Nucleoside/nucleotide analogues in the treatment of chronic hepatitis B

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The current available agents for the treatment of chronic hepatitis B (CHB) include immunomodulatory agents, such as interferon-α and pegylated interferon-α, and oral nucleoside/nucleotide analogues (NAs), including lamivudine, adefovir, telbivudine, entecavir and tenofovir. The NAs work mainly by inhibiting hepatitis B virus (HBV) DNA polymerase activity and thus suppress HBV replication. Oral NAs have become the mainstay of CHB treatment, mainly due to their profound viral suppressive effects and also due in part to the ease of single daily dosing and lack of significant side effects. One major drawback of NA therapy is the development of drug resistance mutations with long-term treatment. Lamivudine, the first oral NA approved for CHB patients, is associated with high rates of drug resistance, with resultant virological relapse and biochemical flare. Fortunately, newer and more potent NAs, such as entecavir and tenofovir, have very low resistance rates, with potent and durable viral suppression. This review is aimed at the current developments in NAs for CHB treatment, detailing the mechanisms of antiviral activity of the different agents, the efficacy of viral suppression, the achievement of treatment endpoints, the development of drug resistance and the optimal strategies for using these drugs.

Keywords: oral therapy, hepatitis B virus, efficacy, resistance

Introduction

Chronic hepatitis B (CHB) is a major health burden, with an estimated 400 million people affected globally. Up to 40% of those with CHB may develop complications, including cirrhosis, decompensated liver disease and hepatocellular carcinoma (HCC). Transmission of hepatitis B virus (HBV) occurs by the parenteral route. The HBV binds onto surface receptors of the host hepatocytes and is internalized via the process of endocytosis. Once the HBV enters the hepatocyte, the partially double-stranded viral relaxed circular DNA undergoes repair within the nuclei, with the formation of covalently closed circular DNA (cccDNA). cccDNA serves as a transcriptional template for the host RNA polymerase II enzyme. The resulting RNA transcripts are then transported to the cytoplasm, where they are translated into viral envelope, core and polymerase proteins. A single strand of pre-genomic RNA is then packaged into the core during assembly of viral nucleocapsids, which is subsequently reverse-transcribed into the first negative strand of the HBV DNA. After completion of the second positive strand of HBV DNA, the resultant double-stranded DNA can either be exported as new HBV progeny or be recycled back to the nucleus to form cccDNA. The life cycle of HBV is depicted in Figure 1.

The importance of HBV DNA levels in determining long-term outcomes in CHB patients has been demonstrated in several large prospective studies. In a study of 2763 CHB patients, the relative risk of mortality from HCC was 11.2 in patients with HBV DNA levels of ≥10⁵ copies/mL, compared with 1.7 in those with HBV DNA levels of 1.6×10³–10⁵ copies/mL. In the REVEAL study of 3653 CHB patients, HBV DNA levels ≥2000 IU/mL were associated with significantly higher risk of HCC. This same cohort also showed that the level of HBV DNA was an independent risk factor for the development of cirrhosis. The levels of HBV DNA have been shown to correlate well with liver histological activity and with fibrosis stages. HBV can cause HCC both directly via integration of its DNA into the host genome and indirectly by inciting chronic injury to the hepatocytes, leading to an increase in cellular turnover.

There are currently two major classes of antiviral agents approved for the treatment of CHB: immunomodulatory agents (including conventional and pegylated interferon-α) and oral nucleotide/nucleoside analogues (NAs). The exact mechanism by which interferon exerts its antiviral effect is unclear. Interferon may have a weak direct antiviral effect on HBV. Other potential major mechanisms include the up-regulation of class I major histocompatibility complex (MHC) antigen expression in infected hepatocytes and activation and modulation of various immune pathways and cytokines that inhibit viral replication. The advantages of interferon-based therapy include the finite duration of therapy and the lack of drug resistance. However, a
significant proportion of patients will not respond to treatment or will still require long-term treatment with NAs upon completion of interferon therapy.11 Currently there are five NAs approved for the treatment of CHB, including three nucleoside analogues (lamivudine, telbivudine and entecavir) and two nucleotide analogues (adefovir and tenofovir).12 A sixth NA, clevudine, has been approved in South Korea and the Philippines for CHB. The structures of the available NAs are illustrated in Figure 2. In contrast to the immunomodulatory effect of interferon, the currently available NAs target HBV polymerase. HBV polymerase is a multifunctional protein with RNA- and DNA-dependent DNA polymerase functions that are essential for viral replication. It contains four domains: the terminal protein (important for initiating HBV
replication and nucleocapsid packaging), the spacer protein, the reverse transcriptase and the RNaseH domain (important for degradation of pre-genomic RNA template). The NAs act by direct inhibition, through competitive binding with endogenous substrates or through incorporation into the viral DNA to act as chain terminators (Figure 1).

The ultimate goal of antiviral therapy in CHB is eradication of HBV from the host. Unfortunately, this is difficult to achieve because of the existence of extremely stable cccDNA residing within the hepatocyte nuclei. Each infected hepatocyte contains an estimated 1–50 copies of cccDNA, which are pivotal for the persistence of HBV infection. The cccDNA acts as a template that utilizes the transcriptional processes of the host to produce the viral RNAs, which are required for viral replication. The amount of cccDNA correlates with hepatitis B s-antigen (HBsAg) levels, but the correlation varies depending on the different disease phases. In hepatitis B e-antigen (HBeAg)-positive patients, a positive correlation is observed between cccDNA, HBsAg and HBV DNA levels. In contrast, the correlation is lost in HBeAg-negative patients, whereas the HBsAg levels are maintained relative to the HBV DNA and cccDNA levels.

The reason for this remains unclear.

Given that complete eradication of HBV is not possible with the currently available therapies, the achievable goals of therapy include the prevention of liver fibrosis/cirrhosis, compensated liver disease and the development of HCC. However, these long-term goals are difficult to evaluate, especially in the absence of large, randomized, placebo-controlled trials with the newer NAs. The efficacy of the NAs from clinical trials have been reported mostly in the context of short-term goals, including normalization of alanine aminotransferase (ALT), HBV DNA suppression, HBeAg seroclearance/seroconversion and the reversal of histological activity.

Since not all HBV carriers require antiviral therapy, there are established regional treatment guidelines to select patients who are most likely to benefit from treatment. The American Association for the Study of Liver Diseases (AASLD) treatment guidelines recommend treatment for those with HBV DNA ≥10⁵ copies/mL and ALT ≥2x the upper limit of normal (ULN). In contrast, the European Association for the Study of the Liver (EASL) guidelines recommend treatment for those with HBV DNA ≥10⁴ copies/mL and ALT >ULN. The Asia Pacific Association for the Study of the Liver (APASL) guidelines recommend treatment for those with ALT >2x ULN and HBV DNA ≥10⁵ or ≥10⁴ copies/mL in HBeAg-positive and HBeAg-negative patients, respectively. For patients who are immunocompromised or undergoing chemotherapy, pre-emptive treatment is recommended regardless of viral load or ALT levels. This review will discuss the NAs that are currently available for the treatment of CHB.

**Lamivudine**

Lamivudine [2′,3′-dideoxy-3′thiacytidine (3TC)] was approved in 1998 for the treatment of CHB at a dose of 100 mg/day and is the first oral NA available for CHB. It is an analogue of cytidine, and is phosphorylated to its active metabolites, acting as a chain terminator after competing for incorporation into viral DNA. Lamivudine is effective in the normalization of ALT, HBeAg seroconversion, suppressing HBV DNA and reversing fibrosis. In addition, lamivudine has been shown to decrease the rate of complications, including liver decompensation and HCC, in both cirrhotic and pre-cirrhotic patients when compared with patients without treatment. The major drawback of lamivudine is the high rate of drug resistance mutations, with 76% having genotypic resistance after 8 years of treatment.

The main mutations responsible for lamivudine resistance include rtM204V and rtM204I. The rtM204V mutation usually occurs concomitantly with the rtL180M mutation, whereas rtM204I can occur alone or together with rtL180M. It has been postulated that rtL180M can restore the replication competency of rt204 mutants and increase drug resistance. Another compensatory mutation, rtV173L, occurs with rt204 and rt180 mutations, and may enhance viral replication. Other mutations shown to confer resistance to lamivudine include rtA181T/V, a mutation with cross-resistance to adefovir.

**Adefovir dipivoxil**

Adefovir, a nucleotide analogue of adenosine monophosphate, was approved in 2002 for treating CHB at a dose of 10 mg/day. In two large Phase III trials, adefovir was shown to significantly improve histological, virological and biochemical parameters compared with placebo. However, the 10 mg dose is suboptimal (see below) and the rate of viral suppression is relatively slow. One of the advantages of adefovir is its efficacy against lamivudine-resistant mutants. HBV harbouring the rtM204V/I mutations remain susceptible to adefovir. The two major mutations of the HBV polymerase gene responsible for adefovir resistance are rtA181V/T and rtN36T. A genotypic resistance rate of 29% is observed after 5 years of treatment with adefovir in HBeAg-negative patients. Another mutation, rt1233V, has been associated with primary adefovir resistance.

Adefovir is associated with dose-dependent nephrotoxicity. The initial development for use in patients infected with HIV was abandoned because of the high rate of renal toxicity observed with higher doses. In CHB patients, a lower dose is used. The nephrotoxicity is commonly manifested as a mild increase in serum creatinine and a decrease in serum phosphate levels. This usually occurs at 4–12 months after commencing therapy, and is reversible when the drug is discontinued. The exact mechanism of renal injury is unknown, although it is postulated that depletion of mitochondrial DNA and a change in multidrug resistance protein 4 expression in the proximal tubules are involved.

**Entecavir**

In 2005, entecavir was approved at a dose of 0.5 mg/day and 1 mg/day for treatment of treatment-naive and lamivudine-resistant CHB, respectively. Entecavir is a carboxylate analogue of guanosine that undergoes intracellular phosphorylation to its active 5′ triphosphate metabolite. This competes with the natural substrate deoxyguanosine triphosphate and inhibits HBV DNA polymerase. In vitro studies have shown that entecavir inhibits HBV DNA polymerase priming, which involves guanosine (an additional antiviral mechanism compared with other NAs), reverse transcription of the pre-genomic messenger RNA and synthesis of the positive-stranded HBV DNA. In contrast...
to other NAs that are obligate chain terminators, the active moiety in entecavir contains a 3′-hydroxyl group that allows a few additional nucleotides to be incorporated prior to chain termination.\textsuperscript{62,64} Therefore entecavir is a non-obligate chain terminator.

Compared with lamivudine, entecavir demonstrated a 30- to 2200-fold efficacy in reducing viral DNA replication \textit{in vitro}.\textsuperscript{29,65} In an early Phase II trial comparing entecavir with lamivudine, higher rates of virological suppression were observed in those treated with entecavir.\textsuperscript{46} The subsequent Phase III trial of 715 treatment-naive HBeAg-positive patients showed a significantly higher rate of histological, virological and biochemical improvement in those treated with entecavir compared with lamivudine.\textsuperscript{47} Similar results were obtained in the Phase III study with 648 treatment-naive HBeAg-negative CHB patients.\textsuperscript{48}

Entecavir has a high barrier to resistance. Therefore the development of drug resistance is rare, occurring at a rate of only 1.2% after 5 years of therapy in treatment-naive patients.\textsuperscript{59,50} The reason for the high genetic barrier of entecavir is because it requires a combination of three mutations before resistance develops.\textsuperscript{51} In addition to the rtM204V and rtL180M mutations that are responsible for resistance to lamivudine, an additional mutation at rtT184I, rtL234I or rtM235V is required for entecavir resistance.\textsuperscript{52,53} The presence of lamivudine resistance mutations thus increases the risk of developing entecavir resistance.\textsuperscript{54,55} In lamivudine-refractory patients, the resistance rate to entecavir is 51% after 5 years of therapy.\textsuperscript{69}

### Telbivudine

In 2006, telbivudine [(\textit{l}-deoxythymidine (LdT)], a synthetic unsubstituted \textit{l}-nucleoside analogue of thymidine, was approved for the treatment of CHB at a dose of 600 mg/day. \textit{l}-Nucleosides differ in their stoichiometric configuration compared with natural nucleosides, with their sugars and base moieties arranged in the \textit{l} configuration rather than the \textit{d} configuration. Telbivudine undergoes phosphorylation to its triphosphate form by cellular kinases, which can inhibit HBV DNA polymerase by competing with thymidine 5′-triphosphate.\textsuperscript{55} Incorporation of telbivudine leads to premature chain termination.

Telbivudine has been demonstrated to be more potent than lamivudine against HBV. In an early Phase Iib trial, telbivudine showed significantly greater virological and biochemical responses compared with lamivudine.\textsuperscript{56} In the double-blind Phase III trial, telbivudine was shown to be superior to lamivudine in HBV reduction and suppression, with lower rates of drug resistance.\textsuperscript{57} A higher therapeutic and histological response was also observed in the telbivudine-treated HBeAg-positive patients compared with those treated with lamivudine. Another Phase III trial of Chinese CHB patients demonstrated greater antiviral and clinical efficacy compared with lamivudine, and with a lower rate of resistance.\textsuperscript{58} In an open-labelled trial of 135 treatment-naive HBeAg-positive patients, telbivudine showed greater HBV DNA suppression compared with adefovir after 24 weeks of treatment.\textsuperscript{59}

Despite the high antiviral potency of telbivudine, the resistance rate is still relatively high, at 25.1% and 10.8% for HBeAg-positive and HBeAg-negative patients, respectively, after 2 years of treatment.\textsuperscript{50,56} An additional 11.3% and 6.5% in HBeAg-positive and HBeAg-negative patients, respectively, developed resistance during the third year.\textsuperscript{61} After 3 years of treatment with telbivudine, the rate of resistance is expected to be in excess of 30%.\textsuperscript{62} rtM204I is the main mutation conferring primary resistance to telbivudine, with or without concomitant rtL80I/V and rtL180M mutations. Other mutations associated with telbivudine resistance include mutations at rtA181T/V and rtL229W/V.

Telbivudine has been shown to have a significantly higher incidence of severe (grade 3 or 4 elevation) creatine phosphokinase (CPK) elevations compared with lamivudine (12.9% versus 4.1%, respectively, \textit{P} < 0.001) after 2 years of therapy in the Phase III study of 680 patients.\textsuperscript{57,60} Myopathy was reported in two of these cases. Myalgia and general weakness were the most common adverse reactions noted in another study of 105 patients treated with telbivudine.\textsuperscript{63} Therefore it is recommended that CPK levels and muscularkeletal symptoms should be monitored regularly in patients treated with telbivudine. Peripheral neuropathy has also been reported in patients receiving a combination of telbivudine and pegylated interferon-\alpha2a, therefore this combination should be avoided.\textsuperscript{64} The mechanism of telbivudine-induced myopathy and neuropathy remains unclear. Further details of muscle histology, nerve conduction studies or electromyography are required to determine the cause. In vitro studies have demonstrated no inhibition of human polymerase \(\alpha\), \(\beta\) or \(\gamma\) by telbivudine after phosphorylation, and no evidence of mitochondrial toxicity.\textsuperscript{65}

### Tenofovir

Tenofovir disoproxil fumarate (9-[(\textit{R})-2-(phosphonomethoxy)-propyl]adenine (PMPA)) is a prodrug of tenofovir that undergoes phosphorylation to competitively inhibit the natural substrate deoxyadenosine 5′-triphosphate. Once incorporated into HBV DNA polymerase, it functions as a chain terminator. Although only approved in 2008 for the treatment of CHB at a dose of 300 mg/day, tenofovir has been available since 2002 for the treatment of HIV infection. Tenofovir has been shown to be superior to adefovir in terms of suppression of HBV DNA, HBeAg seroconversion and normalization of ALT.\textsuperscript{66,67} In vitro studies have demonstrated that tenofovir and adefovir have comparable antiviral activity on a molar basis, thus explaining the higher antiviral efficacy of tenofovir compared with adefovir at clinical doses of 300 mg and 10 mg, respectively.

To date, there are no reported cases of primary resistance to tenofovir in patients with CHB mono-infection. An early study of CHB patients co-infected with HIV suggested a possible role of rtA194T mutation in tenofovir resistance.\textsuperscript{68} HBV with the rtA194T mutation shows reduced susceptibility to tenofovir when combined with lamivudine resistance rtM204V and rtL180M mutations in vitro. The clinical impact of the rtA194T mutation remains to be determined; tenofovir has been shown to be effective in viral suppression in patients with the rtA194T mutation. In vitro studies have also demonstrated that the rtA181T and rtN236T mutations associated with adefovir resistance are associated with decreased susceptibility to tenofovir.\textsuperscript{60,69} In clinical settings, the significance of these adefovir resistance mutations remains to be fully elucidated since multiple studies have shown mixed results.\textsuperscript{70–72}
Tenovir has been shown to be nephrotoxic in patients with HIV infection.\textsuperscript{73–76} In CHB, the renal toxicity observed appears to be mild, and less severe than that observed with adefovir.\textsuperscript{57,77} Nevertheless, renal function and serum phosphate levels should be monitored regularly for patients on tenovir. There is also a potential for bone mineral density loss and osteomalacia with long-term tenovir treatment, which is observed in patients with HIV infection.\textsuperscript{78–80} It is not known whether CHB patients will also develop these complications, therefore monitoring of both renal and non-renal adverse effects of tenovir in CHB patients is essential.

**Clevudine**

Clevudine [2′-fluoro-5-methyl-β-L-arabinofuranosyl-uracil (L-FMAU)] is a thymidine analogue, and therefore is structurally similar to telbivudine. Clevudine has a fluoride group at the 2′ position on the furanose moiety in place of hydrogen which is found in telbivudine. It undergoes stepwise phosphorylation to its active triphosphate metabolite. Based on two Phase III trials with only 6 months of treatment,\textsuperscript{81,82} clevudine was approved in South Korea in 2006 and was approved in the Philippines in 2009. Its proposed mechanism of action includes targeting HBV DNA polymerase, reverse transcriptase and the conversion of partially double-stranded DNA into cccDNA.\textsuperscript{83,84} This reduction in cccDNA combined with its long half-life may contribute to the unique post-treatment effect of clevudine whereby viral suppression is maintained for a period of time even after stopping therapy.\textsuperscript{85}

Unfortunately, with the termination of an international multicentre Phase III study, further global development of clevudine has been halted due to the associated severe myopathy (with biopsy-proven myonecrosis) and mitochondrial toxicity occurring after several months in patients treated with clevudine.\textsuperscript{86–88}

**Treatment efficacy**

There are limited direct head-to-head trials comparing the different antiviral agents, and placebo-controlled trials are even rarer. The registration trials of lamivudine and adefovir were compared with placebo, whereas telbivudine and entecavir were compared with lamivudine, and tenofovir with adefovir.\textsuperscript{24,25,31,32,47,48,57,66} The rates of ALT normalization, viral load reduction and HBeAg seroconversion after 1 year of treatment are summarized in Table 1. In general, newer and more potent antiviral agents have greater HBV DNA suppression. They also have a higher rate of ALT normalization in HBeAg-positive patients, although the rates of HBeAg seroconversion were similar. It is important to emphasize that HBeAg seroconversion is not an adequate treatment endpoint. In a recent study of 42 patients with NA-induced HBeAg seroconversion, only 13 (31%) showed a durable remission.\textsuperscript{39} In another study of lamivudine-induced HBeAg seroconversion, those who stopped treatment had a cumulative incidence of flare of 44% at 5 years, and none of the patients had undetectable HBV DNA.\textsuperscript{30}

In addition to HBeAg seroconversion, loss of HBsAg can also occur with NA treatment. Recent studies have also shown that treatment with NAs using lamivudine, adefovir, telbivudine, entecavir and tenofovir in HBeAg-positive patients can achieve HBsAg seroclearance, with reported rates of 2.8%, 2%, 0.5%, 5% and 8%, respectively.\textsuperscript{38,61,67,91,92} The HBsAg seroclearance rate with pegylated interferon is also reported to be 7%.\textsuperscript{11} Reported rates in HBeAg-negative subjects treated with NAs are much lower in the previously mentioned studies. Kinetic studies of HBsAg levels have shown that in patients treated with NA, there was no decline noted in HBeAg-negative patients, which may explain the lower rates of HBsAg seroclearance observed in this group of patients.\textsuperscript{93,94} Despite HBsAg seroclearance, complete eradication may still not be possible. There is still a risk of reactivation, especially in immunocompromised patients. In addition, there is a risk of HCC even after HBsAg seroclearance in cirrhotic and non-cirrhotic patients.\textsuperscript{95–97} Furthermore, achievement of HBsAg seroclearance by NAs or interferon does not necessarily indicate viral clearance, as there may be emergence of S gene mutants, with active underlying viral replication.\textsuperscript{98,99} For immunocompetent patients with evidence of HBsAg seroclearance and undetectable viral load, treatment can be stopped with close monitoring after cessation of therapy.

The long-term goal of antiviral therapy is the prevention of cirrhosis, liver decompensation and HCC. Prolonged treatment using the currently approved NAs has also been shown to improve liver histology by reducing the grades of inflammation and stages of fibrosis and by reversal of cirrhosis. This beneficial effect was observed in studies using lamivudine,\textsuperscript{100–102} adefovir,\textsuperscript{31,32,38,103} telbivudine,\textsuperscript{57} entecavir\textsuperscript{57} and tenofovir.\textsuperscript{66} However, there remains a paucity of studies showing improvement of survival by preventing liver decompensation and HCC. In an early pivotal study of 651 patients with advanced liver disease, lamivudine significantly reduced the rate of hepatic decompensation and risk of HCC compared with placebo.\textsuperscript{26} This is confirmed by two case–control studies.\textsuperscript{27,108} Other studies have also shown improvement of liver function in patients with compensated liver disease receiving lamivudine.\textsuperscript{109–111} These results are likely related to the long-term suppression of HBV replication. Although further studies comparing NAs with placebo are unlikely to be carried out for ethical reasons, it is reasonable to extrapolate that newer and more potent antiviral NAs have similar, possibly greater, long-term benefits.

It is important to emphasize that in patients with established cirrhosis, effective viral suppression will not eliminate the risk of developing HCC. In a large retrospective analysis of 818 patients,
the risk was high in those with cirrhosis, older age and male gender. Therefore these patients warrant close monitoring and regular HCC surveillance in spite of effective antiviral therapy.

### Antiviral resistance

The major disadvantage of using NAs is the potential for the development of drug resistance mutations. HBV replication occurs at a very high rate, with approximately $1 \times 10^{11}$ virus particles being released every day. This high replication rate, coupled with the absence of an effective proofreading mechanism in the HBV polymerase enzyme, contributes significantly to the development of mutations. The error rate of HBV polymerase is estimated to be $1.4 - 3.2 \times 10^{-5}$ nucleotide substitutions per site per cycle, leading to the emergence of many natural mutants, including those conferring drug resistances. It is possible to have drug resistance mutations present already in the HBV population of treatment-naive patients. Exposure to NAs provides selective pressure favouring HBV that harbours the drug resistance mutations over wild-type HBV.

Mutations for the different NAs were described in the preceding sections and are summarized in Table 2. Drug resistance mutations can alter the interaction between the binding sites of HBV polymerase and antiviral agents. Lamivudine binds at a pocket on the surface of the polymerase formed partly by the rt204 residue. The rtM204V/I mutation responsible for lamivudine resistance causes steric hindrance between the side chain of the rtM204V/I moiety and the sulphur atom in the oxathiolane ring of lamivudine. The compensatory rtL180M mutation, in addition to restoring replication capacity to the mutants close to wild-type levels, may also indirectly affect the binding of lamivudine by introducing a side chain close to the oxathiolane ring of lamivudine. By itself, rtL180M does not lead to lamivudine resistance. However, both mutations do not affect the binding of the natural substrate, cytosine, and therefore viral replication remains active.

The rtI169T, rtS184G, rtS202G/I and rtM250V mutations (responsible for entecavir resistance) likely act in conjunction with the lamivudine resistance mutations to alter the binding of entecavir to HBV polymerase. Mutations at rt169 and rt250 may affect the primer binding region of the reverse transcriptase, and may affect elongation of the DNA strand. For agents such as adefovir and tenofovir, the acyclic structure is less affected by steric hindrance, with fewer atoms involved in the acyclic phosphonate linkage. In addition, the acyclic structure is highly flexible, allowing the drugs to bind to the mutant HBV polymerase despite minor alterations to the binding pocket.

Drugs with a low resistance barrier (such as lamivudine or telbivudine) or those with low antiviral potency at the recommended doses (such as adefovir) are associated with higher rates of resistance (see Table 3). The high barrier to resistance and antiviral potency of entecavir and tenofovir account for their extremely low resistance rates. The course of drug resistance follows a step-wise progression, with the emergence of genotypic resistance, followed by virological breakthrough, and finally culminating in biochemical breakthrough with evidence of hepatitis flares. These flares can be severe enough to cause liver decompensation and, less commonly, death.

Once resistance is evident, a second antiviral agent without cross-resistance should be added as early as possible. Previous experience with adefovir rescue therapy for lamivudine resistance has shown that add-on therapy is superior to switching therapy, with subsequent lower risk of development of resistance to adefovir. For lamivudine and telbivudine resistance, either adefovir or tenofovir is effective. Telbivudine is not recommended in the setting of lamivudine resistance, as they share the same drug resistance mutations. Although entecavir at a daily dose of 1 mg is effective against lamivudine-resistant HBV, it is not recommended, as there is a high rate of subsequent resistance to entecavir. The barrier to entecavir resistance is lowered with pre-existing lamivudine resistance mutations, since only a further single signature mutation is required for entecavir resistance.

### Combination therapy using NAs

The rationale for using a combination of NAs in treatment-naive patients can be extrapolated from the experience with HIV treatment, where combination therapy has become the cornerstone of the highly active antiretroviral therapy (HAART) regimen. In CHB, however, combination therapy has thus far not shown any conclusive additive benefit in viral suppression. In a study of 115 HBeAg-positive CHB patients randomized to receive lamivudine plus placebo or lamivudine plus adefovir, the biochemical response, virological suppression and serological response were similar between the two treatment arms. The lamivudine

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resistance rate at 2 years was lower in the combination group compared with those treated with lamivudine alone (15% versus 43%, respectively). Therefore the combination of NAs from different classes may reduce the subsequent rate of resistance to either drug. The combination of NAs from the same structural class has also been studied in patients on lamivudine and telbivudine as mono- or combination therapy.\textsuperscript{56} Drug resistance was observed in 5% of patients assigned to telbivudine monotherapy, compared with 12% of patients on combination lamivudine and telbivudine. Therefore combinations of NAs from the same structural class does not confer any benefits in lowering resistance.

In view of the very low rate of resistance development with entecavir and tenofovir in treatment-naive patients, long-term monotherapy with either agent as first-line treatment is an effective approach.\textsuperscript{131}

**Sequential therapy using NAs**

There are only a few studies looking at sequential therapy in the use of NAs. In this era of highly potent antiviral agents, there is now the option to switch to another antiviral agent if the response to the initial agent is suboptimal. In a Phase IIb trial of 246 CHB patients with persistent viraemia under lamivudine therapy, patients were randomized to continue lamivudine or switch to telbivudine.\textsuperscript{132} Compared with patients who continued lamivudine, patients switching to telbivudine had a higher mean reduction in HBV DNA from study entry to week 24 (−1.9 versus −0.9 log copies/mL, P<0.001) and a lower rate of treatment failure (5% versus 20%, P<0.001). In a small study of 44 patients on lamivudine who were switched to entecavir therapy, those patients with suboptimal viral response with lamivudine were at risk of developing subsequent entecavir resistance.\textsuperscript{133} Therefore, in patients with suboptimal response, add-on therapy using a second agent with a different resistance profile to the initial agent is likely the best approach. In the absence of documented resistance to lamivudine, and in the presence of complete viral suppression, patients can be safely switched from lamivudine to entecavir if long-term resistance remains a concern.\textsuperscript{134} In patients with suboptimal response to previous NA therapy, the use of tenofovir has been shown to be effective. The cumulative rate of complete viral suppression with tenofovir in this setting continued to increase beyond the first 24 weeks, and most with a suboptimal response at 48 weeks will likely achieve undetectability at 72 weeks or later.\textsuperscript{135,137}

A theoretical approach to sequential therapy is to achieve optimal viral suppression with a potent antiviral agent and switch to a less potent agent such as lamivudine for long-term maintenance to reduce the cost of therapy. In a study of 50 patients who achieved optimal viral suppression with entecavir, patients were randomized to continue entecavir or switch to lamivudine.\textsuperscript{135} Switching to lamivudine resulted in a virological rebound rate of 24% after 96 weeks. Therefore prior optimal viral suppression with entecavir does not confer any significant advantage if the patients continued treatment with a less potent antiviral agent, and thus this approach is not recommended.

**Safety**

NAs have become the mainstay of CHB therapy, in part due to the minimal risk of significant adverse effects. The side effects pertaining to each of the NAs were discussed previously in their respective sections. As a class effect, NAs reduce viral replication by inhibition of HBV polymerase activity. As they are synthetic analogues of naturally occurring nucleos(t)ides, there is the potential for NAs to interfere with human nuclear and mitochondrial DNA (mtDNA) polymerases, which are responsible for gene replication/repair and maintenance of mitochondrial function, respectively. Therefore all NAs carry a ‘Black Box’ warning regarding potential mitochondrial toxicity. Inhibition of mtDNA can deplete intracellular mtDNA levels, resulting in impaired oxidative phosphorylation and cellular damage.\textsuperscript{136} This may be manifested in a variety of clinical presentations, including myopathy, neuropathy, lactic acidosis, macrocytosis, nephrotoxicity, pancreatitis and steatosis.\textsuperscript{137} Fortunately the NAs approved for CHB treatment are associated with minimal mtDNA polymerase inhibition. Of all the available NAs, entecavir displayed the least evidence of mitochondrial toxicity, even at dosages exceeding 100 times the maximum concentration observed in humans.\textsuperscript{138}

**Conclusions**

Prior to the availability of NAs, there was no effective oral agent for treating CHB. NAs have revolutionized the way in which clinicians manage CHB since the introduction of lamivudine in 1998. However, the use of lamivudine is limited by the high rate of resistance occurring within a few years of treatment. Without an effective rescue agent, the decision to treat has to be weighed against the risk of developing resistance. It was not until 2002 that adefovir was approved and became available as an effective rescue agent for lamivudine-resistant HBV. Despite this, the risk of developing drug resistance remained a significant problem in the first 5 years of the new millennium.

The approvals of highly potent NAs with high barriers to resistance, such as entecavir and tenofovir, have been further milestones in CHB therapy. Although development of resistance remains a potential threat, the decision to commence NAs is now no longer heavily dependent on resistance risk. As the majority of patients will require prolonged therapy, on-going monitoring of these newer agents is essential both for long-term safety and resistance risk. With several NAs currently now available, the currently recommended strategy for treatment-naive patients is to start therapy using an NA with a low resistance rate. Both entecavir and tenofovir are recommended as first-line treatment agents. Current evidence does not recommend combination therapy for treatment-naive patients.

In spite of the rapid advances made with NAs over the last decade, complete eradication of HBV remains an elusive goal. The current long-term goals should be the prevention of cirrhosis, liver decompensation and HCC. In patients with established fibrosis/cirrhosis, long-term goals should also include regression of histological stage. Future potential agents targeting different aspects of the HBV replication cycle, including entry of HBV, viral packaging, secretion of HBV and clearance of cccDNA, may eventually lead to complete clearance of HBV.
Transparency declarations
None to declare.

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