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Systematic review: combination therapies for treatment-naïve chronic hepatitis B

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SUMMARY

Background
There is a renewed interest in use of combination therapies in treatment-naïve chronic hepatitis B (CHB) because of limitations of monotherapies.

Aim
To discuss the current status of combination therapies in treatment-naïve CHB.

Methods
PubMed search was done using 'combination', 'sequential' and 'chronic hepatitis B' as the search terms.

Results
The two most popular combination therapies include 'combination of nucleos(t)ide analogues' and 'combination of interferons and nucleos(t)ide analogues'. Combination therapies using two nucleos(t)ide analogues do not lead to higher long-term efficacy. However, addition of a nucleos(t)ide analogue with a good resistance profile to a nucleos(t)ide analogue with a lower genetic barrier to resistance decreases the risk of emergent resistance to the latter. Greater sustained virological, biochemical and seroconversion rates are observed with addition of lamivudine to conventional interferon, but pegylated-interferon monotherapy is equally effective as combination with lamivudine. Again, resistance to lamivudine is lower with its combination with interferons.

Conclusions
The answer to the question whether hepatitis B can be treated better with combination or monotherapy remains largely unknown. Additional trials are warranted of combination therapies of peginterferon and potent nucleos(t)ide analogues or therapies with the combined use of nucleos(t)ide analogues or immunomodulators.

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INTRODUCTION

More than 400 million people worldwide are chronically infected with hepatitis B virus (HBV).[1] Effective therapy is necessary to prevent the progression of chronic hepatitis B (CHB) to cirrhosis, hepatocellular carcinoma and death.

Agents currently used for the treatment of chronic HBV infection are divided into two main groups based on their primary mode of action: the immunomodulators e.g. interferons (IFNs), thymosin, interleukins, etc. and the nucleos(t)ide analogues.

IFNs [including standard IFN and pegylated IFN (peg-IFN)] and other immunomodulators act by promoting cytotoxic T-cell activity for lysis of infected hepatocytes and by stimulating cytokine production for control of viral replication. The introduction of nucleos(t)ide analogues (lamivudine, adefovir, entecavir, telbuvidine, tenofovir, etc.) heralded a new era in the treatment of CHB, and provided a safe, effective and well-tolerated alternative to IFN. Nucleos(t)ide analogues target the reverse transcriptase of HBV and are potent inhibitors of viral replication. Although treatment with nucleos(t)ide analogues profoundly suppresses serum HBV DNA levels and response can be maintained over prolonged periods with ongoing therapy, response to treatment may not be durable in a large proportion of patients after discontinuation of therapy, indicating the necessity for a long-term, and maybe indefinite, treatment.[2, 3] However, development of anti-viral resistance is a major limitation to long-term efficacy of nucleos(t)ide analogues.[4]

HBV therapy must provide potent long-term viral suppression and at the same time avoid development of resistance. To prevent development of anti-viral drug resistance, a judicious use of nucleos(t)ide analogues in patients with chronic HBV infection is needed.[5] The first manifestation of anti-viral resistance is virological breakthrough, which is defined as $a > 1\ \log_{10}$ increase in serum HBV DNA from nadir during treatment in a patient who had an initial virological response. It is usually also followed by a biochemical breakthrough. Emergence of anti-viral resistance can eventually lead to reversion of virological and histological improvement, and enhance the rate of disease progression.[6] The best strategy to avoid the emergence of drug resistance during therapy is to suppress viral replication strongly.[7] A diversity of viruses (quasispecies), including mutants with mutations potentially associated with drug resistance, may exist prior to therapy.[8] Moreover, development of mutant populations is replication-dependent, and resistance emerges only when replication occurs in the presence of the drug selection pressure. Complete suppression of viral replication therefore allows little opportunity for resistance to develop.[9] Several studies have shown that an initial virological response is associated with lower rates of anti-viral drug resistance in HBV patients in the long term (see below). Therefore, anti-viral therapy, once initiated, should aim to suppress viral replication as quickly and completely as possible.

EARLY HBV DNA RESPONSES TO PREDICT LONG-TERM RESPONSES AND RESISTANCE

Recently, the importance of HBV DNA responses to nucleos(t)ide analogues early during the therapy in predicting sustained response and development of resistance has become known. In a recently published study, to determine the optimal time and HBV DNA level during an early treatment period for the prediction of the response after a 5-year lamivudine treatment, HBV DNA levels at various time periods until year 5 were measured in 74 HBeAg-positive chronic HBV patients receiving lamivudine treatment. Seventeen patients achieved an ideal response [HBV DNA level <2000 copies/mL (400 IU/mL), HBeAg seroconversion, normal alanine aminotransferase levels and absence of YMDD mutations] at year 5. Receiver operating characteristic curves showed good predictions as early as week 4. The areas under the curve for weeks 4 and 16 were 0.89 and 0.94, respectively. Predictive indices revealed 4 and 3.6 log-copies/mL (2000 and 800 IU/mL, respectively) to be the best cut-off HBV DNA levels for these two times, respectively. All patients with HBV DNA levels lower than these respective cut-off levels at the two times achieved an ideal response at year 5. Patients with HBV DNA levels above these cut-off values had 83.8% and 87.7% chances of not achieving an ideal response at year 5, respectively.[10]

HBV DNA responses at week 24 have also been assessed for predicting long-term responses and resistance development. A phase 3 telbivudine vs. lamivudine study showed that low HBV DNA levels at week 24 were associated with favourable 1-year efficacy outcomes. Of HBeAg-positive patients at week 24, 41% with undetectable levels of HBV DNA on PCR

underwent seroconversion by week 52 as compared with 4% of patients with more than 4 $\log_{10}$ copies of HBV DNA per millilitre. At week 24, in HBeAg-positive patients in both treatment groups combined, resistance occurred in only 2% of patients who were negative for HBV DNA on PCR, compared to 15% of patients with viral loads above 4 $\log_{10}$ copies/mL. A similar pattern was evident for HBeAg-negative patients.[11]

Adefovir dipivoxil leads to a slower suppression of viraemia than other nucleoside analogues, i.e. lamivudine, entecavir or telbivudine. Therefore, the week 48 time point may be used for predicting resistance to adefovir therapy.[12] In HBeAg-negative patients treated with adefovir dipivoxil for 192 weeks, patients with HBV DNA levels 1000 copies/mL after 48 weeks of therapy had a higher risk (49%) of developing adefovir resistance at week 192 than patients with a viral load <1000 copies/mL at week 48 (6%).

Recently, the 'roadmap concept has been proposed', which recommends monitoring of serum HBV DNA levels to identify outcomes of therapy during nucleos(t)ide analogue therapy. Early monitoring of the virological response to therapy in CHB treated with oral nucleos(t)ides is essential to identify primary treatment failure at week 12 and suboptimal responses at week 24 to modify management accordingly. This roadmap suggests assessment of primary nonresponse at week 12 and of early predictors of efficacy at week 24. The failure to achieve a 1 $\log_{10}$ copies/mL decline in viral load after 12 weeks of therapy is considered a primary nonresponse. It indicates that either there is a compliance issue or that the medication does not exhibit its anti-viral activity in a given patient. When a suboptimal response is identified, anti-viral treatment should be modified. Many experts would choose to switch to a more potent nucleos(t)ide analogue at this interval. The week 12 time point is therefore important to determine the anti-viral activity of the treatment regimen. Assessment of early predictors of efficacy has been suggested at week 24. If at week 24, complete virological response (PCR negative) is achieved, therapy should be continued with the same drug; if there is a partial virological response (HBV DNA ≥300 to <10 000 copies/mL), a second drug with a different genetic mutation profile should be added if the original drug had a low genetic barrier or treatment should be continued beyond 48 weeks with monitoring every 3 months; if there is an inadequate virological response (≥10 000 copies/mL), more potent drug should be added and monitoring should be continued every 3 months.[13] However, prospective testing of this strategy is necessary; also, the timing of treatment modification may depend on the drug used and on the kinetics of viral load decay (patients starting from very high viral load may need additional weeks of therapy to reach the threshold of HBV DNA for treatment modification). Also, whether such a strategy may be applicable to drugs other than nucleos(t)ide analogues like immunomodulators is uncertain. Further studies are needed to clarify these issues.

LIMITATIONS OF CURRENTLY ESTABLISHED APPROACHES

Limitations with regard to virological responses

(i) Potent nucleos(t)ide analogues have recently become available. For example, the 4-year data on entecavir suggest cumulative rates of HBV DNA undetectability exceeding 90% in HBeAg-positive patients after four years and, a cumulative resistance rate of <1%.[14] Tenofovir also demonstrates potent suppression, with 93% and 76% of HBeAg-negative and HBeAg-positive patients having undetectable HBV DNA, respectively, after 1 year, and no genotypic resistance.[15, 16] However, even when HBV DNA becomes undetectable, HBeAg seroconversion does not ensue at proportional frequency. Discrepancies exist between the degree of viral suppression and HBeAg seroconversion. These agents have proven to be effective in suppressing HBV DNA, but, HBeAg seroconversion rates have not improved substantially relative to those obtained with earlier treatments.

(ii) An nucleos(t)ide analogue(s) that allows for cessation of therapy with an acceptable rate of subsequent relapse is still lacking, especially for HBeAg-negative patients.

(iii) The rates of inducing HBsAg loss, with the current nucleos(t)ide analogues are low, with 3% of HBeAg-positive patients receiving tenofovir[16] and 5% of HBeAg-positive patients receiving entecavir[17] achieving this milestone after 1 and 2 years, respectively.

Limitations with regard to development of resistance

All long-term nucleos(t)ide analogues are capable of selecting for resistance, although the resistance

rates with drugs having a high genetic barrier to resistance have been low in the follow-up till now. Nevertheless, these drugs are not entirely free from a risk of resistance and more resistance might be encountered as the duration of follow-up and experiences with these drugs further increase.

COMBINATION THERAPIES

All the above limitations have led to an interest in combination regimens for CHB. In theory, at least, combination therapy might improve upon monotherapy with regard to any or all of the above limitations. Agents acting through different mechanisms can provide a more effective viral suppression leading to more seroconversion, more HBsAg clearance and more durable off-treatment response and reduce the risk of viral mutations. This synergy may lead to a more effective eradication, a shorter duration of therapy and dose reductions resulting in fewer drug side-effects. Support for these concepts comes from the studies in human immunodeficiency virus (HIV) and chronic hepatitis C (CHC), where combination therapies have been proven to be more effective than monotherapy. The potential disadvantages could be higher costs, increased toxicity and drug interactions. Many options are available for combination therapies (Table 1).

Table 1. Types of combination therapies

Two or more nucleos(t)ide analogues
Simultaneous (all nucleos(t)ide analogues started simultaneously)
Add-on (adding another nucleos(t)ide analogue when there has been suboptimal response to initial monotherapy at a specified time point)
Immunomodulators plus nucleos(t)ide analogues
IFNs (standard or peg-IFN) plus nucleos(t)ide analogues
Simultaneous (no precombination phase) with or without anti-viral continuation in postcombination phase.
Sequential (a precombination phase) with or without anti-viral continuation in postcombination phase.
Interleukin 12 plus lamivudine
IFNs plus ribavirin
Thymosine plus IFN plus lamivudine
Two immunomodulators

COMBINATION THERAPY USING NUCLEOS(T)IDE ANALOGUE(S)

Hypothesis

Although all available nucleos(t)ide analogues target the viral polymerase, they have different mechanisms of action on the viral genome replication machinery.[18] Depending on the drug, this inhibitory activity can affect the priming of reverse transcription, viral minus-strand DNA synthesis or plus-strand DNA synthesis (Figure 1). Lamivudine is mainly an inhibitor of minus-strand formation, while clevudine has been shown to affect both minus- and plus-strand DNA synthesis.[19] Adefovir and tenofovir are active on the priming of reverse transcription as well as on elongation of viral minus-strand DNA.[20, 21] Entecavir inhibits both minus- and plus-strand DNA synthesis.[22] Telbivudine is also supposed to inhibit all three enzymatic activities.[23] It is unknown if anti-viral potency is affected by more than one site of inhibition.

The premise of using nucleos(t)ide analogue combinations is that the combination of nucleos(t)ide analogues with different sites of actions could enhance the response rates, decrease the anti-viral resistance, prevent the formation of covalently closed circular DNA (cccDNA) in newly infected cells, decrease the pool of cccDNA in already chronically infected cells in a more effective way than monotherapy and all these effects on HBV replication could result in restoration of immune response to achieve a sustained control of viral replication.

Combination of nucleos(t)ide analogues can be used in two ways:

(i) *Simultaneous combination therapy*. Both the nucleos(t)ide analogues are started simultaneously.

(ii) *Add-on combination therapy*. Adding another nucleos(t)ide analogue when there has been suboptimal response to initial monotherapy at a specified time point (see above).

Experimental data

It has been shown that anti-viral synergy could be obtained by combining adefovir, lamivudine and penciclovir in duck HBV-infected primary hepatocyte cultures.[24] In a hepatoma cell line expressing wild-type HBV, an additive effect was also observed with the combination of adefovir and thymidine analogues (lamivudine, emtricitabine and telbivudine).[25]

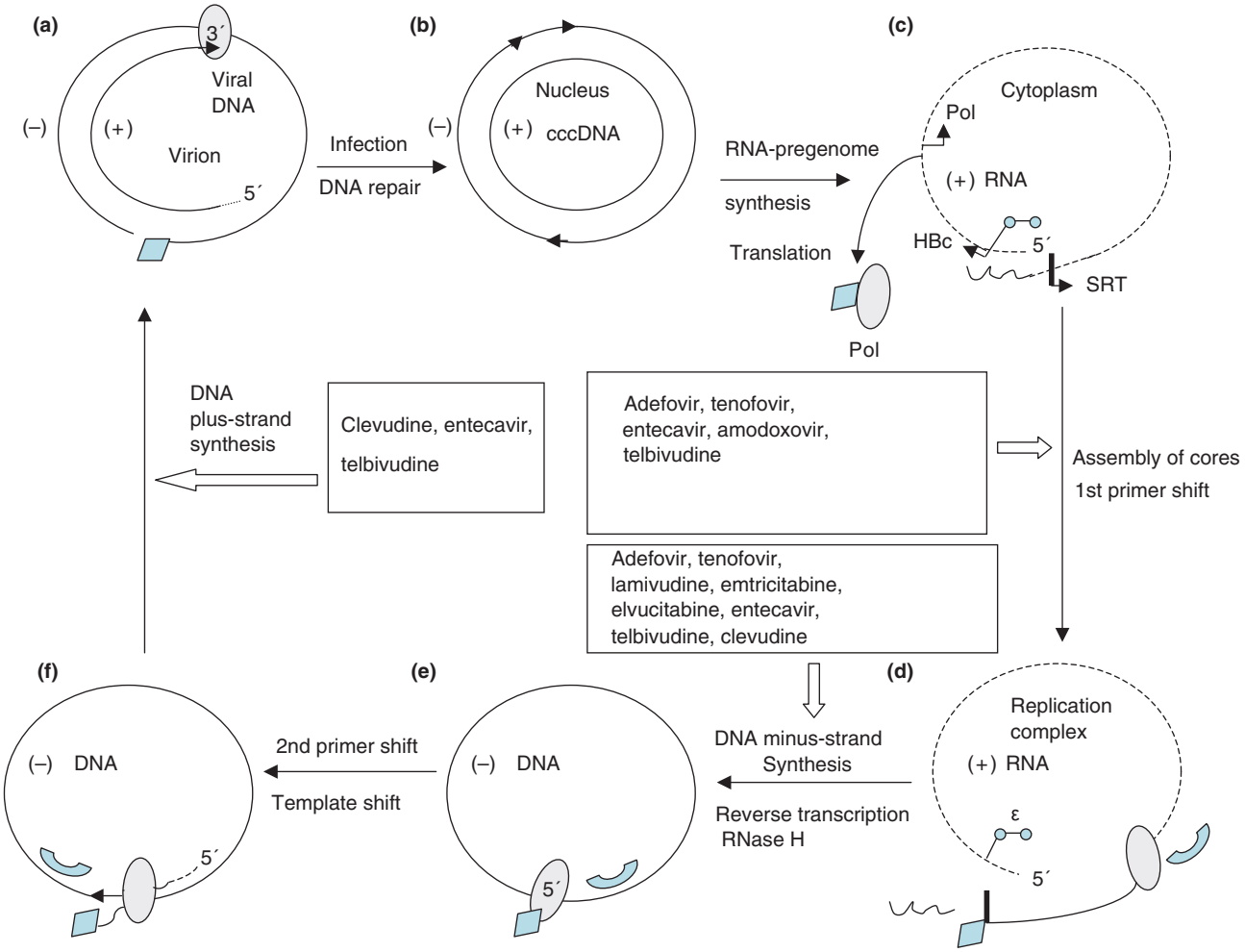

Figure 1. Replication of HBV genome and site of action of different anti-viral agents: the virion DNA after entering the cell nucleus (a) is converted to covalently closed DNA (cccDNA) (b). This episomal DNA is transcribed to various RNAs, one of which serves as a template for the polymerase protein and HBc protein. These two proteins assemble together with their mRNA to the replication complex. The encapsidation signal ε at its 5′ end governs the packaging of the RNA and the priming of the minus-strand DNA (c). The redundant part at 3′ end serves as a signal for reverse transcription after priming. The primase domain of the polymerase serves as a primer for the reverse transcription. Thus, the growing minus-DNA strand is linked at its 5′ end to the primase (d). The reverse transcription proceeds until the 5′ end of the RNA template is reached (e). Thus a short redundancy is generated in the minus-strand. The RNase H activity associated with the reverse transcriptase degrades the RNA template and leaves at its 5′ end an 18-base-long capped RNA fragment, which functions as primer for the plus-strand DNA (f). The DNA polymerase is able to cross the discontinuity in the minus-strand template because of its short terminal redundancy. Thereafter, the structure of virion DNA is reproduced. Various nucleoside and nucleotide analogues act on three different sites: the priming of reverse transcription, elongation of minus-strand DNA or elongation of plus-strand DNA.

One of the major problems of anti-viral therapy of chronic HBV infection is the effect of anti-HBV agents on *de novo* cccDNA formation after viral entry. Even the combination of amdoxovir, emtricitabine and clevudine could not prevent the formation of cccDNA in experimentally infected primary hepatocytes.[26]

Another issue is to determine whether anti-viral therapy may have an effect on already formed cccDNA in chronically infected cells, thus resulting in cell curing. It was shown that a combination of nucleoside analogues may have an additive effect on the intracellular cccDNA levels in already infected cells, which suggests that the additive effect observed on viral

DNA synthesis may also result in an additive effect on cccDNA levels.[26] Therefore, this implies an important role of combination therapy to decrease intrahepatic viral load during long-term therapy.

Clinical data

Clinical responses. In a viral dynamics study comparing the effectiveness of HBV viral suppression by lamivudine monotherapy with that of lamivudine plus famciclovir simultaneous combination therapy in Chinese patients with chronic HBV infection, 21 Chinese HBeAg-positive patients, were randomized to receive either lamivudine 150 mg/day orally (n = 9) or lamivudine 150 mg/day plus famciclovir 500 mg three times a day orally (n = 12) for 12 weeks, with a follow-up period of at least 16 weeks. It was found that the viral load decay was biphasic in both groups of patients. Mean $\log_{10}$ HBV viral decline at week 12 were 1.8 ± 0.2 for lamivudine alone group and 2.5 ± 0.8 for the combination group. The mean antiviral efficacy was significantly poorer with lamivudine alone than for lamivudine plus famciclovir (0.94 ± 0.03 vs. 0.988 ± 0.012, $P = 0.0012$). The fraction of baseline viral production persisting during therapy was 1.2% for the combination compared to 6% for lamivudine alone, a difference of fivefold. This increased efficacy of combination therapy translates into a larger first phase of 1.9 $\log_{10}$ compared to 1.1 $\log_{10}$ for lamivudine alone. The $t_{1/2}$ (days) of free HBV virus and infected cells were similar in both groups (2.3 ± 1.4 vs. 1.8 ± 1.1 and 37 ± 21 vs. 47 ± 52, P = not significant).[27]

The phase 2 trial in nucleos(t)ide naïve HBeAg-positive patients of telbivudine featured three arms: lamivudine alone, telbivudine alone and telbivudine combined with lamivudine.[28] The degree of viral suppression after 1 year was no greater with combination therapy than with telbivudine, although each was superior to lamivudine, and combination therapy was actually slightly inferior to telbivudine monotherapy in attaining therapeutic response (HBV DNA <5 $\log_{10}$ copies/mL coupled with HBeAg loss or normalization of ALT levels) (53%, 77% and 63% for lamivudine, telbivudine and the combination arms, respectively). Although the reasons for these observations are not clear, it seems wise to avoid using drugs with cross-resistance, such as lamivudine and telbivudine, in combination.

A study assessing the combination of emtricitabine plus clevudine (n = 82) for 24 weeks found that, after 24 weeks post-treatment, higher proportion (30% vs. 14%, $P = 0.007$) of patients in the combination arm had HBV DNA<4700 copies/mL along with normal ALT compared to emtricitabine alone (n = 81). In this study, 52% of patients were HBeAg-positive and 34% were treatment-naïve. The safety profile was similar between arms during treatment, with less post-treatment exacerbation of hepatitis B in the combination arm.[29]

In a placebo-controlled study in nucleos(t)ide naïve HBeAg-positive patients of lamivudine monotherapy (n = 57) vs. lamivudine plus adefovir (n = 54), reductions in HBV DNA were comparable between the two treatment arms at week 16 and during the first 52 weeks, but after 104 weeks, median HBV DNA reductions were −3.41 log and −5.22 log, respectively. Similarly, HBV DNA was <200 copies/mL in 41% and 40% at 52 weeks, 14% vs. 26% at 104 weeks and 5 vs. 6% at week 128. HBeAg seroconversion was found in 17% and 10% at 52 weeks, 20% vs. 13% at 104 weeks and 17 vs. 23 at week 128.[30, 31]

In yet another study,[32] comparing adefovir (n = 16) alone to a combination of adefovir plus emtricitabine (n = 14), in treatment-naïve HBeAg-positive patients for 96 weeks, a significant advantage for combination therapy was achieved, with median HBV DNA declines of −3.98 $\log_{10}$ copies/mL vs. −5.30 $\log_{10}$ copies/mL for monotherapy and combination therapy, respectively, at 96 weeks ($P = 0.05$), and HBV DNA <300 copies/mL in 37.5% vs. 78.5%. There was no difference in the incidence of HBeAg seroconversion despite the difference in viral suppression.

Therefore, combination therapies using nucleos(t)ide analogues lead to higher viral suppression although it may not be sustained in long-term therapy or post-therapy cessation. Also higher suppression of the virus does not translate into higher rates of seroconversion in HBeAg-positive patients.

Breakthrough and development of resistance. In the placebo-controlled study in nucleos(t)ide naïve HBeAg-positive patients of lamivudine monotherapy vs. lamivudine plus adefovir,[30, 31] a higher rate of viral breakthrough was seen in the monotherapy group than in the combination group (44% vs. 19%). In the lamivudine monotherapy group, the M204V/I mutation was detected in 20% and 43% at weeks 52 and 104, compared to 9% and 15% at the same time points in the combination therapy group.

The N236T mutation was noted in only one adefovir recipient.

In the study[32] comparing adefovir alone to a combination of adefovir plus emtricitabine, in treatment-naïve HBeAg-positive patients for 96 weeks, the four viral breakthroughs in the study occurred at 64 weeks and beyond; three of these occurred in the combination group but none was associated with drug-resistance mutations. Thus, in this study, there was a complete absence of resistance to emtricitabine. In contrast, in the emtricitabine monotherapy trial, resistance occurred in 13% at 48 weeks.[33]

The results of these trial indicate that the addition of a nucleos(t)ide with a good resistance profile to a nucleos(t)ide with a lower genetic barrier to resistance effectively decreases the risk of emergent resistance to the latter drug.

Summary and future strategies

The situation of anti-HBV therapy is different from the antiretroviral therapy of HIV infection. The antiretroviral drugs belong to several different classes of compounds, which target different steps of the viral life cycle and HIV drug resistance emerges very rapidly during monotherapy. The beneficial effect of combinations therefore could be shown in short-term trials in terms of viral load decline, prevention of drug resistance and decrease in mortality rate.[34] In contrast, in the setting of CHB, anti-virals belong to the same class of nucleos(t)ide analogues and target the viral polymerase (see above). This might be the reason why the combination of nucleos(t)ide analogues did not show any long-term additive effect in terms of viraemia decline compared to the most potent anti-viral drug in the combination. HBV drug resistance emerges relatively slowly compared to HIV infection. Several drugs with different cross-resistance profiles are now available. Clinical experience has shown that the combination of nucleoside analogues with complementary cross-resistance profile is an effective strategy to manage patients developing resistance during nucleos(t)ide therapy.[35] Obviously, these observations cannot be extrapolated to the question of how frequently resistance will emerge when the two drugs are co-administered to treatment-naïve patients. The newer nucleos(t)ide analogues (entecavir, tenofovir, etc.) have a robust resistance profile in nucleos(t)ide naive patients during the first few years of therapy. The benefit of combination therapy in terms of decreasing the resistance development will therefore be difficult to demonstrate in short-term trials. Therefore, future trials of combination therapy should target the following three groups of patients. (i) Patients with the highest risk of resistance development during therapy with nucleos(t)ide analogues; for example, patients with long-standing infection and high viraemia levels associated with more complex viral populations (quasispecies), which are associated with a more rapid hepatocyte turn-over, in turn generating a wider replication space.[36, 37] (ii) Patients who can least afford to develop anti-viral drug resistance from a clinical perspective; for example, patients with liver cirrhosis and/or with HBV recurrence after liver transplantation. (iii) When there has been suboptimal response to initial monotherapy at a specified time point, such as 24 weeks of a drug with a low genetic barrier to resistance or 1 year of a drug with a high barrier (i.e. add-on combination therapy). Individual features and limitations of the individual agents incorporated into combination regimens need to be considered. Additional trials of combination therapy of anti-virals using potent nucleos(t)ide analogues with robust long-term resistance profiles, for treatment-naïve HBV infection are warranted, emphasizing on serological and virological endpoints, such as greater rates of HBV DNA suppression, HBeAg seroconversion, HBsAg clearance, accelerated cccDNA clearance, development of resistance and the capacity to stop therapy without virological relapse at a definable time point. Also, trials comparing combination therapy vs. early add-on therapy in case of partial response should be conducted and both these strategies should be compared to monotherapy with the most potent nucleos(t)ide analogues, such as entecavir and tenofovir.

Combinations of nucleos(t)ide analogues with drugs with other mechanisms of action, such as immunomodulatory agents, small interfering RNAs (siRNAs), etc., have also been tried as combination therapies in CHB.

IFNS PLUS NUCLEOS(T)IDE ANALOGUES

Hypothesis

IFNs have only mild virus-suppressive activity, but can induce an effective host immune response in susceptible patients, whereas nucleos(t)ide analogues have a marked virus-suppressive activity in a majority of patients, but have not been shown convincingly to have clinically relevant immunomodulatory effects.

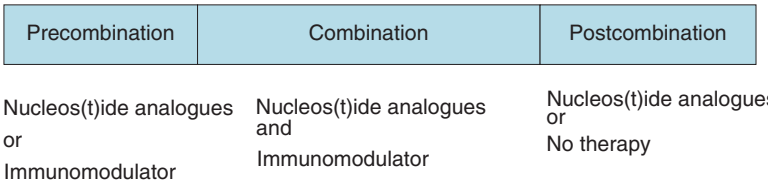

Figure 2. The three phases of combination therapies with anti-viral and immunomodulators.

Therefore, the combination of the two could possibly provide both viral suppression and immunmodulation and hence increase the response rate. The results of such combination therapies have been mixed. Variability of the results could be due to variability in the three potential phases of the combination therapies (Figure 2).

Combination therapies of nucleos(t)ide analogues and immunomodulators can have three phases: Precombination phase, combination phase and postcombination phase (Figure 2). One or more of them could be clubbed to achieve the desired results.

Precombination phase. Adding a precombination phase could qualify for a sequential therapy. During this phase of treatment, either of the drugs (i.e. nucleos(t)ide analogues or immunomodulator) is used alone, at least once.

Combination phase. During this phase, both the nucleos(t)ide analogues and the immunomodulator are used together throughout the treatment period.

Postcombination phase. In this phase, either nucleos(t)ide analogues are continued or no therapy is given.

When there is no precombination phase, it is called simultaneous combination therapy. The approach in combination therapy with the precombination phase merits to be called 'sequential combination therapy'. With the 'sequential combination therapy', an important issue remains unanswered; whether to use a nucleos(t)ide analogue or an immunomodulator in the precombination phase. The hypothesis for the use of sequential combination therapy using nucleos(t)ide analogues in the precombination phase is that a low pre-treatment serum HBV DNA level is associated with an increased probability of response to IFN-α. The most important factor known to predict favourable response to IFN-α is low baseline HBV DNA levels.[18, 19] It has been shown that the decrease in viral load induced by lamivudine therapy is associated with the subsequent restoration of the CD4 and then the CD8 cellular immune response against HBV.[38] Enhanced T-cell reactivity is observed with rapid and profound suppression of HBV DNA levels.[39] Lowering HBV DNA levels by lamivudine before starting peg-IFN has been shown to be superior to using peg-IFN alone.[40]

Another important issue is whether to continue nucleos(t)ide analogues in the postcombination phase. The premise of continuing anti-virals in the postcombination phase is that prolonged use of nucleos(t)ide analogues has been shown to improve therapeutic response, suppress and eliminate the cccDNA and maintain seroconversion.[41]

Theoretically, the ideal combination therapy could be one that uses nucleos(t)ide analogues in precombination phase to reduce the circulating virus prior to introducing immunomodulators, immunomodulate to kill cells having virus and continue viral suppression with anti-virals in the postcombination phase to reduce reinfection of new hepatocytes.

Clinical data

Clinical responses. Efficacy of simultaneous standard IFN plus lamivudine in HBeAg-positive treatment-naïve CHB patients. Trials examining use of standard IFN- and lamivudine in simultaneous combination, in treatment-naïve HBeAg-positive patients have shown that combination therapy had a greater on-treatment viral suppression and higher rates of sustained off-treatment response than lamivudine alone, but no difference in sustained off-treatment response compared to IFN-α alone (Table 2).

Efficacy of sequential lamivudine and standard IFN in HBeAg-positive treatment-naïve CHB patients. There are only a few clinical trials, which have used sequential therapy with lamivudine in the precombination phase.[42, 47] Similarly, there are limited trials which have used a maintenance phase of lamivudine treatment following the combination therapy.[47] The trial of

Table 2. Combination of lamivudine and standard IFN in HBeAg-positive chronic hepatitis B

Author (reference)	Type of combination	location	Combination regimen	Comparison regimen	Response definition	Sustained response		
						Combination arm (%)	Comparison arms (%)	*P*
Schalm *et al.*[42]	Sequential	North Europe North America	L 100 mg/day × 8 weeks, followed by L and α-IFN 10 MU TIW × 16 weeks (n = 75)	α-IFN monotherapy: 8 weeks oral placebo, followed by 10 MU TIW × 16 weeks (n = 69) L 100 mg/day × 52 weeks (n = 82)	HBeAg(−), anti-HBe(+) and undetectable HBV DNA*	29	19 18	0.12 0.10
Barbaro *et al.*[43]	Simultaneous	Mediterranean	IFN-α-2b 9 MU TIW and L 100 mg/day × 24 weeks (n = 76)	L × 52 weeks (n = 75)	HBeAg(−), anti-HBe(+) and undetectable HBV DNA	33	15	0.014
Yalcin *et al.*[44]	Simultaneous	Middle East	L 100 mg/day and IFN-α-2b 10 MU TIW × 12 months (n = 33)	IFN α-2b 10 MU TIW × 12 months (n = 16)	HBeAg(−), anti-HBe(+) and undetectable HBV DNA	45	19	N.S.
Hasan *et al.*[45]	Sequential	Middle East	IFN-α-2a 4.5 MU/day × 16 weeks and L 100 mg/day starting from week 5 and contin uing for 48 weeks (n = 32)	L 100 mg/day (n = 29)	HBeAg(−), anti-HBe(+) and undetectable HBV DNA	6.2	0	N.S.
Deng *et al.*[46]	Simultaneous	China	IFN-α 10 MU TIW and L 100 mg/day, followed by 6 months of L (n = 30)	L 100 mg/day (n = 30) IFN-α × 6 months (n = 30)	HBeAg/anti-HBe seroconversion	46.7	13.3 33.3	0.005
Sarin *et al.*[47]	Sequential	India	L 100 mg/day × 52 weeks and IFN-α 5 MIU/day added for 16 weeks after the first 8 weeks (n = 38)	L 100 mg/day × 52 weeks (n = 37)	HBeAg(−), anti-HBe(+) and undetectable HBV DNA	36.8	10.8	0.026
Ayaz *et al.*[48]	Simultaneous	Turkey	IFN-α-2a 9 MU TIW and L 100 mg/day × 12 months (n = 33)	IFN-α-2a alone × 12 months (n = 35)	ALT normalization, HBeAg(−) and undetectable HBV DNA	13	12	N.S.
Asik *et al.*[49]	Simultaneous (paediatric)	Turkey	IFN-α-2a 5 MU/m^2 TIW × 6 months and L 4 mg/kg/day × 1 year given simultaneously (n = 27)	–	Seroconversion of HBeAg/anti-HBe	39.1		
Kansu *et al.*[50]†	Simultaneous (paediatric)	Turkey	IFN-α-2a 9 MU/m^2 × 6 months plus and L 4 mg/kg simultaneously (n = 112)	IFN-α-2a 9 MU/m^2 × 6 months plus and L 4 mg/kg started 2 months prior to IFN-α-2a (n = 65)	Loss of HBeAg, development of antibodies to HBeAg and undetectable HBV DNA	55.3	27.6	<0.01

Table 2. Continued

Author (reference)	Type of combination	location	Combination regimen	Comparison regimen	Response definition	Sustained response		P
						Combination arm (%)	Comparison arms (%)	
Dikici *et al.*[51]	Simultaneous (paediatric)	Turkey	IFN-α 10 MU/m^2 and L 4 mg/kg simultaneously × 6 months, then L alone continued for 6 months (n = 17)	L alone for the first 2 months, then IFN-α added to L for 6 months and L alone continued for 4 months (n = 15)	Loss of HBeAg, development of antibodies to HBeAg and undetectable HBV DNA	47	46	N.S.
Dikici *et al.*[52]	Simultaneous (paediatric)	Turkey	IFN-α-2b 10 MU/m^2 and L 4 mg/kg/day given synchronously × 6 months (n = 30)	IFN-α-2b 10 MU/m^2 and L 4 mg/kg/day given synchronously × 12 months (n = 27)	Hepatitis Be antigen clearances	37	56	N.S.
Dikici *et al.*[53]	Simultaneous (paediatric)	Turkey	Simultaneous IFN-α-2b 10 MU/m^2, TIW s.c. plus L 4 mg/kg/day × 12 months (n = 27)	IFN-α × 12 months (n = 13)	Loss of HBeAg, development of antibodies to HBeAg and undetectable HBV DNA	37	30.7	N.S.
Ozgenc *et al.*[54]	Simultaneous (paediatric)	Turkey	5 MU/m^2 IFN-α-2a s.c TIW × 6 months plus L 4 mg/kg/day × 12 months (n = 29)	5 MU/m^2 IFN-α-2b s.c TIW × 6 months plus L 4 mg/kg/day × 12 months (n = 34)	ALT normalization, HBV DNA clearance and HBe/anti-HBe seroconversion	44.8	47.1	N.S.
Akman *et al.*[55]	Sequential vs. simultaneous (paediatric)	Turkey	IFN-α 5 MU/m^2 s.c., TIW × 6 months, followed by L 4 mg/kg/day for an additional 6 months (sequential therapy group) (n = 24)		ALT normalization, eradication of HBV DNA and HBe/anti-HBe seroconversion	25		N.S.
			IFN-α and L simultaneously for 6 months and then continue L alone for another 6 months (simultaneous therapy group) (n = 21)			33.3		
Kuloglu *et al.*[56]	Simultaneous (paediatric)	Turkey	IFN-α-2a 9 MU/m^2 s.c., TIW × 6 months, plus L 4 mg/kg/day × 24 months (n = 15)	IFN-α-2a 9 MU/m^2 s.c., TIW × 6 months (n = 16)	ALT normalization, eradication of HBV DNA and HBe/anti-HBe seroconversion	40	37.5	N.S.

Table 2. Continued

Author (reference)	Type of combination	location	Combination regimen	Comparison regimen	Response definition	Sustained response		
						Combination arm (%)	Comparison arms (%)	*P*
Yilmaz[57]	Simultaneous vs. sequential (paediatric)	Turkey	L 3 mg/kg/day × 3 months followed by IFN-α-2a 10 MU/m^2 s.c., TIW × 6 months, then L × additional 6 months (n = 21) L and IFN-α-2a, started simultaneously (n = 12)		HBeAg seroconversion with ALT normalization	66.7		N.S.
						50		

L, lamivudine
* Response assessed at the end of lamivudine treatment for lamivudine monotherapy group.
† Lamivudine was continued for 6 months after anti-HBe seroconversion or stopped at 24 months in nonresponders. Anti-HBe seroconversion was higher and earlier, and HBV DNA clearance was earlier in combination group ($P < 0.05$).

Schalm *et al.*,[42] which used sequential therapy without continuing lamivudine in the postcombination phase, found higher rates of sustained off-treatment response compared with lamivudine alone, but no difference in the sustained off-treatment response compared to IFN-α alone. The trial by Sarin *et al.*,[47] which used sequential therapy with continued lamivudine in the postcombination phase, did not have a treatment arm of IFN alone for the sake of comparison to answer whether continuing anti-virals in the postcombination phase is useful or not.

Efficacy of simultaneous peg-IFN plus lamivudine in HBeAg-positive CHB patients. The simultaneous combination of lamivudine and peg-IFN has shown higher rates of sustained off-treatment response than lamivudine alone, but with no statistical difference in the sustained off-treatment response compared to peg-IFN alone (Table 3). In one study,[58] peg-IFN monotherapy was compared to 48 weeks of lamivudine monotherapy or the combination of lamivudine and peg-IFN. The end of treatment decline in HBV DNA was more robust in patients treated with the combination therapy the values being −7.2 log, −4.5 log and −5.8 log, respectively, for combination therapy, peg-IFN alone and lamivudine monotherapy. However, combination therapy was not more effective in achieving sustained virological response at the end of a 24-week follow-up period. HBeAg seroconversion occurred more frequently in patients with on treatment ALT flares.[62]

In another study,[59] peg-IFN-α-2b was given in a dose of 100 μg weekly for 32 weeks followed by 50 μg weekly until completion of 52 weeks of treatment. This treatment arm was compared to the identical dose and duration of peg-IFN given simultaneously with 52 weeks of lamivudine. There was a greater decline in HBV DNA in the combined group (approximately −5 log vs. −2 log) as well as a higher rate of HBeAg loss (44% vs. 29%) at the end of treatment. However, these differences were not sustained during a 26-week follow-up period. The reasons for this discrepancy are unclear but the modification in dosage at the 32-week treatment interval could have been a contributing element. HBsAg loss occurred in 5% of the peg-IFN monotherapy group and 7% of the combined therapy patients. Further analyses of the data demonstrated that the rate of HBeAg clearance was the highest (58%, $P = 0.008$) in patients who experienced ALT flares.[63]

Similarly, in a study evaluating the effect of peg-IFN or its combination with lamivudine on liver histology of 110 patients with HBeAg-positive CHB,

Table 3. Pegylated-IFN plus lamivudine in HBeAg-positive chronic hepatitis B

Author (reference)	Study design	Location	Regimens	ETR				
				Undetectable levels of serum HBV DNA	HBeAg(−) and anti-HBe(+)	ALT normalization	HBeAg(−), serum HBV DNA <5 log-copies/mL and ALT normalization	Histologic response
Lau *et al.*[58]	RCT	Multi	Simultaneous	HBV DNA <400 copies/mL				
			Peg-IFN-α-2a 180 μg/weeks + placebo (n = 271) × 48 weeks	25	27	39	10	
			Peg-IFN-α-2a 180 μg/weeks + L 100 mg/day (n = 271) × 48 weeks	69	24	46	15	
			L 100 mg/day (n = 272) × 48 weeks	40	20	62	18	
Janssen *et al.*[59]	RCT	Multi	Simultaneous	DNA < 400 copies/mL				
			Peg-IFN-α-2b 100 μg/week × 32 weeks, then 50 μg/week till 52 weeks + L 100 mg/day × 52 weeks (n = 130)	33	25	51		48
			Peg-IFN-α-2b + placebo × 52 weeks (n = 136)	10 (P < 0.001)	22 (P = 0.52)	34 (P = 0.005)		53 (P = 0.57)
Chan *et al.*[60]	RCT	China	Sequential					
			Peg-IFN-α-2b 1.5 μg/kg/weeks × 8 weeks, then peg-IFN + L 100 mg/day × 24 weeks, then L × 28 weeks (n = 48)		60			
			L 100 mg/day × 52 weeks (n = 47)		28 (P = 0.001)			
Chan *et al.*[61]	RCT	China	Sequential	HBeAg loss, anti-HBe appearance and HBV DNA, 500 000 copies/mL				
			Peg-IFN-α-2b 8 weeks administered, then peg-IFN + L 100 mg/day × 24 weeks, then L × 28 weeks (n = 50)	60		90		
			L 100 mg/day × 52 weeks (n = 50)	28 (P < 0.001)		78		

Only significant P-values are indicated (as compared to L). ETR, end of treatment responses; L, lamivudine; SVR, sustained viral responses.

				SVR				
YMDD	Viral resistance	Primary treatment failure	F/U	Undetectable serum HBV DNA	HBeAg(−) and anti-HBe(+)	ALT normalization	HBeAg(−), serum HBV DNA <5 log-copies/mL and ALT normalization	Histological response
			24 weeks					
				14 ($P < 0.001$)	32 ($P < 0.001$)	41 ($P = 0.002$)	23 ($P < 0.001$)	49
4 ($P < 0.001$)				14 ($P < 0.001$)	27 ($P = 0.02$)	39 ($P = 0.006$)	21 ($P < 0.001$)	52
27				5	19	28	10	51
			26 weeks	DNA < 400 copies/mL				
11				9	29	35		
				7 (0.43)	29 ($P = 0.92$)	32 ($P = 0.60$)		
				Sustained response: HBeAg loss and HBV DNA <100 000 copies/mL from treatment cessation until the end of follow-up				
			Combination: 117 ± 34 weeks	29.2				
			L: 124 ± 29 weeks	8.5 ($P < 0.05$)				
			24 weeks	HBeAg loss, anti-HBe appearance and HBV DNA 500 000 copies/mL				
				36			50	10
				14 ($P = 0.011$)			30	9

treated for 52 weeks with peg-IFN-α-2b in simultaneous combination with either lamivudine or placebo found that treatment with peg-IFN therapy improved liver necro-inflammation and fibrosis in HBeAg-positive CHB patients, particularly in responders to therapy. The addition of lamivudine to peg-IFN did not further improve the histological outcome. In the peg-IFN/lamivudine combination therapy group, no significant association between virological and biochemical end-points and histological improvement was observed.[64]

Efficacy of sequential peg-IFN plus lamivudine in HBeAg-positive CHB patients. Chan *et al.*[60] using sequential approach of peg-IFN and lamivudine with peg-IFN alone for 8 weeks of precombination phase with a total of 32-week course of peg-IFN-α-2a combined with 52 weeks of lamivudine found a sustained response of 36% in combination arm and 14% in lamivudine alone arm. Unfortunately, this study also did not have a peg-IFN alone limb.

Efficacy of simultaneous standard IFN plus lamivudine in HBeAg-negative CHB. Trails of simultaneous standard IFN plus lamivudine in HBeAg-negative CHB have also yielded negative results (Table 4).

Table 4. Standard IFN plus lamivudine in HBeAg-negative chronic hepatitis B

Author (reference)	Type of combination	Location	Combination regimen	Comparison regimen	Response definition	Sustained response Combination arm (%)	Sustained response Comparison arms (%)	*P*
Manesis *et al.*[65]	Sequential	Greece	L 100 mg/day from 1 to 12 months and IFN-α-2b from 7 to 18 months (n = 36)	IFN-α-2b (historical control) (n = 36)	ALT normalization and serum HBV DNA levels ≤30 000 copies/mL	22.2	13.9	N.S.
Tatulli *et al.*[66]	Simultaneous	Italy	IFN-α 6 MU TIW + L × 52 weeks (n = 29)	–	Undetectable serum HBV DNA and ALT normalization.	14		
Economou *et al.*[67]	Simultaneous	Greece	IFN-α-2b 5 MU TIW and L 100 mg/day × 24 months (n = 24)	L 100 mg/day × 24 months (n = 26)	Undetectable serum HBV DNA and ALT normalization	21	12	N.S.
Yurdaydin *et al.*[68]	Sequential	Turkey	L × 2 months, then L and IFN 9 MU, TIW × 10 months	L 100 mg/day × 12 months	Undetectable HBV DNA	27	25	N.S.
Santantonio *et al.*[69]	Simultaneous	Italy	IFN-α 5 MU TIW and L 100 mg/day × 12 months (n = 24)	L 100 mg/day × 12 months (n = 26)	Undetectable serum HBV DNA and ALT normalization	17	19	N.S.
Karabay *et al.*[70]	Simultaneous	Turkey	IFN-α 9 MU TIW × 24 weeks and L 100 mg/day × 1 year (n = 14)	IFN-α 9 MU TIW × 24 weeks (n = 13)	Undetectable HBV DNA	50	38	N.S.

L, lamivudine.

However, in a recent study from Greece, 36 anti-HBe-positive patients were treated with IFN (3 MU subcutaneously three times weekly) and lamivudine (100 mg orally once a day) for 12 months. After completion of the combined treatment, all patients continued to receive lamivudine monotherapy indefinitely. Overall, 35 patients (97%) showed virological response at 12 months. Four patients (11%) cleared HBsAg and developed anti-HBs. During the follow-up time, after the discontinuation of IFN, of 30 ± 12 months, 13 patients (36%) exhibited 'break-through' infection. The cumulative rates of break through infection at the end of 1, 2, 3 and 4 years of treatment were 0%, 14%, 32% and 59%, respectively. Combination therapy appeared to be effective and may also delay the selection of lamivudine-resistant variants.[71]

Efficacy of sequential lamivudine plus standard IFN in HBeAg-negative CHB. Manesis *et al.*[65] in a sequential study design with lamivudine first but without postcombination phase with lamivudine found similar responses with the sequential vs. IFN monotherapy. Yurdaydin *et al.*[68] in a sequential study design with lamivudine first but without postcombination phase with lamivudine found similar responses with the sequential vs. lamivudine alone therapy.

Efficacy of simultaneous peg-IFN plus lamivudine in HBeAg-negative CHB. Marcellini *et al.* found that a simultaneous combination of peg-IFN plus lamivudine for 48 weeks was better than peg-IFN alone or lamivudine alone.[72] However, another small study found that simultaneous combination of peg-IFN plus lamivudine for 48 weeks was no better than peg-IFN alone.[73]

Efficacy of sequential peg-IFN plus lamivudine in HBeAg-negative CHB. In a small study sequential therapy with lam and peg-IFN was found to be no better than lam alone (Table 5).[74]

In a meta-analysis, comparing IFN and lamivudine vs. IFN for HBeAg-positive patients, it was found that greater sustained virological, biochemical and seroconversion rates were observed with addition of lamivudine to conventional [odds ratio (OR) = 3.1, 95% confidence intervals (CIs): 1.7–5.5, $P < 0.0001$; OR = 1.8, 95% CI: 1.2–2.7, $P = 0.007$ and OR = 1.8, 95% CI: 1.1–2.8, $P = 0.01$, respectively], although not pegylated (OR = 1.1, 95% CI: 0.5–2.3, $P = 0.8$; OR = 1.0, 95% CI: 0.7–1.3, $P = 0.94$ and OR = 0.9, 95% CI: 0.6–1.2, $P = 0.34$, respectively) IFN-α, with no significant effect on HBeAg clearance rates (OR = 1.6, 95% CI: 0.9–2.7, $P = 0.09$ and OR = 0.8, 95% CI: 0.6–1.1, $P = 0.26$, respectively). This meta-analysis, which was not restricted to treatment-naïve subjects, concluded that, in comparable populations, peg-IFN monotherapy is likely to be equally or more efficacious than conventional IFN and lamivudine combination therapy, thus constituting the treatment of choice, with no added benefit with lamivudine addition. However, when conventional IFN is used, its combination with lamivudine should be considered.[75]

In another meta-analysis, comparing lamivudine and lamivudine vs. IFN for HBeAg-positive patients, it was found that greater sustained virological, biochemical and seroconversion rates with the addition of conventional (OR = 4.5, CI: 2.2–9.4, $P < 0.001$; OR = 2.1, 95% CI: 1.3–3.2, $P = 0.002$ and OR = 2.6, 95% CI: 1.4–4.8, $P = 0.001$, respectively) and pegylated (OR = 2.0, 95% CI: 1.1–3.6, $P = 0.02$; OR = 1.8, 95% CI: 1.3–2.6, $P < 0.001$ and OR = 1.6, 95% CI: 1.1–2.3, $P = 0.03$, respectively) IFN-α to lamivudine, with the former also yielding greater hepatitis Be antigen clearance rates (OR = 2.6, 95% CI: 1.3–5.2, $P = 0.008$). Peg-IFN monotherapy and its combination with lamivudine were comparable; the use of this combination is not justified. In contrast, when conventional IFN is used, its combination with lamivudine should be considered.[76]

Breakthrough and development of resistance. The combination of peg-IFN and nucleos(t)ide analogues seems to reduce the rates of viral resistance. Lau *et al.*[58] found that 27% of those receiving a 48-week course of lamivudine monotherapy had detectable YMDD mutations vs. 4% of those on both peg-IFN-2a plus lamivudine. Janssen *et al.*[59] found that 6% of patients receiving combination therapy who entered the trial without a pre-existing YMDD mutant developed such a mutation by the end of the 48-week treatment period. This is substantially lower than the 15–32% rates of viral resistance at 1 year observed in patients receiving lamivudine monotherapy. A recent Korean study investigated the effects of IFN-α combined with lamivudine on the occurrence of viral breakthrough during long-term lamivudine therapy. Eighty-three patients with CHB were randomly allocated to a combination of lamivudine and IFN-α ($n = 41$) or lamivudine only ($n = 42$), and then followed up for >12 months. There was no difference in cumulative rates of undetectable serum HBV DNA (100% vs. 100% at 24 months, $P = 0.13$) and

Table 5. Pegylated-IFN plus lamivudine in HBeAg-negative chronic hepatitis B

Author (reference)	Study design	Location	Regimens	ETR		
				Undetectable serum HBV DNA	ALT normalization	Serum HBV DNA <400 copies/mL and ALT normalization
Marcellin *et al.*[72]	RCT	Multi	Stimultaneous	HBV DNA <400 copies/mL		
			Peg-IFN-α-2a 180 μg/weeks + placebo (n = 177) × 48 weeks	63	38	27
			Peg-IFN-α-2a 180 μg/weeks + L 100 mg/day (n = 179) × 48 weeks	87	49	46
			L 100 mg/day (n = 181) × 48 weeks	73	73	60
Kaymakoglu *et al.*[73]	RCT	Turkey	Simultaneous	HBV DNA <4 pg/mL		
			Peg-IFN-α-2b 1.5 μg/kg/weeks × 48 weeks (n = 19)	63	53	
			Peg-IFN-α-2b 1.5 μg/kg/weeks + L 100 mg/day × 48 weeks (n = 29)	79	66	
Vassiliadis *et al.*[74]	RCT	Greece	Sequential	HBV DNA <400 copies/mL		
			L 100 mg/day alone for 3 months, then L and peg-IFN-α-2b (100 mg s.c. once weekly) for 3 months and then peg-IFN-α-2b alone for 9 months (n = 18)	88*	72.2	
			L100 mg/day × at least 15 months (n = 24)	70.8	70.8	

Only significant P-values are indicated (compared to L); L, lamivudine.
* Response occurred significantly earlier in the sequential combination treatment group (median time to response, 6 months vs. 12 months, $P < 0.05$.

					SVR			
Histological improvement	YMDD	Viral resistance	Primary treatment failure	F/U	Undetectable serum HBV DNA	ALT Normalization	Serum HBV DNA <400 copies/mL and ALT normalization	Histologic response
				24 weeks	HBV DNA <400 copies/mL			
					19 ($P < 0.001$)	59 ($P = 0.004$)	15 ($P = 0.007$)	55
	1 ($P < 0.001$)				20 ($P < 0.001$)	60 (0.0003)	16 ($P = 0.003$)	46
	18				7	44	6	46
				24 weeks	HBVDNA <400 copies/mL			
					26	42		
					24	48		
				12 months	HBV DNA <400 copies/mL			
					33.3	72.2	33.3	
					16.7	25 ($P < 0.01$)	12.5	

cumulative rates of serum HBeAg loss between the combination and the lamivudine group (49%, 61% and 67% vs. 31%, 39% and 42%, respectively, at 12, 24 and 36 months; $P = 0.07$). The cumulative occurrence rate of viral breakthrough, however, was significantly lower in the combination group than in the lamivudine group (5%, 20% and 30% vs. 10%, 55% and 58%, respectively, at 12, 24 and 36 months; $P = 0.006$). From the patients with viral breakthrough, YMDD mutants were detected in 82% of the lamivudine group in contrast with 56% of the combination. Thus IFN-α combined with lamivudine may reduce viral breakthrough during long-term lamivudine therapy, probably by suppressing the appearance of YMDD mutants.[77]

Summary and future strategies

Greater on treatment viral suppression occurs when peg-IFN and lamivudine are taken together compared with that of either agent alone. As far as response rates are concerned, peg-IFN monotherapy is likely to be equally or more efficacious than conventional IFN and lamivudine combination therapy, with no added benefit with lamivudine addition. However, when conventional IFN is used, its combination with lamivudine may be considered at least in HBeAg-positive CHB patients. A separate analysis comparing simultaneous vs. sequential combination therapies is warranted. The resistance to lamivudine is lower with combination therapy. A high rate of HBsAg clearance has been reported when adefovir is used with peg-IFN in a small pilot study. In this study (adefovir was combined with peg-IFN-α-2b, both administered for 48 weeks), the rate of HBeAg seroconversion (53%) as well as that of HBsAg seroconversion (15%) were higher when compared with historical cohorts.[78]

Whether the lack of synergy between IFNs and lamivudine against HBV is specific to this drug combination or reflects a more fundamental flaw in the rationale of combining an immunomodulatory agent and a nucleoside analogue is yet to be explored. Because nucleos(t)ide analogues work slowly in promoting HBeAg seroconversion, the results of combination therapy should be compared with the nucleoside analogue alone given for a suitably long period of time. Studies combining peg-IFN with other more potent anti-virals such as entecavir, and tenofovir in different types of combination regimens, are needed to address these issues.

INTERLEUKIN-12 (IL-12) PLUS LAMIVUDINE

Hypothesis

The diversity of clinical out comes after exposure to HBV is determined primarily by the host immune response.[79] In contrast to the strong anti-viral T-cell reactivity in acute hepatitis B, patients with chronic HBV infection have weak or undetectable T-cell reactivity to HBV. T cells control HBV replication by noncytolytic anti-viral effects primarily mediated by IFN-gamma (IFN-γ).[80] IL-12 stimulates natural killer cells and T-lymphocytes to produce IFN-γ, promotes T-helper 1 responses, and enhances CD8 cytotoxic T-cell activity. These unique properties of IL-12 indicate that it might have an important role in achieving sustained control of HBV replication.[81]

Experimental data

The administration of recombinant IL-12 to HBV transgenic mice resulted in complete inhibition of HBV replication in the liver and undetectable viraemia, mediated through IFN-γ induction.[82] In addition, IL-12 restored *in vitro* the hypo-responsiveness to viral antigens of T cells obtained from patients with CHB.[83]

Clinical data

In a pilot study, 15 patients with HBeAg-positive CHB were randomized to receive either lamivudine alone for 24 weeks (group 1); combination of lamivudine for 16 weeks and recombinant human-interleukin-12 (rhIL-12) (200 ng/kg twice weekly), starting 4 weeks after initiation of lamivudine, for 20 weeks (group 2), or the same schedule as for group 2, with lamivudine and a higher dose of rhIL-12 (500 ng/kg, group 3). Lamivudine plus higher dose rhIL-12 showed a greater anti-viral activity than lamivudine monotherapy. However, after stopping lamivudine in groups 2 and 3, serum HBV DNA increased significantly despite continuing rhIL-12 administration. Lamivudine plus rhIL-12 treatment was associated with a greater increase in virus-specific T-cell reactivity, IFN-γ production. Therefore, the addition of IL-12 to lamivudine enhances T-cell reactivity to HBV and IFN-γ production.[84]

STANDARD IFN PLUS RIBAVIRIN

Hypothesis

A major advancement in treating hepatitis C virus infection has been the development of combination therapy with IFN and ribavirin. IFN monotherapy is limited by poor sustained virological responses, even when higher doses are used. In contrast, IFN/ribavirin combination therapy results in much-improved sustained remission rates.[85, 86] Ribavirin and IFN in combination has been used for the treatment of dual CHB and CHC.[87] After about 2 year post-treatment follow-up, 21% of the responsive patients also cleared hepatitis B surface antigen.[88] It has recently been shown that ribavirin and IFN-α combination therapy induces $CD4^+$ T-cell proliferation and Th1 cytokine secretion in patients with CHB.[89]

Clinical data

However, in a study, for the treatment of HBeAg-positive CHB, adding ribavirin did not increase the efficacy of IFN.[90]

THYMOSIN PLUS IFN PLUS LAMIVUDINE

In a study in China, 74 patients with HBeAg-positive CHB were divided into three groups: sequential combination group (patients received 8 weeks of thymosin α1, 6 months of IFN begun in the fifth week, and lamivudine begun 2 months later after HBeAg seroconversion or just after the withdrawal of IFN, to more than 18 months =30), simultaneous combination group (patients received 6 months IFN and thymosin α1 simultaneously in the same manner as in sequential anti-viral group; $n = 14$) and lamivudine group (patients received more than 18-month treatment with lamivudine = 30). HBeAg seroconversion, undetectable HBV DNA and normalization of ALT were seen in 76.7%, 57.1% and 16.7% among the three groups, respectively ($P < 0.01$). Sequential anti-viral therapy had much higher rates of long-term efficacy. Mechanisms to promote the anti-viral effect might be dependent on the immunoregulatory action of thymosin α1 in the earlier period and the specific inhibition of HBV DNA replication by lamivudine in the later period of the therapeutic course.[91]

COMBINATION THERAPY USING SIRNAS

Hypothesis

siRNAs are a class of 20–25 nucleotide-long double-stranded RNA molecules that play a variety of roles in biology. These are involved in the RNA interference (RNAi) pathway where the siRNA interferes with the expression of a specific gene, and also act in RNAi-related pathways, e.g. as an anti-viral mechanism or in shaping the chromatin structure of a genome. A combination of siRNAs targeting different sites of HBV transcripts could have additive effects.

Experimental data

In a study to evaluate the inhibitory effect mediated by combination of siRNAs targeting different sites of HBV transcripts on the viral replication and antigen expression *in vitro*, seven siRNAs targeting surface (S), polymerase (P) or precore (PreC) region of HBV genome were designed and chemically synthesized. HBV-producing HepG2.2.15 cells were treated with or without siRNAs for 72 h. Synthetic siRNAs targeting S and PreC gene could efficiently and specifically inhibit HBV replication and antigen expression. The expression of HBsAg and HBeAg and the replication of HBV could be specifically inhibited in a dose-dependent manner by siRNAs. Furthermore, the combination of siRNAs targeting various regions could inhibit HBV replication and antigen expression in a more efficient way than the use of single siRNA at the same final concentration.[92]

COMBINATION THERAPY USING TWO IMMUNOMODULATORS

Hypothesis

Immunomodulators other than IFNs such as thymosine α and anti-HBV vaccine have been found to be useful in treatment of CHB. It is possible that a combination of two immunomodulators would be more effective than either of them alone.

Thymosin α1 plus IFN

A study from Singapore compared the combination of thymosin α1 and lymphoblastoid IFN (combination

therapy; $n = 48$) with thymosin alone (monotherapy; $n = 50$), for 24 weeks in naïve HBeAg-positive CHB patients with raised ALT. The HBeAg loss at 72 weeks was 45.8% and 28.0% for combination therapy and monotherapy, respectively ($P = 0.067$) showing a trend towards HBeAg loss when using combination therapy. There were no statistically significant differences between the two therapies with respect to HBeAg seroconversion, changes in histology, normalization of ALT or loss of HBV DNA.[93]

Vaccine plus IFNs

In a Turkish study, 50 treatment-naïve children with CHB were randomly assigned to receive either IFN-α-2b for 9 months, and pre-S2/S vaccine at the beginning and 4 and 24 weeks after initiation of IFN therapy ($n = 25$) or recombinant IFN-α-2b alone for 9 months ($n = 25$). The mean HBV DNA values were significantly reduced in combination group at the end of the therapy ($P = 0.004$), but no statistically significant difference was found 6 months after the end of treatment. Sustained HBeAg seroconversion with clearance of HBV DNA was obtained in 13 (52%) treated with combination therapy, and in eight (32%) treated with IFN monotherapy ($P = 0.251$).[94]

SUMMARY

In summary, the answer to the question whether hepatitis B can be treated better with combination or monotherapy remains largely unknown. There is however no doubt that today we have entered an era where monotherapies alone seem to have plateaued in their efficacy. There is an urgent need to evaluate combination of peg-IFN and potent oral nucleos(t)ide analogues or the combined use of nucleos(t)ide analogues or immunomodulators. The possibility of multidrug combinations such as in HIV should also be explored. These strategies and newer drugs targeting alternative sites of viral replication and their combinations will ultimately allow us to treat CHB with more confidence.

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