Review article: nucleoside analogues for the treatment of chronic hepatitis B

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SUMMARY
Current accepted treatment for chronic hepatitis B uses either the immunomodulator interferon alpha or nucleoside analogues lamivudine or adefovir. Interferon has side effects which mean it is often poorly tolerated. Long-term use of lamivudine is associated with increasing viral resistance for each year it is taken and the rebound viraemia that can occur when the drug is stopped is also of concern to many. Adefovir appears to have less of the resistance issues of lamivudine but is still a relatively new drug and at present its use is principally limited to patients with lamivudine-resistant disease.

A number of other nucleoside analogues are currently being developed with some now at the stage of early clinical trials. A proportion share the significant resistance problems of lamivudine but many appear to have more potent anti-viral effect than the drugs currently available. If some of these newer anti-viral agents are approved for use in chronic hepatitis B, the potential for prolonged suppression of hepatitis B virus replication with resultant stabilization or improvement in liver disease may be achieved.

INTRODUCTION
Chronic Hepatitis B (CHB) affects 350 million people worldwide and the long-term sequelae of CHB infection, cirrhosis and hepatocellular carcinoma (HCC) are responsible for 1 million deaths per year.¹

Current guidelines independently published by American and European Associations for Study of the Liver (AASLD and EASL) and a recently published algorithm for treatment of CHB in the United States recommend treatment of patients who are either HBeAg positive or HBeAg negative, with hepatitis B virus (HBV) DNA levels >10⁵ copies/mL and who have evidence of moderate to severe hepatitis. The severity of hepatitis can be evaluated by the presence of serum alanine aminotransferase (ALT) levels greater than twice the upper limit of normal (>2 × ULN) for at least 3 months.²⁻⁵ Recently updated AASLD guidelines do however recommend liver biopsy in these patients in order to fully assess the extent of liver disease prior to treatment being commenced.⁵

The aim of treatment is to reduce liver damage either by directly suppressing viral replication using nucleoside analogues or indirectly, modulating the host immune response using interferon (IFN). The response to therapy can be described as biochemical – a return of serum transaminases to the normal range – virological and histological.⁶ A virological response is currently defined as a loss of HBeAg in those who were positive when treatment was initiated and a decrease in serum HBV DNA to below detectable levels using non-amplified assays (i.e. <10⁵ copies/mL).² The increasing use of PCR techniques and the uncertainty of the clinical significance of virus below these levels is now complicating this definition. A histological response is a decrease of at least two points in the pathological activity index compared with the pre-treatment score.⁶

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EASL guidelines define a beneficial response to therapy as a combined response consisting of both biochemical and virological responses, along with a histological improvement where evidence of this is available. It has also described a complete response as referring to loss of HBsAg and development of antiHBs. AASLD guidelines differ slightly in describing complete response to treatment as a biochemical and virological response with loss of HBsAg but not necessarily seroconversion to antiHBs positive.

It is important to distinguish between therapeutic responses that occur while being maintained on treatment, at the end of treatment and a period of time after therapy has been completed. A sustained response is the goal of treatment and is defined as the maintenance of a response to treatment six or twelve months after therapy is discontinued.

The AASLD and EASL guidelines also recommend treatment with nucleoside analogues of clinically evident HBV-related cirrhosis with HBV DNA levels >10^5 copies/mL, irrespective of serum transaminase levels. However both acknowledge the difficulty in deciding optimal timing for treatment in these patients in order to minimize the risk of developing drug resistant HBV. Lamivudine resistant HBV has been reported to cause hepatic decompensation in this group of patients. Drug resistance may also limit therapeutic options in patients in whom liver transplantation is being considered. European guidelines specifically recommend initiation of anti-viral therapy in liver transplant candidates. Reduction of HBV DNA to low levels prior to transplantation reduces the risk of graft reinfection but the risk of development of drug resistant HBV means ideal timing for commencement of therapy may depend on donor organ availability in individual transplant centres.

The AASLD guidelines recommend stopping lamivudine therapy after 1 year in HBeAg positive patients if they have demonstrated seroconversion to anti-HBe positive on at least two occasions 2 months apart. However treatment can be continued for longer in patients who have yet to demonstrate a serological response. It should probably also be continued in patients who have lost HBeAg but have yet to become anti-HBe positive. Other guidelines suggest continuing treatment after HBeAg seroconversion until HBV DNA is negative for 6 months using PCR techniques. At this stage seroconversion should be reconfirmed before considering stopping the drug. If HBV does not become negative by PCR but remains stable for 6 months along with stable HBeAg seroconversion this can also be an indication for stopping treatment.

There are no clear guidelines as to how long nucleoside analogues should be continued in patients with eAg negative CHB. Despite demonstrating biochemical and virological responses to lamivudine while being actively treated, a sustained response after stopping treatment is only seen in around 10% of patients. Long-term nucleoside analogue therapy is increasingly being recognized as necessary for this group of patients but drug resistance will become almost inevitable with longer duration of therapy, presenting a real management problem for a large proportion of the HBeAg negative cohort. For this reason it has recently been recommended that adefovir might be considered first-line therapy in this group of patients.

With the exception of patients with decompensated HBV-related cirrhosis, neither set of guidelines make strong recommendations as to which particular treatment modality should be used for the patient groups described above, only that treatment should be instituted. Treatment options for patients with CHB currently employ two different approaches. The immunomodulator interferon alpha (IFN-α) was the first therapy to be licensed for use. Unfortunately, it has some unpleasant side effects and its efficacy is limited, particularly in those with HBeAg negative disease. Nucleoside analogues are drugs designed to inhibit the intracellular replication of HBV virions. Two drugs are currently licensed, lamivudine in 1997 and adefovir in 2002 with (unlicensed) famciclovir being used before these. Lamivudine has revolutionized the treatment of HBeAg negative CHB in particular, but is far from perfect. Adefovir has only received its FDA license recently and its use at present tends to be limited to patients who have lamivudine-resistant disease. A number of other nucleoside analogues are currently being developed with several in Phase II and III trials. An understanding of the efficacy and mode of action of these drugs as well as information regarding drug resistance helps to explain the direction HBV treatment may take in the future.

STRUCTURE AND REPLICATION

Hepatitis B virus is a member of the hepadnaviridae family, a group of small DNA viruses capable of causing acute and chronic hepatic infection in a variety of
species. It is a circular, partially double-stranded 3.2 kilobase DNA molecule that contains four overlapping open reading frames (ORF).\textsuperscript{1, 10}

Hepadnaviridae also exist in mammals and birds, including woodchucks (WHV) and ducks (DHBV).\textsuperscript{1, 10} WHV in particular is structurally very similar to HBV and has a comparable natural history, in that almost all woodchucks with WHV will develop HCC by 4 years of age.\textsuperscript{11} Both animals are used as models for HBV drug development. In addition, hepatocytes from chronically infected animals are used in cell culture systems for in vitro testing, as are transfected human liver cells.

The ORF encode the main HBV proteins, P, S, C and X. The P ORF encodes the viral polymerase that is essential for replication. C or core gene encodes the nucleocapsid protein and the S and pre-S regions carry genetic information for surface glycoprotein production, most notably HBsAg. The precore part of the genome, in conjunction with the core gene is responsible for production of eAg. The X ORF is not present in all members of the hepadnaviridae family and is notably absent in the avian viruses. Protein X in HBV is a complex regulatory protein required for the infectivity of the HBV. It is also thought to modulate gene expression.\textsuperscript{10}

Replication of HBV is not via the conventional process of semi-conservative DNA synthesis. Instead it involves synthesis of an RNA intermediate (pregenomic RNA, pgRNA) that then undergoes reverse transcription to synthesize a negative DNA strand before formation of the positive DNA chain. HBV polymerase is essential for this reverse transcription process, adhering to the pgRNA encapsidation signal and then binding with free nucleotides inside the nucleocapsid to incorporate them into the DNA strands.\textsuperscript{10, 12}

After uptake into a hepatocyte the HBV is converted within the nucleus to covalently closed circular DNA (cccDNA), a step thought to be catalysed by hepatocyte DNA polymerases (see Figure 1). This cccDNA permanently infects the cell and is used as a template for viral replication. Cellular RNA polymerase II catalyses the formation of pgRNA from the cccDNA template. The RNA for all four HBV genes is then transported to the cytosol where their translation to proteins allows the continuation of viral replication within the nucleocapsid, catalysed by newly translated HBV viral polymerase. Much of the HBV is then packaged within an envelope protein and then exported from the cell. It is thought a smaller proportion is retained and ‘recycled’ in the nucleus to maintain the pool of cccDNA.\textsuperscript{10, 12}

Nucleoside analogues bear a close resemblance to naturally occurring nucleosides adenosine, guanine, thymidine and cytosine that make up DNA. The drugs exert their anti-viral effect through integration in HBV DNA, causing termination of chain synthesis. Ideally the nucleoside analogue should show a higher affinity for the viral polymerase than the natural nucleoside so that the analogue is preferentially bound by the polymerase and incorporated into the DNA molecule.\textsuperscript{12} The structure of the nucleoside analogue may lack a hydroxyl group and so cannot form a bond between itself and any subsequent nucleotides, terminating DNA elongation. Alternatively its incorporation may cause chain termination two to three nucleotides downstream because of structural changes in the molecule.\textsuperscript{12}

Concern for therapeutic safety arises when the nucleoside analogue’s affinity for host cellular polymerases approaches that for the viral polymerase. This can lead to their integration into cellular or mitochondrial polymerase causing drug-related toxicity.\textsuperscript{12}

cccDNA

cccDNA remains within infected hepatocytes and acts as a template for pregenomic RNA formation. One major drawback with current nucleoside analogue therapy is the rebound viraemia that occurs when the drug is stopped.\textsuperscript{6, 13} In a proportion of patients this can cause an increase in serum ALT levels and in a small number has been reported to cause fatal reactivation.\textsuperscript{14} It is thought this rebound viraemia is because of the persistence of cccDNA within hepatocytes. To date, none of the drugs that have been developed show unequivocal evidence of being able to target cccDNA\textsuperscript{12, 15–21} although some of the newer drugs may be having some effect, as the latent phase between cessation of the drug and rebound viraemia lengthens.\textsuperscript{11, 22}

DRUG RESISTANCE IN HBV

Hepatitis B virus is unusual in that it replicates by reverse transcription. Like human immunodeficiency virus (HIV) which also uses this method, the viral reverse transcriptase does not have proof-reading ability. In HIV this results in a large number of circulating quasispecies in each individual, some of which are preferentially selected under the immune pressure of drug therapy, resulting in these
drug-resistant genotypes of HIV predominating. A similar theory has been postulated for HBV. Resistance to anti-HBV therapy is clearly documented with use of both lamivudine and famciclovir and is theoretically possible with any nucleoside analogue that targets the viral polymerase.

Genotypic resistance is determined by examining the DNA sequence of the reverse transcriptase part of the HBV polymerase gene. Resistance is usually detected using hybridization techniques probing for previously recognized resistance mutations. Alternatively the whole polymerase gene can be individually sequenced before therapy and again in patients who experience viral breakthrough while being compliant with anti-viral therapy. This is the technique often employed when exploring genotypic resistance for new anti-viral agents.

Clinically phenotypic resistance is recognized when a patient who has initially had a good response to anti-viral treatment experiences HBV DNA breakthrough while remaining compliant with therapy. The assumption is that the predominant HBV at that time is genotypically resistant to the anti-viral agent being used.

Phenotypic resistance can be confirmed in cell culture systems that measure the inhibitory concentration of a drug; that is the dose of drug required to reduce viral replication by 50% or more. Cell culture systems are set up with cells infected with the strain of virus thought to be drug resistant and the inhibitory concentrations are determined. Drug resistance is usually associated with at least a 10-fold increase in inhibitory concentration. These systems also allow in vitro testing of cross-resistance between drugs.

The DNA analysis of HBV has revealed areas in the reverse transcriptase (rt) part of the polymerase gene that appear to confer phenotypic resistance to nucleoside analogues. Within this section are six highly conserved domains A to F, with the principal mutations relating to famciclovir and lamivudine therapy in domains B and C respectively.

Until recently the numbering system used to identify HBV mutations was complicated as numbering of amino acids began from the beginning of the polymerase gene. As genome length varies slightly between genotypes this resulted in the classical lamivudine-resistant mutation at the ‘YMDD’ locus described as being at amino acid positions 552, 550, 539 or 549 depending on the dominant genotype in the study population. A consensus meeting in 1999 proposed a different, genotype-independent system based on subdivision of the polymerase gene into four regions: terminal protein (tp), spacer domain, cccDNA, and reverse transcription of DNA strands.
reverse transcriptase (rt) and ribonuclease H (rh).\textsuperscript{38} Numbering of amino acids begins from one at the start of each section. It is this new system (with the older, more familiar one in parentheses) that will be employed in this article when referring to amino acid substitutions.

| New    | Old     |
|--------|---------|
| Lamivudine |         |
| rt180  | 528,526,515,525 |
| rt204  | 552,550,539,549 |
| Famciclovir |     |
| rt173  | 521,519,508,518 |
| rt207  | 555,553,542,552 |
| rt180  | 528,526,515,525 |

The principal polymerase gene mutations associated with lamivudine and famciclovir have been determined using a combination of polymerase gene sequencing and cell culture systems. Famciclovir-resistant mutations have been identified mainly in the B domain, the principal substitution being rtL180M (previously L528M).\textsuperscript{24, 29, 39, 40} In contrast most lamivudine-related resistance mutations are located in the C domain, principally in what has become known as the ‘YMDD motif’.\textsuperscript{6, 13} In reality only the ‘M’ of this region changes, to either isoleucine (I) or valine (V) – the so-called YIDD or YVDD variants. However this nomenclature can be confusing and the preferred term is rtM204I/V (M552I/V).

Lamivudine-resistant mutations tend to fall into one of two groups. The first group has mutations in both B and C domains – rtL180M and rtM204V, while the second has only the C domain mutation rtM204I.\textsuperscript{6} Given that rtL180M is also associated with famciclovir resistance it is not surprising that HBV with this B domain mutation is inherently phenotypically resistant to famciclovir therapy as well. In patients first treated with famciclovir who are changed to lamivudine because of treatment failure, lamivudine resistance has been shown to develop more quickly than in those who are treatment naïve when started on lamivudine. This is thought to be due to the pre-existence of the rtL180M mutation at the beginning of lamivudine treatment.\textsuperscript{29}

In vitro studies have allowed cross-resistance testing of known lamivudine-resistant HBV with other anti-viral agents. These have shown significant resistance to emtricitabine and clevudine,\textsuperscript{30, 33} clearly important in a clinical setting. In vitro studies have also consistently shown adefovir to be active against lamivudine-resistant mutations \textsuperscript{30–33, 36} and this property has also been proven clinically.\textsuperscript{41–43}

The clinical importance of the development of drug-resistant HBV is still under debate. In vitro work suggests that the replication competency of HBV with these specific mutations is reduced when compared with wild type.\textsuperscript{30, 33} Clinical studies have shown that while elevated HBV DNA levels with lamivudine resistance is essentially universal, continuation of treatment under these circumstances is not associated with biochemical or histological deterioration in the majority of patients.\textsuperscript{6} Given that a significant proportion of patients even without lamivudine-resistant mutations will experience a rebound viraemia on stopping the drug, some experts would advise that such patients continue to take lamivudine while being closely monitored, while recent studies from Asia have suggested the drug may be stopped safely in lamivudine-resistant disease.\textsuperscript{6}

One study from Asia has demonstrated deterioration in histological indices in around 20% of patients after 3 years of lamivudine treatment when 50% of the cohort had detectable lamivudine-resistant HBV.\textsuperscript{13} There are also reports in patients after OLT\textsuperscript{7, 44} and in the immunocompetent\textsuperscript{13} of clinical deterioration, sometimes to the point of the development of fulminant hepatic failure, where the HBV detected was predominantly of a drug-resistant genotype. Certainly in a transplant setting and even with Hepatitis B immunoglobulin (HBIG) therapy now being used routinely, no centre would be keen to transplant a patient with high levels of lamivudine-resistant HBV DNA without access to a suitable alternative treatment because of the risk of developing graft reinfection, particularly in the form of fibrosing cholestatic hepatitis.

Modern-day treatment of HIV involves use of a combination of usually three or four antiretroviral medications, expressly because this has been shown to reduce the development of drug resistant strains. The similarities between the replication and treatment of these two viruses is clear, leading many to predict that combination therapy may also be the only way to curb the rate of HBV mutation and allow effective viral suppression. Low levels of viral replication should then encourage stable HBeAg seroconversion and the improved prognosis associated with this.
FAILED THERAPIES

Several anti-HBV drugs did not fulfill their early potential, with the most memorable and tragic being fialuridine (FIAU). This nucleoside analogue reached the stage of Phase II trials before its toxic side effects were discovered. The study was terminated as an emergency at week 13 when seven of 15 patients were discovered to have developed severe hepatotoxicity and progressive lactic acidosis that then progressed despite the drug being stopped. Other serious adverse events included pancreatitis, myopathy and neuropathy. Three other patients showed signs of mild hepatotoxicity while the other five were symptom-free, having only been exposed to FIAU for a short time. Of the seven worst affected, only two survived, following OLT. Three died despite receiving a liver transplant and the other two succumbed before they could be transplanted.45

Electron microscopy carried out on postmortem specimens showed grossly abnormal mitochondria. This case illustrates well the toxicity risks involved when using nucleoside analogues as anti-viral agents. If the drug has relatively similar specificities for viral and host polymerases it may also be integrated into cellular or, as is believed in the case of FIAU, mitochondrial DNA with disastrous consequences.12

Ara-AMP (vidarabine) and lobucavir were also withdrawn at the clinical trial stage because of safety concerns. Vidarabine was found to cause significant neuromuscular toxicity while lobucavir was found to be teratogenic in animal models.13

FAMCICLOVIR

Famciclovir is the oral form of the guanosine analogue penciclovir and was developed initially for its action against herpes viruses. As with lamivudine, penciclovir requires to be transformed to its active triphosphate metabolite. It competes with dGTP for incorporation into the viral DNA molecule, resulting in structural changes that cause chain termination a few nucleotides downstream.12

In the mid-1990s, OLT for HBV-related decompensated liver disease was becoming more common because of the use of perioperative HBlg to reduce graft reinfec­tion rates. Case reports and small series from single centres began to appear of patients who had sustained graft reinfec­tion or reactivation of HBV following immunosuppression for other organ transplants. Many of these patients appeared to have been successfully treated with famciclovir, often with histological as well as biochemical and virological improvement. However clinical responses did not appear to be consistent, with some patients having no response at all to the drug, while others had virological relapse while on treatment.56–52

In vitro and animal studies were used to confirm these and other incidental clinical findings. The work showed that penciclovir had an anti-viral effect on DHBV,19, 53, 54 WHV 55 and HBV in cell culture 56. Soon clinical studies designed to assess the effect of famciclovir on patients with CHB were producing promising results.57–59 However, with prolonged use it became apparent that not only did a percentage of patients fail to respond initially to therapy but relapse on treatment was common, usually secondary to drug resistance.40, 52, 60 The advent of a licence for lamivudine in CHB provided an alternative to famciclovir as lamivudine’s consistent effects on HBV DNA levels, ALT and histological indices had already been proven in large multicentre trials. A recent study carried out in Asian patients directly compared the anti-viral effects of famciclovir and lamivudine and appear to confirm what has long been suspected from in vitro studies and clinical experience, that lamivudine has a superior effect on HBV DNA in patients treated with either drug for 12 weeks.61

Current interest in famciclovir is now principally in its role in combination with other anti-viral agents – either immunomodulatory,62, 63 lamivudine 55, 64–66 or both.67

LAMIVUDINE (2’3’-DIDECOXY-3’-THIACYTIDINE, 3TC)

Lamivudine is the (−) enantiomer form of the cytosine analogue 3TC. It requires phosphorylation to 3TC triphosphate before competing with dCTP for incorporation into HBV polymerase DNA. It lacks a hydroxyl group and so causes immediate DNA chain termination.12

Lamivudine’s efficacy as an antiHBV agent has been confirmed using randomized controlled trials in a wide variety of clinical situations. It has been shown to be effective in reducing HBV DNA levels, normalising serum transaminases and improving histological indices in patients with both HBeAg positive and negative disease.6, 13 In those who are HBeAg positive, seroconversion can reach rates of 50% with five years of therapy.6, 13 It has similar effects on laboratory values and improves clinical outcome in patients with
HBV-related decompensated cirrhosis. In combination with HBIG therapy lamivudine has reduced graft reinfection in OLT from around 90% to <10%. Lamivudine therapy is associated with a number of problems such as rebound viraemia which is now known is likely to be common to all nucleoside analogues. However, the development of drug resistant HBV with lamivudine monotherapy has become a particular problem that increases with each year of additional therapy. It has been shown that after 12 months of treatment, 14–32% of patients will have detectable mutations in the C domain of the reverse transcriptase segment of the polymerase gene. This rate increases to around 70% after 5 years of therapy.

Lamivudine monotherapy is therefore not ideal for the treatment of CHB. The work being carried out on the development of other nucleoside analogues should increase therapeutic options, particularly with regard to combination therapy.

ADEFORVIR (9-[2-(PHOSPHONOMETHOXYL)ETHYL]-ADENINE, PMEA)

Adefovir is an analogue of the nucleotide adenosine. It already contains a phosphate group and requires only a final phosphorylation step before competing with adenosine for integration into HBV DNA. There, as with nucleoside analogues it targets HBV polymerase and causes termination of DNA strand synthesis. It may exert an additional anti-viral effect through stimulation of natural killer cell activity and induction of IFN production. It is taken in its oral prodrug form, adefovir dipivoxil.

Adefovir was initially developed for use against HIV in doses of 60 mg a day. However its additional anti-hepadnaviral properties were observed in co-infected individuals and Phase I and II trials soon confirmed the drug’s anti-HBV effect at doses much lower than were required in HIV. This significantly reduced the problems with renal toxicity that were being recognized with higher doses.

A series of studies using animal models as well as cell culture systems confirmed adefovir’s effect on both wild type and drug-resistant forms of HBV. These studies were then corroborated by case reports of adefovir being used to good effect in patients with lamivudine-resistant HBV, in both the transplant setting and in hepatic decompensation. A number of smaller studies have also emerged from cohorts of patients co-infected with HIV and HBV, with lamivudine-resistant HBV developing as a result of lamivudine being used as part of an antiretroviral regime. These studies have confirmed a reduction in HBV DNA and normalization of transaminases levels in this sub-group of patients.

Two large international double-blinded placebo-controlled Phase III trials have recently been published, examining the effects of treatment in HBeAg positive and negative patients as separate groups of patients. Both trials have shown that treatment with adefovir 10 mg once a day for 48 weeks causes significant decreases in HBV DNA (median 3.91 log10 in eAg negative and 3.52 log10 in eAg positive), serum transaminases and significant histological improvement, when compared with placebo. In the HBeAg positive group only 12% seroconverted with a further 12% losing HBeAg without gaining anti-HBe by the end of the 48-week treatment period. Data from extension of the HBeAg positive trial for a further 48 weeks shows these biochemical and virological benefits appear to continue. Dose-dependent nephrotoxicity is infrequent when using adefovir 10 mg/day in patients with well-compensated liver disease. However reversible renal toxicity occurs in a greater percentage of both transplant recipients (13%) and those with decompensated cirrhosis (12%).

As with lamivudine and famciclovir, the major concern regarding use of any nucleoside analogue as monotherapy is the development of drug-resistant mutations. Adefovir resistance-related mutations in the HIV reverse transcriptase gene have been well documented, increasing concern that its use as long-term therapy for HBV will result in the development of similar mutations. There has also been concern that use of what would for HIV be a sub-therapeutic dose of adefovir in co-infected patients would lead to the development of HIV resistance to adefovir. One study has shown this does not appear to be the case.

Phase III trials investigating the efficacy of adefovir reported no evidence of drug resistance after 48 weeks of treatment. Continuation of therapy in a significant proportion of these patients for up to 144 weeks has revealed adefovir-related resistance at two novel sites. Both mutations, rtA181V in the B domain and rtN236T in the D domain of the reverse transcriptase gene, have been phenotypically and genotypically related to adefovir resistance. In contrast to lamivudine,
resistance rates are low, calculated to be approximately 4% after 3 years of therapy in the reported study population.84

NEWER NUCLEOSIDE ANALOGUES

A number of nucleoside analogues are currently being investigated as potential anti-HBV agents. This review will concentrate on those that have reached the stage of inclusion in clinical trials.

ENTECAVIR (BMS-200475)

Entecavir is a carboxylic 2′-deoxyguanosine analogue that is metabolized rapidly to its active triphosphate metabolite.85 In vitro studies have demonstrated that entecavir acts as an inhibitor at three stages of HBV replication: priming, reverse transcription and DNA-dependent DNA synthesis.86 This compares favourably with lamivudine that has no effect on priming and requires greater drug concentrations to inhibit the second two steps.87 In vitro work has also shown that not only does wild-type HBV polymerase have a greater affinity for entecavir than the natural dGTP,88 it appears to have no effect on mitochondrial metabolism.89

Work carried out using entecavir in animal models has been very encouraging. Early studies in woodchucks has shown that therapy of up to 12 weeks could result in a 7–8 log10 reduction in WHV DNA.18 Although this study also suggested an overall decrease in hepatic cccDNA in entecavir-treated woodchucks, these animals still experienced rebound viraemia on stopping the drug, albeit with a longer latent phase in the group treated with a higher dose.18 A more recently published trial used entecavir treatment in woodchucks for up to 3 years (although using a once-weekly dosing regimen that is unlikely to give optimal inhibition of viral replication) and produced very promising results. It showed a significant improvement in survival in animals treated for 14 and 36 months compared with historical controls and demonstrated that in most animals WHV DNA levels stayed at or below the level of detection by PCR for the duration of treatment. In eight of nine animals with prolonged exposure to the drug, cccDNA was undetectable in liver samples taken at the end of treatment and remained negative for at least 5 months without treatment. No clinical or genotypic evidence of drug resistance was found with this prolonged treatment regime.11

In vitro89, 90 duck and now Phase II human studies87 appear to show that the anti-viral effects of entecavir are superior to those of lamivudine, particularly in the first phase of viral loss. A significant improvement in the reduction of HBV DNA has been demonstrated with both 100 and 500 μg daily doses when compared with lamivudine 100 mg od.87

In vitro86, 88 and clinical studies91–93 also illustrate entecavir’s good effect against lamivudine-resistant HBV. Phase II trials have shown not only a reduction in HBV DNA levels by a mean of 4.46–5.10 log10 (dose dependent) over 48 weeks of treatment, but also a normalization of serum transaminase levels.91 It may also be the case that, unlike other nucleoside analogues, entecavir’s anti-viral effect is unaffected by baseline ALT values.94 The drug is well tolerated87, 95 although the degree of post-treatment rebound viraemia and hepatitis in humans has yet to be clarified.87, 95 Its effect on seroconversion may also not be as dramatic as was hoped.87 There have been no reports of viral breakthrough on therapy and HBV genome sequencing has so far failed to show evidence of genotypic resistance to entecavir after 48 weeks of treatment.96 The results of Phase III trials are awaited with interest.

EMTRICITABINE ((-)CIS-5-FLUORO-1-[2(HYDROXYMETHYL)-1,3-OXANTHIOLAN-5YL]CYTOSINE, -FTC)

Emtricitabine is a cytosine analogue that is structurally very similar to lamivudine. As with other nucleoside analogues its anti-HBV effect has been proven in vitro33, 97 and in animal models21, 98, 99 before progressing to clinical trials in humans. These preclinical studies have shown a significant effect with FTC on HBV DNA levels but suggest its effect on cccDNA may be minimal.21

Human Phase I/II clinical trials have shown an average 3.3 log10 reduction in HBV DNA levels in patients treated with emtricitabine for only 8 weeks.100 Extension of the treatment period up to 2 years has shown normalization of ALT in 76% and undetectable HBV DNA using hybridization techniques (limit of detection 4700 copies/mL) in 41% of patients, with a median decrease in HBV DNA of around 3 log10. Twenty-nine per cent of patients seroconverted to anti-HBe positive although this was not always stable. Importantly and not altogether surprising given its structural similarity to lamivudine, 6% of patients who received 1 year of treatment with emtricitabine and
19% after 2 years had the C-domain (+/- B-domain) mutations known to be associated with lamivudine resistance. A small proportion (<10%) of patients experienced rebound viraemia after stopping emtricitabine. All remained clinically well. In vitro work has confirmed cross-resistance to emtricitabine with lamivudine-resistant HBV. As with lamivudine it is likely that in the future emtricitabine will have restricted use as anti-HBV monotherapy because of these problems with resistance. However it would appear to be a candidate for combination treatment.

CLEVUDINE (L-FMAU, 2'-FLUORO-5-METHYL-β-L-ARIBINOFURANOSYL)

Clevudine is a l-thymidine analogue that also has demonstrable anti-HBV effects in vitro and in vivo. It works by inhibiting DNA-dependent positive strand DNA synthesis alone rather than reverse transcription. Woodchuck studies have shown clevudine to be highly effective, reducing WHV DNA by up to 8 log_{10} after 28 days of therapy. This was accompanied by a reduction in replicative intermediates and WHsAg levels. cccDNA was also decreased in the highest dose group where 50% of animals also had no evidence of viral rebound up to 12 weeks after stopping therapy.

Phase II clinical studies also show a profound reduction in HBV DNA levels of approximately 4.5–5.0 log_{10} with only 28 days of treatment, and the results of further human studies are awaited. Unfortunately a limiting factor in the use of clevudine may be its apparent cross-resistance to lamivudine-resistant HBV, which has been demonstrated in vitro. Clevudine itself has been shown in woodchucks to cause viral resistance after 6–12 months of use, with mutations principally in the B-domain of the polymerase gene, which can also confer resistance to famciclovir and lamivudine.

TELIVUDINE (L-DT, β-L-DEOXYTHYMIDINE)

Telbivudine is a further nucleoside analogue currently entering Phase III trials. In vitro studies suggest its active triphosphate form is safe, with no adverse effects on mitochondrial function or morphology. Its actions are specific for hepadnaviridae and its effect on woodchucks is similar to that of entecavir and clevudine with a reduction in WHV DNA of up to 8 log_{10} with only 28 days of treatment.

Phase I/II trials suggest the reduction in viraemia over a relatively short time-course may not be quite as dramatic in humans, although a certain amount of dose-adjustment appears to still be taking place. Certainly the drug appears to be safe and well-tolerated and Phase IIb study results suggest that telbivudine has superior anti-viral efficacy to lamivudine. Unfortunately telbivudine does appear to exhibit cross-resistance to lamivudine-resistant HBV with rtM204I and rtM204V/rtL180M genotypes. This is likely to limit its application in treating lamivudine-resistant disease but with Phase III trials currently underway these should provide useful information regarding de novo telbivudine resistance.

TENOFOVIR

Tenofovir is a nucleoside analogue already licensed for use in HIV treatment. To date no randomized controlled trials have been carried out using this drug in HBV infection. In vitro studies have shown its efficacy in cell culture systems and a number of case reports have appeared in the literature documenting the successful treatment of (usually lamivudine-resistant) HBV in HIV co-infected individuals. One pilot study has also been carried out in this population that showed a mean 4.3 log_{10} reduction in HBV DNA with 24 weeks of therapy. Two of six patients became HBV DNA negative by PCR. Reports of its success in treating liver

| Nucleoside analogue | Mean drop HBV DNA | Duration of therapy |
|---------------------|-------------------|---------------------|
| Lamivudine          | 3–4 log_{10}      | 24–48 weeks         |
| Adefovir (HBCAg +ve) | 3.52 log_{10}     | 48 weeks            |
|                     | 3.91 log_{10}     |                    |
| Entecavir           | 4.46 log_{10}     | 48 weeks            |
| Emtricitabine       | 3.04 log_{10}     | 56 days             |
| Clevudine           | 4.82 log_{10}     | 28 days             |
| Telbivudine         | 6.09 log_{10}     | 52 weeks            |
| Tenofovir*          | 4.26 log_{10}     | 24 weeks            |

Wherever possible when using data from Phase I/II trials the figure quoted is for the dose that is currently being used in Phase IIb and III clinical trials.

* In patients co-infected with human immunodeficiency virus and hepatitis B virus (HBV). Treatment was added to lamivudine and famciclovir combination when this was virologically ineffective.
decompensation secondary to lamivudine-resistant HBV infection alone also exist \(^{121, 122}\) although this is less likely to be a common indication at present following the licensing of adefovir.

Tenofovir has demonstrated dose-dependent nephrotoxicity in patients being treated for HIV.\(^ {123}\) In addition its safety and efficacy have not been formally assessed in CHB, nor have formal dose-escalation trials been carried out for wild-type and lamivudine-resistant HBV. For these reasons, despite tenofovir having a license for treatment of HIV, tenofovir cannot currently be recommended for treatment of CHB (Table 1).

**COMBINATION THERAPY**

Early *in vitro* work using human hepatoblastoma cell lines provided the earliest indication that combining individual drugs with anti-HBV activity may produce a synergistic anti-viral effect.\(^ {124}\) The discovery of lamivudine’s effect on HBV production and subsequent licensing as treatment for CHB provided a real opportunity for the investigation of potential combination therapy regimes in a clinical setting.

**Lamivudine and α-IFN combination (see Table 2)**

Most substantial trials have been carried out combining α-IFN with lamivudine in eAg positive patients. Three studies have been carried out in cohorts of more than 100 patients, all with elevated ALT (>1.3 × ULN) and high HBV DNA levels.\(^ {125-127}\) However they vary in the therapies the patients had received prior to entry into the reported studies and these differences make direct comparison of the trials difficult. The studies also do not always use directly comparable treatment regimes. As the characteristics of the treatment groups vary between studies it is difficult to determine whether the differences in treatment regime are significant.

The study examining the response to treatment in previous IFN non-responders showed lamivudine monotherapy had a significant biochemical, histological and virological response when compared with placebo.\(^ {127}\) Results were disappointing for combination therapy, showing only histological improvement at the end of treatment, with no difference from placebo using biochemical and virological parameters.\(^ {127}\)

The other two studies were more encouraging with significantly better eAg seroconversion in treatment naïve patients given combination therapy compared with either monotherapy.\(^ {125, 126}\) The third study was weakened somewhat by its lack of an α-IFN monotherapy arm, however it did show significant virological and serological improvement in the combination group compared with lamivudine monotherapy.\(^ {125}\) Serological response to treatment was also impressively sustained in the combination group.\(^ {125}\) However this trial has been noted for the surprisingly low histological and biochemical response rate reported for the lamivudine monotherapy arm when compared with historical cohorts previously reported in the literature.\(^ {128}\)

Two smaller studies in eAg negative patients have also been reported. Once again the patients represent a heterogeneous mix with regard to prior α-IFN therapy, with 18 of 29\(^ {129}\) and 21 of 50\(^ {130}\) having previously failed treatment. Both studies show good initial biochemical and virological responses with combination therapy when treated for up to 12 months but unfortunately this response could not be sustained after stopping treatment. One interesting observation from the larger study that compared combination therapy with lamivudine monotherapy was that combination therapy may help protect against reverse transcriptase mutations as none were apparent in this group compared with 31% in the lamivudine arm.\(^ {130}\)

Combination therapy with standard α-IFN may therefore confer some additional benefit to patients who are eAg positive and treatment naïve. Pegylated IFN is used widely in the treatment of chronic Hepatitis C and has recently been shown to be of greater efficacy than standard IFN when used as monotherapy in eAg positive CHB.\(^ {131}\)

A number of studies are being carried out evaluating the effect of combination pegylated IFN and lamivudine in eAg positive and negative CHB.\(^ {132-135}\) Results from two studies comparing combination pegylated IFN and lamivudine with lamivudine monotherapy have shown promising results.\(^ {134, 135}\) One other suggests a good response in HBeAg positive patients to pegylated IFN although any additional effect of lamivudine has still to be confirmed.\(^ {132}\) A fourth study with over 500 HBeAg negative patients included pegylated IFN and lamivudine monotherapy arms. It has suggested the sustained response to combination therapy, while significantly better than lamivudine alone, is no different to peg-IFN monotherapy.\(^ {133}\)

Combination IFN/lamivudine therapy is not recommended in any of the current guidelines for treatment of CHB. At present, other than in treatment of naïve eAg
| Study            | No of patients (% treatment naïve) | HBeAg status | Treatment regime                                                                 | Percentage with sustained viral response (SVR)* | Comments                                                                 |
|------------------|-------------------------------------|--------------|----------------------------------------------------------------------------------|-------------------------------------------------|-------------------------------------------------------------------------|
| Barbaro et al.   | 151 (87%)                           | Positive     | IFNα 9 MU tiw + lam 100 mg od 24/52 vs. Lam 100 mg od 52/52                      | 33% combination                                 | 48/52 follow-up                                                        |
| Schalm et al.    | 226 (100%)                          | Positive     | Lam 100 mg od for 8/52 then + IFNα 10 MU tiw for 16/52 vs. IFNα 10 MU tiw for 16/52 vs. Lam 100 mg od for 52/52 | 36% combination                                 | Surprisingly poor response to lamivudine                               |
| Schiff et al.    | 238 (0%)                            | Positive     | Lam 100 mg od for 52/52 vs. Placebo for 52/52 vs. Lam 100 mg od for 8/52 then + IFNα 10 MU tiw for 16/52 | 24% lamivudine                                  | Benefit only on per-protocol analysis, not intention to treat.           |
| Tatulli et al.   | 29 (38%)                            | Negative     | Lam 100 mg od + IFNα 6 MU tiw for all patients for 52/52                        | 14% total                                       | 52/52 follow-up                                                        |
| Sanantonio et al.| 50 (64%)                            | Negative     | Lam 100 mg od for 52/52 vs. Lam 100 mg od + IFNα 5 MU tiw for 52/52             | 19% lamivudine                                  | No difference between IFN-failed and naïve groups                      |
| Janssen et al.   | 137 (%)                             | Positive     | PEG-IFNα-2b 100 µg/week for 8/12 then 50 µg/week for 3/12 vs. PEG-IFNα-2b as above + lam 100 mg od for 52/52 | 44% for all patients                            | 24/52 follow-up. Interim results only, effect of additional lamivudine not known. |
| Sung et al.      | 40 (100%)                           | Positive     | PEG-IFNα-2b 1.5 µg/kg/week for 32/52 + lam 100 mg od after first 8/52 then lam alone 28/52 vs. Lam 100 mg od for 52/52 | 50% combination                                 | 24/52 follow-up                                                        |
| Chan et al.      | 100 (100%)                          | Positive     | PEG-IFNα-2b 1.5 µg/kg/week for 32/52 + lam 100 mg od for 52/52                 | 36% combination                                 | High relapse rate in combination group during follow-up. (24/52)       |
| Marcellin et al. | 546 (86%)                           | Negative     | PEG-IFNα-2a 180 µg/week vs. PEI-IFNα-2a 180 µg/week + lam 100 mg od vs. Lamivudine 100 mg od all for 48/52 | 44% combination                                 | 24/52 follow-up                                                        |

IFNα, interferon alpha; PEG-IFNα, pegylated interferon alpha (2a or 2b as shown in the table); Lam, lamivudine; MU, million units (of interferon or pegylated interferon, administered subcutaneously); kg, kilograms; mg, milligrams; µg, micrograms; tiw, three times a week; od, once a day; /-52, weeks; /-12, months.

* HBeAg seroconversion (HBeAg positive patients) or HBV DNA <10^5 copies/mL (HBeAg negative patients)
positive disease there is no evidence for the superiority of any of these regimes to those currently in use. However the full results of studies carried out using pegylated IFN are awaited with interest, particularly for those with eAg negative disease.

**Nucleoside analogue combination therapy**

In *vitro* and animal studies have suggested that a combination of penciclovir, the oral form of famciclovir, and lamivudine have at least an additive effect on HBV DNA suppression.\(^{55, 74, 116}\) Human studies have also shown a greater reduction of HBV DNA with a combination of these two drugs.\(^{65, 66}\) However no effect on HBeAg seroconversion has been demonstrated, although this may be due to the short duration of treatment in this study.\(^{65}\)

The advent of adefovir has led to famciclovir becoming almost redundant because of its relatively poor efficacy as well as its cross-resistance with lamivudine. Adefovir has already been proven highly effective when used sequentially for lamivudine-resistant HBV.\(^{42, 43}\) It has a much lower rate of resistance than either famciclovir or lamivudine and so far the reverse transcriptase gene mutations are novel to adefovir with little theoretical risk of cross-resistance with lamivudine.\(^{26, 32, 36, 84}\)

In *vitro* studies have again been useful in investigation of the anti-viral effects of adefovir as part of combination therapy. One report has suggested the addition of adefovir to lamivudine in DHBV-infected duck hepatocytes has a synergistic effect on DHBV DNA production.\(^{74}\)

One study in treatment naïve patients evaluating a combination of adefovir and lamivudine compared with lamivudine monotherapy has shown in its 1 year interim analysis that viral breakthrough is greatly reduced in the combination group. However ALT normalization at weeks 48 and 52 is significantly better in the lamivudine monotherapy group.\(^{117}\) The study is continuing at present and it may be that a second year of treatment is required for the clinical benefit of reduced drug resistance to manifest.

**Sequential nucleoside analogue therapy**

The effectiveness of sequential therapy with different nucleoside analogues depends very much on the agents used. The assumption is made that a second drug is given when treatment with the first fails and the most likely reason for this is the development of drug resistance. As described above, early clinical experience suggested that famciclovir-resistant HBV was sensitive to lamivudine. However resistance to lamivudine often developed relatively quickly after being started.\(^{29}\) This is because the rtL180M (L528M) mutation that usually confers resistance to famciclovir is also present in group one lamivudine-resistant HBV in association with rtM204V (M552V).\(^{24, 29}\)

Data from *in vitro* studies suggest clevudine,\(^ {30, 107}\) telbivudine\(^ {113}\) and emtricitabine\(^ {109}\) demonstrate cross-resistance with lamivudine. It would therefore be anticipated these drugs would have no significant clinical effect on lamivudine-resistant HBV. However similar *in vitro* studies did demonstrate the effectiveness of adefovir\(^ {30, 107, 109, 113}\), entecavir\(^ {88, 113}\) and tenofovir\(^ {114, 138}\) against lamivudine-resistant CHB. This anti-viral action has now been shown in a clinical setting for all three drugs.\(^ {42, 43, 91, 115, 119}\) As entecavir is being prescribed in the setting of clinical trials and tenofovir is only licensed for use against HIV, choice of therapy in patients with lamivudine-resistant HBV is currently very limited. Adefovir does provide a treatment option for patients with lamivudine-resistant disease. However after the lessons of HIV drug resistance, prescription of sequential monotherapy for patients with CHB should not be seen as a long-term solution.

Few believe treatment of CHB with monotherapy, either immunomodulatory or with nucleoside analogues, is ideal. Suppression of viral reproduction is not optimal and the inevitability of viral breakthrough because of drug resistance makes combination of two or more agents an obvious next step. However much more evidence is required before this approach can be concluded as effective.

**HIV CO-INFECTION**

In the pre-highly active antiretroviral therapy (HAART) era (before 1997) patient survival in HIV/HBV co-infected patients was poor. Evaluation of anti-HBV therapies in this population has therefore become important relatively recently. As survival in patients with HIV improves\(^ {139}\) so the management of CHB, including end-stage liver disease, in the cohort which constitutes approximately 10% of the HIV-positive population, becomes critical.

An early pilot study treated 40 co-infected patients with advanced HIV with lamivudine for 12 months.
At the end of the treatment period 26 of 27 patients had HBV DNA levels less than the limit of detection using hybridization techniques, five of 27 had lost HBeAg and three of these patients had seroconverted to being anti-HBe positive.\textsuperscript{140}

The CAESAR study was a large multicentre randomized double-blind placebo-controlled trial that proved the efficacy of the addition of lamivudine to HIV treatment regimes. A retrospective subgroup analysis of 122 HIV/HBV co-infected patients included in this study was carried out. This showed a significant decrease in HBV DNA levels in lamivudine-treated patients compared with controls.\textsuperscript{141}

Prolonged treatment with lamivudine results in the same mutations in the HBV reverse transcriptase gene in this population as it does in immunocompetent individuals.\textsuperscript{142} The mutations also appear to develop at approximately the same rate as they do in patients infected with only Hepatitis B.\textsuperscript{143} Rapid deterioration in HIV-positive patients as a result of decompensated or accelerated lamivudine-resistant HBV has been described.\textsuperscript{144, 145} Work has therefore been carried out in HIV/HBV positive patients with lamivudine-resistant disease to establish the safety and efficacy of some of the more novel nucleoside analogues.

Many studies describe the effects of tenofovir, a drug licensed for use in HIV, on lamivudine-resistant HBV in co-infected individuals. All prove the drug reduces HBV DNA levels in a variety of patient groups, however the cohorts contain very small numbers of patients.\textsuperscript{115–117, 120, 146, 147} In vitro studies have also supported clinical data, showing tenofovir is effective against wild type and lamivudine-resistant HBV.\textsuperscript{114, 138} A larger trial has recently reported significant virological HBV responses in 87 co-infected patients treated for a median of nine months with tenofovir as part of HAART.\textsuperscript{146} However, randomized controlled trials evaluating tenofovir in the longer term and in larger cohorts in co-infected patients are lacking but are necessary if the potential of this drug is to be realized.

The efficacy of adefovir in HIV/HBV co-infected patients with lamivudine-resistant HBV has also been illustrated in a pilot study involving 35 patients. Ten milligrams adefovir daily was added to pre-existing HAART that also included lamivudine 150 mg twice a day. All patients had proven genotypic lamivudine-resistant HBV and treatment with adefovir continued for 144 weeks in 29 of the 35 patients.\textsuperscript{148} These patients showed a highly significant decrease in HBV DNA and ALT at weeks 48, 96 and 144. Two patients seroconverted to anti-HBe by week 48 and there was no evidence of phenotypic or genotypic resistance to adefovir.\textsuperscript{148}

Early studies with α-IFN showed poor response in those who were HIV positive.\textsuperscript{149} This was confirmed a few years later when HIV negativity was shown to be a strong positive predictive predictor for response to α-IFN.\textsuperscript{150} However little information is available in these studies regarding the stage of HIV infection of the patients and both predate the advent of HAART. The majority of nucleoside analogues used in the treatment of HBV in co-infected individuals have the benefit of being active against both HIV and HBV and are therefore usually integrated in the HAART regime.

CHILDREN

The α-IFN is licensed for use in children with CHB. Its efficacy is similar to adults with a recent meta-analysis suggesting eAg clearance is around 30% compared with 12% in placebo controls.\textsuperscript{151} The long period of treatment required with α-IFN (up to 12 months in some paediatric studies) along with its unpleasant side effects make it far from ideal in patients under the age of 12 years.

Two studies have been carried out investigating lamivudine in a paediatric population. The first explored pharmacokinetics and safety and established a dose of 3 mg/kg/day up to 100 mg a day as being equivalent to 100 mg daily in adults.\textsuperscript{152} The second was a multicentre placebo-controlled trial investigating the efficacy of this dose of lamivudine in patients aged 2–17 years, treated for 48 weeks. This did show significantly better virological responses in the lamivudine compared with placebo group.\textsuperscript{153} However the response rate was only 23%, less than with α-IFN.\textsuperscript{151, 153} As in adult studies better response rates were seen in those with ALT levels greater than twice the upper limit of normal, lower HBV DNA and higher histological activity indices on liver biopsy. Mutations in the C-domain of the reverse transcriptase gene were detected in 19% of patients after 48 weeks of therapy.\textsuperscript{153}

Extension of this study randomized children at 48 weeks according to their HBeAg status at that time. Those who were HBeAg positive were given open-label
lamivudine for a further 48 months. Thirty per cent of those treated for 2 years had loss of HBeAg and HBV DNA negativity (<0.7 millequivalents/mL). An additional 21% of children treated with lamivudine who had not had a virological response at the end of the initial 48-week treatment period showed a virological response after a total of 36 months of therapy. In children who had a virological response, this was sustained in around 90%. Lamivudine resistance rates with the extension of the study were not reported.

One recent pilot study evaluating a combination of α-IFN and lamivudine in 17 children has shown promising results, suggesting seroconversion rates of over 40%. However, treatment with the combined therapy lasted up to 3 years in some patients and long-term follow-up of this study as well as extension to a larger patient group is required.

CIRRHOSIS AND LIVER TRANSPLANTATION

Nucleoside analogues have a particular role to play in patients who have Child-Pugh grade B or C cirrhosis because of CHB. α-IFN is not used in this group due to reports of clinical deterioration as a result of therapy. It would appear that lamivudine, as the nucleoside analogue with which there is most experience, can bring about significant clinical improvement in this group of patients.

Early reports were of relatively small groups of patients all with clinically significant cirrhosis and HBV DNA levels higher than $10^5$ copies/mL. These patients were shown to experience a significant decline in bilirubin levels and Child-Pugh score and improvement in albumin levels as a result of lamivudine therapy. In contrast to α-IFN, therapy was tolerated well.

One larger study of 154 patients identified a biphasic survival pattern where many patients with very severe liver failure died from complications of liver disease within 6 months of being commenced on lamivudine. However if a patient survived this first crucial period, projected 3-year survival on continued therapy was 88%. Predictors for mortality before 6 months were elevated baseline bilirubin and creatinine levels as well as higher HBV DNA.

A study carried out to investigate the use of lamivudine before and after liver transplantation also showed significant benefit. Patients were commenced on lamivudine at the time of listing for transplantation. Of 77 patients treated, only one was formally removed from the transplant waiting list because of clinical improvement. However even in the 30 patients who were not transplanted, presumably because of lack of available donor organs, survival was 70% at 4 years. There was no control arm of the study but this compared favourably with historical controls. After 2 years of treatment a significant drop in ALT and increase in albumin was documented in these patients, along with a trend to lower bilirubin levels.

In the group who received lamivudine and then were transplanted a median of 38 months after starting treatment, 60% were HBsAg negative after 12 weeks of follow-up post-transplant. Around half of these patients had been followed for a further 3 years and 59% remained sAg negative. None of the patients received HBlg after transplantation. This trial also illustrated the importance of baseline HBV DNA levels as higher titres were associated with higher rates of Hepatitis B recurrence post transplant.

Consideration of the implications of viral resistance is even more crucial in Child-Pugh grades B and C cirrhotics than in any other group of patients with CHB. There are case-reports of potentially fatal hepatic decompensation as a result of a flare of lamivudine-resistant HBV in patients with HBV-related cirrhosis. When considering liver transplantation nucleoside analogues are used to reduce HBV DNA to low or undetectable levels prior to surgery in order to minimize the risk of reinfection. However prolonged exposure to lamivudine before listing increases the chance of a patient developing high titres of drug-resistant HBV prior to transplantation. Cases of fibrosing cholestatic hepatitis because of reinfection of a graft with lamivudine-resistant HBV have been reported.

In cases such as these adefovir has proven efficacy in both the pre- and post-transplant setting. As more nucleoside analogues are licensed for use drug resistance should become less of an issue. Sequential therapy will be used to treat drug resistant virus and combination therapy should be used to prevent the development of resistance in treatment-naïve patients. At present however, the AASLD recommendation to discuss these high-risk patients with a transplant centre before commencing anti-viral therapy should be seriously considered.

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CONCLUSION

This is by no means an exhaustive catalogue of anti-HBV agents, particularly regarding drugs still in the earlier stages of development. It should however, familiarize the reader with the anti-virals that are likely to become increasingly important in the treatment of CHB in the coming few years. It also illustrates some of the difficulties still being experienced with this class of drugs as a whole.

Nucleoside analogue therapy for Hepatitis B has not quite reached the complex stage of combination therapy that has shown its effectiveness in the treatment of HIV, but increasing numbers of drugs are being added to the hypothetical armamentarium. The option of immunomodulatory therapy as part of this combination is also being actively pursued. It is likely that in 10 or 15 years time the concept of treatment of chronic HBV with lamivudine as a single agent will be regarded as foreign as the use of zidovudine (AZT) monotherapy for HIV today.

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