibitor.
[c]CHB, chronic hepatitis B; HDV, hepatitis D virus; HAART, highly active antiretroviral therapy. Ages listed are the populations in which treatment is approved.
[d]Duration studied in clinical trials cited. SC, subcutaneously; PO, oral. Indicates normal adult dose, given normal renal function.
[e]HBV DNA measured by PCR. Lower limit of detection: Peg-IFN/ETV/LAM/TBV studies <300 copies/mL, ADV/TDF studies: <400 copies/mL.
[f]Histologic improvement defined as at least a two-point reduction in the Knodell necroinflammatory score and no worsening in the Knodell fibrosis score.
[g]When specifically defined, viral resistance was defined as virologic breakthrough with treatment-emergent resistance mutations confirmed by genetic sequencing.
[h]C/I, contraindicated.
[i]Interferon alfa-2b (IFN-α) also approved for CHB, but use has been largely supplanted by pegIFN-α: viral parameters, resistance, and side effects/safety are similar or inferior to those seen with peginterferon alfa-2a.
[j]Histologic data for peg-IFN is from 72 weeks (end of follow-up); not available from end of treatment.
[k]Lamivudine data for seroconversion, viral response, histology include data from Lai et al 1998,[65] Dienstag et al 1999,[66] and control data from ETV, TBV, and peg-IFN trials cited; data from Hann et al 2008[52] included in resistance rates.

resistance (at rtT184, rtS202, or rtM250) before exposure to entecavir. None of the 7 patients who had virus with baseline substitutions normally associated with entecavir resistance experienced virologic breakthrough at 48 weeks; however, two patients (1.4%) did develop virologic rebound associated with entecavir-resistant mutations at one year.[42]

Cumulative long-term data on LAM-refractory patients treated with entecavir yielded significantly higher rates of resistance compared to nucleoside-naive patients: 1- and 5-year probabilities of genotypic entecavir resistance were 6% and 51%, respectfully, and corresponding probabilities of genotypic entecavir resistance associated with virologic breakthrough were 1% and 43%.[57] Preliminary 6-year data demonstrated a continued increase in genotypic entecavir resistance and virologic breakthrough (57% and 50%, respectively). However, entecavir resistance was rare among LAM-refractory patients who achieved HBV DNA levels of <300 copies/mL on entecavir; at year 6, of 74 LAM-refractory patients who achieved an undetectable HBV DNA on entecavir, 5 (7%) developed entecavir resistance.[58] All patients with entecavir virologic breakthrough at year 5 had HBV virus with three or more resistance mutations, including both rtM204V and rtL180M, in addition to rtT184, rtS202, and/or rtM250 substitutions.[57] Changes at amino acid positions I169, A181, S78, and V84 were also seen in the ETV-resistant population.[57] Given the high rates of emergence of entecavir resistance in LAM-refractory patients, entecavir is generally not recommended in this setting.

Role of Entecavir in HBV Therapy

Currently, when treatment is indicated based on HBV viral loads, HBeAg positivity, liver histology, and serum ALT, seven agents are approved for the treatment of chronic hepatitis B: interferon alfa-2b, pegylated interferon alfa-2a, lamivudine, telbivudine, entecavir, adefovir, and tenofovir (Table 2). Important considerations in the choice of antiviral for HBV treatment include efficacy in reducing HBV DNA, improvement in liver histology, and HBeAg seroconversion, as well as risk of emergent resistance, tolerability, durability, ease of dosing, and cost. Based on the results of pre-clinical and clinical trials, entecavir is seen as a preferred agent for the treatment of HBeAg-positive and HBeAg-negative

chronic hepatitis B in nucleoside-naive patients. As the primary goal of HBV therapy is the achievement of complete virologic suppression with the long-term goals of reducing HCC, progressive liver disease and death, the potency, favorable tolerability profile, and high barrier to resistance of entecavir make it a first-line agent in this population.

As pre-existing resistance to lamivudine facilitates the development of entecavir resistance, entecavir use in LAM-refractory patients must be used with more caution. Frequent monitoring for virologic response and breakthrough are important, although long-term data to date suggests low frequency of emergent resistance in patients who achieve and maintain virologic suppression.

Treatment of decompensated cirrhosis is recommended to attempt clinical improvement, prevent recurrent reactivations, and—if disease is so advanced that only liver transplantation will be of benefit—reduce risk of HBV recurrence in the transplant graft. In this tenuous and pre-transplant population, use of antivirals with potency and favorable resistance profiles—such as entecavir and tenofovir—is particularly important. While only adefovir and lamivudine are currently licensed for use in patients with HBV-related decompensated cirrhosis, study data to date suggests entecavir is safe and effective in this population,[50] and post-hoc analysis of the three primary phase 3 trials of entecavir revealed similar efficacy and safety in patients with advanced liver fibrosis/cirrhosis compared to the overall population.[60] Thus, some guidelines already recommend entecavir as a first-line option in nucleos(t)ide-naive patients with decompensated cirrhosis.[18] However, many of these patients may be treatment-experienced; entecavir monotherapy in the setting of previous lamivudine resistance is not recommended due to high probabilities of entecavir resistance and virologic failure.[57] Regardless of antiviral history, close monitoring for virologic failure or breakthrough, resistance, or exacerbations of liver disease is recommended in this population.[18] Results of ongoing trials on the safety of entecavir in decompensated cirrhosis and post-transplant patients are needed; data on entecavir's potential to initiate carcinogenesis will also be relevant in cirrhotic patients, who have increased baseline risk of HCC.[61] However, entecavir may have an important role in the treatment of patients with advanced liver disease.

As the potential for nephrotoxicity is often a concern in this population, pre- or post-transplant, entecavir is an attractive option over nucleotide analogs tenofovir and adefovir due to its high potency, high barrier to resistance, and lack of renal toxicity. In addition, while long-term safety data in the post-transplant setting is needed, the use of HBV immunoglobulin (HBIg) and oral nucleos(t)ide antivirals after liver transplantation has markedly diminished HBV recurrence and mortality post-transplant, and is now standard of care.[18,61]

While entecavir may also be effective in some LAM-refractory patients, use in this population is limited by pre-existing LAM resistance mutations; caution should be used when M204+/− L180 mutation(s) are present at baseline, as 5-year risk of developing entecavir resistance is high in these patients. Thus, current recommendations state that entecavir should be avoided in LAM-refractory patients; instead, either tenofovir or adefovir should be added to lamivudine in these patients or they should be switched to tenofovir/emtricitabine.[17,18,62] However, in patients without lamivudine-resistant HBV who fail to seroconvert from HBeAg positive to negative at one-year on lamivudine therapy, switching to entecavir may be a potent, well-tolerated option. Continuing lamivudine in addition to entecavir may lead to increased risk of entecavir resistance.

As previously discussed, entecavir—like tenofovir, lamivudine, and higher doses of adefovir—has some anti-HIV activity; in addition, co-infected patients may be currently or previously treated with lamivudine or emtricitabine for their HIV infection, so there is risk of cross-resistance if entecavir is administered. In general, entecavir should not be used as a first-line agent in HIV/HBV co-infected patients. In certain cases, if entecavir is used in HIV/HBV co-infected patients, it should only be administered with a fully suppressive HIV antiretroviral regimen.

Further clinical studies of entecavir in patients in expanded populations—including patients of Black/African-American race or of Hispanic ethnicity, patients with decompensated HBV-related cirrhosis, orthotopic liver transplant recipients, HIV/HBV co-infected patients, and pediatric populations—and studies of entecavir in combination with and compared to other HBV antivirals are underway.

Conclusions

Hepatitis B virus continues to be a prevalent cause of chronic liver disease worldwide. Effective, durable suppression of HBV viremia has been shown to be crucial in preventing the significant morbidity and mortality caused by chronic HBV infection. Thus, availability of potent, specific antivirals with high barrier to resistance will be central in improving clinical outcomes and survival. Entecavir, with its favorable efficacy, tolerability, and resistance profile, is an attractive first-line option for the treatment of chronic HBV in treatment-naive patients, and can be effective in some LAM-refractory patients. In addition, though it is not currently licensed for this use, it may be one of the preferred agents for select patients with HBV-related decompensated cirrhosis or orthotopic liver transplant. Future studies will reveal important information on entecavir combination therapy and use in special populations.

Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

References

1. Centers for Disease Control and Prevention. Recommendations for identification and public health management of persons with chronic hepatitis B infection. *Morbidity and Mortality Weekly Reports*. 2008;57(RR-8):1–20.
2. World Health Organization. Hepatitis B vaccines: WHO position paper. *Weekly Epidemiologic Record*. 2009;40(84):405–20.
3. Sorrell M, Belongia E, Costa J, et al. National Institutes of Health Consensus Development Conference Statement: management of hepatitis B. *Annals of Internal Medicine*. 2009;150(2):104–10.
4. Lin SM, Yu ML, Lee CM, et al. Interferon therapy in HBeAg positive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. *J Hepatol*. 2007;46(1):45–52.
5. Chen YC, Chu CM, Yeh CT, Liaw YF. Natural course following the onset of cirrhosis in patients with chronic hepatitis B: a long-term follow-up study. *Hepatol Int*. 2007;1(1):267–73.
6. Lok AS. Chronic hepatitis B. *N Engl J Med*. 2002;346(22):1682–3.
7. Chan HL, Tse CH, Mo F, et al. High viral load and hepatitis B virus subgenotype ce are associated with increased risk of hepatocellular carcinoma. *J Clin Oncol*. 2008;26(2):177–82.
8. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA*. 2006;295(1):65–73.
9. Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med*. 2004;351(15):1521–31.

10. Hui CK, Leung N, Yuen ST, et al. Natural history and disease progression in Chinese chronic hepatitis B patients in immune-tolerant phase. *Hepatology*. 2007;46(2):395–401.

11. Chang MH, Hsu HY, Hsu HC, Ni YH, Chen JS, Chen DS. The significance of spontaneous hepatitis B e antigen seroconversion in childhood: with special emphasis on the clearance of hepatitis B e antigen before 3 years of age. *Hepatology*. 1995;22(5):1387–92.

12. Liaw YF. Hepatitis flares and hepatitis B e antigen seroconversion: implication in anti-hepatitis B virus therapy. *J Gastroenterol Hepatol*. 2003;18(3):246–52.

13. Liaw YF, Chu CM, Huang MJ, Sheen IS, Yang CY, Lin DY. Determinants for hepatitis B e antigen clearance in chronic type B hepatitis. *Liver*. 1984; 4(5):301–6.

14. McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of. 1536 Alaska Natives chronically infected with hepatitis B virus. *Ann Intern Med*. 2001;135(9):759–68.

15. Kao JH. Role of viral factors in the natural course and therapy of chronic hepatitis B. *Hepatol Int*. 2007;1(4):415–30.

16. Hsu YS, Chien RN, Yeh CT, et al. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. *Hepatology*. 2002;35(5):1522–7.

17. Keeffe EB, Dieterich DT, Han SH, et al. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States:. 2008 update. *Clin Gastroenterol Hepatol*. 2008;6(12):1315–41; quiz 286.

18. European Association For The Study Of The Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol*. 2009;50(2): 227–42.

19. Tuttleman JS, Pourcel C, Summers J. Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. *Cell*. 1986; 47(3):451–60.

20. 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. *Journal of Virology*. 1997;71(12):9392–9.

21. Werle-Lapostolle B, Bowden S, Locarnini S, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. *Gastroenterology*. 2004;126(7):1750–8.

22. Baraclude [package insert]. Princeton, NJ: Bristol-Myers Squibb Company;. 2009.

23. Innaimo SF, Seifer M, Bisacchi GS, Standring DN, Zahler R, Colonno RJ. Identification of BMS-200475 as a potent and selective inhibitor of hepatitis B virus. *Antimicrobial Agents and Chemotherapy*. 1997;41(7):1444–8.

24. Yamanaka G, Wilson T, Innaimo S, et al. Metabolic studies on BMS-200475, a new antiviral compound active against hepatitis B virus. *Antimicrob Agents Chemother*. 1999;43(1):190–3.

25. Seifer M, Hamatake RK, Colonno RJ, Standring DN. In vitro inhibition of hepadnavirus polymerases by the triphosphates of BMS-200475 and lobucavir. *Antimicrob Agents Chemother*. 1998;42(12):3200–8.

26. Langley DR, Walsh AW, Baldick CJ, et al. Inhibition of hepatitis B virus polymerase by entecavir. *J Virol*. 2007;81(8):3992–4001.

27. Ono SK, Kato N, Shiratori Y, et al. The polymerase L528M mutation cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. *Journal of Clinical Investigation*. 2001;107(4):449–55.

28. Levine S, Hernandez D, Yamanaka G, et al. Efficacies of entecavir against lamivudine-resistant hepatitis B virus replication and recombinant polymerases in vitro. *Antimicrob Agents Chemother*. 2002;46(8):2525–32.

29. Lin PF, Nowicka-Sans B, Terry B, et al. Entecavir exhibits inhibitory activity against human immunodeficiency virus under conditions of reduced viral challenge. *Antimicrob Agents Chemother*. 2008;52(5):1759–67.

30. Jakobsen MR, Arildsen H, Krarup HB, Tolstrup M, Ostergaard L, Laursen AL. Entecavir therapy induces de novo HIV reverse-transcriptase M184V mutation in an antiretroviral therapy-naive patient. *Clin Infect Dis*. 2008;46(9):e88–91.

31. Sasadeusz J, Audsley J, Mijch A, et al. The anti-HIV activity of entecavir: a multicentre evaluation of lamivudine-experienced and lamivudine-naive patients. *AIDS*. 2008;22(8):947–55.

32. European Medicines Agency. Baraclude: product information [online]. Available from: http://www.ema.europa.eu/humandocs/Humans/EPAR/baraclude/baraclude.htm.

33. Yan JH, Bifano M, Olsen S, et al. Entecavir pharmacokinetics, safety, and tolerability after multiple ascending doses in healthy subjects. *J Clin Pharmacol*. 2006;46(11):1250–8.

34. Genovesi EV, Lamb L, Medina I, et al. Efficacy of the carbocyclic 2'-deoxyguanosine nucleoside BMS-200475 in the woodchuck model of hepatitis B virus infection. *Antimicrob Agents Chemother*. 1998;42(12): 3209–17.

35. Marion PL, Salazar FH, Winters MA, Colonno RJ. Potent efficacy of entecavir (BMS-200475) in a duck model of hepatitis B virus replication. *Antimicrob Agents Chemother*. 2002;46(1):82–8.

36. Colonno RJ, Genovesi EV, Medina I, et al. Long-term entecavir treatment results in sustained antiviral efficacy and prolonged life span in the woodchuck model of chronic hepatitis infection. *The Journal of Infectious Diseases*. 2001;184(10):1236–45.

37. Lai CL, Rosmawati M, Lao J, et al. Entecavir is superior to lamivudine in reducing hepatitis B virus DNA in patients with chronic hepatitis B infection. *Gastroenterology*. 2002;123(6):1831–8.

38. Chang T-T, Gish R, Hadziyannis S, et al. A dose-ranging study of the efficacy and tolerability of entecavir in Lamivudine-refractory chronic hepatitis B patients. *Gastroenterology*. 2005;129(4):1198–209.

39. Tenney DJ, Levine SM, Rose RE, et al. Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to Lamivudine. *Antimicrob Agents Chemother*. 2004;48(9): 3498–507.

40. Chang TT, Gish RG, de Man R, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med*. 2006; 354(10):1001–10.

41. Lai CL, Shouval D, Lok AS, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med*. 2006; 354(10):1011–20.

42. Sherman M, Yurdaydin C, Sollano J, et al. Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. *Gastroenterology*. 2006;130(7):2039–49.

43. Yao G, Chen C, Lu W, et al. Efficacy and safety of entecavir compared to lamivudine in nucleoside-naïve patients with chronic hepatitis B: a randomized double-blind trial in China. *Hepatology International*. 2007; 1(3):365–72.

44. Gish R, Lok A, Chang T-T, et al. Entecavir therapy for up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. *Gastroenterology*. 2007;133(5):1437–44.

45. Chang T-T, Lai C-L, Yoon S, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. *Hepatology*. 2010;51(2):422–30.

46. Sherman M, Yurdaydin C, Simsek H, et al. Entecavir therapy for lamivudine-refractory chronic hepatitis B: improved virologic, biochemical, and serology outcomes through 96 weeks. *Hepatology*. 2008;48(1): 99–108.

47. McMahon MA, Jilek BL, Brennan TP, et al. The HBV drug entecavir—effects on HIV-1 replication and resistance. *N Engl J Med*. 2007; 356(25): 2614–21.

48. Pessoa M, Gazzard B, Huang A, et al. Efficacy and safety of entecavir for chronic HBV in HIV/HBV coinfected patients receiving lamivudine as part of antiretroviral therapy. *AIDS*. 2008;22(14):1779–87.

49. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. December 1,. 2009;1–161. Available from: http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf [Accessed 2010] Apr 30.

50. Shim J, Lee H, Kim K, et al. Efficacy of entecavir in treatment-naïve patients with hepatitis B virus-related decompensated cirrhosis. *Journal of Hepatology*. 2010;52(2):176–82.

51. Lok AS, Zoulim F, Locarnini S, et al. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. *Hepatology*. 2007;46(1):254–65.

52. Hann H-W, Gregory V, Dixon J, Barker K. A review of the one-year incidence of resistance to lamivudine in the treatment of chronic hepatitis B : Lamivudine resistance. *Hepatology International*. 2008;2(4):440–56.

53. Liaw Y-F, Gane E, Leung N, et al. 2-Year GLOBE trial results: telbivudine Is superior to lamivudine in patients with chronic hepatitis B. *Gastroenterology*. 2009;136(2):486–95.

54. Tenney DJ, Rose RE, Baldick CJ, et al. Two-year assessment of entecavir resistance in Lamivudine-refractory hepatitis B virus patients reveals different clinical outcomes depending on the resistance substitutions present. *Antimicrob Agents Chemother*. 2007;51(3):902–11.

55. Baldick C, Tenney D, Mazzucco C, et al. Comprehensive evaluation of hepatitis B virus reverse transcriptase substitutions associated with entecavir resistance. *Hepatology*. 2008;47(5):1473–82.

56. Baldick CJ, Eggers BJ, Fang J, et al. Hepatitis B virus quasispecies susceptibility to entecavir confirms the relationship between genotypic resistance and patient virologic response. *J Hepatol*. 2008;48(6):895–902.

57. Tenney DJ, Rose RE, Baldick CJ, 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(5):1503–14.

58. Tenney DJ, Pokornowski KA, Rose RE, et al. Entecavir maintains a high genetic barrier to HBV resistance through 6 years in naive patients. *Journal of Hepatology*. 2009;50 Suppl 1:S10.

59. Kobashi H, Fujioka S, Kawaguchi M, et al. Two cases of development of entecavir resistance during entecavir treatment for nucleoside-naive chronic hepatitis B. *Hepatol Int*. 2009;3(2):403–10.

60. Schiff E, Simsek H, Lee WM, et al. Efficacy and safety of entecavir in patients with chronic hepatitis B and advanced hepatic fibrosis or cirrhosis. *Am J Gastroenterol*. 2008;103(11):2776–83.

61. Papatheodoridis G, Cholongitas E, Archimandritis A, Burroughs A. Current management of hepatitis B virus infection before and after liver transplantation. *Liver International*. 2009;29(9):1294–305.

62. Ghany MG, Doo EC. Antiviral resistance and hepatitis B therapy. *Hepatology*. 2009;49(5 Suppl):S174–84.

63. Suzuki F, Toyoda J, Katano Y, et al. Efficacy and safety of entecavir in lamivudine-refractory patients with chronic hepatitis B: randomized controlled trial in Japanese patients. *Journal of Gastroenterology and Hepatology*. 2008;23(9):1320–6.

64. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med*. 2005;352(26):2682–95.

65. Marcellin P, Lau GK, Bonino F, 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(12):1206–17.

66. Lai CL, Chien RN, Leung NW, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med*. 1998;339(2):61–8.

67. Dienstag JL, Schiff ER, Wright TL, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med*. 1999;341(17):1256–63.

68. Lai CL, Gane E, Liaw YF, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. *N Engl J Med*. 2007;357(25):2576–88.

69. Marcellin P, Chang TT, Lim SG, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *N Engl J Med*. 2003;348(9):808–16.

70. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. *N Engl J Med*. 2003;348(9):800–7.

71. Marcellin P, Heathcote EJ, Buti M, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. *N Engl J Med*. 2008;359(23):2442–55.

72. Rajendra A, Wong J. Economics of chronic hepatitis B and hepatitis C. *Journal of Hepatology*. 2007;47(4):608–17.

73. Entecavir (*Baraclude)* for chronic hepatitis B infection. *The Medical Letter on Drugs and Therapeutics*. 2005;47(1210):47–8.

74. Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. *Hepatology*. 2007;45(4):1056–75.