Hepatol Int (2008) 2:440–456
DOI 10.1007/s12072-008-9105-y

REVIEW ARTICLE

A review of the one-year incidence of resistance to lamivudine in the treatment of chronic hepatitis B

Lamivudine resistance

Hie-Won Hann · Vicki L. Gregory · Jonathan S. Dixon · Keith F. Barker

Received: 2 July 2008 / Accepted: 14 September 2008 / Published online: 21 October 2008

Abstract
Purpose The development of antiviral resistance is a recognized challenge to successful treatment of chronic hepatitis B (CHB), but it has been difficult to establish an accurate estimate of its incidence due to a number of factors: (a) lack of an accepted definition of antiviral resistance; (b) lack of a standardized assay to assess resistance; and (c) lack of consensus on patient selection criteria for resistance testing. Lamivudine, an effective and well-established antiviral agent, has been reported to show one-year resistance rates in CHB ranging from 6% to 32%, but methodologies used to calculate these rates vary considerably. This article reviews the clinical, statistical, and laboratory methodologies of clinical studies reporting one-year rates of antiviral resistance to lamivudine in CHB.
Methods Studies reporting one-year resistance rates to lamivudine in CHB were analyzed for methodologic differences and their influence on reported resistance rates.
Results Studies using only a genotypic definition of resistance reported one-year rates ranging from 14% to 32%. Studies assessing genotypic resistance in patients with evidence of virologic breakthrough reported much lower one-year resistance rates of 6.4–15.4%.
Conclusions It is important when comparing resistance rates to antiviral drugs in CHB to consider the methodology and definition of resistance used because this can dramatically influence the results.

Keywords Chronic hepatitis B · Hepatitis B virus · Lamivudine · Resistance · Virological resistance · Antiviral resistance · Genotypic resistance · Phenotypic resistance

H.-W. Hann (✉)
Division of Gastroenterology and Hepatology, Department of Medicine, Jefferson Medical College, 1025 Walnut Street, Philadelphia, PA 19107, USA
e-mail: Hie-Won.Hann@jefferson.edu

V. L. Gregory
Writeology, 19 Riddy Lane, Bourn, Cambridge CB23 2SP, UK
e-mail: vicki.gregory@writeology.co.uk

J. S. Dixon · K. F. Barker
GlaxoSmithKline, 980 Great West Road, Brentford, Middlesex TW8 9GS, UK
e-mail: jon.s.dixon@gsk.com

K. F. Barker
e-mail: keith.f.barker@gsk.com

Introduction

Chronic hepatitis B (CHB) is a serious global health problem, affecting more than 400 million people worldwide [1] and causing an estimated 1 million deaths each year from cirrhosis and hepatocellular carcinoma [2]. Effective treatment is, therefore, essential to prevent the long-term consequences of the disease. At present, there are two major treatment options for CHB: immune modulators (interferon [IFN] α-2b and pegylated IFN α-2a) and antiviral agents (nucleoside or nucleotide analogs: lamivudine, adefovir dipivoxil [ADV], entecavir, and telbivudine) [3]. Combination antiviral therapy (e.g., tenofovir plus emtricitabine) can be employed but is neither approved by regulatory agencies nor widely available in areas where CHB is endemic.

In the past, immune modulators were the only option available to treat CHB. However, their efficacy is variable, with rates of HBeAg loss ranging from 15% to 47% [4–7].

They are also inconvenient to administer, and adverse effects, including flu-like symptoms, fatigue, anorexia, anxiety, and depression, can affect up to 90% of patients [3]. The advent of convenient, well-tolerated, oral antiviral agents in the last decade has revolutionized the management of CHB, but has introduced a new set of management issues. To achieve durable viral suppression, antiviral therapy must be continued long-term, and indefinitely in some patients. However, a longer duration of treatment is associated with an increasing risk of developing antiviral resistance, which, in turn, can lead to treatment failure and disease breakthrough.

It is difficult to establish an accurate estimate of the incidence of antiviral resistance in CHB for a variety of reasons. First, antiviral resistance is an imprecise term with no accepted, standardized definition. Historically, it has encompassed several different concepts, including genotypic resistance, phenotypic resistance, virologic breakthrough, and clinical resistance. Second, there is no standardized assay for determining antiviral resistance, which has led to considerable variability in reported resistance rates. Third, the various assays that are available to detect and quantify antiviral resistance demonstrate different limits of detection. Finally, different studies use different criteria to select patients for resistance testing, making accurate comparisons very difficult. These factors have resulted in large variations in reported resistance rates to anti hepatitis B agents in published literature.

Lamivudine is the most extensively studied antiviral licensed to treat CHB. It is a well-established nucleoside analog that is particularly widely used in Asia Pacific regions, where it represents a cost-effective treatment option [8]. It is recommended by the Asian Pacific Association for the Study of the Liver (APASL) as a first-line therapy for CHB and as the drug of choice for: patients with imminent or overt hepatic decompensation; short-term prophylaxis of patients undergoing chemotherapy or immunosuppression; and long-term prophylaxis in liver transplant patients [9, 10]. In many of these patient populations, data using other antivirals are limited.

Lamivudine has been reported to have resistance rates ranging from 6% to 32% after 1 year of therapy [11–25], but the methodology used to calculate these rates varies considerably between studies. This article provides an in-depth review of clinical studies reporting antiviral resistance rates to lamivudine in the treatment of CHB and discusses the reasons contributing to such large discrepancies in resistance rates between different studies.

Antiviral resistance

The term "antiviral resistance" encompasses several concepts, including genotypic resistance, phenotypic resistance, virologic resistance, and clinical resistance. It is important to define these terms accurately to compare scientific literature on antiviral resistance.

Genotypic resistance

The National Institutes of Health (NIH) has defined genotypic resistance to antiviral therapy for CHB as [26]:

> "Detection of viral populations bearing amino acid substitutions in the reverse transcriptase region of the HBV genome that have been shown to confer resistance to antiviral drugs in phenotypic assay, during antiviral therapy. These mutations are usually detected in patients with virologic breakthrough but they can also be present in patients with persistent viraemia and no virologic breakthrough."

The hepatitis B virus has a particularly high mutation rate because HBV DNA polymerase lacks a "proofreading" mechanism to detect and excise incorrectly incorporated nucleotides [27]. This results in a high rate of nucleotide substitutions that leads to variations in the HBV polymerase amino acid sequence, which, in turn, may lead to structural or functional alterations. Often these variants have no clinical impact, but certain strains of HBV variant may affect therapeutic response. The presence of antiviral agents exerts selection pressure that may lead to the dominance of viral variants associated with antiviral resistance [28].

Several mutations in the HBV polymerase genome have been identified that confer resistance to antiviral agents, either alone or in combination (Table 1). Some of these confer primary antiviral resistance (mutations that directly reduce antiviral susceptibility), whereas some confer secondary resistance by restoring functional defects (e.g., replication fitness) in primary resistant strains [29].

Table 1 HBV polymerase mutations associated with antiviral resistance in vitro and in vivo [29]

Antiviral agent	Mutations documented to confer resistance
Lamivudine	rtM204V
	rtM204I
	rtL180M
ADV	rtN236T
	rtA181T
	rtA181V
Entecavir	rtL180M + rtM204V + rtT184G + rtS202I
	rtL180M + rtM204V + rtI169T + rtM250V
Telbivudine	rtM204I
	rtM204V + rtL180M

Cross-resistance

Cross-resistance is decreased susceptibility to more than one antiviral drug conferred by the same amino acid substitution or combination of amino acid substitutions [26].

Cross-resistance can occur between antiviral agents used in the management of CHB. For example, the lamivudine rtM204I mutation confers cross-resistance to telbivudine and the ADV rtA181T mutation may confer cross-resistance to lamivudine [29]. In addition, some resistant mutations increase the probability of developing resistance to other antiviral agents by reducing the number of additional mutations required to confer resistance to the other agent. An example of this is entecavir: a combination of three to four different mutations is required to confer entecavir resistance, but two of these are associated with lamivudine resistance (the primary rtM204V mutation and the secondary rtL180M mutation, which restores replication fitness in rtM204V variants) [30]. Strains of HBV that exhibit this pattern of lamivudine resistance, therefore, need to acquire only one to two new mutations to develop entecavir resistance rather than the three to four mutations that would be required in wild-type strains, thus accelerating the development of resistance [30]. Cross-resistance is particularly important when selecting rescue therapy for patients who have developed resistance to a given antiviral agent. For example, a patient with lamivudine resistance due to the rtM204I mutation would not benefit from switching to telbivudine. Similarly, entecavir would be an unwise choice of salvage therapy for a patient with the rtM204V + rtL180M pattern of lamivudine resistance because this would increase the potential to develop entecavir resistance [30]. In both these cases, add-on therapy with ADV would be a more appropriate treatment option because strains of HBV with primary lamivudine-resistant mutations remain sensitive to ADV [29].

In clinical research, genotypic resistance raises a number of practical issues, including the selection of suitable methods for identifying amino acid substitutions, assessing in vitro susceptibility to antiviral agents, and selecting patients for detailed genotypic testing. These factors have been discussed later in the article.

Phenotypic resistance

The NIH has defined phenotypic resistance to antiviral therapy for CHB as [26]:

> "Decreased susceptibility of an HBV polymerase to an antiviral treatment in vitro."

Confirmation of phenotypic resistance requires demonstration that a given amino acid substitution reduces the susceptibility of HBV to an antiviral agent compared with wild-type virus. The molecular mechanisms by which these amino acid substitutions confer resistance are as follows: structural changes that physically hinder the binding of nucleotide/nucleoside analogs to HBV polymerase (steric hindrance); indirect disruption of the HBV polymerase triphosphate binding site; reduced catalytic efficiency of the HBV polymerase enzyme; and active excision of the nucleotide/nucleoside analog from transcribed HBV DNA [28].

As these antiviral resistance mechanisms affect the function of HBV polymerase, the resulting resistant strains often replicate less efficiently than wild-type virus. However, under the selective pressure of antiviral therapy, secondary compensatory mutations can arise that restore replication fitness [28].

Virologic resistance

The development of genotypic resistance can result in a loss of virologic response to an antiviral agent (i.e., an increase in viral load). This is virologic breakthrough, which has been defined by the NIH as [28]:

> "An on-treatment increase in serum HBV DNA of ≥ 1 $\log_{10}$ IU/ml from nadir on two consecutive occasions one month apart, in a medication compliant patient who initially responded to treatment."

Virologic breakthrough can be due to factors such as noncompliance with medication, so to confirm virologic resistance, the presence of resistant mutations must also be demonstrated. Virologic resistance is indicative of resuming HBV replication and precedes loss of biochemical and histologic responses to antiviral treatment [28].

Clinical implications of virologic resistance

Clinically, virologic resistance can have a number of consequences associated with the loss of benefits of antiviral therapy.

Biochemical breakthrough (clinical resistance) Biochemical breakthrough, sometimes called clinical resistance, is defined as an on-treatment elevation in serum alanine aminotransferase (ALT) in a medication-compliant patient who initially achieved ALT normalization [26]. Biochemical breakthrough is indicative of resumed liver damage and follows virologic breakthrough by anything from a few weeks to several years [29]. Biochemical breakthrough can sometimes result in a significant increase in ALT beyond baseline levels and can occasionally lead to hepatic decompensation and liver failure.

Reduction in histologic improvement A reduction in the histologic consequences of CHB is the ultimate goal of antiviral therapy. Virologic resistance can lead to a worsening in initial improvements in fibrosis and cirrhosis and may even increase the risk of developing hepatocellular carcinoma compared with ongoing viral suppression [28].

Serologic consequences Rates of HBeAg seroconversion, which are indicative of long-lasting suppression of HBV, are reduced in patients with antiviral-resistant HBV compared with those with ongoing antiviral suppression [28].

Other potential consequences Further consequences of virologic resistance include the potential for transmission of resistant strains to drug-naive patients, reducing the treatment options available for CHB; and potential vaccine failure due to mutations in the hepatitis B surface antigen (the target of vaccine-stimulated antibodies) associated with drug resistance. However, these are theoretical risks that have not been proven in clinical practice.

Measuring virologic resistance

Several methods are available to monitor the development of virologic resistance. An increase in viral load is usually the first indication that resistance may have developed, although this can also be due to noncompliance with medication.

Viral load assays

To detect virologic breakthrough, it is necessary to measure viral load accurately using HBV DNA assays. Many assays are able to detect HBV DNA, but in order to monitor viral load over time, it is necessary to use a quantitative measure.

Quantitative HBV DNA assays

Several quantitative DNA assays are available, each employing different principles to detect serum HBV DNA and each having a different range of detection. The following range of assays has been used in clinical studies reporting virologic resistance.

Solution or direct membrane hybridization HBV DNA from a serum sample is enzymatically denatured and incubated, either in solution or on a fixed membrane, with an RNA or a DNA probe specific for a highly conserved region of the HBV genome [31]. The resulting hybridized product can be detected and quantified by antibody capture, fluorescence, chemiluminescence, or radioactive probes [32].

Branched-chain DNA hybridization HBV DNA from a serum sample is enzymatically denatured and captured by incubating it in wells coated with oligonucleotide probes specific for conserved sequences within the HBV genome. Several different oligonucleotide target probes are then added, which bind to specific regions of the hybridized DNA. Branched DNA amplifiers are added that bind to the target probes. The free end of each amplifier is capable of binding a number of labeled probes, amplifying the signal and increasing the sensitivity of hybridization techniques [31]. Sensitivity can be further increased by the use of "preamplifiers," which are capable of binding a number of amplifiers [33].

Quantitative PCR In the presence of DNA polymerase, HBV DNA from a serum sample is thermally denatured and incubated with primers corresponding to the 3′ and 5′ ends of a specific DNA sequence within a conserved region of the HBV genome. This amplifies the target segment of HBV DNA such that it can be detected and quantified using fluorescent hybridization probes [34]. This can be done in real time. Quantitative PCR is considerably more sensitive than either direct hybridization techniques or branched-chain hybridization [35, 36].

Units of measurement

Different assays of viral load have historically used different units to quantify HBV DNA levels including copies/ml, mEq/ml (millions of genome equivalents), and pg/ml. In 2001, the WHO established an international standard for HBV DNA reporting, using a reference sample with high viral load that was analyzed using several different viral load assays [37]. On the basis of these analyses, the sample was assigned a viral load of 10^6 IU/ml and all HBV DNA levels should now be reported using these units, using conversion factors if necessary to convert from alternative units. These conversion factors vary between different assays and manufacturers [28]. It is important to note that IU/ml is an arbitrary unit that does not correspond to the number of viral particles.

HBV DNA data should be presented on a logarithmic scale to represent reductions of a large magnitude accurately.

Dynamic ranges of detection

The viral load of patients chronically infected with hepatitis B can range from 0 to approximately 10^{11} IU/ml. No assay currently available is able to cover the entire dynamic range, but some are more sensitive than others (Table 2). Assays with a low limit of detection are able to quantify levels of HBV DNA that would be undetectable

by less sensitive assays. Conversely, those with a higher upper limit of detection are able to quantify viral loads that would have to be measured by dilution methods using techniques with a lower upper limit of detection.

Genotype mutation analyses

An increase in viral load is not necessarily indicative of the development of virologic resistance. To confirm whether a resistant mutation has arisen, genotypic analysis is required to detect nucleotide substitutions in the reverse transcriptase region of the HBV genome. A number of methods can be used to achieve this.

PCR amplification and sequence analysis

Sample HBV DNA can be amplified by PCR and genetically sequenced to detect any mutations compared with a pretreatment sample or a published sequence of the same HBV genotype. PCR sequencing is capable of identifying all mutations within the genome regardless of whether they have been previously identified as resistant mutations. However, it is not suitable for high-throughput screening [29].

Reverse hybridization line probe assay

Line probe assay involves the use of a series of highly specific oligonucleotide probes covering polymorphisms at codon positions known to be associated with virologic resistance. The probes are applied to membrane strips in lines and hybridized with denatured HBV DNA (or PCR fragments) from the sample to be tested. The resulting hybridized products can be analyzed by chromogenic comparison of the membrane strip against a reference strip from wild-type or pretreatment HBV DNA. This can be used to determine any differences in amino acids at each codon position between wild-type or pretreatment virus and posttreatment virus [38].

Line probe assays are capable of detecting single nucleotide mismatches but require specific probes for each mutation of interest [29].

Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) uses endonucleases to digest sample DNA (or PCR products) into small fragments at restriction sites specific to the enzymes used. The resulting restriction fragments are separated according to length by gel electrophoresis and the gel can be analyzed against a pretreatment or a wild-type sample to identify any mutations that alter the pattern of fragments such as alterations of the bases at the restriction sites, insertions, deletions, or transversions. The RFLP requires the use of specific endonucleases for each mutation of interest [29].

Restriction fragment mass polymorphism

Like RFLP, restriction fragment mass polymorphism (RFMP) uses specific endonucleases to digest sample DNA (or PCR products) into restriction fragments. These fragments are analyzed according to mass by specialized mass spectrometry techniques and compared with pretreatment or wild-type samples to identify mutations that alter the pattern of fragments. Like RFLP, specific endonucleases are required for each mutation of interest [29].

Limits of detection

As virologic resistance emerges, the ratio of mutant to wild-type virus increases. Different methods of genotypic analysis have different sensitivities to detect emerging resistant mutations (Table 3).

Phenotype assays

Genetic techniques are capable of identifying single-nucleotide mutations within the HBV genome. However, the hepatitis B virus has a high rate of spontaneous

Table 2 Dynamic range of viral load assays [28]

Viral load assay	Approximate dynamic range	
	Lower limit (IU/ml)	Upper limit (IU/ml)
Solution hybridization	10^5–10^6	10^9
"Ultrasensitive" solution hybridization	10^4	10^8
Branched-chain hybridization: high viral load	10^6	10^{10}
Branched-chain hybridization: low viral load	10^2–10^3	10^7
Quantitative PCR	10^2–10^3	10^5–10^7
Real-time quantitative PCR	30	10^8

Table 3 Limit of detection of genotype mutation analyses [28]

Genotype mutation analysis	Sensitivity (%)[a]
PCR sequencing	20
Line probe assay	5
RFLP	5
RFMP	<1

[a] Percentage of mutant population required to be detectable

mutations, so phenotypic testing is required to determine the clinical significance of identified mutations.

Enzymatic assay

Enzymatic analysis of HBV DNA polymerase activity in the presence or the absence of antiviral agent is an indicator of the antiviral susceptibility of different HBV DNA strains. Enzymatic analysis can be conducted by measuring the incorporation of radiolabeled nucleotides into HBV reverse transcription products in an in vitro cell line. To assess the effect of specific mutations on HBV DNA polymerase activity and antiviral susceptibility, the mutated gene must be transduced into the cell line using a viral vector and enzymatic activity, both with and without the antiviral agent, compared with a reference control.

HBV replication assays

The ultimate aim of antiviral agents is to suppress HBV replication to prevent the biochemical and histologic consequences of CHB infection. In vitro HBV replication assays in hepatocyte-derived cell lines or stable HBV-expressing cell lines can be used to determine the antiviral susceptibility of HBV strains.

To measure in vitro replication of specific HBV mutants, hepatocyte-derived cell lines are transfected either with the entire amplified mutant HBV genome or with laboratory-engineered HBV created by site-directed mutagenesis or recombinant techniques to express the mutant of interest. Alternatively, specific mutations can be introduced into a stable HBV-expressing cell line. Replication of these mutant HBV strains can be quantified with and without the antiviral agent and compared with that of wild-type virus. However, differences between laboratory-based cell lines and in vivo cell lines may affect cellular function, so the results of in vitro testing do not always correspond well with clinical findings and a small decrease in antiviral susceptibility in vitro may result in clinical resistance in vivo [29].

Patient populations

With the availability of so many different assays, some of which are expensive and time-consuming, it is not practical to test all measures of resistance in all patients. However, selecting patient populations for assessment can result in widely differing reported resistance rates. It is recommended that all patients should be assessed at three-monthly intervals for serum HBV DNA and ALT levels, and any patients with evidence of virologic breakthrough should ideally be tested for genotypic or phenotypic resistance [29].

Reported genotypic resistance rates

Many different studies have examined the development of resistance to antiviral agents in CHB, but differences in clinical, statistical, and laboratory methodologies between studies have resulted in considerable differences in reported resistance rates and made it difficult to compare studies in an accurate, like-for-like manner. Table 4 provides a list of the key features of pivotal studies of antiviral therapy that have reported genotypic resistance rates in CHB. These studies have been discussed in detail in the next section.

A common feature of these studies is that they use a purely genotypic definition of resistance and all patients in the given treatment group are assessed for resistant mutations regardless of virologic or clinical response to treatment. This may lead to an overestimate of resistance rates because the clinical significance of resistant mutations is not considered.

Lamivudine in Chinese patients with HBeAg-positive CHB: Lai et al. [11]

In this double-blind Phase III trial, 335 of 358 patients were evaluated for genotypic resistance. No resistant mutations were detected in patients treated with placebo. In the combined lamivudine groups, the incidence of resistant mutations after 52 weeks of treatment was 14% (9% mixed wild-type and mutant populations and 5% mutant populations only). The specific rate for each dose of lamivudine is not reported. As the development of resistance is related to the degree of viral suppression [28], the overall rate of resistance to lamivudine reported by Lai et al. might be affected by incomplete viral suppression in the lamivudine 25-mg group, which would bias the results toward a higher than expected resistance rate. However, it is not possible to determine this from the reported data.

The article states that the development of resistant mutants was associated with an increase in ALT and HBV DNA levels above nadir but below pretreatment levels. However, the increase in viral load is not quantified, so it is not possible to determine the virologic or clinical

Table 4 Reported one-year genotypic resistance rates to lamivudine in published literature[a]

First author and year	Treatment arms (n)	Clinical population	Population subject to genotypic resistance testing[b]	Genotype mutation analysis	Lower limit of detection of genotype mutation analysis	HBV DNA assay	Lower limit of detection of HBV DNA assay[c]	One-year genotypic resistance rate
Lai et al. 1998 [11]	• Lamivudine 25 mg ($n = 142$) • Lamivudine 100 mg ($n = 143$) • Placebo ($n = 73$)	Chinese patients with HBeAg-positive CHB	All evaluable patients	PCR and RFLP	100,000 copies/ml or 5% of viral population	Solution hybridization	1.6 pg/ml	• Lamivudine (25 mg and 100 mg) 14% • Placebo 0%
Dienstag et al. 1999 [12]	• Lamivudine 100 mg ($n = 71$) • Placebo ($n = 72$)	US patients with HBeAg-positive CHB	All evaluable patients treated with lamivudine	PCR	Not reported	Solution hybridization	1.6 pg/ml	• Lamivudine 32%
Schalm et al. 2000 [13]	• Lamivudine 100 mg ($n = 84$) • Lamivudine + IFN ($n = 75$) • IFN monotherapy ($n = 69$)	Treatment-naive patients with HBeAg-positive CHB	All evaluable patients	PCR and RFLP	500 copies/ml	Solution hybridization	3.0 pg/ml (8×10^6 copies/ml)	• Lamivudine 31% • Lamivudine + IFN 0% • IFN 0%
Schiff et al. 2003 [14]	• Lamivudine 100 mg ($n = 119$) • Lamivudine 100 mg + IFN ($n = 63$) • Placebo ($n = 56$)	IFN nonresponders with HBeAg-positive CHB	All evaluable patients	PCR, sequence analysis, and RFLP	750 genomes/ml	Solution hybridization	10^6 genomes/ml (3.0 pg/ml vs. US sample or 1.6 pg/ml vs. European sample)	• Lamivudine 27% • Lamivudine + IFN 0% • Placebo 0%
Lai et al. 2003 [15]	• Lamivudine ($n = 468$)	All patients from [8–11]	Integrated analysis of all evaluable patients from [8–11]	PCR and RFLP	Integrated analysis of various assays	Solution hybridization	Integrated analysis of various assays	• Lamivudine 24%
Yao 2000 [16]	• Lamivudine 100 mg ($n = 322$) • Placebo for 12 weeks and then lamivudine 100 mg ($n = 107$)	Chinese patients with HBeAg-positive CHB	All evaluable patients	Not reported	Not reported	Solution hybridization	1.6 pg/ml	• Lamivudine 15% • Placebo and then lamivudine 5%

Table 4 continued

First author and year	Treatment arms (*n*)	Clinical population	Population subject to genotypic resistance testing[b]	Genotype mutation analysis	Lower limit of detection of genotype mutation analysis	HBV DNA assay	Lower limit of detection of HBV DNA assay[c]	One-year genotypic resistance rate
Tassopoulos et al. 1999 [17]	• 52 weeks of lamivudine 100 mg ($n = 60$) • 26 weeks of placebo ($n = 65$)	Patients with HBeAg-negative CHB	All evaluable patients	RFLP	100,000 copies/ml or 5% of viral population	Branched chain DNA assay	2.5 pg/ml	• Lamivudine 27% • Placebo not reported
Jonas et al. 2002 [18]	• Lamivudine 3 mg/kg ($n = 191$) • Placebo ($n = 97$)	Children aged 2–17 years with HBeAg-positive CHB	All evaluable patients	PCR and RFLP	Not reported	Branched chain DNA assay	0.7 mEq/ml	• Lamivudine 19% • Placebo not reported
Lau et al. 2005 [19]	• Lamivudine 100 mg ($n = 272$) • Lamivudine + pegylated IFN ($n = 271$) • Pegylated IFN + placebo ($n = 271$)	HBeAg-positive CHB	All evaluable patients in the lamivudine groups	Reverse hybridization line probe assay	Not reported	Quantitative PCR	200 copies/ml ($\sim$36 IU/ml)	• Lamivudine 27% • Lamivudine + pegylated IFN 9%
Marcellin et al. 2004 [20]	• Lamivudine 100 mg ($n = 181$) • Lamivudine + pegylated IFN ($n = 179$) • Pegylated IFN + placebo ($n = 177$)	HBeAg-negative CHB	All evaluable patients in the lamivudine groups	Reverse hybridization line probe assay	Not reported	Quantitative PCR	200 copies/ml ($\sim$36 IU/ml)	• Lamivudine 18% • Lamivudine + pegylated IFN 1%

[a] All studies used genotypic mutation assay to detect genotypic resistance

[b] Evaluable patients = patients with adequate serum samples at time of analysis

[c] Where possible, limits of detection of HBV DNA assay are converted into IU/ml

significance of the genotypic resistance rate reported in this study.

Lamivudine in US patients with HBeAg-positive CHB: Dienstag et al. [12]

In this US double-blind Phase III trial, 44 of 66 patients in the lamivudine group had adequate serum samples at week 52 to be evaluated for genotypic resistance. No resistance testing was performed in the placebo group. In the lamivudine group, resistant mutations were detected in 14 of 44 patients (32%); the ratio of mixed to variant-only populations is not reported. This is higher than any other reported genotypic resistance rate for lamivudine. However, the small number of patients tested for resistant mutants and the lack of a placebo control for this assay make it difficult to assess the significance of the result.

In this study, the development of mutants was associated with an increase in serum HBV DNA to a mean level of 47 pg/ml. This is significantly lower than mean baseline levels (142 pg/ml), but nadir values are not reported, so it is not possible to determine whether the development of genotypic resistance led to the development of virologic resistance.

Lamivudine and IFN α in treatment-naive HBeAg-positive CHB: Schalm et al. [13]

In this Phase III trial, 230 treatment-naive patients with HBeAg-positive CHB were randomized to receive treatment with lamivudine 100 mg daily for 1 year; lamivudine 100 mg daily for 8 weeks followed by lamivudine + IFN α 10 million units three times per week for 16 weeks; or placebo daily for 8 weeks followed by placebo daily + IFN α 10 million units three times per week for 16 weeks.

All patients were followed up off treatment to week 64 (i.e., 12 weeks after lamivudine discontinuation and 40 weeks following IFN α discontinuation).

No resistant mutations were detected in serum samples from patients in the IFN α monotherapy or lamivudine + IFN α groups, although the number evaluated is not reported. However, the samples analyzed for mutations were taken 28 to 40 weeks after treatment discontinuation. Given that wild-type virus is known to re-emerge after treatment discontinuation, the lack of resistant mutants in these samples does not necessarily indicate a lack of development of resistance during the treatment phase of the study.

In the lamivudine group, 61 patients were evaluated for resistant mutations at week 52. In total, resistant mutations were detected in 31% (19/61) of the patients, but the ratio of mixed to mutant-only populations is not reported.

The relationship between the development of resistant mutations and clinical measures of viral breakthrough is not reported in this study.

Lamivudine and IFN α in HBeAg-positive IFN nonresponders: Schiff et al. [14]

In this Phase III trial, patients with HBeAg-positive CHB and previous treatment failure on IFN α were randomized to receive treatment with lamivudine 100 mg daily for 52 weeks; lamivudine 100 mg daily for 8 weeks followed by lamivudine + IFN α 10 million units three times per week for 16 weeks; or placebo daily for 52 weeks ($n = 56$). After 52 weeks, the lamivudine group was re-randomized to lamivudine or placebo for a further 16 weeks to compare the effect of continuing antiviral therapy with treatment discontinuation.

At week 52, 99 of 119 patients in the lamivudine group, 48 of 63 patients in the lamivudine + IFN α group, and 47 of 56 patients in the placebo group were evaluated for resistant mutations. No resistant mutations were detected in the placebo group or the lamivudine + IFN α group. However, the samples analyzed for mutations were taken 28 weeks after treatment discontinuation in the combination group, so the possibility that mutant virus emerged during treatment but had been replaced by wild-type virus by the time of analysis could not be discounted. Resistant mutations were detected in 27% (27/99) of patients in the lamivudine monotherapy group. The ratio of mixed to mutant-only populations is not reported.

In this article, it is stated that HBV DNA and ALT levels remained below baseline for patients with resistant mutations, but change from nadir values (if any) is not reported, so it is not possible to determine the clinical impact of the development of mutant virus or whether it led to virologic resistance.

Lamivudine combined analysis: Lai et al. [15]

A combined analysis of the four Phase III trials of lamivudine described investigated the development of resistant mutations in a total of 794 patients with HBeAg-positive CHB with evaluable serum samples, 468 of whom were treated with lamivudine monotherapy. Using this integrated data, the one-year incidence of the development of resistant mutations in patients treated with lamivudine monotherapy was 24% (10% mixed wild-type and mutant populations and 14% mutant populations only). This analysis also included data collected from a long-term continuation of the Chinese study [11, 39, 40] and determined that the rate of development of resistant mutations in patients receiving continuous treatment with lamivudine monotherapy was 42% after 2 years (9% mixed populations and 32% mutant

populations); 53% after 3 years (20% mixed populations and 33% mutant populations); and 70% after 4 years (28% mixed populations and 42% mutant populations).

However, the same limitations apply to this analysis as to the individual studies: quantitative increases from nadir in clinical measures of hepatitis B activity (HBV DNA and ALT levels) are not reported, so it is not possible to determine whether the development of resistant mutations was accompanied by virologic or clinical resistance.

Lamivudine in patients with HBeAg-positive CHB and elevated ALT: Yao [16]

In this study, Chinese patients with HBeAg-positive CHB were randomized to 12 weeks of double-blind treatment with lamivudine 100 mg daily or placebo. The study was unblinded at the end of 12 weeks, and all patients were offered open-label treatment with lamivudine for up to 2 years.

At 1 year, resistant mutants were detected in 43 of 295 (15%) patients treated with lamivudine and 5 of 101 (5%) patients treated with placebo followed by lamivudine. The study reports that most of these patients (42/48) had serum HBV DNA and ALT levels that were lower than baseline, but the rate of virologic or clinical breakthrough is not reported.

Lamivudine in patients with HBeAg-negative CHB: Tassopoulos et al. [17]

In this placebo-controlled study of patients with HBeAg-negative CHB, the incidence of resistant mutations in patients treated with lamivudine was 2% (1/53 patients) at week 26 and 27% (11/41 patients) at week 52. However, nine patients who were lamivudine responders at week 52 (HBV DNA negative by branched-chain assay plus normal ALT) did not have adequate samples for resistance testing and were therefore excluded from the analysis. Given the small number of patients involved in this study, this could have skewed the results, leading to a deceptively high genotypic resistance rate. Resistance rates in patients treated with placebo are not reported.

Of the 11 patients with resistant mutations at week 52, eight had undetectable HBV DNA by branched-chain assay and therefore did not demonstrate evidence of virologic resistance. Only two had elevated ALT levels at week 52, but it is not clear whether the elevation occurred after development of resistant mutants or whether it was persistently elevated throughout the study. Overall, median HBV DNA was undetectable and median ALT within the normal range in patients with resistant mutants. This suggests that the high genotypic resistance rate reported in this study was not accompanied by development of clinical resistance.

Lamivudine in children: Jonas et al. [18]

In this study, 166 of 191 patients in the lamivudine group and 86 of 96 patients in the placebo group had adequate serum samples at week 52 to be evaluated for genotypic resistance. In the lamivudine group, resistant mutations were detected in 19% (31/166) of patients; the rate in the placebo group is not reported and assumed to be zero.

Patients who developed resistant mutations had a substantially higher median viral load at baseline than those who did not (1648 vs. 753.2 mEq/ml). Median HBV DNA remained significantly below baseline values despite the emergence of resistant mutations (6.75 mEq/ml), but as nadir values are not reported, it is not possible to determine the impact of genotypic resistance on virologic response.

Lamivudine versus pegylated IFN α-2a in HBeAg-positive CHB: Lau et al. [19]

In this multicenter trial, 814 patients with HBeAg-positive CHB were randomized to receive 48 weeks of treatment with lamivudine 100 mg daily; lamivudine 100 mg daily plus pegylated IFN α-2a 180 μg per week; or pegylated IFN α-2a 180 μg per week plus oral placebo.

In the lamivudine monotherapy arm, resistant mutants were detected in 27% (69/254) of patients at week 48. In the combination lamivudine/pegylated IFN group, lamivudine resistance was detected in 9% (9/256) of patients. Patients treated with pegylated IFN monotherapy did not undergo resistance testing. The authors propose that the lower rate of resistance in the combination group may be in the more complete HBV DNA suppression than due to lamivudine monotherapy group.

Although the rate of genotypic resistance to lamivudine monotherapy in this study is high, all evaluable lamivudine-treated patients were assessed regardless of response to treatment. The impact of the development of resistant mutations on viral load or ALT levels is not reported, and it is therefore impossible to determine the clinical significance of the genotypic resistance rate in this study.

Lamivudine versus pegylated IFN α-2a in patients with HBeAg-negative CHB: Marcellin et al. [20]

In this international study, 537 patients with HBeAg-negative CHB were randomized to receive 48 weeks of treatment with lamivudine 100 mg daily; lamivudine 100 mg daily plus pegylated IFN α-2a 180 μg per week; or pegylated IFN α-2a 180 μg per week plus oral placebo.

At week 48, genotypic resistance was detected in 18% (32/179) of patients in the lamivudine monotherapy arm and <1% (1/173) of patients in the combination lamivudine/pegylated IFN group. Resistance was not assessed in the pegylated IFN monotherapy arm.

The virologic impact of the development of resistant mutants was not reported in this study, which might overestimate the rate of clinically significant resistance.

Reported virologic resistance rates

Genotypic resistance assays detect the presence of mutations known to be associated with virologic resistance but give no indication of the clinical significance of the mutants detected. To overcome this, several more recent studies have included virologic measures of resistance to provide a more relevant assessment of virologic resistance. Table 5 provides a list of the key features of such studies, which have been discussed in detail in the next section.

The important feature of these studies is that they all used a robust definition of resistance that included both virologic and genotypic assays. Virologic breakthrough was accurately quantified and only patients with evidence of virologic breakthrough were assessed for genotypic resistance. This perhaps better reflects clinically significant resistance. However, it is important to note that definitions of virologic breakthrough differed among the studies.

These studies did not assess resistant mutations in the absence of virologic breakthrough and might underestimate the genotypic resistance rate.

Lamivudine cohort study: Thompson et al. [21]

This prospective study followed a cohort of 85 CHB patients for a median of 19 months (range = 6–54 months). The study was specifically designed to investigate the incidence of lamivudine resistance, which was defined as:

- an increase in viral load from nadir *or* reappearance of HBV DNA in a patient who initially achieved undetectable HBV DNA; *and*
- detection of known lamivudine resistant mutations on genotypic analysis.

The rate of development of resistance to lamivudine was modeled using Kaplan–Meier analysis.

In this study, 26 of 85 patients developed virologic lamivudine resistance during follow-up. The proportion of patients experiencing an increase in viral load without evidence of lamivudine resistance is not reported. The rate of virologic resistance to lamivudine was reported to be 6% after 1 year, 31% after 2 years, and 51% after 4 years. The presence of precore-variant HBV genotype, high baseline ALT, and persistent viremia at 6 months were found to be independent predictors of early development of lamivudine resistance.

Lamivudine versus telbivudine: Lai et al. [22]

In this Phase IIb trial, 104 patients with HBeAg-positive CHB were randomized to receive 1 year of treatment with lamivudine (100 mg), telbivudine (400 or 600 mg), or lamivudine (100 mg) + telbivudine (400 or 600 mg).

By week 48, virologic breakthrough had occurred in 15.4% (3/19) of patients treated with lamivudine, 12.2% (5/41) of patients treated with lamivudine + telbivudine, and 4.5% (2/44) of patients treated with telbivudine (p = not significant). All of these patients were confirmed to have resistant mutations by genotype mutation analysis, except for one patient in the combination group, who experienced virologic breakthrough with wild-type virus.

No mention is made in this study of assessment of patient compliance, which might have affected virologic breakthrough rates (particularly in the patient with wild-type virus and virologic breakthrough).

Lamivudine versus telbivudine: Lai et al. [23]

This is a detailed data analysis of the first 48 weeks of the Phase III GLOBE registration trial of telbivudine versus lamivudine to determine the rate of virologic breakthrough and genotypic resistance in the intent-to-treat population.

Virologic breakthrough was defined per protocol as outlined in Table 5. Using this definition, 11.0% of HBeAg-positive patients treated with lamivudine and 5.0% of those treated with telbivudine developed virologic breakthrough by week 48 ($p < 0.001$). In HBeAg-negative patients, the rates were 10.7% and 2.2%, respectively.

In the lamivudine group, resistant mutations rtM204I (±rtL180M) and rtM204V + rtL180M were detected in HBV DNA from patients with virologic breakthrough. In patients experiencing breakthrough on telbivudine, rtM204I was the predominant mutation, accompanied by the secondary mutations rtL180I/V in 2.2% and rtL180I/V+rtL180M in 0.3% of telbivudine-treated patients.

Lamivudine versus entecavir in HBeAg-positive patients: Chang et al. [24]

In this entecavir Phase III trial, genotypic mutation analysis and phenotypic resistance testing were performed in all patients for whom paired samples were available (baseline and week 52) in the entecavir group. In the lamivudine group, genotypic mutation analysis and phenotypic

Table 5 Reported one-year virologic resistance rates to lamivudine in published literature[a]

First author and year	Treatment arms (*n*)	Clinical population	Definition of resistance	Definition of virologic breakthrough	Population subject to genotypic resistance testing	HBV DNA assay	Lower limit of detection of HBV DNA assay[b]	One-year virologic resistance rate
Thompson et al. 2006 [21]	• Lamivudine 100 mg ($n = 85$)	HBeAg-positive and HBeAg-negative patients with elevated ALT	Virologic breakthrough *and* detection of resistant mutations	Increase in viral load from nadir *or* reappearance of HBV DNA	Patients with protocol-defined virologic breakthrough	Solution hybridization	0.5 pg/ml (10^5 copies/ml)	• Lamivudine 6%
Lai et al. 2005 [22]	• Lamivudine ($n = 19$) • Lamivudine + telbivudine ($n = 41$) • Telbivudine ($n = 44$)	Patients with HBeAg-positive CHB	Virologic breakthrough	Increase in HBV DNA to: • >5 $\log_{10}$ copies/ml *or* • within 1 $\log_{10}$ of baseline in patients who did not achieve <5 $\log_{10}$ copies/ml	Patients with protocol-defined virologic breakthrough	PCR	200 copies/ml (~36 IU/ml)	• Lamivudine 15.4% • Lamivudine + telbivudine 12.2% • Telbivudine 4.5%
Lai et al. 2007 [23]	HBeAg-positive patients • Lamivudine 100 mg daily ($n = 463$) • Telbivudine 600 mg daily ($n = 458$) HBeAg-negative patients • Lamivudine 100 mg daily ($n = 224$) • Telbivudine 600 mg daily ($n = 222$)	HBeAg-positive and HBeAg-negative patients with CHB	Virologic breakthrough *and* detection of resistant mutations	Increase in serum HBV DNA of ≥1 $\log_{10}$ copies/ml from nadir on at least two consecutive occasions	Patients with protocol-defined virologic breakthrough	PCR	300 copies/ml (~54 IU/ml)	HBeAg-positive patients: • Lamivudine 11.0% • Telbivudine 5.0% HBeAg-negative patients: • Lamivudine 10.7% • Telbivudine 2.2%
Chang et al. 2006 [24]	• Lamivudine 100 mg ($n = 358$) • Entecavir 0.5 mg ($n = 355$)	Treatment-naive patients with HBeAg-positive CHB	Detection of resistant mutations (entecavir group); detection of resistant mutations *and* virologic breakthrough (lamivudine group)	Increase in serum HBV DNA of ≥1 $\log_{10}$ IU/ml from nadir	All evaluable patients (entecavir group); patients with protocol-defined virologic breakthrough (lamivudine group)	PCR	300 copies/ml (~54 IU/ml)	• Lamivudine 12.6% • Entecavir 0%

Table 5 continued

First author and year	Treatment arms (*n*)	Clinical population	Definition of resistance	Detection of resistant mutations *and* virologic breakthrough	Definition of virologic breakthrough	Population subject to genotypic resistance testing	HBV DNA assay	Lower limit of detection of HBV DNA assay[b]	One-year virologic resistance rate
Lai et al. 2006 [25]	• Lamivudine 100 mg ($n = 317$) • Entecavir 0.5 mg ($n = 331$)	Treatment-naive patients with HBeAg-negative CHB	Detection of resistant mutations *and* virologic breakthrough	Increase in serum HBV DNA of ≥ 1 $\log_{10}$ IU/ml from nadir	Patients with protocol-defined virologic breakthrough (both groups); 211 randomly selected patients (entecavir group)	PCR	300 copies/ml (~54 IU/ml)	• Lamivudine 6.4% • Entecavir 0%

[a] In all studies, genotype mutation analysis was by PCR amplification and sequence analysis of the reverse transcriptase domain of the HBV genome, with an unreported lower limit of detection

[b] Where possible, limits of detection of HBV DNA assays are converted into IU/ml

resistance assays were performed only for those patients with NIH-defined evidence of virologic breakthrough.

In total, 339 of 355 patients in the entecavir group were evaluated for genotypic resistance. Although several nucleotide substitutions occurred during treatment, none was found to be associated with reduced susceptibility to entecavir in vitro, although it is known that in vitro susceptibility testing does not correlate well with in vivo results [29]. Virologic breakthrough occurred in 2% (6/355) entecavir-treated patients but was not associated with the emergence of any nucleotide substitutions in the reverse transcriptase region of the HBV genome. The rest of the HBV genome was not sequenced, so it is possible that mutations occurred elsewhere that contributed to virologic rebound. However, this has not been investigated in clinical trials.

Virologic breakthrough occurred in 18% (63/358) of patients treated with lamivudine. Of these, 45 had genotypic evidence of resistant mutations, giving a one-year incidence of NIH-defined resistance of 12.6%.

Lamivudine versus entecavir in HBeAg-negative patients: Lai et al. [25]

In this study, genotypic and phenotypic resistance assays were performed for all patients with NIH-defined evidence of virologic breakthrough. In addition, genotypic resistance was assessed in 211 randomly selected patients in the entecavir group.

In these randomly selected entecavir-treated patients, several nucleotide substitutions were detected, but none was associated with an in vitro reduction in entecavir susceptibility. This, however, does not necessarily correlate well with in vivo efficacy. Mutations in the HBV genome of the remaining 114 entecavir-treated patients were not assessed.

Virologic breakthrough occurred in 8% (25/313) of patients treated with lamivudine and 2% (5/325) of patients treated with entecavir. In the lamivudine group, 20 of these had genotypic evidence of resistant mutations, giving a one-year incidence of NIH-defined resistance of 6.4%. In the entecavir group, none of the patients with virologic breakthrough had evidence of mutations in the reverse transcriptase region of the HBV genome or in vitro evidence of entecavir resistance.

Cross-resistance: lamivudine and entecavir

Although Phase III clinical trials of entecavir have reported a very low rate of the development of resistance [24, 25], there is evidence that pre-existing lamivudine-resistant mutations accelerate the development of resistance to entecavir. Entecavir resistance has been reported in

patients who had previously experienced disease breakthrough on lamivudine owing to rtL180M and rtM204V substitutions [30]. These patients had a reduced clinical response to entecavir from the outset and developed frank virologic breakthrough during prolonged entecavir therapy. Detailed genotypic and phenotypic analyses determined that the development of the additional substitutions rtM250V or rtT184G plus rtS202I, in the presence of existing rtL180M and rtM204V mutations, conferred resistance to entecavir in vitro [30]. Other mutations were detected that reduced the susceptibility to entecavir even further (rtT184S and rtI169T), but the clinical significance of these was not clear [30].

The contribution of pre-existing lamivudine-resistant mutations to the development of entecavir resistance was confirmed in a comprehensive analysis of HBV DNA from patients involved in three studies of entecavir in lamivudine-resistant CHB [41]. Patients included in the analysis had documented lamivudine-resistant HBV at baseline (rtL180M, rtM204V, or rtM204I mutations) and were treated with entecavir 1.0 mg daily for at least 48 weeks [41]. Some patients were enrolled into a continuation study in which they received either entecavir 1.0 mg daily or a combination of lamivudine 100 mg daily + entecavir (0.5 or 1.0 mg) daily, but these were included in the analysis only if they received 1.0-mg dose of entecavir and if treatment was considered to be continuous (treatment interruptions lasting no more than 35 days) [41].

In this analysis, NIH-defined virologic breakthrough, assessed by quantitative PCR analysis with a lower limit of detection of 300 copies/ml (~54 IU/ml; conversion factor 1 IU = 5.6 copies/ml), occurred in 5 of 187 patients within 1 year and 21 of 151 patients within 2 years of entecavir treatment. Of these, lamivudine- and entecavir-resistant mutations were detectable by PCR and sequence analysis in 2 and 14 patients, respectively. This translates into a frequency of 1% at 1 year and 9% at 2 years for virologic resistance to entecavir in lamivudine-resistant patients [41]. All patients with virologic breakthrough and genotypic evidence of resistance in this analysis harbored T184, S202, or M250 substitutions, all being documented entecavir-resistant mutations [41]. There was evidence that some of these mutations might have been pre-existing at baseline and emerged during treatment owing to selective pressure [41]. It is important to note that some patients included in the year 2 analysis were receiving a combination of entecavir and lamivudine treatment, which might have affected the development of resistance.

A further analysis has compared the emergence of entecavir resistance in nucleoside-naive patients and those with pre-existing lamivudine resistance during up to 4 years of treatment, using similar methods to the study described above [42]. Of the 663, 278, 149, and 120 nucleoside-naive patients treated with entecavir for 1, 2, 3, and 4 years, respectively, a total of three patients developed entecavir-resistant mutations, two of whom experienced virologic breakthrough. This corresponds to a cumulative probability of developing virologic entecavir resistance of 0.8% over 4 years [42]. Of the 187, 146, 80, and 53 lamivudine-resistant patients treated with entecavir for 1, 2, 3, and 4 years, however, virologic breakthrough associated with resistant mutations was observed in 1%, 10%, 16%, and 15%, respectively [42]. This corresponds to a cumulative probability of developing virologic entecavir resistance of 39.5% over 4 years [42]. Again, some lamivudine-resistant patients included in the analysis from 1 year onward were receiving a combination of lamivudine and entecavir, which might have affected the results.

Discussion

Lamivudine is a well-established antiviral agent in the management of CHB and has been used as a comparator for newer nucleoside and nucleotide analogs. The one-year incidence of the development of resistance to lamivudine is often quoted at 24% [15], but rates ranging from 6% to 32% have been reported [11, 25]. One of the reasons for the discrepancies in reported resistance rates is the difficulty in establishing a standardized definition of virologic resistance that uses standardized quantification methods.

Resistance to antiviral agents can be defined genotypically, virologically, or clinically. Genotypic resistance is the emergence of viral mutations during antiviral therapy that have been shown to confer resistance to antiviral drugs in phenotypic assay. Chronologically, genotypic resistance precedes loss of virologic and clinical response to antiviral agents, sometimes by months or even years [29]. Assessing resistance genotypically, therefore, gives no indication of the clinical significance of the mutants detected and may result in an overestimation of clinically relevant resistance. Many early trials of lamivudine used a purely genotypic definition of resistance and reported one-year resistance rates of 14% to 32% [11, 14, 16–20].

Virologic resistance is an increase in viral load due to the emergence of resistant mutations, and perhaps better reflects clinical resistance. Studies that have assessed virologic resistance to lamivudine have reported one-year rates of lamivudine resistance ranging from 6% to 15.4% [22–25], considerably lower than those using genotypic methods alone[11, 14, 16–20]. Although these studies may underestimate the emergence of resistant mutants, they provide a far more relevant measure of resistance in clinical practice. However, even among these studies, the exact definition of virologic breakthrough varies and makes comparisons of results among studies difficult.

In several studies, virologic breakthrough occurred in the absence of detectable resistant mutations [22–25]. There are two possible explanations for this. First, non-compliance with treatment could lead to virologic rebound that is unrelated to antiviral resistance. Second, the methods used to detect resistant mutations might not have been sufficiently sensitive in patients harboring a small population of mutant virus among a predominantly wild-type strain.

It is interesting to note that virologic resistance to lamivudine tended to be lower in patients with HBeAg-negative CHB than those with HBeAg-positive CHB (6.4% vs. 12.6–15.4%) [22, 24, 25]. This might be due to baseline HBV DNA, which is generally lower in HBeAg-negative patients, so antiviral treatment is more likely to suppress viral replication below threshold levels, thereby reducing the potential for resistance to develop.

In one study, development of virologic resistance to lamivudine was associated with high baseline HBV DNA levels [18]. This is supported by a retrospective analysis of the emergence of virologic resistance among 79 patients treated with lamivudine for a median of 26 months [43]. In this study, Kaplan–Meier analysis of virologic response was performed for a subset of patients with HBeAg-positive CHB to determine the probability of developing virologic breakthrough according to baseline HBV DNA [43]. In patients with baseline HBV DNA levels of 6.6 $\log_{10}$ copies/ml or less ($n = 15$), the cumulative rate of virologic resistance to lamivudine was reported to be 6.7% after 1 year, 18% after 2 years, and 39% after 3 years, compared with 19%, 45%, and 88%, respectively, in those with baseline HBV DNA levels of more than 6.6 $\log_{10}$ copies/ml ($n = 17$) [43]. Again, this might be due to more rapid suppression of viral replication below threshold levels in patients with lower viral load at baseline.

In addition to different definitions of resistance, studies of antiviral agents have suffered from the lack of a standardized method to quantify measures of resistance, in particular viral load. Available techniques range from solution hybridization assays with a lower limit of detection of 10^5 IU HBV DNA/ml to real-time PCR assays capable of detecting just 30 IU HBV DNA/ml [28]. This is particularly problematic when comparing results from older studies, such as the Phase III lamivudine studies, with more recent trials using new technologies. The Phase III lamivudine studies all used solution hybridization to quantify viral load because the more sensitive quantitative PCR methods were not available at the time the studies were designed. This means that some patients were reported as HBV DNA negative who would have had detectable HBV DNA, using more modern methods. However, as the Phase III lamivudine studies tested *all* patients for evidence of genotypic resistance, regardless of virologic status, and used qualitative PCR to amplify HBV DNA prior to genotypic assay, this is unlikely to have resulted in a lower-than-anticipated resistance rate.

The head-to-head studies of lamivudine with telbivudine and entecavir used sensitive, PCR-based assays to determine viral load and defined resistance using a combination of virologic and genotypic parameters that better reflect clinically significant resistance than genotypic measures alone [22–25]. In the entecavir studies, populations tested for resistance varied among the treatment groups: all patients treated with entecavir were analyzed for resistant mutations (genotypic resistance), whereas only those with evidence of virologic breakthrough underwent genotypic testing in the lamivudine group (virologic resistance) [24, 25]. However, as no resistant mutations were observed in the entecavir group, this is unlikely to have influenced the study findings. These head-to-head studies found that lamivudine had a higher rate of resistance than either telbivudine or entecavir, but the rates reported were nevertheless considerably lower than the 24% usually quoted for lamivudine [15].

The data in this review suggest that the rate of emergence of clinically significant resistance to lamivudine may be lower than is often reported, but can nevertheless be problematic. However, effective treatment options are available for patients who develop resistance to lamivudine. The APASL guidelines recommend the addition of ADV for patients with lamivudine resistance [9]. Lamivudine-resistant mutations remain sensitive to ADV, and adding, rather than switching to, ADV, reduces the potential for the subsequent development of ADV resistance [44–46]. Switching to entecavir is also an option [9], although the development of entecavir resistance is accelerated in patients with pre-existing lamivudine-resistant mutations [30]. Additional or alternative therapy should be initiated as soon as resistance is detected to achieve maximal therapeutic response [47].

There has been some debate on predicting the development of resistance based on a patient's initial response to antiviral treatment. An analysis of combined lamivudine and telbivudine data indicated that a poor initial response to therapy (HBV DNA > 4 $\log_{10}$ copies/ml after 24 weeks of treatment) is a predictor of the subsequent development of resistance [23]. This has led to the development of the "roadmap" concept, which suggests that patients who fail to achieve an adequate virologic response to therapy by week 24 should either have a second antiviral added or switch to an alternative treatment [48]. However, there are no data supporting the best course of action in such patients, and, as yet, it is unclear whether this strategy affects the long-term outcome.

In conclusion, thorough review of studies investigating the emergence of resistance to lamivudine during CHB

therapy has revealed a diverse range of methodologies to assess resistance. Studies that use purely genotypic methods report resistance rates at 1 year ranging from 14% to 32% [11, 14, 16–20]. However, these may overestimate clinically relevant resistance. Studies that use virologic assays of resistance report lower one-year resistance rates, ranging from 6.4% to 15.4% [22–25], and may provide a more relevant measure of resistance. It is important when comparing resistance rates with antiviral drugs in CHB to consider the methodology and definition of resistance used because this can dramatically influence the results.

References

1. Lai C, Ratzui V, Yuen M-F, Poynard T. Viral hepatitis B. Lancet 2003;362:2089–2094
2. World Health Organization. Hepatitis B. Geneva: World Health Organization; 2000. Factsheet no. 204
3. Conly J, Johnston B. Treatment options for hepatitis B. Can J Infect Dis Med Microbiol 2007;18(3):173–176
4. Di Bisceglie AM, Fong TL, Fried MW, Swain MG, Baker B, Korenman J. A randomized, controlled trial of recombinant alpha-interferon therapy for chronic hepatitis B. Am J Gastroenterol 1993;88:1887–1892
5. Perrillo RP, Schiff ER, Davis GL, Bedenheimer HC, Lindsay K, Payne J. A randomised, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. N Engl J Med 1990;323:295–301
6. Perez V, Tanno H, Vilamil F, Fay O. Recombinant interferon alpha-2b following prednisone withdrawal in the treatment of chronic type B hepatitis. J Hepatol 1990;11(Suppl):S113–S117
7. Lok AS, Lai CL, Wu PC, Leung EK. Long-term follow-up in a randomized controlled trial of recombinant alpha 2-interferon in Chinese patients with chronic hepatitis B infection. Lancet 1988; 2:298–302
8. Lacey L, Gane E. The cost-effectiveness of long-term antiviral therapy in the management of HBeAg-positive and HBeAg-negative chronic hepatitis B in Singapore. J Viral Hepatitis 2007; 14:751–766
9. Liaw Y-F, Leung N, Kao J-H, Piratvisuth T, Gane E, Han K-H, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update [online]. Hepatol Int 2008. doi: 10.1007/s12072-008-9080-3
10. Katz L, Fraser A, Gafter-Gvili A, Leibovici L, Tur-Kaspa R. Lamivudine prevents reactivation of hepatitis B and reduces mortality in immunosuppressed patients: systematic review and meta-analysis. J Viral Hepat 2008;15:89–102
11. Lai C-L, Chien R-N, Leung N, Chang T-T, Guan R, Tai D-I, et al. A one-year trial of lamivudine for chronic hepatitis B. N Engl J Med 1998;339:61–68
12. Dienstag J, Schiff E, Wright T, Perrillo R, Han H-W, Goodman Z, et al. Lamivudine as initial treatment for CHB in the United States. N Engl J Med 1999;341:1256–1263
13. Schalm S, Heathcote J, Cianciara J, Farrell G, Sherman M, Willems B, et al. Lamivudine and alpha interferon combination treatment of patients with CHB infection: a randomised trial. Gut 2000;46:562–568
14. Schiff E, Dienstag J, Karayalcin S, Grimm I, Perrillo R, Husa P, et al. Lamivudine and 24 weeks of lamivudine/interferon combination therapy for hepatitis B e antigen-positive chronic hepatitis B in interferon nonresponders. J Hepatol 2003;38:818–826
15. Lai C-L, Dienstag J, Schiff E, Leung N, Atkins M, Hunt C, et al. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis 2003;36:687–696
16. Yao G. Management of hepatitis B in China. J Med Virol 2000;61:391–397
17. Tassopoulos N, Volpes R, Pastore G, Heathcote J, Buti M, Goldin R, et al. Efficacy of lamivudine in patients with hepatitis B e antigen-negative/hepatitis B virus DNA-positive (precore mutant) chronic hepatitis B. Hepatology 1999;29:889–896
18. Jonas M, Kelley D, Mizerski J, Badia I, Areias J, Schwarz K, et al. Clinical trial of lamivudine in children with chronic hepatitis B. N Engl J Med 2002;346(22):1706–1713
19. Lau G, Piratvisuth T, Luo K, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352(26):2682–2695
20. Marcellin P, Lau G, Bonino F, Farci P, Hadziyannis S, Jin R, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351(12):1206–1217
21. Thompson A, Ayres A, Yuen L, Bartholomeusz A, Bowden D, Iser D, et al. Lamivudine resistance in patients with chronic hepatitis B: role of clinical and virological factors. J Gastroenterol Hepatol 2006;22:1078–1085
22. Lai C-L, Leung N, Teo E-K, Tong M, Wong F, Hann H-W, et al. A 1-year trial of telbivudine, lamivudine and the combination in patients with hepatitis B e antigen-positive chronic hepatitis B. Gastroenterology 2005;129:528–536
23. Lai C-L, Gane E, Liaw Y-F, Hsu C-W, Thongsawat S, Wang Y, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 2007;357:2576–2588
24. Chang T-T, Gish R, de Man R, Gadana A, Sollano J, Chao Y-C, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354(10):1001–1010
25. Lai C-L, Shouval D, Lok A, Chang T-T, Cheinquer H, Goodman Z, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006;354(10):1011–1020
26. Hoofnagle J, Doo E, Liang T, Fleischer R, Lok A. Management of hepatitis B: summary of a clinical research workshop. Hepatology 2007;45:1056–1075
27. Gunther S, Sommer G, Plikat U, Iwanska A, Wain-Hobson S, Will H, et al. Naturally occurring hepatitis B virus genomes bearing the hallmarks of retroviral G to A hypermutation. Virology 1997;235:104–108
28. Locarnini S, Hatzakis A, Heathcote J, Keeffe E, Liang T, Mutimer D, et al. Management of antiviral resistance in patients with chronic hepatitis B. Antiviral Ther 2004;9:679–693
29. Lok A, Zoulim F, Locarnini S, Bartholomeusz A, Ghany M, Pawlotsky J-M, et al. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. Hepatology 2007;46:254–265
30. Tenney D, Levine S, Rose R, Walsh A, Weinheimer S, Discotto L, et al. Clinical emergence of entecavir-resistant hepatitis B requires additional substitutions in virus already resistant to lamivudine. Antimicrob Agents Chemother 2004;48(9):3498–3507
31. Zaaijer H, Ter Borg F, Cuypers H, Hermus M, Lelie P. Comparison of methods for detection of hepatitis B virus DNA. J Clin Microbiol 1994;32(9):2088–2091
32. Ho S, Chan T, Cheng I, Lai K. Comparison of second-generation Digene hybrid capture assay with the branched-DNA assay for measurement of hepatitis B virus DNA in serum. J Clin Microbiol 1999;37(8):2461–2465

33. Collins M, Irvine B, Tyner D, Fine E, Zayati C, Chang C-A, et al. A branched DNA signal amplification assay for quantification of nucleic acid targets below 100 molecules/ml. Nucleic Acid Res 1997;25(15):2979–2984
34. Ho S, Yam W-C, Leung E, Wong L-P, Leung J, Lai K-N, et al. Rapid quantification of hepatitis B virus DNA by real-time PCR using fluorescent hybridization probes. J Med Microbiol 2003;52:397–402
35. Zanella I, Rossini A, Domenighini D, Albertini A, Cariani E. Quantitative analysis of hepatitis B virus DNA by real-time amplification. Eur J Clin Microbiol Infect Dis 2002;21(1):22–26
36. Chen T, Luk J, Cheung S-T, Yu W-C, Fan S-T. Evaluation of quantitative PCR and branched-chain DNA assay for detection of hepatitis B virus DNA in sera from hepatocellular carcinoma and liver transplant patients. J Clin Microbiol 2000;38(5):1977–1980
37. Saldanha J, Gerlich W, Lelie N, Dawson P, Heerman K, Heath A. An international collaborative study to establish a World Health Organization standard for hepatitis B virus DNA nucleic acid amplification techniques. Vox Sanguinis 2001;80:63–71
38. Stuyver L, Van Geyt C, De Gendt S, Van Reybroeck G, Zoulim F, Leroux-Roels G, et al. Line probe assay for monitoring drug resistance in hepatitis B virus-infected patients during antiviral therapy. J Clin Microbiol 2000;38(2):701–707
39. Liaw Y, Leung N, Chang T, Guan R, Tai D, Ng K, et al. Effects of extended lamivudine therapy in Asian patients with CHB. Gastroenterology 2000;119:172–180
40. Leung N, Lai C-L, Chang T-T, Guan R, Lee C-M, Ng K-Y, et al. Extended lamivudine treatment in patients with CHB enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology 2001;33:1527–1532
41. Tenney D, Rose R, Baldick C, Levine S, Pokornowski K, Walsh A, 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):902–911
42. Colonno R, Rose R, Pokornowski K, Baldick C, Eggers B, Yu D, et al. Four year assessment of ETV resistance in nucleoside-naive and lamivudine refractory patients [abstract]. J Hepatol 2007; 46(1):S294. (abstract 781)
43. Chae H, Hann H-W. Baseline HBV DNA is the most important factor associated with virologic breakthrough in chronic hepatitis B treated with lamivudine. World J Gastroenterol 2007;13(30): 4085–4090
44. Liaw Y, Lee C, Chien R, Yeh C. Switching to adefovir monotherapy after emergence of lamivudine resistant mutations in patients with liver cirrhosis. J Viral Hepat 2006;13:250–255
45. Rapti I, Dimou E, Mitsoula P, Hadziyannis S. Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. Hepatology 2007;45:307–313
46. Lampertico P, Vigano M, Manenti E, Iavarone M, Sablon E, Colombo M. Low resistance to adefovir combined with lamivudine: a 3-year study of 145 lamivudine-resistant hepatitis B patients. Gastroenterology 2007;133:1445–1551
47. Liaw Y. Rescue therapy for lamivudine-resistant chronic hepatitis B: when and how? Hepatology 2007;26:266–268
48. Gane E. The Roadmap concept: using early on-treatment virologic responses to optimize long-term outcomes for patients with chronic hepatitis B [online]. Hepatol Int 2008. doi:10.1007s12072-008-9083-0