Antiviral Therapy of Chronic Hepatitis B

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Abstract
Since the licensing of lamivudine in 1999, the treatment of chronic hepatitis B has been revolutionized by the introduction of oral nucleoside and nucleotide analogues (NAs), which act as inhibitors of the HBV polymerase. The effectiveness of the first of these substances was limited by incomplete response and resistance development in many patients, but today, highly potent substances are available that make a reliable and durable suppression of HBV replication, a reduction of necroinflammatory activity in the liver, and even a reversion of liver fibrosis achievable for almost all patients. Beyond that, NA treatment can prevent the development of hepatocellular carcinoma in many patients. HBeAg seroconversion appears in approximately 50% of all HBeAg-positive patients during NA treatment. However, the ideal treatment endpoint, the serologic loss of HBsAg, remains a rare event almost exclusively achievable for HBeAg-positive patients. After cessation of the treatment, HBV replication tends to relapse in most patients, which is why the duration of NA treatment is indefinite. Future treatment strategies should aim at tailoring individual NA treatment regimens to increase HBs loss rates and optimize treatment duration.

Introduction
Nucleos(t)ide analogues (NAs) represent two different subclasses of polymerase inhibitors; while both are based on purines or pyrimidines, acyclic nucleotide analogues have an open (acyclic) ribose ring that confers greater binding capacity to resistant hepatitis B virus (HBV) polymerase variant (fig. 1). Due to their high effectiveness, safety, and uncomplicated once daily oral fixed dosage, NAs have become the global mainstay of HBV treatment [1–3].

The decision to use NAs instead of pegylated interferon-α (PEG-IFNα) for treatment of chronic hepatitis B can be based on many factors. Among these are contraindications to or poor tolerability of PEG-IFNα, as well as the presence of negative predictors for response to PEG-IFNα such as high viral load, negative HBeAg, or presence of HBV genotypes C or D. In HBeAg positive patients, the serologic end points of HBV treatment as HBeAg seroconversion and HBsAg loss or seroconversion can be achieved in a similar proportion of patients by both treatment options, so the treatment duration will be finite. In contrast, in HBeAg negative patients, there is no defined treatment end point. However, new treatment approaches with NA treatment of a defined duration are currently investigated, and it can be assumed that discontinuation of NA treatment can increase the rate of serologic responders among HBeAg negative patients.
Mechanism of Action

NAs inhibit HBV DNA synthesis by competitive interaction with the natural substrates of the HBV polymerase, as in the case of adefovir and tenofovir deoxyadenosine triphosphate. Once integrated, they lead to termination of the nascent DNA chain. Their effectiveness is determined by their bioavailability, specific binding affinity to the HBV polymerase, and genetic barrier against resistance of the HBV, and varies between substances (fig. 2). Beyond their antimetabolite activity, NAs have been described to exert immunostimulatory effects in vitro [4, 5]. However, it is unclear if and to what extent these effects contribute to their antiviral potency.

Treatment Options

The nucleoside analogues lamivudine, telbivudine, and entecavir, and the acyclic nucleotide analogues adefovir dipivoxil and tenofovir disoproxil fumarate are currently licensed for the treatment of chronic hepatitis B. Owing to their strong antiviral potency and high genetic barrier against resistance, current guidelines for the treatment of chronic hepatitis B recommend entecavir and tenofovir as preferred first-line NAs; however, according to their availability, all licensed NAs may be used for treatment [1–3]. Lamivudine, a (−) enantiomer of 2′-3′ dideoxy-3′-thiacytidine, is a nucleoside analogue that was approved for the treatment of chronic HBV infection in 1999 with a daily dose of 100 mg. Long-term lamivudine treatment is associated with an increasing rate of antiviral drug resistance reaching approximately 70% after 5 years in patients with HBeAg-positive HBV infections. Therefore, lamivudine is no longer considered a first-line treatment [1–3].

Adefovir dipivoxil is an oral diester prodrug of adefovir, an acyclic nucleotide adenosine analogue that is active in its diphosphate form. Adefovir was approved as the first substance with simultaneous activity against wild-type and lamivudine-resistant HBV variants in the USA in 2002 and in Europe in 2003. It is active in vitro against a number of DNA viruses other than HBV and retroviruses (e.g. HIV). The antiviral effect of adefovir in the licensed dosage of 10 mg/day is rather low in comparison to other available antivirals (fig. 2), a disadvantage making it vulnerable for resistance development and inappropriate for the use as first-line monotherapy [1–3].

Telbivudine is a thymidine analogue which is active against HBV, but at least in vitro not active against other viruses, including HIV. At a dose of 600 mg/day telbivudine has higher antiviral activity compared to either lamivudine 100 mg/day or adefovir 10 mg/day. More patients achieved an HBeAg loss within 48 weeks as compared to other NAs (fig. 2) [6].

Entecavir, a cyclopentyl guanosine nucleoside analogue, is a highly selective inhibitor of HBV replication which was licensed in 2006. Entecavir is a potent inhibitor of wild-type mutants, but less effective against lamivudine-resistant HBV mutants. Treatment-naïve HBeAg-
positive patients achieved undetectable HBV DNA levels in 67 and 74% after 1 and 2 years, respectively, of ETV treatment, reaching up to 94% after 5 years [7]. So far, the rate of resistance at 6 years of treatment is approximately 1.2% for treatment-naïve patients [8]. Loss of HBsAg occurred in 5% of treatment-naïve individuals after 2 years of ETV therapy [9].

Fig. 2. One-year efficacy of NAs currently approved for the treatment of chronic HBV infection [3]. Treatment efficacy is defined by suppression of serum HBV DNA levels below the limit of detection, ALT normalization and rates of HBeAg seroconversion. Differences in antiviral efficacies of the substances have to be interpreted with caution (no head-to-head studies). LAM = Lamivudine; ADV = adefovir dipivoxil; ETV = entecavir; L-dT = telbivudine; TDF = tenofovir disoproxil fumarate.

Tenofovir disoproxil fumarate, an ester prodrug form of tenofovir [(R)-9-(2-phosphonomethoxypropyl)], is an acyclic nucleoside phosphonate, or nucleotide analogue, closely related to adefovir. Tenofovir has selective activity against retroviruses and hepadnaviruses, and is approved for the treatment of HIV infection and chronic hepatitis B. Tenofovir showed sustained antiviral ef-
Efficacy over 7 years (HBV DNA <400 copies/ml) in almost all treatment-naïve HBeAg-negative and -positive patients, and also in patients with prior treatment failures (up to 99%) [10–12]. HBeAg loss and HBeAg seroconversion were found in 55 and 40% of treatment-naïve patients, respectively. Of the HBeAg-positive patients, 12% experienced HBsAg loss. Development of HBV resistance against tenofovir has not yet been observed [10, 13].

Endpoints of Treatment with NAs

The aim of treatment of HBV infections is to prevent the development of long-term complications such as liver cirrhosis and hepatocellular carcinoma (HCC), and to increase survival [1–3]. The main goal is the sustained suppression of HBV replication (fig. 3). The success of antiviral treatment can be monitored by viral and patient-related surrogate parameters (table 1). The rationale for these endpoints is described below.

Suppression of HBV Replication

The natural course of HBV infections was assessed in 3,774 untreated HBV-infected patients over a mean period of 11.4 years in Taiwan in the REVEAL study [14, 15]. The strongest predictor for development of cirrhosis and HCC was found to be the level of HBV DNA at the start of observation. In multivariate models, the relative risk of cirrhosis increased when HBV DNA reached levels >300 copies/ml. Individuals with HBV DNA levels ≥10⁴ copies/ml (or ≥2,000 IU/ml) were found to have a 3- to 15-fold greater incidence of HCC as compared to those with a viral load <10⁴ copies/ml. On the other hand, the risk for these complications was shown to be decreased by treatment with NAs [16, 17]. The complete and sustained suppression of HBV replication as measured by HBV DNA in serum has therefore become the key endpoint for the treatment of chronic HBV infection in recent treatment guidelines [1–3]. Because highly potent

Table 1. Summary of response criteria for treatment with NAs

<table>
<thead>
<tr>
<th>Virologic response</th>
<th>Biochemical response</th>
<th>Histologic response</th>
<th>Long-term effects</th>
</tr>
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<tbody>
<tr>
<td>Sustained decrease of HBV DNA to levels &lt;2,000 IU/ml</td>
<td>Sustained ALT normalization</td>
<td>Reversion of fibrosis</td>
<td>Avoidance of liver cirrhosis and HCC</td>
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<tr>
<td>(or &lt;10,000 copies/ml), ideally to &lt;60 IU/ml (or &lt;300 -</td>
<td></td>
<td>Reduction of inflammatory activity</td>
<td></td>
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<tr>
<td>copies/ml), as measured by a highly sensitive assay</td>
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<tr>
<td>Sustained HBeAg seroconversion in HBeAg-positive patients</td>
<td>Loss of HBsAg with or without appearance of anti-HBs</td>
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<tr>
<td>Biochemical response</td>
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NAs with reliable and safe long-term performance like entecavir and tenofovir have become available, this endpoint is achievable for the vast majority (>95%) of patients [7, 9, 10].

**HBeAg Seroconversion**

Seroconversion from detectable serum HBeAg to undetectable HBeAg and detectable anti-HBe is an immunologic event, which is followed by a period of inactive carrier status. This period is characterized by very low or undetectable HBV replication and normal serum transaminases, and antiviral treatment is often no longer necessary [18]. HBeAg seroconversion will be achieved by increasing frequency over 5–7 years of treatment with NAs in up to 40–50% of patients [7, 10]. Immunotolerant patients who are characterized by positive HBeAg, high HBV DNA levels, and normal ALT levels show equally strong suppression of HBV DNA levels during treatment as compared to patients with increased inflammatory activity; however, only 5% of patients showed HBeAg seroconversion after 192 weeks of treatment with tenofovir or tenofovir plus emtricitabine [19]. Some observations, however, reveal that HBeAg reversion may occur in up to 30% of patients after cessation of NA treatment [20, 21]. Therefore, according to the current guideline, patients who achieved HBeAg seroconversion should receive consolidation treatment after HBeAg seroconversion for at least 1 year before the treatment is stopped. Another concept would be to continue NA treatment until the occurrence of HBeAg loss. Even after HBeAg seroconversion or HBsAg loss, those patients should still be monitored for HBV replication and progression of liver disease on a regular basis [1]. For the probability of the development of complications, it seems to be important at which point in time HBeAg seroconversion occurs. In a recent long-term observational study in 483 HBeAg-positive patients achieving spontaneous HBeAg seroconversion, it was shown that the incidence of cirrhosis and HCC was lower for patients who had achieved HBeAg seroconversion at the age <30 years compared to patients who achieved seroconversion at an age >40 years [18].

The mechanism underlying the immunologic phenomenon of HBeAg seroconversion is not yet fully understood, and it has also not been shown how suppression of HBV replication by NAs can trigger or accelerate this phenomenon. It has, however, been demonstrated that the T cell response against HBV can be impaired by viral proteins, and the assumption was made that long-term treatment with NAs may restore the T cell function and support immune control of the infection [22, 23].

**HBsAg Loss**

Because HBsAg loss or seroconversion are associated with a complete and definitive remission of the activity of chronic hepatitis B and an improved long-term outcome, it is regarded as a cure for chronic hepatitis B and an ideal endpoint of antiviral treatment [1–3].

**Regression of Liver Fibrosis**

Very recently, regression of fibrosis was demonstrated in a subanalysis of the tenofovir trials 102 and 103 evaluating 348 patients who provided paired biopsies before and after 5 years of treatment [24]. The majority (88%) of these patients had an improvement in overall liver histology as defined by an improvement of at least 2 points in the Knodell score of histologic activity index. Even in a subgroup of patients who had liver cirrhosis at the start of treatment, a reversion to lower degrees of fibrosis was observed in 70%. A decrease of 2 points or greater in the Knodell necroinflammatory score was also found in 96% of 59 patients providing paired biopsies before and after 3–7 years of treatment with entecavir [7]. Therefore, it can be assumed that effective treatment with NAs generally leads to similar improvements in liver fibrosis.

**Prevention of Complications**

Effective long-term suppression of HBV replication with NAs reduces complications such as liver cirrhosis and the development of HCC. This effect is especially pronounced in patients with liver cirrhosis [16, 25, 26]. However, a period of effective suppression over 4–5 years seems to be a prerequisite before the incidence in HCC starts to decrease, as it was recently shown for treatment with tenofovir [16]. Treatment with entecavir was also shown to decrease the incidence of liver decompensations and HCC in a retrospective European cohort study of 372 patients [27]. However, even under complete suppression, the risk for HCC development remains elevated, especially in those patients with liver cirrhosis.

**Treatment Indication**

The most important parameters for the indication are serum levels of HBV DNA and ALT activity; however, recent treatment guidelines describe different conditions in which a treatment should be started (table 2). All patients with liver cirrhosis or high-grade liver fibrosis and any measurable serum HBV DNA should be considered for long-term antiviral therapy with a highly potent NA [1–3].

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Prediction of Response to NA Treatment

Prediction of HBV DNA Suppression
The serum level of HBV DNA was shown to be associated with the treatment duration necessary to suppress HBV DNA to undetectable levels [10]. Incomplete suppression is characterized by persistent HBV replication during antiviral therapy. Ongoing HBV replication during treatment with NAs should be avoided to prevent the selection of resistant HBV strains; however, this risk seems negligible during first-line treatment with entecavir or tenofovir, and suppression to undetectable levels during follow-up treatment can be expected [7, 9, 10].

Prediction of HBeAg Seroconversion
Several baseline factors including low HBV DNA levels, high ALT levels (above 2–5 × ULN) and presence of HBV genotype A are predictive for HBeAg seroconversion [28–31]. Additionally, an early decrease in HBV DNA levels during treatment is associated with a higher rate of patients losing HBeAg [29–31]. As HBsAg loss during NA treatment occurs almost exclusively in patients who were initially HBeAg positive, HBeAg seroconversion can be considered a predictor for HBsAg loss [10].

Prediction of HBsAg Loss
HBsAg loss or seroconversion seems to occur only in a limited number of patients receiving NA treatment [32]. During long-term treatment with tenofovir, these patients were characterized by an early decrease in HBsAg levels and HBsAg loss for up to 5 years of subsequent treatment. In all other patients, HBsAg levels decreased only gradually [10, 32]. It therefore seems rather unlikely that a prolongation of NA treatment beyond 5 years will be associated with a linear increase of the rates of HBsAg loss. Also, HBsAg loss was almost exclusively observed in patients with genotypes A or D [10].

Experimental Markers for Response to NA Treatment
Using highly potent NAs, suppression of serum HBV DNA can be achieved in almost all patients, including those with and those without long-term response. Reliable markers for treatment response could help to tailor NA treatments and to estimate their duration. Markers for response to NAs which are currently investigated are host or virus related. Thus, HBeAg seroconversion was shown to be predictable by a decrease in the levels of HBeAg after 6 months of entecavir treatment [33]. Higher serum levels of the interferon-inducible protein 10 have recently been shown to be associated with a greater...
decrease in HBsAg levels during treatment with entecavir in a cohort of 114 European patients, an observation which had also been previously made in a smaller cohort of patients receiving treatment with different NAs [34, 35]. Other viral markers like core-related antigen or cytokines such as interleukin-22 which show an association to response to NA treatment are currently being investigated [36].

As for HBeAg seroconversion, variants within the host’s innate immune system were shown to be associated with HBsAg loss [37, 38]. Thus, it could be shown that the single nucleotide polymorphism TLR4 rs4986790 is significantly associated with spontaneous HBsAg loss [37]. Novel treatment approaches targeting TLR 7 have been shown to induce strong decreases in serum HBsAg levels, which supports the concept of an important role of the innate immune system in HBsAg clearance [39].

**HBV Resistance against NAs**

If a confirmed re-increase of HBV DNA >1 log during ongoing treatment with NAs is observed, HBV resistance development can be suspected [1–3]. This virologic breakthrough is often followed by a biochemical breakthrough, which is characterized by a re-increase of formerly normal ALT levels [1–3]. Resistance of HBV against NAs is mediated by variants in the HBV polymerase gene, which decrease the susceptibility of the virus. NAs show similar mutation pathways and cross-resistance within their group, which is due to their conformational similarities (fig. 1). Mono-therapy with lamivudine, adefovir, or telbivudine is frequently associated with spontaneous resistance development and should therefore be avoided [1, 3]. Entecavir has a very high genetic barrier against resistance, but resistance occurs often in patients with preexisting lamivudine resistance. Tenofovir is the only NA for which no resistance development was shown, even though there seems to be a certain cross-resistance to adefovir, which may lead to a slightly reduced susceptibility, leading to a slower decline in HBV DNA and to incomplete virologic responses in some patients [40–42].

**Combination of NAs versus Monotherapy for the Management of HBV Resistance**

The recent treatment guidelines tend to recommend combination regimes including one nucleotide and one nucleoside analogue for patients with HBV resistance against NAs [1–3]. Patients with resistance to lamivudine should be treated with add-on combination of tenofovir; however, tenofovir has also been shown to be effective as monotherapy in patients with lamivudine resistance [1, 3, 12]. The AASLD guidelines also give the option of switching these patients to entecavir, but this is associated with an increased risk of entecavir resistance [3]. Resistance to adefovir should be treated by adding lamivudine, switching or adding to entecavir according to AASLD, and by switching to tenofovir plus a nucleoside analogue according to the EASL guidelines (in the EASL guidelines, a more detailed treatment algorithm taking into account different HBV mutations is given) [1]. A recent study, however, found monotherapy with tenofovir is not inferior to a combination of tenofovir and the nucleoside analogue emtricitabine in patients with incomplete response or resistance to adefovir [11]. Suppression of HBV DNA under the limit of detection was observed in 84 and 82% of the patients after 168 weeks, respectively, and no viral breakthrough was observed in either group. The combination of tenofovir and emtricitabine was also not superior to tenofovir in patients with lamivudine resistance, leading to suppression of HBV DNA to undetectable levels in 86 and 89% of the patients after 96 weeks of treatment, respectively [12]. In two uncontrolled studies, some patients with a high viral load and resistance to adefovir showed an incomplete response to tenofovir monotherapy, but no viral breakthrough was observed in these studies [41, 42].

Resistance to entecavir should be treated by adding tenofovir. According to these observations, tenofovir seems to be able to suppress HBV replication under the limit of detection as monotherapy in the presence of most HBV variants, including those conferring resistance to lamivudine and adefovir. A combination treatment might still be safe and timely if an incomplete response to tenofovir is suspected.

**Combination Treatments beyond Resistance Management**

**Combination of NAs in Treatment-Naïve Patients**

A combination therapy with lamivudine and adefovir showed no difference in the virologic and biochemical response when compared to a monotherapy with lamivudine in studies of treatment-naïve patients with intermediate-to-high viral loads [43]. The combination of lamivudine with telbivudine also showed no benefit compared to a monotherapy with either drug in this setting [44]. However, in immune-tolerant patients with a mean viral load >8 log10 IU/ml, stronger suppression of HBV
DNA to undetectable levels after 192 weeks was found in patients receiving tenofovir plus emtricitabine as compared to those receiving tenofovir alone (76 vs. 55%; p = 0.011) [19]. The combination of tenofovir plus entecavir could also show a stronger antiviral efficacy than a monotherapy with entecavir over a duration of 100 weeks in patients with HBV DNA ≥ 10⁸ IU/ml who were noncirrhotic, treatment naïve, and HBeAg positive; however, in patients with lower viral loads there was no benefit for this combination treatment and longer follow-up in these patients might adjust the rate of viral suppression between the two regimens [45]. The same combination treatment was shown to be safe and effective in 24 patients with advanced fibrosis, so it might be used in patients with liver cirrhosis and high viral replication in which a fast suppression of HBV replication is needed [46].

**Combination of NAs and PEG-IFNα**

Treatments which were started as a combination of PEG-IFNα with lamivudine, adefovir, or entecavir could not show a benefit in suppression of HBV DNA levels or serologic response [47–49]. The combination treatment with telbivudine and PEG-IFNα was shown to lead to peripheral neuropathy in 9 of 48 (18.8%) patients, and only in 10 of 3,500 (0.28%) patients who received telbivudine as monotherapy – therefore, it should not be applied [50]. However, other combination therapies of PEG-IFNα and NAs may be promising. Recently, a switch to PEG-IFNα after 4 years of complete response to entecavir was shown to be associated with higher HBeAg and HBsAg loss and seroconversion rates as compared to a control group in which entecavir was continued as monotherapy [51]. Similar studies are currently being undertaken to investigate combination treatment of PEG-IFNα and tenofovir.

**Future Directions**

Current NA-based treatments for chronic hepatitis B are effective and safe, but many clinical needs are unmet. A treatment that can clear HBsAg or even cccDNA would be the ultimate goal. According to our knowledge, HBV infections require lifelong NA treatment for most patients; however, finite treatment strategies have shown that some HBeAg-negative patients may benefit from treatment termination after long-term response, and those strategies should be more refined in future studies [55, 56]. Add-on of or switch from NAs to PEG-IFNα may induce a decrease in HBsAg levels in patients with a suppression of HBV DNA, but stable HBsAg levels during long term treatment. New concepts combining immune modulation and antiviral activity are currently under investigation (i.e. agonists of Toll-like receptors), and also an inhibitor of HBV entry, the lipoprotein Myrcludex-B is currently investigated for its clinical value. In this context, novel response markers may help to tailor individual treatment regimes. These improvements of the management of HBV infections are within reach and should be studied. A new formulation of tenofovir alafenamide (TAF) has probably a better renal safety profile and may replace TDF in the future; however, novel NAs or treatment strategies targeting beyond NAs will probably not be available in the close future.

**Safety and Renal Side Effects of NA Treatment**

NAs are generally well tolerated. Adefovir and tenofovir may damage renal tubular cells and cause nephrotoxicity, but the risk seems to be low and the damage is mostly reversible [10, 52]. Nevertheless, patients treated with these drugs should be monitored for renal dysfunction [1]. Tenofovir has been reported to cause loss in bone mineral density in patients infected with human immunodeficiency virus, though this phenomenon has not been noted in patients with HBV monoinfection [53]. The same phenomenon has not been reported in patients with chronic hepatitis B [10]. Entecavir may cause severe lactic acidosis in patients with impaired liver function and a MELD score of ≥ 20 points [54]. Interestingly, some patients receiving telbivudine showed an increase in glomerular filtration rates, especially those with preexisting mild renal impairment [36]. However, it is not clear if this potential benefit can outweigh adverse effects of telbivudine, which are neuropathy and creatine kinase elevation [6].

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