Recent studies suggest that long-term suppression of viral replication is critical to reducing the complications of chronic hepatitis B virus (HBV) infection. Monitoring for continued virological response during and after treatment is essential because current treatment options have limited success in achieving durable endpoints, and antiviral resistance may emerge during long-term therapy. Methods of monitoring treatment response include tests for serum aminotransferase levels, HBV DNA level, hepatitis B e antigen (HBeAg) and antibody (anti-HBe), hepatitis B surface antigen (HBsAg) or antibody (anti-HBs), and liver histology. Virological suppression and loss of HBeAg or HBsAg with or without seroconversion play a prominent role in decision-making regarding the success and duration of antiviral therapy. Guidelines recommend that testing for serum markers be repeated every 12-24 weeks during antiviral therapy and every 6-12 months afterward. Recent data also suggest that serum HBV DNA levels should be assessed at weeks 12 and 24 of therapy, because early viral response may predict the likelihood of sustained response and antiviral resistance. The use of serum HBV DNA levels for this purpose requires an assay with a wide range of quantification, such as real-time polymerase chain reaction assays, which have a 7-8 log\text{10} dynamic range. Newer, investigational methods for monitoring treatment response include quantitative measurement of HBsAg, HBeAg, and intrahepatic covalently closed circular DNA. Conclusions: Better methods for defining durable treatment endpoints are needed. Other areas requiring further research include the optimal treatment duration and the establishment of the optimal use of early viral kinetics for decision-making during antiviral therapy. (HEPATOLOGY 2009;49:S166-S173.)
Methods of Monitoring Treatment Response

Antiviral therapy for chronic hepatitis B appears to reduce the risk of long-term complications such as cirrhosis and hepatocellular carcinoma. Because complications tend to occur after decades of infection and often long after treatment has been initiated, clinical trials and practicing clinicians utilize surrogate, short-term markers of risk and treatment benefit. Each of the surrogate markers, including serum aminotransferase levels, serum HBV DNA level, hepatitis B e antigen (HBeAg) or antibody to HBeAg (anti-HBe), hepatitis B surface antigen (HBsAg) or antibody to HBsAg (anti-HBs), and liver histology, has been used as a measure of the response to antiviral therapy. Indeed, several definitions of treatment response have been enumerated at previous National Institutes of Health conferences and have been used variably in clinical trials of antiviral therapy: biochemical response (normalization of serum alanine aminotransferase [ALT]); virological response (decrease in serum HBV DNA or loss of HBeAg with or without the development of anti-HBe); histological response (improvement in the histology activity index by at least two points without worsening of fibrosis score as compared to pretreatment biopsy); and complete response (biochemical and virological response with loss of HBsAg). Recent studies favor using durable HBV DNA suppression as the primary measure of therapeutic success.

The serum ALT level is the most simple and accessible means of evaluating treatment response. Biochemical response is the most frequently achieved treatment response in clinical trials, occurring in 40%-70% of patients on antiviral therapy. The serum ALT level is commonly used as an indirect marker of histological activity, with normalization of the ALT level during the course of antiviral therapy thought to reflect improvement in hepatic necroinflammation. Serial measurement of ALT levels may reflect treatment-associated hepatitis flares or the emergence of antiviral resistance. Serum ALT is one of the few measures of early treatment response in patients with HBeAg-negative chronic hepatitis B.

The primary drawback of using serum ALT as a marker of treatment response is its limited predictive value. In the early trials of lamivudine therapy, not all patients achieving biochemical response (70%) had improvement on histological evaluation (56%). A recent retrospective study of more than 1000 patients with chronic hepatitis B revealed that a substantial proportion of patients with persistently normal ALT levels (<40 U/L) have significant fibrosis or inflammation on biopsy. In that study, 40% of patients with HBeAg-positive chronic hepatitis B and 13% of those with HBeAg-negative disease with normal serum ALT had a fibrosis stage greater than 2. The normal range of serum ALT (30-50 U/L) used in clinical trials of antiviral therapy may be too high to identify at-risk patients accurately because the standard reference rates do account for such factors as alcohol habits, diabetes, or obesity. Lower thresholds (19 U/L for women and 30 U/L for men) may be more appropriate. However, one retrospective study found that the prevalence of advanced histology (defined as stage 2 fibrosis or stage 1 fibrosis with at least grade 2 inflammation) in patients with HBeAg-positive or HBeAg-negative chronic hepatitis B and an ALT level 1 to 1.5 times the upper limit of the more stringent normal range was 20% and 27%, respectively. These data suggest that serum ALT does not alone suffice as a marker of disease activity and should be interpreted in the context of serum HBV DNA level and histology. The utility of ALT is also limited for patients with a normal ALT level at the start of therapy.

Reduction in the level of serum HBV DNA or virological response is the earliest and perhaps most appropriate measure of treatment response. HBV DNA reduction during antiviral therapy appears to be a good surrogate for histological improvement. In a review of 26 prospective studies of treatment of chronic hepatitis B, investigators found a significant correlation between HBV DNA levels and histological grading at the end of treatment in patients with HBeAg-positive chronic hepatitis B treated with nucleoside analogs. Specifically, they noted that a 1 log_{10} reduction in median serum HBV DNA level corresponded with a two-point decrease in median histological grading, meeting criteria for histological response. Other studies have shown that early reduction in serum HBV DNA predicts the likelihood of sustained virological response to antiviral therapy. Of note, maintained HBV DNA suppression is the only reliable measure of ongoing treatment response in the majority of patients who do not achieve durable serological endpoints.

The use of serum HBV DNA level as a method of assessing treatment response has been complicated by a lack of standardization in the unit of measure and in the criteria for treatment response used in clinical trials. To reconcile the heterogeneity in assays, the World Health Organization created the international unit (IU/mL), a standardized unit of measure (corresponding to approximately 5-6 copies/mL for most amplification assays), which should now be used in all commercial HBV DNA quantitative assays and reported in clinical trials.

The commercially available HBV DNA assays differ in their limits of detection and dynamic range, as summarized in Table 1. The use of serum HBV DNA level as a measure of treatment response requires quantification...
over a wide range, usually at least 5 \( \log_{10} \) units. Real-time polymerase chain reaction (PCR) is a technique that amplifies and simultaneously quantifies a DNA sample. Current real-time PCR assays possess a 7 or 8 \( \log_{10} \) dynamic range, which permits accurate characterization of pretreatment viral loads that may be in the hundreds of millions, as well as the low viral loads (as low as 30 IU/mL) that may be seen in treatment-induced suppression or in inactive carriers.\(^{17,18}\) This wide range of detection allows accurate quantification of the early response to therapy. For assays with more limited range, specimens at the upper end may need to be diluted to extend their range. Consistent use of the same assay is recommended in assessing treatment response. Eventually, the primary goal of therapy will likely be to achieve undetectable serum HBV DNA by the most sensitive assays.

The serological markers of treatment response tend to correlate with improvement in other surrogate markers, including serum ALT and HBV DNA levels. For example, anti-HBe seroconversion is more common in patients experiencing full suppression on therapy and rare in those with persistent viremia.\(^{19,20}\) The most durable treatment endpoint is HBsAg loss with or without anti-HBs seroconversion. However, this serological endpoint occurs in fewer than 2% of patients taking nucleoside analogs for 1 year and 3%-8% of patients receiving interferon or peginterferon.\(^{7}\) Unfortunately, in HBeAg-negative disease, HBsAg loss is the only meaningful serological endpoint. In HBeAg-positive disease, serological markers of treatment response include both HBeAg and HBsAg loss. Treatment cessation is possible after these endpoints occur because they are more durable than HBV DNA suppression alone.

Histological response, defined as an improvement in the histology activity index (HAI) of two points or more or improvement in the fibrosis score, has been used as the benchmark for treatment response in clinical trials. Antiviral therapy has been shown to retard the progression or cause regression of fibrosis in chronic hepatitis B.\(^{21,22}\) The threshold for improvement used in clinical trials has not been demonstrated to be predictive of long-term outcomes prospectively. However, one retrospective study suggests that changes in liver histology of this magnitude may be a valid surrogate endpoint for antiviral therapy. For 10 years, investigators followed 89 patients who were treated with interferon alfa and underwent liver biopsies either 6 or 12 months after the completion of therapy. As a group, patients who experienced liver-related complications had a median one-point rise in the modified HAI score, whereas patients without complications had a median reduction of one point in the modified HAI score at the end of therapy.\(^{23}\) Because sampling error, intraobserver variability, and discrepancies in pretreatment and posttreatment biopsy size may account for some of the differences observed in pretreatment and posttreatment biopsies, a better surrogate for long-term risk reduction is a combination of biochemical, virological, and histological improvement. Indeed, in view of the risks of liver biopsy and the demonstrated predictive strength of virological endpoints, histology now appears to be a less useful endpoint in clinical practice.

### Treatment Endpoints

Each of the surrogate markers of treatment response can be considered endpoints of therapy, although virological suppression and loss of HBeAg and HBsAg play a more prominent role in decision-making with respect to antiviral therapy.

In HBeAg-positive disease, short-term treatment endpoints include HBeAg loss with or without anti-HBe seroconversion. Seroconversion occurs in approximately 20% of patients in the first year of antiviral therapy and increases with longer duration of therapy. It is sustained in 50%-90% of treated patients depending on the duration of treatment after seroconversion, patient age and serum HBV DNA level at the end of treatment.\(^{24}\) Treatment-induced seroconversion is associated with suppressed HBV DNA and a reduced risk of cirrhosis.\(^{25}\)

In a small proportion of patients experiencing HBeAg loss, loss of HBsAg or anti-HBs seroconversion also occurs, often after the treatment period. In long-term follow-up of patients who received standard or pegylated interferon, HBsAg loss occurred in 11% of patients over 3-5 years.\(^{25,26}\) In another, smaller study of patients who experienced seroconversion during interferon therapy,
HBsAg loss continued after therapy at a rate of approximately 8% per year for a cumulative rate of 86% by 9 years. Long-term complications occur at a negligible rate after the development of anti-HBs.

For patients with HBeAg-positive chronic hepatitis B in whom seroconversion does not occur but HBV DNA is fully suppressed on therapy, continued normalization of the ALT and sustained HBV DNA suppression are the only markers of successful treatment and the absence of antiviral resistance, as observed in HBeAg-negative hepatitis.

In HBeAg-negative disease, loss of HBsAg is the only clear indication for stopping therapy. It tends to occur after long-term HBV DNA suppression. Among patients receiving adefovir therapy, 5% had HBsAg loss over a 5-year period. Short of this endpoint, treatment goals in HBeAg-negative chronic hepatitis B include HBV DNA suppression and normalization of ALT levels.

For both HBeAg-negative and HBeAg-positive disease, sustained virological suppression is a critical on-treatment endpoint. Indeed, a recent phase 3 trial comparing tenofovir and lamivudine for the treatment of chronic hepatitis B used reduction in serum HBV DNA as a primary treatment outcome. However, questions remain regarding the optimal use of serum HBV DNA for decision-making during therapy. Clinical trials have used inconsistent target levels for response in part based on the HBV DNA assay utilized, and a discriminatory threshold for HBV DNA in predicting risk of progression remains to be established, particularly with the use of highly sensitive assays. Evidence-based guidelines for starting and stopping therapy based on serum HBV DNA levels are needed.

The two remaining treatment endpoints, normalization of serum ALT levels and histological improvement, are not particularly helpful for decision-making during antiviral therapy for the reasons discussed already.

**On-Treatment Milestones and Their Predictive Value**

Serum HBV DNA level and its decline at various time points during antiviral therapy are likely to play an increasing role in dictating the course of therapy. Because its levels reflect viremia, reduction in HBV DNA is the most direct marker of antiviral effect. Recent studies suggest that early viral kinetics during antiviral therapy may predict the likelihood of sustained virological response in chronic hepatitis B, as it does in chronic hepatitis C.

The Globe Study Group evaluated 1370 patients with chronic hepatitis B randomly assigned to receive telbivudine (600 mg daily) or lamivudine (100 mg daily). During therapy, patients were categorized as having undetectable HBV DNA (<300 copies/mL) or having varying degrees of residual viremia. Patients with undetectable serum HBV DNA at 24 weeks had an HBeAg seroconversion rate of 41% at 1 year, compared to just 4% in those with HBV DNA in excess of 4 log_{10} copies/mL. Resistance rates for the same groups at 1 year were 2% and 15%, respectively. In multivariate analysis, undetectable HBV DNA at week 24 was the best predictor of clinical and virological efficacy (independent of HBeAg status).

These findings have been confirmed by several smaller studies, which are summarized in Table 2. In 56 patients receiving adefovir therapy after the development of lamivudine resistance, 87% of patients who experienced a decline in serum HBV DNA by more than 3 log_{10} at 3 months lost HBeAg, as compared to only 24% of patients who had less than a 3 log_{10} decline. A study evaluating the predictive value of HBV DNA levels at numerous time points during lamivudine therapy found that HBV DNA levels at week 4 (<2000 IU/mL) and week 16 (<800 IU/mL) were the best predictors of a combined endpoint of anti-HBe seroconversion, ALT normalization, and absence of lamivudine-resistance mutations after 5 years of treatment. All patients with HBV DNA levels lower than these cutoffs achieved the combined endpoint. In contrast, the probability of failing to meet this endpoint at year 5 in patients with HBV DNA levels above these cutoffs were 84% and 88%, respectively. In a study of 66 patients receiving peginterferon and lamivudine (with the majority starting peginterferon 8 weeks prior to lamivudine), 27% had a sustained virological response, defined as loss of HBeAg, anti-HBe seroconversion, and HBV DNA <10,000 copies/mL at 1 year.

### Table 2. Predictive Value for Outcomes of Early HBV DNA Measurements During Antiviral Therapy

<table>
<thead>
<tr>
<th>Time</th>
<th>Antiviral</th>
<th>Predictive Level</th>
<th>Serocconversion</th>
<th>Resistance Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 4</td>
<td>LMV</td>
<td>HBV DNA &lt;2000 IU/mL</td>
<td>100% PPV for HBeAg seroconversion</td>
<td>–</td>
</tr>
<tr>
<td>Week 8</td>
<td>PEG/LMV</td>
<td>HBV DNA &gt; 10^6 copies/mL</td>
<td>92% PPV for HBeAg seroconversion</td>
<td>–</td>
</tr>
<tr>
<td>Week 12</td>
<td>ADV</td>
<td>&lt;3 log_{10} decline in HBV DNA</td>
<td>87% HBeAg seroconversion (vs. 24% if &lt;3 log_{10})</td>
<td>–</td>
</tr>
<tr>
<td>Week 24</td>
<td>TEL/LMV</td>
<td>HBV DNA &lt;300 copies/mL</td>
<td>41% HBeAg seroconversion (vs. 4% if &gt;10^4 copies/mL)</td>
<td>2% (vs. 15% if HBV DNA &gt;10^4 copies/mL)</td>
</tr>
<tr>
<td>Week 24</td>
<td>LMV</td>
<td>HBV DNA &lt;200 copies/mL</td>
<td>–</td>
<td>8% (vs. 64% if HBV DNA &gt;10^4 copies/mL)</td>
</tr>
</tbody>
</table>

ADV, adefovir; IU, international units; LMV, lamivudine; NPV, negative predictive value; PEG, pegylated interferon; PPV, positive predictive value; SC, seroconversion; TEL, telbivudine.
Receiver operating characteristic (ROC) curves were used to identify predictors of sustained response. The area under the ROC curve for HBV DNA level was greatest at week 8 and yielded a negative predictive value of 92%, meaning that patients without early virological response were highly unlikely to experience sustained response.

Of great concern, patients who do not experience early and significant HBV DNA suppression are far more likely to develop nucleoside antiviral resistance. In one study, patients with serum HBV DNA levels in excess of $10^3$ copies/mL after 6 months of lamivudine therapy had a 63% chance of developing YMDD-resistance mutations. Although these data do not yet permit definitive decisions regarding treatment duration to be made based on early virological response, they highlight the importance of assessing early predictors of sustained virological response prospectively in large clinical trials.

A group of expert hepatologists recently proposed a “roadmap” for antiviral therapy based on early on-treatment response. They recommended measurement of HBV DNA at 12 and 24 weeks to predict the likelihood of sustained virological response. The panel recommended that patients with full HBV DNA suppression (<60 IU/mL) at 24 weeks continue the same antiviral agent. They recommended that patients with a partial response to antiviral therapy (60-2000 IU/mL) have an agent with a different resistance profile added if the initial drug had a low genetic barrier to resistance, or undergo further monitoring without a change in therapy until 48 weeks if they were receiving an agent with a high genetic barrier. For patients with an inadequate response to therapy at 24 weeks (>2000 IU/mL), the panel recommended adding a more potent drug. The proposed roadmap applies to patients receiving nucleoside analogs, but an analogous approach may offer a stopping rule for peginterferon. The applicability of the current roadmap to entecavir and tenofovir, which have excellent suppressive efficacy and little long-term resistance to date, is unclear.

**Duration of Therapy Based on Treatment Response**

Early studies of antiviral therapy in HBeAg-positive chronic hepatitis B suggested that HBeAg loss (or HBeAg seroconversion) was sustained in more than 70% of patients. Later studies showed relapse rates as high as 50%, which was attributed to duration of therapy after HBeAg loss. Subsequently, one group reported results of treating 85 patients with an additional 24 months of lamivudine therapy following HBeAg seroconversion: the rates of relapse following cessation of therapy were 13% at 1 year and 16% at 2 years, suggesting that long-term administration of antiviral therapy might enhance the durability of response. A second group evaluated patients randomized to 6 or 12 months of lamivudine consolidation therapy following HBeAg seroconversion and reported no difference in relapse rates between the two groups, with a 59% posttreatment virological relapse rate at 2 years in the 6-month group and 50% in the 12-month group. The investigators reported that HBV DNA level at the time of treatment cessation was the strongest predictor of relapse: patients with DNA less than 200 copies/mL had a 37% relapse rate compared to 73% in those with HBV DNA levels above $10^3$ copies/mL. Guidelines now generally recommend either 6 or 12 months of consolidation therapy following seroconversion.

In patients with chronic hepatitis B who are HBeAg-negative, the optimal duration of therapy is unknown. Although clinical trials have typically treated patients for 48 weeks, more than 90% of patients with complete HBV DNA suppression on nucleoside therapy relapse within 1 year after discontinuing therapy. As a result, treatment of HBeAg-negative disease with oral antiviral agents is often continued until HBsAg loss occurs or, more commonly, until antiviral resistance and biochemical relapse develops. The duration of interferon therapy in HBeAg-negative disease is typically predetermined rather than based on clinical endpoints. In general, longer duration of therapy appears to be better than shorter duration, but the value of extending therapy from 24 to 48 weeks and to beyond 48 weeks is unknown. The rate of sustained virological response following interferon therapy appears to be better than that of nucleoside therapy, with one study showing 17% of patients having undetectable HBV DNA (<400 copies/mL) after 4 years of follow-up.

**Frequency and Timing of Monitoring**

The optimal frequency and timing of monitoring during and after antiviral therapy is not known. The American Association for the Study of Liver Diseases guidelines recommend that during the course of antiviral therapy, patients undergo assessment of liver tests every 12 weeks and HBV DNA levels every 12-24 weeks. The guidelines also recommend that HBeAg and anti-HBc testing be performed every 24 weeks in HBeAg-positive chronic hepatitis B, and HBsAg testing every 6-12 months in patients who are HBeAg-negative with persistently undetectable levels of HBV DNA. According to the guidelines, patients receiving interferon or peginterferon should have regular assessment for safety with complete blood count checked each month and a test for thyroid stimulating hormone every 12 weeks. Safety issues also dictate that serum creatinine (with or without phosphate levels) be monitored every 12 weeks.
tested every 12 weeks in patients taking adefovir or tenofovir.

The guidelines from European Association for the Study of the Liver suggest that following antiviral therapy, serum studies such as liver biochemistries, HBV DNA level, HBeAg, and anti-HBe be checked every 1-3 months for the first 12 months after treatment cessation and then every 6-12 months. They also recommend yearly HBsAg tests in patients with sustained virological response to monitor for late clearance. In the long-term, the single most important test for demonstrating sustained response is the serum HBV DNA level. The recommendations for the frequency of serum monitoring during and after antiviral therapy are summarized in Table 3.

### Monitoring Tools that Require Further Study

Other serum markers may eventually prove useful for monitoring the effect of antiviral therapy. Serum hepatitis B core antigen (HBcAg) measurement is less expensive and easier to perform than HBV DNA measurement, and may be a reliable method of predicting treatment response. A recent study compared HBcAg levels in 54 untreated individuals and 39 patients treated with either lamivudine or entecavir for 48 weeks and found that the logarithmic reduction in HBcAg correlated with logarithmic reduction of the serum HBV DNA, total intrahepatic DNA, and hepatic covalently closed circular DNA (cccDNA) levels. Patients with a baseline HBcAg concentration of <40,000 kU/mL or a concentration <200 kU/mL at week 24 of treatment were more likely to have undetectable HBV DNA at week 48.

Similarly, quantitative measurement of serum HBcAg may be used to predict seroconversion. In 271 patients who received 48 weeks of peginterferon in a phase 3 trial, HBcAg levels declined consistently throughout the course of therapy in patients who experienced HBeAg seroconversion. After 24 weeks of treatment, patients with the highest HBcAg levels (>100 Paul Ehrlich Institute unit [PEIU]/mL) had a 4% chance of seroconversion. The negative predictive value (96%) of high week 24 HBcAg was superior to that of HBV DNA (86%).

HBcAg quantitation may also be a useful method for predicting sustained virological response. In 386 patients treated with peginterferon or lamivudine, investigators observed a significant association between on-treatment HBsAg decline (>1 log_{10}) and HBcAg clearance 3 years after the cessation of therapy. Similarly, in another 48 patients receiving peginterferon, a decrease in HBsAg levels by 0.5 log_{10} at 12 weeks had positive and negative predictive values of 89% and 90%, respectively, for sustained response. The predictive values of a 1 log_{10} decline in HBsAg at week 24 were even higher. These findings raise the possibility of using quantitative HBsAg as an alternative to serum HBV DNA as an early on-treatment response predictor.

Intrahepatic cccDNA level may be a superior predictor of treatment response compared to serum HBV DNA level because it accurately reflects viral persistence during antiviral therapy. In 47 patients with HBeAg-positive chronic hepatitis B who were receiving lamivudine monotherapy or lamivudine and peginterferon combination therapy, the positive and negative predictive values of a −0.8 log_{10} genome copies/cell decline in cccDNA were 56% and 86%, respectively, for sustained virological response. Similarly, 32 patients receiving 48 weeks of adefovir therapy experienced a significant decline in cccDNA of −0.8 log genomes/cell. The decline in cccDNA correlated with reduction in the serum HBsAg titer. Prospective studies are needed to define the cccDNA threshold under which host-mediated control of viral replication is possible. Unfortunately, quantitation of cccDNA by real-time PCR is not yet standardized and requires a liver biopsy.

### Conclusions and Needs for Future Research

Periodic serum HBV DNA monitoring should now be viewed as the principal means of monitoring therapy and assessing response and will, therefore, play an increasing role in the management of chronic hepatitis B.
role in decision-making during antiviral therapy. Serological endpoints are the most desirable durable endpoints. Histological endpoints will likely play a supportive role in management of chronic hepatitis B, although the utility of cccDNA measurement in predicting sustained virological response merits further evaluation. Future research should be aimed at defining criteria for starting and stopping therapy based on HBV DNA level; how to use on-treatment HBV DNA values at defined time points as predictors of therapeutic outcome; and defining threshold levels that predict lack of disease progression and permit rational cessation of therapy. In coming years, the use of standardized measures of HBV DNA is critical, as is the use of consistent measures of outcome in prospective clinical trials so that treatment and monitoring guidelines can be clearly defined and universally applicable. In addition, better methods for defining durable endpoints for antiviral therapy should be explored. The optimal duration of therapy after seroconversion in HBeAg-positive disease, particularly in those with residual viremia, and an appropriate treatment endpoint, short of HBsAg loss, in HBeAg-negative disease must be defined so that indefinite therapy can be averted in large numbers of patients.

References
