



Antiviral Therapy for AECHB and Severe Hepatitis B (Liver Failure)

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Abstract

This chapter describes the principles of antiviral therapy, treatment strategies, medications and recommendations for AECHB, HBV-ACLF, HBV-related liver cirrhosis, HBV-related HCC, and liver transplantation.

1. Severe exacerbation of chronic hepatitis B is closely related to continuous HBV replication. Therefore, inhibiting HBV replication to reduce viral load may block disease progression and improve the quality of life of these patients. ETV or TDF has been recommend first-line drug for the treatment of AECHB.
2. A hyperactive immune response due to continuous HBV replication is the main mechanism for development of severe hepatitis B. In addition to comprehensive treatment, early administration of potent nucleoside analogs can rapidly reduce HBV DNA concentration, relieve immune injury induced by HBV, and reduce liver inflammation and patient mortality. Antiviral agents have become important in the treatment of severe exacerbation of chronic hepatitis B.
3. Long-term antiviral treatment with nucleoside analogs can delay or reverse the progress of liver cirrhosis. Virologic response, viral resistance and adverse drug reactions should be closely monitored during treatment. The treatment should be optimized for maximum effect based on each patient's responses.

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4. Effective antiviral therapy can suppress HBV replication and reduce the incidence of HBV-related HCC. Patients with HBV-related HCC should receive individualized and optimal multidisciplinary comprehensive treatment. Anti-viral drugs with high efficacy, low resistance and low adverse drug reactions should be selected to improve the patient's quality of life and prolong survival time.
5. Methods to prevent HBV reinfection after liver transplantation include passive immunization (HBIG), antiviral treatment (nucleoside analogs) and active immunization (hepatitis B vaccine).
6. Clinical trials involving sequential combination therapy with NUC and Peg-IFN have shown statistically significant decline in HBsAg levels on treatment and high rates of sustained post-treatment serologic response. Combination therapy with novel DAA and immunotherapeutic approach may hold promise to overcome both cccDNA persistence and immune escape, representing a critical step towards HBV cure.

5.1 Basic Principles and Strategies of Antiviral Therapy

Ting Wu and Qin Ning

CHB is a progressive disease, and its development is the interaction between HBV and the host's immune response. Host's immune system eliminates HBV through cytolysis and non-cytolysis mechanism, which leads to hepatic inflammation, hepatocyte necrosis and apoptosis. Continuous hepatic inflammation can lead to liver fibrosis, liver cirrhosis, liver failure, and even liver cancer gradually. HBV replication plays a key role in the disease evolution. Therefore, suppressing HBV replication is crucial in treatment of CHB.

The development of continuous liver inflammation, cirrhosis and liver cancer is closely related to the sustainable HBV replication in CHB patients. Hence, inhibiting HBV replication and reducing HBV DNA load is the key to block the disease progression and improve the survival quality in the treatment of CHB [1].

5.1.1 The Goal of Antiviral Therapy for Chronic Hepatitis B

The fundamental goal of CHB treatment is to eliminate HBV or suppress HBV permanently, thus to reduce viral pathogenicity and infectivity, relieve or inhibit hepatic necroinflammation. However, the existing antiviral drugs cannot remove the intercellular covalently closed circular DNA (cccDNA). Therefore, the goal of antiviral treatment is to sustainably suppress the virus, reduce or prevent hepatic injury and disease progression. The short-term goal of clinical practice is sustainable HBV suppression, ALT normalization and prevention from decompensated liver cirrhosis (initial response), releasing the hepatic necroinflammation and liver fibrosis during and after treatment. The long-term and ultimate goal is prevention from liver decompensation, relief or halt of the progression to liver cirrhosis or HCC, and prolonged survival period (sustained response) [2–7].

5.1.2 Approved Anti HBV Drug

HBV is difficultly to be eliminated, and there is no drug can absolutely eradicate HBV infection so far. Many drugs can function against HBV, but only two classes are internationally recognized as antiviral drugs: interferon and nucleoside analogues (Fig. 5.1).

5.1.3 Treatment Strategies for Chronic Hepatitis B in Guidelines

5.1.3.1 Common Guidelines for Management of CHB

Common guidelines for management of CHB (Table 5.1).

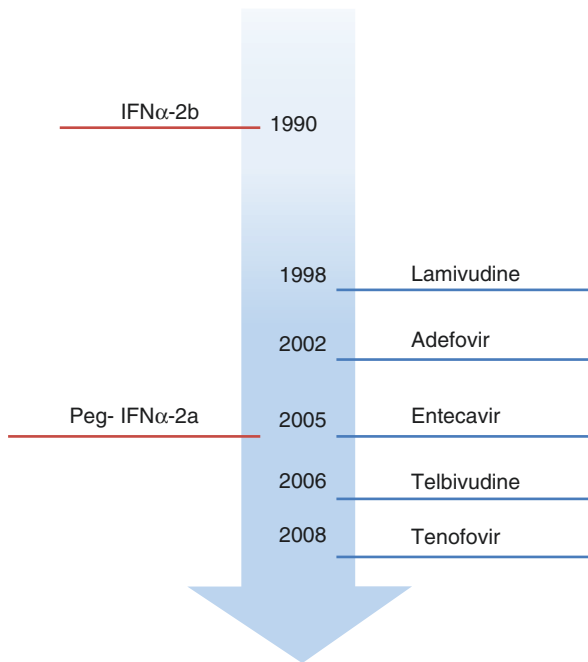


Fig. 5.1 Approved therapy for chronic hepatitis B over time

Table 5.1 Common guidelines for management of CHB

Publication date	Organizations	Abbreviation of guidelines
2015	The Asian Pacific Association for the Study of the Liver	APASL guideline
2017	European Association for the Study of the Liver	EASL guideline
2018	American Association for the Study of Liver Diseases	AASLD guideline
2015	Chinese Society of Liver disease/Chinese Society of Infectious disease	CSLD/CSID guideline
2015	World Health Organization	WHO guideline

5.1.3.2 The Recommendations for the Treatment of HBeAg Positive Patients with CHB in Guidelines

The recommendations for the treatment of HBeAg positive patients with CHB in guidelines (Table 5.2) [3–6, 8].

5.1.3.3 The Recommendations for the Treatment of HBeAg Negative Patients with CHB in Guidelines

The recommendations for the treatment of HBeAg negative patients with CHB in guidelines (Table 5.3) [3–6, 8].

Table 5.2 The recommendations for the treatment of HBeAg positive patients with CHB in guidelines

HBV DNA	ALT	Recommendations
APASL 2015		
>20,000 IU/mL	>2 × ULN	Start antiviral treatment, if HBV DNA <2 × 10 ⁶ IU/mL without the possibility of decompensation, temporarily monitor serological conversion closely for 3 months instead of antiviral treatment
>20,000 IU/mL	<2 × ULN	If there is moderate to severe biopsy proved inflammation/fibrosis or noninvasive diagnosed moderate to severe fibrosis, start antiviral treatment
<20,000 IU/mL	Any ALT	If there is moderate to severe biopsy proved inflammation/fibrosis or noninvasive diagnosed moderate to severe fibrosis, start antiviral treatment
EASL 2017		
>2,000 IU/mL	Normal	If there is moderate to severe biopsy proved inflammation or fibrosis, start antiviral treatment If there is moderate to severe noninvasive diagnosed fibrosis, start antiviral treatment For patients with immune tolerance, if there is no family history of liver cirrhosis or liver cancer, temporarily monitor closely
>2,000 IU/mL	>1 × ULN	Start antiviral treatment
AASLD 2018		
>20,000 IU/mL	>2 × ULN	If there is no spontaneous HBeAg clearance in 3–6 months, start antiviral treatment
>20,000 IU/mL	≤2 × ULN	If there is moderate to severe inflammation or significant fibrosis proven by biopsy, start antiviral treatment
CSLD/CSID 2015		
>20,000 IU/mL	>2 × ULN	Start antiviral treatment
	<2 × ULN	Knodell HAI ≥4, or necroinflammation ≥2, or fibrosis ≥2
<20,000 IU/mL	>1 × ULN	Age >40 years, start antiviral treatment
	<1 × ULN	Age <40 years, monitor closely and biopsy is recommended, if Knodell HAI ≥4, or necroinflammation ≥2, or fibrosis ≥2, start antiviral treatment
WHO 2015		
>20,000 IU/mL	>1 × ULN	Age >30 years, start antiviral treatment
Extra-hepatic manifestations		May start antiviral treatment

Table 5.3 The recommendations for the treatment of HBeAg negative patients with CHB in guidelines

HBV DNA	ALT	Recommendations
APASL 2015		
>2000 IU/mL	>2 × ULN	If last for 3–6 months or there is a possibility of decompensation, start antiviral treatment
>2000 IU/mL	<2 × ULN	If there is moderate to severe biopsy proved inflammation/fibrosis or noninvasive diagnosed moderate to severe fibrosis, start antiviral treatment
EASL 2017		
>2,000 IU/mL	Normal	If there is moderate to severe biopsy proved inflammation or fibrosis, start antiviral treatment If there is moderate to severe noninvasive diagnosed fibrosis, start antiviral treatment For patients with immune tolerance, if there is no family history of liver cirrhosis or liver cancer, temporarily monitor closely
>2,000 IU/mL	>1 × ULN	Start antiviral treatment
AASLD 2018		
>2000 IU/mL	>2 × ULN	Start antiviral treatment
>2000 IU/mL	<2 × ULN	If there is moderate to severe inflammation or significant fibrosis proven by biopsy, start antiviral treatment
CSLD/CSID 2015		
>2000 IU/mL	>2 × ULN	Start antiviral treatment
	<2 × ULN	Knodell HAI ≥4, or necroinflammation ≥2, or fibrosis ≥2
<20,000 IU/mL	>1 × ULN	Age >40 years, start antiviral treatment
	<1 × ULN	Age <40 years, monitor closely and biopsy is recommended, if Knodell HAI ≥4, or necroinflammation ≥2, or fibrosis ≥2, start antiviral treatment
WHO 2015		
>20,000 IU/mL	>1 × ULN	Age >30 years, start antiviral treatment
Extra-hepatic manifestations		May start antiviral treatment

5.1.3.4 The Recommendations for the Treatment of Cirrhotic Patients with CHB in Guidelines

The recommendations for the treatment of cirrhotic patients with CHB in guidelines (Table 5.4) [3–6].

5.1.3.5 The Recommendations for Liver Biopsy in Guidelines

The recommendations for liver biopsy in guidelines (Table 5.5) [3–6].

Table 5.4 The recommendations for the treatment of cirrhotic patients with CHB in guidelines

HBV DNA	HBV DNA	Recommendations
APASL 2015		
Compensation	>2000 IU/mL	Antiviral treatment
Decompensation	Detectable	Antiviral treatment, consider liver transplantation
EASL 2017		
Compensation	Detectable	Antiviral treatment although with normal ALT and/or HBV DNA load lower than 2000 IU/mL
Decompensation	Detectable	Antiviral treatment immediately, consider liver transplantation
AASLD 2018		
Compensation	>2000 IU/mL	Start antiviral treatment
	<2000 IU/mL	Start antiviral treatment
Decompensation	HBsAg positive	Antiviral treatment immediately, ready to liver transplantation
	HBsAg negative	Ready to liver transplantation
CSLD/CSID 2015		
Compensation	>2000 IU/mL	Start antiviral treatment for HBeAg positive patients Start antiviral treatment for HBeAg negative patients with detectable HBV DNA
Decompensation	Detectable	Start antiviral treatment to avoid liver transplantation

5.1.3.6 The Recommendations for CHB Initial Treatment Options in Guidelines

The recommendations for CHB initial treatment options in guidelines (Table 5.6) [3–6, 8].

5.1.3.7 The Recommendations for CHB Cirrhosis Initial Treatment Options in Guidelines

The recommendations for CHB cirrhosis initial treatment options in guidelines (Table 5.7) [3–6].

5.1.3.8 The Recommendations for CHB Treatment Duration in Guidelines

The recommendations for CHB treatment duration in guidelines (Table 5.8) [3–6, 8].

5.1.3.9 The Conclusion of Recommendations for CHB Treatment Duration in Guidelines

1. Antiviral treatment strategies for HBeAg positive patients (Fig. 5.2).
2. Antiviral treatment strategies for HBeAg negative patients (Fig. 5.3).

5.1.3.10 The Recommendations for Management of Lamivudine Drug-Resistance in Guidelines

The recommendations for management of lamivudine drug-resistance in guidelines (Table 5.9) [3–6, 8].

Table 5.5 The recommendations for liver biopsy in guidelines

HBsAg	HBV DNA	ALT	Recommendations
APASL 2015			
Positive	>20,000 IU/mL	<2 × ULN	If age >35 years, perform biopsy
Negative	>2000 IU/mL	<2 × ULN	If age >35 years, perform biopsy
EASL 2017			
Positive	>2000 IU/mL	>1 × ULN	If age >30 years and/or with history of cirrhosis or liver cancer, consider biopsy
Negative	>20,000 IU/mL	>1 × ULN	Consider biopsy
AASLD 2018			
Positive	>20,000 IU/mL	>2 × ULN	If compensated, consider biopsy before antiviral treatment
	>20,000 IU/mL	≤2 × ULN	If age >40 years, ALT between 1–2 × ULN and with history of HCC, consider biopsy
Negative	>2000 IU/mL	<2 × ULN	Consider biopsy
CSLD/CSID 2015			
Positive	>20,000 IU/mL	1–2 × ULN	Consider biopsy
		<1 × ULN	If age >30 years, consider biopsy
Negative	>2000 IU/mL	1–2 × ULN	Consider biopsy
		<1 × ULN	If age >30 years, consider biopsy

Table 5.6 The recommendations for CHB initial treatment options in guidelines

Abbreviation of guidelines	Recommended drugs
APASL 2015	ALT 2–5 × ULN: based on IFN-α, or NAs (entecavir and tenofovir are preferred) ALT >5 × ULN: based on IFN-α, or NAs (entecavir and tenofovir are preferred, especially when hepatic decompensation)
EASL 2017	Entecavir and tenofovir as the first-line choice, or pegylated interferons
AASLD 2018	Pegylated interferon-α, entecavir or tenofovir are preferred; Interferon-α/pegylated interferon-α, lamivudine, adefovir, entecavir, tenofovir or telbivudine can be used; Adefovir or entecavir for interferon nonresponders or patients with interferon contraindication.
CSLD/CSID 2015	Pegylated interferon-α, entecavir
WHO 2015	Tenofovir and entecavir as the first-line choice IFN may be considered when HBV DNA viral load and genotyping are available, or co-infection with HDV

Table 5.7 The recommendations for CHB cirrhosis initial treatment options in guidelines

Abbreviation of guidelines	Compensatory situation	Recommended drugs
APASL 2015	Compensation	Entecavir or tenofovir, therapy based on interferon- α can also be used when ALT $<5 \times$ ULN
	Decompensation	Entecavir or tenofovir
EASL 2017	Compensation	Entecavir or tenofovir; Pegylated interferon can be used for cirrhotic patients with compensated liver function; Lamivudine is not recommended
	Decompensation	Entecavir (1 mg) or tenofovir
AASLD 2018	Compensation	Entecavir (1 mg) or tenofovir first; Lamivudine, adefovir, entecavir, tenofovir or telbivudine are not recommended
	Decompensation	Entecavir or tenofovir
CSLD/CSID 2015	Compensation	NAs with low resistance first; Use interferon with caution, start from small doses
	Decompensation	NAs with low resistance first; Interferon is forbidden

Table 5.8 The recommendations for CHB treatment duration in guidelines

Drugs	HBeAg(+)	HBeAg(-)	Compensated cirrhosis	Decompensated cirrhosis
APASL 2015				
IFN	48 weeks	At least 1 year	-	-
NUCs	HBeAg seroconversion and a consolidation therapy for 1-3 year	Uncertain; HBV DNA undetectable for three times continuously with interval of 6 months	No recommendations	No recommendations
EASL 2017				
IFN	48 weeks Consider to stop drug if HBV DNA not lower than 20,000 IU/mL or HBsAg not decline significantly at 12-week	48 weeks Consider to stop drug if HBV DNA not decline $\geq 2 \log_{10}$ IU/mL and HBsAg not decline significantly at 12-week	No recommendations	-
NUCs	HBeAg seroclearance or seroconversion	Long-term treatment	Long-term treatment	Long-term treatment
AASLD 2018				
IFN	Interferon for 12-24 weeks; Pegylated interferon for 48 weeks, extension may be more effective	Interferon for 12-24 weeks; Pegylated interferon for 48 weeks, extension may be more effective	-	-

Table 5.8 (continued)

Drugs	HBeAg(+)	HBeAg(-)	Compensated cirrhosis	Decompensated cirrhosis
NUCs	HBeAg seroconversion and an extension of at least 12 months after HBV DNA negative	Continue treatment to HBeAg seroclearance	Long-term treatment HBeAg seroconversion and HBsAg loss for HBeAg positive patients HBsAg loss seroclearance for HBeAg negative patients	Life-long treatment
CSLD/CSID 2015				
IFN	Interferon for 6 months; and Pegylated interferon for 1 year	Interferon for 1 year; Pegylated interferon for 1 year	No recommendations	—
NUCs	ALT normalization, HBeAg seroconversion and HBV DNA undetectable, two times continuously with interval of 6 months, total duration more than 2 years	ALT normalization and HBV DNA undetectable and a consolidation therapy for 1 year and a half, three times continuously with interval of 6 months, total duration more than 2 years and a half	Long-term treatment	Long-term treatment
NUCs	If HBV DNA testing is available: HBeAg loss and seroconversion, after completion of at least 1 additional year of treatment, Persistently normal ALT, Persistently undetectable HBV DNA levels	If HBV DNA testing is available: After completion of at least 1 additional year of treatment, Persistently normal ALT, Persistently undetectable HBV DNA levels	Life-long treatment	Life-long treatment
	If HBV DNA testing is not available: Persistent HBsAg loss, After completion of at least 1 additional year of treatment	If HBV DNA testing is not available: Persistent HBsAg loss, After completion of at least 1 additional year of treatment		

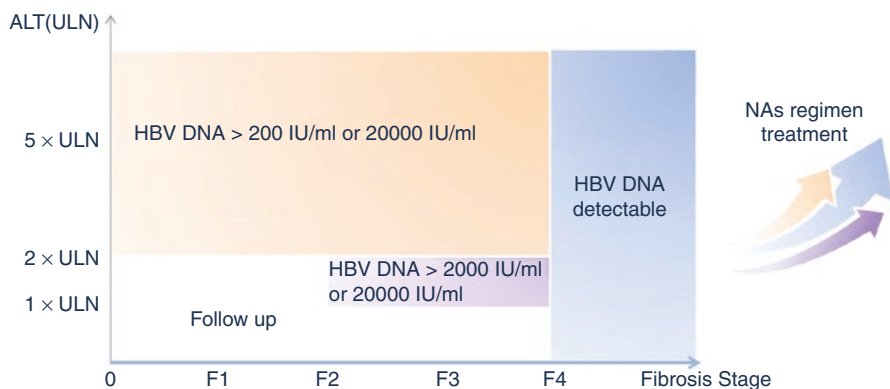


Fig. 5.2 Antiviral treatment strategies for HBeAg positive patients

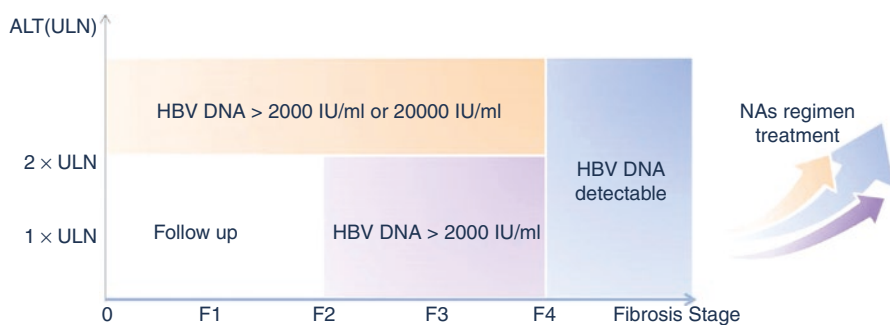


Fig. 5.3 Antiviral treatment strategies for HBeAg negative patients

Table 5.9 The recommendations for management of lamivudine drug-resistance in guidelines

Abbreviation of guidelines	Recommendations
APASL 2015	Switch to tenofovir Add adefovir
EASL 2017	Add tenofovir
AASLD 2018	Add tenofovir Switch to tenofovir
CSLD/CSID 2015	Switch to tenofovir
WHO 2015	Switch to adefovir

5.1.3.11 The Recommendations for Management of Adefovir Drug-Resistance in Guidelines

The recommendations for management of adefovir drug-resistance in guidelines (Table 5.10) [3–6, 8].

Table 5.10 The recommendations for management of adefovir drug-resistance in guidelines

Abbreviation of guidelines	Recommendations
APASL 2015	Switch to entecavir Switch to tenofovir
EASL 2017	If LAM-naïve: switch to ETV or TDF If LAM-resistance: switch to TDF or TAF If HBV DNA plateaus: add ETV*** or switch to ETV
AASLD 2018	Add entecavir Switch to tenofovir or entecavir
CSLD/CSID 2015	Add or entecavir Switch to entecavir or tenofovir
WHO 2015	Switch to adefovir

5.1.4 Antiviral Treatment in Special Populations

Consensus of Antiviral Treatment in Special Populations with chronic hepatitis B was put forward by China expert committee of antiviral treatment in special population with chronic hepatitis B in 2010.

5.1.4.1 The Choice of Antiviral Drugs for Fulminant Hepatitis B

In China, HBV infection is one of the main causes of liver failure. HBV related liver failure can be further divided into acute liver failure, subacute liver failure, acute-on-chronic liver failure and chronic liver failure. Nucleoside analogues can be safely used in the treatment of HBV related liver failure, and improve the prognosis of patients.

Nucleoside analogues treatment can improve the survival rate and reduce the incidence of complications in HBV related acute and subacute liver failure patients. Therefore, nucleoside analogues treatment should be early applied in HBsAg positive and HBV DNA detectable patients with acute and subacute liver failure, and nucleoside analogues can quickly inhibit virus, including lamivudine, entecavir and tenofovir, are recommended for these patients. Drug resistance should be monitored in long-term nucleoside analogues treatment. Remaining HBV cannot be completely excluded even when HBsAg and HBV DNA are undetectable during the treatment, therefore the antiviral treatment should continue to HBsAg seroconversion. Antiviral treatment is unnecessary for anti-HBs positive patients at first visit [9].

5.1.4.2 Patients with HBV Related Primary Liver Cancer

For patients with HBV related primary liver cancer, liver cancer resection, radiofrequency ablation and interventional therapy all can lead to HBV reactivation and liver function damage aggravation, antiviral treatment is decided depending on liver compensatory situation. IFN- α can exhibit both anti-virus and anti-cancer effect, delay the tumor recurrence and prolong the median survival period. IFN- α should be the preference if the patients can tolerate IFN- α . If the patients have

contraindications to IFN- α , lamivudine, adefovir or entecavir can be chosen depending on HBV DNA loads, cirrhosis compensatory situation and kidney function. For patients undergoing hepatic arterial chemotherapy, prophylactic nucleoside analogues treatment should be given before chemotherapy. For patients with advanced liver cancer, portal vein branch thrombosis, but without contraindications to IFN- α , IFN- α treatment can extend survival period [9–15].

5.1.4.3 Patients with HBV Related Liver Transplantation

Patients awaiting liver transplantation because of HBV-related end-stage liver disease or liver cancer should be given nucleoside analogues with strong HBV inhibition and low drug-resistance, or nucleotides analogues combination treatment, in order to reduce viral load and prevent graft re-infection.

Lamivudine and (or) adefovir combination with HBIG can be safely and effectively prevent graft re-infection, and reduce the re-infection rate to below 10%.

HBV-associated liver transplant patients require lifelong treatment of antiviral drugs for the prevention of hepatitis B recurrence. HBsAg-negative patients receiving anti-HBs positive donor liver should also receive long-term treatment of lamivudine or preventive treatment of HBIG [1, 9, 16–27].

5.1.4.4 Elderly CHB Patients

According to WHO, elderly CHB patients refers to CHB patients aged ≥ 60 years old. Generally speaking, according to current guidelines, ≥ 60 years of age is not a contraindication to antiviral therapy, so their treatment can refer to the relevant guidelines, but their desire, risks and benefits of treatment should be comprehensive evaluated. Especially for the patients using IFN- α , the expected survival and liver function compensatory situation, possible side effects, underlying hypertension, diabetes, coronary heart disease, and the improvement of liver function should be comprehensive evaluated. Additionally, the treatment response, side effects, blood sugar, kidney function and occurrence of liver cancer should be closely monitored during and after treatment [9].

5.1.4.5 Children CHB Patients

Children CHB patients are usually in the immune tolerance phase of HBV infection, hence they could not receive antiviral treatment, but should be closely followed up. FDA has approved of IFN- α (2–17 years of age), lamivudine (2–17 years of age), and adefovir (12–17 years of age) for use in children. Recommended IFN- α dose is 6 MIU/m² of body surface area three times per week, and the maximum dose is 10 MIU/m² total body surface area. It is showed that IFN- α effects the same in children as in adult. Recommended lamivudine dose is 3 mg/(kg day) with a maximum dose of 100 mg/day. Recommended adefovir dosage and usage are the same with adult patients [9, 28–33].

5.1.4.6 Pregnant CHB Patients

Mother to child transmission is the main route of transmission of HBV infection in China, in order to block the transmission of HBV, antiviral therapy in pregnant CHB

patients is very important. Firstly, antiviral treatment should be completed before pregnancy as possible. It is recommended to consider pregnant 6 months later after interferon or nucleoside analogs treatment. Secondly, unwanted pregnancies should be terminated during IFN- α treatment because of its pregnancy toxicity. Pregnancy safety of nucleosides analogs has not been proved by any clinical trials, but a large number of studies have shown that lamivudine and tenofovir were safe, so the treatment with lamivudine could continue under the premise of full communication with patients. Telbivudine, adefovir or entecavir treatment may switch to lamivudine treatment.

Pregnant CHB patients with slight ALT elevation, should be monitored closely or given liver protection and symptomatic treatment, and given antiviral treatment after delivery. Pregnant CHB patients with poor liver function could be given lamivudine treatment after full consultations with patients and signed informed consent forms.

Serum HBV DNA load in pregnant CHB patients is the key factors of mother to child transmission, and effective antiviral treatment can significantly reduce the transmission incidence. According to the findings, lamivudine or telbivudine treatment could start in 28–34 week of pregnancy to block transmission, and the withdrawal can refer to the patients with immunosuppressive agents or chemotherapy.

In addition, women with husbands receiving IFN- α antiviral treatment, should only consider pregnancy 6 months later after withdrawal. There is no evidence at present that nucleosides analogs have a negative impact on sperm and embryo, pregnancy could be taken into account under the premise of full communication with patient [9, 34–45].

5.1.4.7 HBV and HCV Co-infected Patients

HBV and HCV co-infection increases the incidence of severe hepatitis, liver cirrhosis and liver cancer. When co-infection, HCV may inhibit HBV generally, and different treatment should be given depending on HBV and HCV viral load and ALT level (Table 5.11) [9, 46–53].

Table 5.11 Reference scenario of antiretroviral therapy for HBV and HCV co-infection

HBV DNA	HCV DNA	ALT	Recommendatory strategy
Undetectable	Detectable	–	Referring to the standard anti-HCV treatment regimens
Detectable	Detectable	<2 × ULN	Referring to the standard anti-HCV treatment regimens
Detectable	Detectable	>2 × ULN	IFN- α + ribavirin \pm NUCs according to the patient's condition ★
Detectable	Undetectable	<2 × ULN	Referring to the carrier management, do not treat, regular follow up.
Detectable	Undetectable	>2 × ULN	Referring to the standard anti-HBV treatment regimens
Undetectable	Undetectable	–	Do not treat, regular followed up.

★ Avoid combination of IFN- α and telbivudine

5.1.4.8 HBV and HIV Co-infected Patients

HBV and HIV co-infection increases HBV DNA load, reduces spontaneous HBeAg seroconversion rate, aggravates liver damage and increases mortality in patients with liver disease. Anti-HBV regimens should combine with highly active antiretroviral therapy (HAART).

If anti-HBV and HIV treatment is needed, anti-HBV drugs such as tenofovir + lamivudine or tenofovir + Truvada could be used in HAART.

If HAART only contains lamivudine as anti-HBV drugs, HBV drug resistance should be closely monitored and treatment should be adjusted in time.

If HAART is unnecessary, adefovir, telbivudine and IFN- α can be used for anti-HBV treatment.

Because lamivudine, tenofovir and entecavir monotherapy induced risk of HIV drug-resistance, lamivudine, tenofovir and entecavir treatment are not recommend to these patients [9, 54–60].

5.1.4.9 CHB Patients Underlying Kidney Disease

Anti-HBV treatment is critical for hepatitis B virus associated Glomerulonephritis (HBV-AG). Patient diagnosed with HBV-AG must start anti-viral therapy as long as HBV DNA is detectable. A number of studies show that kidney disease alleviated significantly after lamivudine treatment, along with HBV DNA decline and HBeAg clearance. Adefovir has been shown to increase serum creatinine level in some patients in clinical trials, therefore should be carefully chosen. There is not enough clinical evidence of telbivudine and entecavir treatment for HBV-AG. There is no consensus of nucleoside analogue treatment for HBV-AG currently. Safety and efficacy of IFN- α and pegylated IFN- α treatment for HBV-AG have not been proved [9, 61–67].

5.1.4.10 Patients Receiving Immunosuppressive Agents or Cytotoxic Therapy

Elevation of HBV DNA can be observed in 20–50% of the HBsAg-positive patients receiving immunosuppressive agents or cytotoxic therapy, including corticosteroids, anti-CD20 and anti-TNF. Some patients suffer from transaminase elevation and jaundice, and severe patients develop to fulminant liver failure even death. Nucleoside analogues prophylactic treatment can decrease HBV reactivation. Regardless of the HBV DNA level, HBsAg carriers should receive nucleoside analogues antiviral treatment 2–3 weeks before immunosuppressive or cytotoxic therapy. Antiviral drugs inhibit HBV DNA rapid, such as lamivudine, telbivudine and entecavir, are preferred for prophylaxis. Most patients cannot tolerate the recurrent aggravations induced by drug-resistance. Prophylaxis decision should be made depending on baseline HBV DNA load and duration of immunosuppressive therapy or cytotoxic therapy. Antiviral drugs with low resistance are recommended if prophylaxis will last more than 12 months. Treatment duration: if baseline HBV DNA $\leq 10^5$ copies/mL, treatment should be continued to 6 months after immunosuppressive therapy or cytotoxic therapy completed; if baseline HBV DNA $> 10^5$ copies/mL, treatment should be continued to the referring withdrawal standard of ordinary

CHB patients. However, IFN- α is not recommended because of the bone marrow suppression.

There is no consensus of prophylactic antiviral treatment for HBsAg-negative and anti-HBc-positive patients during the immunosuppressive or cytotoxic therapy. Serum HBV marker and HBV DNA level should be monitored [9, 68–73].

5.1.4.11 Patients with Autoimmune Thyroid Disease

HBV infection itself is not correlated with thyroid dysfunction. IFN- α can aggravate underlying autoimmune thyroid disease or induce emerging thyroid disease in some patients because of its immunomodulatory activity and direct thyroid toxicity. Uncontrolled thyroid dysfunction contraindicates IFN- α antiviral therapy.

Patients with previous thyroid dysfunction or high titer of thyroid autoantibody (TPO-Ab >18 IU/mL) before treatment should be monitored for thyroid function during IFN- α antiviral treatment. Patients with emerging thyroid dysfunction during treatment should terminate antiviral treatment. Majority of thyroid dysfunction emerged during treatment in patients without history of thyroid dysfunction is reversible, and can restore after IFN- α withdrawal [74–77].

5.1.5 Management of Drug-Resistance

Resistance to nucleoside analogues is a serious problem in CHB treatment, which does make the long-term treatment strategies become a difficulty.

5.1.5.1 Predictors of HBV Resistance Mutations

A variety of factors may be associated with resistance to nucleoside analogues, including nucleoside analogue type, HBV DNA load at initial therapy, liver fibrosis/cirrhosis, and previous nucleoside analogues treatment. In addition, male gender, high body mass index and alcohol abuse are also the risk factors of resistance mutations in antiviral therapy. However, a growing number of studies suggest that early virological response is an important indicator to predict drug resistance [78, 79].

5.1.5.2 Prevention of Drug-Resistance

Select nucleoside analogues treatment indications reasonably. Nucleoside analogues treatment is not recommended for the HBV infected patients in immune tolerant phase or non-active phase, especially those who are younger, if they do not receive immunosuppressive therapy or chemotherapy. For the CHB patients with first flare, especially those who are younger, nucleoside analogues should be given with caution after fully analysis of inductions.

Select nucleoside analogues treatment strategies reasonably. Treatment should be consulted the Guideline on prevention and treatment of chronic hepatitis B in china. For patients with antiviral therapy indications, drugs with strong antiviral activity and low resistance are recommended if nucleoside analogues are chosen. The previous antiretroviral therapy, including drugs, treatment response and resistance mutations, should be understood in order to avoid nucleoside analogues with

cross-resistance. Furthermore, sequential monotherapy treatment should be avoided for multi-drug resistance.

Improve patients' compliance. Prescribed time and adequate medication should be repeatedly emphasized to the patients during nucleoside analogues treatment. According to the clinical trial, more than 30% of cases with virologic breakthrough are resulting from poor compliance. Gradual dose reduction will significantly increase the risk of resistance and is forbidden.

Regularly detect HBV DNA load and genotypic resistance and timely adjust treatment. HBV DNA load is the most important indicators of drug resistance in nucleoside analogue antiviral therapy. HBV DNA levels should be monitored regularly during treatment. Numerous clinical trial data show that early virological response is an important predictor of drug-resistance. Therefore, AASLD and EASL guidelines both recommend adjusting treatment plan based on EVR to improve the efficacy and reduce the incidence of drug resistance [6, 78–81].

5.1.5.3 Management of Emerged Drug-Resistant Mutations

Patients with normal ALT and mild inflammation or fibrosis (<G1S1) before treatment can stop the anti-viral treatment, and need to be monitored closely for antiretroviral therapy again promptly if aura symptoms happened. Most patients with nucleoside analogues resistant, especially the decompensated cirrhotic patients, need rescue therapy timely. Virologic breakthrough usually precede biochemical breakthrough, and rescue therapy before biochemical breakthrough can avoid sudden hepatitis flare and aggravation.

Management of lamivudine and adefovir resistance refers to guide recommendations above. Add adefovir or tenofovir (the latter has not been approved by SFDA, and the safety of combination of entecavir and tenofovir needs further study), or switch to IFN- α or pegylated IFN- α for patients with entecavir resistance. Add adefovir or tenofovir for patients with telbivudine resistance. Tenofovir resistance has not detected, if happened, entecavir, telbivudine, lamivudine or emtricitabine can be added theoretically. For lamivudine and adefovir multidrug resistance, switch to emtricitabine or combination of entecavir and tenofovir (but not been approved by SFDA), or switch to IFN- α or pegylated IFN- α treatment [6, 80, 81].

5.1.6 Difficult-to-Treat Chronic Hepatitis B

Definition of refractory CHB: CHB patients with treatment failure or poor efficacy and CHB patients have been confirmed poor efficacy by evidence-based medicine, using anti-HBV drugs, including nucleoside analogues and (or) interferon, under the existing guidelines or recommendations for various reasons and (or) factors.

Refractory CHB patients include: (1) Patients with primary non-response, partial virological response and (or) virologic breakthrough, drug-resistant HBV mutations and clinical drug-resistant. (2) Patients with serological (HBeAg) no response or partial response, namely HBeAg-positive patients without HBeAg loss or HBeAg seroconversion after initial treatment of more than 1 year. (3) High baseline HBV

viral load: HBeAg-positive patients with HBV DNA $>10^9$ copies/mL, HBeAg-negative patients with HBV DNA $>10^7$ copies/mL. (4) Cirrhotic (compensated and decompensated) patients and AECHB patients. (5) Other patients with HBV reactivation after transplantation, immunosuppressive therapy, combination of other viral infections such as HCV and HIV, combination of metabolic/autoimmune diseases such as insulin resistance, hyperlipidemia and (or) non-alcoholic steatohepatitis and fiber cholestatic hepatitis.

The present guidelines for the treatment of refractory CHB involve virus strains resistant mutations and virologic breakthrough, partial virological response, cirrhosis, virus reactivation after liver transplantation, receiving immunosuppressive therapy, and combination of other viral infections (HCV and HIV). Majority of the treatment recommendations are based on expert consensus, clinical experience, or clinical studies of small sample. However, for patients with serological no-response or partial response, high baseline HBV viral load, AECHB, insulin resistance and non-alcoholic steatohepatitis, and fiber cholestatic hepatitis, there is no clear guidance.

Currently ongoing hepatitis B related clinical researches more concentrated in drug selection and optimization of treatment-naïve patients, and only a small portion and small-scale studies involve refractory CHB.

5.1.7 Antiviral Therapy for AECHB

Anti-HBV treatment was not taken into account in AECDHB in the past. It is thought that immune pathological damage is the key in the development of AECHB, and HBV is just a promoter. The role of hepatitis virus in development of AECHB has not been paid enough attention.

With the study of AECHB mechanism deep going, more and more scholars have realized that constant HBV replication induced hyperactive immune response is a major factor in exacerbation. When HBV induces hypersensitivity, a large amount of immune complexes generate and activate the immune network, leading to serious hepatocyte damage through the following mechanisms: (1) Th1 cells activate, release interleukin-2 (IL-2), and mediate cytotoxicity of cytotoxic T cell (CTL), macrophages and natural killer (NK) cells; (2) macrophages activated by HBV and endotoxin release cytokines (including tumor necrosis factor TNF- α and fgl2), inducing direct hepatocyte damage or secondary damage by microcirculation disturbance; (3) Fas ligands (Fas-L) express increasingly on the surface of infected hepatocytes, conjunct to Fas expressed by CTL, and induced apoptosis. Antiviral therapy can quickly suppress HBV replication, reduce intercellular viral spread, and inhibit the membrane target antigen expression, so as to inhibit CTL attack and relieve hepatocyte injury and necrosis. Antiviral treatment at early stage in disease is the pivot to terminate intense cellular and humoral immunity. Therefore, it is advocated to start antiviral treatment for severe hepatitis patients with HBV replication. Some scholars believe that viral load is an important indicator for AECHB, although it does not directly related to liver damage. HBeAg and HBV DNA

seroclearance is important for remission and positive for improving cure rate of severe hepatitis. Therefore, early effective anti-viral therapy can reduce the viral load, suppress virus generation by infected hepatocytes, decrease infection of newborn hepatocytes, alleviate liver inflammation and is beneficial to liver recovery. Antiviral therapy has become an effective treatment for AECHB [82].

Whether antiviral therapy increase or alleviate immune response in the advanced stage of severe hepatitis had been controversial. However, recent studies reach a consensus that antiviral treatment can slow disease progression and improve recent survival rate.

In most early studies, lamivudine failed to improve liver function and survival in AECHB compared to placebo [83–87]. However, a study from Taiwan showed that lamivudine could improve survival in patients with baseline total bilirubin less than 342 mmol/L (20 mg/dL) [88]. Wong and Chan reported that antiviral therapy in AECHB cannot improve recent survival, but could prevent further deterioration [89]. In early stage, there is intense immune response, high viral load, severe inflammation, and ongoing immune liver damage. Viral load can affect the progression and prognosis. Patients with HBV DNA positive have a relative poor prognosis because of more virus antigen on hepatocyte surface activating immune injury. In middle-advanced stage, the impact of HBV DNA on progression and prognosis would weaken because immune response has alleviated after self-regulation. Therefore, HBV DNA level in early severe CHB is a significant indicator for prognosis, and antiviral therapy is essential. In middle-advanced stage, HBV DNA level has little influence on the prognosis, and antiviral therapy is meaningful to prevent recurrence. In addition, lamivudine can obtain sustained virologic response, but long-term treatment may induce viral resistance and virologic breakthrough, which reduce the efficacy of antiviral therapy [90].

Most recent studies suggest that antiviral therapy for HBV DNA positive patients in early severe hepatitis can postpone disease progression and improve recent survival rate [91]. Ma et al. analyzed 248 cases of HBV-ACLF retrospectively. 124 patients added entecavir on the basis of standard medical treatment, another 124 patients only received standard medical treatment without nucleoside analogues. The 1-month and 3-month survival rate of entecavir-treated patients were 72.58% (90/124) and 61.59% (76/124) respectively, and significantly higher than those of control with 53.23% (66/124) and 61.29% (57/124). Entecavir-treated patients get a significant improved MELD scores compared to control post treatment, suggesting that entecavir can postpone progression of HBV-ACLF and improve recent survival [92]. Lin et al. investigated 120 HBV-ACLF cases with entecavir treatment, and concluded that entecavir can significantly increase the survival rate [93]. Hu et al. investigated the efficacy of lamivudine and entecavir on HBV-ACLF. After 1-month treatment, survival rates are similar, but clinical improvement rate in lamivudine and entecavir group were significantly higher than basic treatment group. After 6-month treatment, the cumulative survival rates of lamivudine and entecavir group were 65.8%, 60.1% respectively and significantly higher than the basic treatment group (42%). In patients with baseline HBV DNA $>10^7$ IU/mL, cumulative survival rate in antiviral treatment group were higher than basic treatment group. Patients

with pretreatment MELD scores >30 had a lower cumulative survival rate than patients with MELD scores ≤ 2 , but obtained better response to antiviral therapy. It also showed that there was no significant difference in efficacy using entecavir and lamivudine treatment for HBV-ACLF [94]. Tillmann et al. summarized 14 cases of AECHB with lamivudine treatment. It suggested that patients with lamivudine treatment had an overall survival rate without transplant of 78.2%, but patients without lamivudine only 45.7% [95]. Garg et al. found that tenofovir can significantly reduce HBV DNA levels in HBV-ACLF, improve CTP and MELD score, and decrease mortality. HBV DNA decrease of more than 2lg copies/mL after 2-week treatment is a predictor of good prognosis.

5.2 Antiviral Therapy for Severe Hepatitis B (Liver Failure)

Hai-Bin Su

Liver failure is a common syndrome, and its incidence is increasing with the use of alcohol and growing epidemic of obesity and diabetes. Liver failure is defined as inability of the liver to perform its normal, metabolic, excretory and biotransformation functions by Chinese Medical Association [96]. Its manifestation includes coagulopathy, jaundice, hepatic encephalopathy (HE) and ascites. Liver failure can be divided into four classes: acute liver failure (ALF), sub-acute liver failure (SALF), acute on chronic liver failure (ACLF), and chronic liver failure (CLF) according to histopathological characteristics and the progression of disease. ALF is a syndrome with liver function deterioration rapidly accompanied with a grade II or higher HE within 2 weeks illness duration. SALF onset is slowly than ALF that symptoms occur within 2–26 weeks. Liver failure occurred with known or unknown chronic liver disease refer to ACLF. CLF is defined as Progressive deterioration and decompensation of liver function in patients with liver cirrhosis, mainly manifested with complications of portal hypertension. Based on the severity of clinical manifestations, sub-acute and acute-on-chronic liver failure can be divided into early, middle, and end stages. Early stage has severe fatigue and gastrointestinal symptoms, total bilirubin level is more than 171 $\mu\text{mol/L}$ or daily increase of total bilirubin is more than 17 $\mu\text{mol/L}$ and prothrombin activity (PTA) is less than 40%. Middle stage has stage II HE and/or ascites and PTA $\leq 30\%$. End stage has refractory complications such as stage III or higher HE, hepatorenal syndrome, massive hemorrhage of the upper alimentary tract, severe infection and refractory fluid and electrolyte imbalance, PTA $\leq 20\%$. Otherwise, Asian Pacific Association for the Study of the Liver (APASL) define ACLF as a severe liver injury, leading to coagulation abnormality usually with an INR ≥ 1.5 , and any degree of mental alteration (HE) in a patient with pre-existing liver disease and with an illness duration less than 4 weeks [97].

Etiology, epidemic and precipitating factors of liver failure are different between western countries and China. Non-infection factors such as alcohol and drug induced liver failure are predominant in western countries [98]. However, hepatitis B virus (HBV) infection is the main reason to induce liver failure [99]. We

retrospective analysis the etiology of 1977 cases of liver failure came from 13 provinces in China northern area from 2002 to 2006 and found HBV infection induced liver failure was about 82.8% [99]. So far, liver transplantation is the most effective way to treat HBV induced liver failure. But due to the cost and shortage of organ donor, liver transplantation can't be used widely. Comprehensive treatment including supportive therapy, antiviral therapy and immunoregulation therapy is the main way to treat HBV related liver failure in China. But mortality of liver failure is high and total curative rate is only about 35.56% because of the complicate pathophysiology in liver failure [100]. The precise mechanism underlying the liver injury caused by HBV-ACLF and the factors contributing to the progression of liver failure remain unknown. Generally, virus factors, host factors, and their interaction determine the outcome of ACLF. HBV DNA replication is one of the key factors causing the progression from liver damage to liver failure. The HBV DNA level is closely associated with the risk of hepatocellular carcinoma development, and HBV DNA suppression significantly improves the prognosis of cirrhosis. Current clinical guidelines advocate oral antiviral treatment in decompensated cirrhosis and sustained HBV DNA suppression to reduce sequelae [4, 96, 97, 101].

5.2.1 Theory Basis of Antiviral Therapy for Severe Hepatitis B (Liver Failure)

5.2.1.1 The Association Between HBV Genotype and Severe Hepatitis B (Liver Failure)

Eight different HBV genotypes (A–H) have been described based on their genomic heterogeneity. Many studies showed that the severity of HBV infection correlated with HBV genotype. In the Asia-Pacific countries, genotype B and C HBV are predominant with genotype C HBV associated with delayed HBeAg seroconversion and more aggressive disease activity as compared to other HBV genotypes [102]. Patients infected with HBV genotype C have high HBV DNA level and high HBeAg positive rate than people infected with other HBV genotypes. These patients have low response to anti-viral therapy and progress to liver failure, particular in patients infected with genotype C2. Zhang et al. analyses 2922 hepatitis B patients and found that the most common HBV genotype was B and C in chronic hepatitis B patients [103]. Patients infected with genotype C was more predisposed to chronic and cirrhosis and hepatic carcinoma. Genotype B and C had no influence on illness progression in acute and mild patients, but HBeAg positive rate and HBV DNA level were high in patients infected with genotype C. Further studies showed that HBV BCP/pre C mutation was associated with HBV genotype. A1846T and C1913 mutation probably associated with ACLF. C1913 was an independent prognostic factor in ACLF patients [104].

5.2.1.2 The Effect of Gene Mutation

HBV DNA polymerase lack proof function which can lead to viral mutation during replication. In addition, HBV exist in many quasispecies. Under select press,

quasispecies variance can cause the change of HBV replication, pathogenicity, antigen epitope which lead to influence immune response and viral resistance. Over immune response can result in severe liver injuries. HBV pro core/core, pre S, P gene and multi gene mutation were found to be associated with liver failure [105].

One of the functions of HBeAg is to induce immune tolerance. In the absence of HBeAg, patients harboring pre-core mutant HBV may have a more rigorous immunological response. Chronic infection with pre-core mutants has been often associated with multiple flares with interspersed asymptomatic periods. Mutations at the basal core promoter (BCP) regions lead to decreased HBeAg synthesis, active liver histology, and increased viral replication. These exacerbations are seen to lead to fulminant hepatic failure [89, 106, 107].

We investigated HBV BCP A1762T/G1764A double mutation in liver failure patients [104]. Longitudinal study showed that nucleotide mutation sites were occurred more in HBV-ACLF than cirrhosis patients among which nt53, nt1846, nt1896 and nt1913 were associated with HBV-ACLF. T1846 mutation was found exist more in genotype B than genotype C (57.1% vs 30.4%), A/G1913 mutation is found frequently in HBeAg negative patient than positive patients (28% vs 13.2%). These indicated that pre core/BCP mutation associated with the occurrence of liver failure and influence patients outcome.

HBx is a multifunction regulatory protein which can influence gene transcription, activate signal transduction, enhance viral replication, accelerate protein degradation and regulate cell apoptosis. HBx can participate in the process of HBV pre S and BCP/pre core mutation

5.2.1.3 Immune Characteristics in Liver Failure

Liver failure caused by chronic HBV infection (i.e., chronic severe hepatitis B) is a common life-threatening disease in China. The pathogenesis of chronic severe hepatitis B is complex and is currently not completely understood. However, one widely accepted mechanism is the induction of cellular immune responses mediated primarily by cytotoxic T lymphocytes (CTLs) and delayed-type hypersensitivity T cells. These immune responses are induced by viral protein antigens expressed in the target cell surface due to the active replication of HBV and eventually result in large areas of liver cell necrosis [108]. The specific mechanisms may involve several factors [109]. Using a hybridization assay for HBV DNA and a conventional enzyme immunoassay to measure the HBeAg level, an earlier study showed significant parallel increases in serum HBeAg and HBV DNA levels and accumulation of intracellular viral proteins several weeks before the hepatitis flare. In addition, there was a subsequent increase in anti-HBe production and HBeAg/anti-HBe immune complex formation, implicating the important role of the immune response to HBV in initiating the hepatitis flare [110]. Immunohistologic studies during the hepatitis flares have shown CD8+ T cells in the mononuclear cell infiltrates, strong membranous expression of human leukocyte antigen class I (HLA-I), and cytoplasmic or membranous/submembranous hepatitis B core antigen (HBcAg) expression [111, 112]. Some findings suggest that hepatitis B flares are the results of dynamic changes of the innate and adaptive immune responses with HLA-I restricted, CTL

mediated immune cytolysis of HBV antigen(s) expressing hepatocytes and its downstream apoptotic mechanisms [113, 114]. The activated Th1 cells release interleukin-2 and excite cytotoxic effects of CTLs, macrophages, Natural Killer cells, and lymphokines [102]. Macrophages, activated by HBV and endotoxins, release various cytokines mainly with tumor necrosis factor (TNF)- α , which directly damage liver cells and also result in secondary injury of liver cells through disturbances in microcirculation. Fas ligands (Fas L) are highly expressed in the surface of HBV-infected liver cells and combine with Fas expressed by CTLs, together inducing hepatocellular apoptosis. Therefore, antiviral therapy early in chronic severe hepatitis B is beneficial for suppressing intense cellular immune responses induced by HBV replication. If HBV replication is suppressed rapidly, the immune pathological responses of chronic severe hepatitis B may be reduced, thus effectively blocking the disease progression.

5.2.2 Clinical Research of Antiviral Therapy for Severe Hepatitis B (Liver Failure)

The administration of anti-HBV therapy in chronic severe hepatitis B (acute- or subacute-on-chronic liver failure) is still undergoing research, and limited data are presently available. So far, anti HBV treatment drugs include interferon and oral antiviral drugs. Interferon application is contraindicated in the treatment of liver failure due to its limited anti-viral efficacy, significant adverse drug effects, and induction of immune enhancement, which can further result in aggravation of liver damage. Oral anti HBV drugs including Adefovir dipivoxil (ADV), Lamivudine (LAM), Telbivudine (TBV), Entecavir (ETV) and Tenofovir (TDF) have few adverse effects. Antiviral therapy may have the advantage of shortening the replication and thereby reduce disease duration without the side effects of interferon. Therefore, many studies have been carried out to find the efficacy of oral antiviral drugs on patients with liver failure.

5.2.2.1 Antiviral Therapy in HBV Related Acute Liver Failure

The effect of antiviral therapy in HBV related acute liver failure is controversial. Some studies reported that antiviral therapy can't improve outcome because HBVDNA can be eradicated spontaneously due to enhanced immune response in liver failure patients. But HBV infection is the initial factor to induce over immune response that can lead to liver damage. Early treatment with antiviral therapy can inhibit HBV DNA replication and attenuate immune reaction which can reduce liver damage, hepatocyte apoptosis and necrosis. Tillmann et al. [115] reported that 17 acute HBV related liver failure patients were treated with LAM and 20 patients were treated with placebo. Encephalopathy occurred in 3 (17.6%) and 11 (68.6%) patients, respectively ($p = 0.005$). It demonstrated that early use of antiviral drugs can reduce the rate of encephalopathy and mortality. In addition, a prospective study about ETV on 6 acute liver failure patients demonstrated that ETV can reduce HBV DNA load significantly and 5 patients achieved anti HBsAg conversion [116].

Therefore, monitor closely and early use antiviral drugs to reduce hepatocyte apoptosis and necrosis on patients with HBV related acute liver failure are very important.

5.2.2.2 Antiviral Therapy in HBV Related Acute on Chronic Liver Failure (HBV-ACLF)

The objective of antiviral treatment for HBV-ACLF is to reduce viral load at an appreciably high rate, thereby promoting reduction in hepatocyte cell death and improved survival outcomes by prevention of decompensation related multiorgan complications in this group of severely ill patients. Several studies have delineated the fact that low pretreatment HBV DNA load and a rapid decrement in viral load improves outcomes in HBV-ACLF [117], whereas a study from India reported that a 2 log decrease in HBV DNA at week 2 improved survival benefit in patients with HBV-ACLF [118]. Antiviral therapy also promotes chances of stabilization to liver transplant time and improves transplant outcomes. Studies have debated on the issue of antiviral therapy related improvement in the long term [119]. LAM decreased viral load significantly, but did not result in significant biochemical or clinical improvement compared with those patients given placebo. Mortality of patients receiving nucleoside analog therapy was significantly lower than the placebo group, which indicated that antiviral therapy improved prognosis of patients with HBV-ACLF if implemented as soon as possible [117]. Even in the age of effective antiviral therapy, early transfer to a transplantation facility should be considered before managing conservatively by medical means. The APASL consensus guidelines on ACLF describe the value of early and prompt institution of antiviral therapy in HBV-ACLF. HBV DNA levels are now not an indication for commencement of antivirals in HBV-ACLF reactivation, as earlier starting of such therapy, even prophylactic, has been found to have great survival benefit in the long run.

From 2006 to 2009, early and middle stage HBV-ACLF patients in our hospital were recruited in a prospective study to evaluate the efficacy and safety of LAM and ETV [120]. No antiviral therapy was used in control group. This study showed that LAM or ETV can reduce 3 and 6 months mortality, improve survival rate on patients with ACLF-HBV. Three and 6 months cumulative survival rate of LAM and ETV therapy were 69.2% and 65.8%, 67% and 60.1%, respectively, which was higher than control group (42% and 42%, $p = 0.045$ and 0.04). No significant difference on survival rate between LAM and ETV ($p = 0.723$). This study also showed that MELD score was an effective prognostic predict factor on patients with ACLF-HBV. Patients with MELD score less than 30 had a good outcome. Another study also demonstrated that For HBeAg-negative patients with HBV-ACLF, when entecavir was added to comprehensive therapy, a MELD score ≥ 30 predicted very poor prognosis due to fatal liver failure [121].

Hu et al. made a survival analysis on 190 HBV-ACLF patients and results indicated that nucleoside analog application in early and middle stage HBV-ACLF can improve survival rate and prolong patients' life. Median survival time was 5.7 and 1.79 months in patients treated with and without antiviral therapy. Another study indicated that patients with MELD score 30–40 and HBV DNA load decrease 2 log

had a better outcome than patients with HBV DNA load decrease less than 2 log within 4 weeks treatment [122]. In addition, mortality had no relationship with HBV DNA load if MELD score >40. Xiao et al. also demonstrated that nucleoside analog treatment is an independent prognosis predict factor in 219 HBV induced liver failure. LAM and ETV had no difference. Although antiviral therapy can improve patients survival rate, treatment with and without nucleoside analog had no difference in late stage liver failure patients.

5.2.2.3 Antiviral Therapy in Chronic Liver Failure

Currently, liver transplantation is the ultimate therapeutic option for decompensated cirrhosis patients. However, liver transplantation can't be used in all patients because of the shortage of donor organs. Therefore, the aim to treat decompensated cirrhosis is to decrease the occurrence of disease associated complications and the liver associated mortality rate [123]. The natural history of decompensated HBV-related cirrhosis is affected by high HBV replication which may exist in some decompensated HBV-related cirrhosis patients [123]. Hepatic necroinflammation and fibrosis progression are improved after sustained viral suppression is achieved which can prevent decompensation in cirrhosis [124]. Oral NAs treatment are strongly recommended in most clinical guidelines for decompensated HBV-related cirrhosis patients no matter what HBV DNA replication level is [109, 110]. Yao et al. [125] found that CTP scores was reduced more than three points in 69% LAM treated patients with severely decompensated cirrhosis. Furthermore, CTP scores was decreased to less than seven point in 38% of these patients, and their statuses on the United Network of Organ Sharing waiting list changed to inactive. A randomized controlled trial in Asia demonstrated less liver-related morbidity in the LAM-treated patients with HBV associated advanced compensated cirrhosis compared to the untreated controls because of the reduced incidence of hepatic decompensation and lower risk of HCC. Increased CTP scores were noted in 3.4% of the patients in the LAM group compared to 8.8% of the patients in the placebo group ($p = 0.02$) [126]. Fontana et al. showed most deaths caused by liver related complications occurred within the first 6 months in patients with decompensated HBV-related cirrhosis treated with LAM. Pretreatment high HBV DNA replication level, serum bilirubin and creatinine were associated with 6-months mortality rates significantly [127]. This finding indicates early antiviral therapy might be important.

5.2.2.4 Antiviral Drugs in HBV Related Liver Failure

Lamivudine

LAM is a nucleoside analogue that inhibits HBV DNA synthesis which was the first oral drug to treat chronic HBV infection in 1998. Its mechanism is to compete with nature cytidine to inhibit HBV polymerase, then terminate HBV replication. Chan et al. [128] studied the effect of lamivudine in treatment of severe hepatitis-B-related acute exacerbations leading to ACLF in 28 patients as against 18 controls. It was concluded that lamivudine had no survival benefit compared with conventional treatment in severe aggravations of chronic hepatitis B and that liver transplantation

should be considered in these patients with thrombocytopenia and high bilirubin. However, another meta study [129] analysis 242 studies to evaluate the short-term effect of lamivudine (LMV) treatment for severe chronic hepatitis B. They found that the survival rates and PTA of the test group were distinctively higher than those of the control group at weeks 4, 8, and 12 of the treatment course. The HBV-DNA negative change rate was distinctively higher throughout the 12 weeks of LMV treatment. For patients who started LMV treatment in the middle stage, the mortality rate of the test group was lower. They concluded that LMV decreased HBV-DNA levels in the serum, improved liver function in patients, and enhanced survival rate during the early and medium stages of severe chronic hepatitis B. Tsubota et al. [130] studied 25 patients with spontaneous severe acute exacerbation treated with lamivudine, and found that lamivudine monotherapy did not prevent hepatic failure deterioration significantly, but it resulted in long-term benefits. Baseline serum bilirubin, pre-existing cirrhosis and baseline PT were independent determinants of prognosis.

Adefovir

ADV is an acyclic nucleotide analogue of adenosine monophosphate. Use of adefovir for HBV-ACLF has been rare. In two case reports, adefovir dipivoxil failed to salvage cases of lamivudine resistance after jaundice and liver failure developed. A lower antiviral potency and the potential risk of nephrotoxicity of ADV remain a problem for routine use as a first-line treatment. It is hence not advisable to use adefovir as a first-line drug in the treatment of acute severe exacerbation. But considered to the high viral resistant to LAM, ADV plus LAM can reduce the incidence of LAM resistance. The combination of ADV and LAM can be used in liver failure patients. 128 patients with decompensated cirrhosis caused by LAM-resistant HBV were treated with ADV and HBV DNA level become undetectable occurred in 81% of patients and CTP scores were improved [131]. But another study focused on long time outcome found that resistance to ADV was 20% and renal toxicity was confirmed in 3% of patients [132].

Entecavir

ETV is a cyclopentyl guanosine analogue that can inhibit HBV polymerase's function. Compared with LAM and ADV, ETV has a more potent activity against wild type HBV [133, 134]. ETV has been studied in in cirrhotic patients. One Korea study showed CTP and MELD scores were improved in 55 patients treated with ETV for 12 month. The 2-year cumulative incidence of HCC was 6.9%, and the cumulative death rate was 17% [90]. Many studies have been carried out to compared the efficacy of ETV and LAM to treat HBV related ACLF. The short-term efficacy of entecavir vs lamivudine was similar and the degree of pretreatment liver failure significantly affected the outcome of treatment [135, 136]. In summary, the pros and cons of LAM vs ETV in decompensated or severe acute exacerbation of chronic hepatitis B were ETV being more effective in promoting faster viral load decrement. Also the available clinical evidence suggests that clinicians treating chronic hepatitis B patients with acute HBV exacerbation or decompensated liver disease should use the most potent nucleoside analogs available [137].

Telbivudine

TBV is a synthetic thymidine nucleoside analogue that has potent antiviral activity against HBV. One study investigated the short-term efficacy and safety of TBV therapy in liver failure patients caused by chronic hepatitis B virus (HBV) infection [138]. In this study, 20 patients were treated with TBV, and the other 18 patients were treated with LAM. HBV DNA levels in the TBV group fell to the lower limit of detection after the fifth week, which was more rapid than in the LAM group. In addition, the total bilirubin and prothrombin time activity of the patients with TBV treatment showed a more significant improvement as compared to the patients treated with LAM from the start of the fifth week. They concluded that TBV treatment is superior to LAM treatment in improving the condition of patients with liver failure as a result of chronic HBV infection in the short term. But viral resistance is also a major concern. A study in decompensated cirrhosis patients with HBV infection showed genotypic resistance was developed in 27% of the TBV patients during the 2-year period [139]. Therefore, TBV used as a first-line treatment has limitations in patients with HBV-related decompensated cirrhosis.

Tenofovir

TDF is an acyclic nucleotide analogue with potent inhibition of HBV polymerase/reverse transcriptase. In a seminal study by Garg et al. [90], consecutive patients with ACLF due to spontaneous reactivation of chronic hepatitis B were randomized to receive either TDF or placebo. The primary endpoint was survival at 3 month. More than 2 log reduction in HBV DNA levels at 2 weeks was associated with survival rate. The authors concluded that TDF had potent activity to reduce HBV-DNA levels, improve CTP and MELD scores, and reduce mortality in patients with severe spontaneous reactivation of chronic hepatitis B presenting as ACLF, and that reduction in HBV-DNA levels at 2 week should be considered a desirable goal and a good predictor of survival. Until now, no studies have reported viral resistance to TDF. Therefore, TDF and ETV can be considered to be the first-line therapy because their potent activity against HBV replication and high resistance barrier. In addition, the data about TDF to treat HBV-ACLF is limited. Thus, larger prospective and multicenter studies are encouraged to evaluate further the effect of TDF on short-term mortality of patients with HBV associated ACLF.

5.2.3 Prospects on Antiviral Therapy for Severe Hepatitis B (Liver Failure)

There still lack multicenter, larger samples, prospective and randomized clinical trial to test the efficacy of antiviral therapy. But it is likely that antiviral therapy with nucleos(t)ide can improve patients survival rate in patients with HBV-related liver failure [140]. Therefore, antiviral therapy is reasonable to try in patients with high HBV DNA replication. In recent year, LAM and other nucleoside analogue have been used in severe hepatitis B wildly.

The efficacy of antiviral therapy is correlated with the time to start. Many clinical trials demonstrated that early antiviral treatment is important for patients with liver

failure [141]. Antiviral therapy used in early and middle stage liver failure can improve patients survival rate. In patients with late stage, such as serum total bilirubin is more than $342 \mu\text{mol/L}$, it's rare that patients can get benefit from antiviral therapy. Therefore, early use antiviral therapy can reduce viral load, inhibit viral replication, reduce new hepatocyte be infected by HBV again and alleviate liver inflammation and all of that are benefit to hepatocyte regeneration.

The criteria for antiviral therapy dependent on HBV DNA level in serum. Some people suggested antiviral therapy should be used in patients with HBeAg positive and HBV DNA $>10^4$ copies/mL or HBeAg negative and HBV DNA $>10^3$ copies/mL. However, take into account over immune response in liver failure patients that associated with viral eradication, even patients with HBsAg positive and HBV DNA undetectable should be considered for antiviral therapy. In our opinion, antiviral therapy should be used in all liver failure patients with HBV replication immediately.

The feature of oral antiviral drugs should be considered when used in liver failure patients. Side effects of nucleos(t)ide, including elevate CK, myopathies and lactate acidosis, can occurred during treatment. We observed the change and effect of elevated CK during the treatment of ETV and LAM in liver failure patients. We found that no different of CK elevation in both drugs. CK elevation was consistence with infection and hepatic renal syndrome. But long term safety of NAs has not been confirmed in liver failure patients.

Antiviral therapy should be used for life because the NAs treatment can't eradicate cccDNA in the hepatocyte. Some cirrhotic patients developed viral resistance to LAM during long-term LAM therapy which cause virologic response loss [142, 143]. In antiviral treatment naïve patient, The LAM resistance rate is up to 70% after 5 years of continuous therapy and the annual resistance rate is up to 20% [6], compared with that ETV resistance rate is less than 0.5% in patients treated with ETV at 4 years [144]. Therefore, LAM should be used with careful monitoring for the development of resistance. And ETV or TDF rather than LAM is recommended to be used as the first-line therapy in patients with HBV infection because of its high genetic barrier and potent activity against HBV replication [145].

Multiorgan failure occurred rapidly frequently in liver failure patients. Although some patients can recover treated by comprehensive and antiviral therapy, all patients should be evaluated for liver transplantation. It's challenge to study the effect of NA, how and when to use NA and how to treat viral resistance to NA in liver failure patients. Further studies are needed to evaluate patient outcomes after antiviral therapy with NA in liver failure patients.

5.3 Antiviral Treatment for Hepatitis B Virus Related Liver Cirrhosis

Ke Ma and Qin Ning

For patients with chronic hepatitis B (CHB), HBV continue replication caused progression of liver disease, eventually lead to cirrhosis or hepatocellular carcinoma.

The principle of treatment for HBV-related cirrhosis is a comprehensive treatment, including antiviral, anti-inflammatory, hepatoprotective treatment and anti-fibrosis, which antiviral treatment is the key point. Since 1999, listed lamivudine, there were many new ideas and concepts on treatment for HBV-related cirrhosis, liver failure. A large number of evidence-based medicine evidence suggests that sustained suppression of HBV by anti-viral treatment can reduce liver inflammation and fibrosis, reduce or delay disease progression, and ultimately improve survival rates and quality of life. Internationally there are many CHB antiviral therapy management guidelines, but antiviral therapy for HBV-related cirrhosis was still a hot and difficult issue in clinic. This article reviews some of the new progress and new perspectives of antiviral therapy liver cirrhosis based on the recent research.

5.3.1 Goals and Endpoints of Antiviral Treatment for HBV-Related Cirrhosis

Management is guided by recommendations from the American Association for the Society of Liver Disease (AASLD) [6], European Association for the Study of Liver (EASL) [4], Asian Pacific Association for the Study of Liver (APASL) [146], and Society of Hepatology and Infectious Diseases of Chinese Medical Association (CMA) have formed a consensus [3]. Guideline 2015 edition issued by the Chinese Medical Association clearly stated that the overall goal of treatment is to CHB was to suppress HBV as long as possible, relieve inflammation or necrosis of liver cells and liver fibrosis, prevent the progression of cirrhosis, reduce and prevent the occurrence of hepatic decompensation, HCC and its complications, thereby improving the quality of life and survival rate.

For patients with cirrhosis, whether compensated or non-compensated, antiviral treatment can delay or reduce hepatic decompensation and HCC occurs, does not change the final outcome of end-stage liver cirrhosis. In the guideline 2010 edition issued by the Chinese Medical Association, the goal of antiviral therapy in HBV compensated cirrhotic patients is to prevent progression of the disease to decompensated cirrhosis, end-stage liver disease, hepatocellular carcinoma. The goal of antiviral therapy in HBV-related decompensated cirrhosis is to improve the hepatic disease severity, improve the clinical symptoms and quality of life, and prolong patient's survival.

For the endpoint of the antiviral treatment, the definition of each guide is slightly different. Guideline 2018 edition issued by AASLD explicitly mentioned these patients with compensated cirrhosis should receive long-term treatment. However, treatment maybe stopped in HBeAg positive patients if they have confirmed HBeAg seroconversion and have completed at least 6 months of consolidation therapy and in HBeAg negative patients if they have confirmed HBsAg clearance. For these patients with decompensated cirrhosis life-long treatment is recommended. Guidelines 2008 issued by American Society of Digestive Disease [147] recommend long-term treatment until negative HBV DNA and HBsAg disappears. In the

guideline 2017 edition issued by EASL, Endpoint of antiviral treatment has even been further subdivided into “ideal endpoint”, “satisfactory endpoint” and “basic endpoint”, where “ideal endpoint” means to achieve HBsAg clearance with/without HBsAg seroconversion.

5.3.1.1 Antiviral Therapeutic Indications

Patients with liver cirrhosis generally have characteristics of longer course of disease, most of mother to child transmission, the large proportion of treated patients, high complexity of quasispecies and higher risk of resistance. The clinical treatment decisions in these patients need to consider a variety of factors, including long-term treatment, delay disease progression, improve histology, low resistance rates, good safety and patient tolerance, etc.

There are differences in current guidelines of countries in antiviral therapy indications for liver cirrhosis, mainly cutoff viral load. For compensated cirrhosis, 2012 APASL guideline pointed HBV DNA >2000 IU/mL, 2009 AASLD guideline that the HBV DNA >2000 IU/mL or HBV DNA <2000 IU/mL but elevated ALT should be treated with antiviral treatment, and 2012 EASL guidelines as long as detectable HBV DNA should be treated. 2010 Chinese guideline point that whether normal or elevated ALT, HBeAg(+) and HBV DNA $\geq 10^4$ copies/mL, HBeAg negative and HBV DNA $\geq 10^3$ copies/mL should be antiviral treated. For decompensated cirrhosis, the recommendations of the guidelines is basically the same: as long as HBV DNA can be detected, even if the viral load is low, it should be treated.

5.3.1.2 Recommendation of Antiviral Drug for HBV-Related Cirrhosis

Currently there are two types of anti-HBV drugs, including interferons and nucleos(t)ide analogues. Among them, decompensated cirrhosis is a contraindication to interferon. Even compensated cirrhosis, due to the risk of hepatic decompensation, the use of interferon should be very careful. Patients need to be closely monitored in the clinical application process, dose adjustments, the injection interval prolonged, and assistant drug to reduce adverse reactions and other measures so that the patient can safely complete drug treatment. On the contrary, the nucleos(t)ide analogues with strong antiviral effect, ease of use, safety, and well tolerance were recommended as the preferred treatment of liver cirrhosis by the major guidelines.

Currently, lamivudine (LAM), adefovir dipivoxil (ADV), telbivudine (LdT), entecavir (ETV), and tenofovir disoproxil fumarate (TDF) have been approved for CHB therapy. Different nucleoside drug efficacy and safety varies. Cirrhotic patients should be treated with potent, fast, direct inhibition of viral replication drugs. In 2018 AASLD 2017 EASL and 2015 APASL guidelines, for decompensated cirrhosis nucleoside (acid) analogues with potent effect and low resistance, such as entecavir or tenofovir should be selected.

5.3.1.3 Efficacy of Nucleos(t)ide Analogue for HBV-Related Cirrhosis

Lamivudine (LAM)

The REVEAL-HBV study of Chen [148] has established an HBV viral load paradigm in the natural history of CHB. Serum HBV DNA level has been shown to be significantly and independently associated with incidence of hepatocellular carcinoma (HCC) and cirrhosis. Liaw et al. [126] evaluated the effectiveness of antiviral therapy in preventing disease progression in patients with CHB and advanced fibrosis or cirrhosis. This is a large-scale, multi-center, randomized, double-blind, placebo-controlled prospective study. The study has changed the concept of the world, especially the Chinese doctors to treat CHB and liver cirrhosis, and promote the development of antiviral treatment for CHB and liver cirrhosis. Results showed that 3-year follow-up hepatocellular carcinoma occurred in 17 patients (3.9%) who received LAM and 16 patients (7.4%) who received placebo ($P = 0.047$). This study also found that LAM therapy reduced the risk of HCC by 51%, the risk of disease progression to fibrosis/cirrhosis by 55%. It first confirmed that antiviral therapy with LAM could delay disease progression, reduce the incidence of HCC.

In the study of patients with decompensated cirrhosis has also been confirmed LAM was well tolerated, could effectively stabilize or improve liver function, and delay progression of liver disease, reduce liver transplantation. Hann et al. [149] conducted a prospective, multicenter study evaluated LAM in 75 decompensated cirrhosis patients, 93% of whom were not waiting for liver transplantation. All 75 patients tested HBsAg(+) and 62% tested positive for HBeAg(+) at baseline. In 64% of patients HBV DNA levels were detectable by the branched chain DNA assay.

The virus in 69% of these patients after 6 months treatment and in 64% overall became undetectable by the bDNA assay. ALT, bilirubin and albumin level improved throughout treatment. From 10 at baseline to 8 at last visit the median CTP score also improved. After a median of 13.1 months of treatment, a virologic breakthrough occurred in only 18% patients. Treatment of LAM can improve liver function in nontransplantation candidates with decompensated cirrhosis. A meta-analysis from Huang et al. [150] indicated that LAM and LdT significantly decrease the mortality rate and disease severity in decompensated cirrhosis patients. Eight studies (total 511 patients) were included. Data showed that LAM and LdT significantly decreased the mortality rate (RR 0.36, 95% CI 0.25–0.54), improved the CTP scores (mean difference -3.23 , 95% CI -3.98 to -2.48).

In the study of patients with liver cirrhosis showed clinical improvement after treatment with LAM 3–6 months. And even in patients with clinical improvement, they may develop to HCC, therefore such patients still need early treatment, and close monitoring of HCC.

It is showed good efficacy and safety in the LAM treatment of HBV related compensated or decompensated liver cirrhosis. However, in the long course of LAM treatment viral resistance could not be ignored. More importantly, if these patients with chronic liver disease for a long time, poor liver reserve function, not promptly be treated, condition will deteriorate or even lead to death due to viral resistance. In

clinical use of LAM, the proportion of viral resistance increased year by year, 14%, 38%, 49%, 66%, the first year, second year, third year, fourth year, respectively. After the occurrence of viral resistance, some patients will be worsening, and even hepatic decompensation. Therefore it is emphasized that patients with compensated or decompensated liver cirrhosis by LAM therapy need to be improved compliance, closely monitoring and follow-up, to be adjusted treatment based on serum HBV DNA response situation. Some patients with decompensated cirrhosis or high viral load should be considered an initial combined with adefovir dipivoxil.

Adefovir (ADV)

The rates of ADV resistance in LAM-resistant subjects with HBV chronic hepatitis and cirrhosis are reported to be 6.4% and 25.4% after 1 and 2 years, respectively [151]. Nevertheless, ADV use as a rescue therapy is affected by a primary non-response in 8–15% of patients. In a study of ADV add-on LAM rescue therapy in lamivudine-resistant patients, Kim et al. [152] reviewed 167 patients with ADV add-on rescue treatment for 5 years. After 5 years treatment 86.9% patients had complete virological response. ALT in 92.5% patients normalized, HBeAg seroconversion occurred in 16.7% patients. A study from Woo et al. [153] was to determine the long-term clinical outcomes after ADV rescue therapy in decompensated patients infected with lamivudine-resistant HBV. In total, 128 patients with a decompensated state and lamivudine-resistant HBV were treated with ADV at a dosage of 10 mg/day for a median of 33 months in this multicenter cohort study. Following ADV treatment, 86 (72.3%) of 119 patients experienced a decrease in Child-Pugh score of at least 2 points, and the overall end-stage liver disease score decreased from 16 ± 5 to 14 ± 10 (mean \pm SD, $P < 0.001$) during the follow-up period. With ADV treatment, 67 patients (56.3%) had undetectable serum HBV DNA (detection limit, 0.5 pg/mL). Virologic breakthrough occurred in 38 patients (36.1%) and 9 patients had a suboptimal ADV response. The overall survival rate was 89.9% (107/119), and a suboptimal response to ADV treatment was associated with both no improvement in Child-Pugh score (≥ 2 points; $P = 0.001$) and high mortality following ADV rescue therapy ($P = 0.012$). Three years of ADV treatment was effective and safe in decompensated patients with lamivudine-resistant HBV.

Telbivudine (LdT)

The GLOBE [154] trial was one of the largest international multi-center clinical trials of LdT treatment of CHB. The safety and efficacy of LdT versus LAM monotherapy has been compared for 2 years in CHB patients. HBeAg(+) and HBeAg(-) patients were treated 104 weeks with LdT or LAM once daily. The patients treated by LdT achieved superior therapeutic response versus these treated by LAM in HBeAg positive (63% vs 48%; $P < 0.001$) and HBeAg negative (78% vs 66%; $P = 0.007$) patients. In both the HBeAg positive and the HBeAg negative groups, Greater HBV DNA suppression and less resistance was observed in patients treated by LdT than LAM. After 52 weeks of therapy in the Phase III GLOBE study, HBV resistance (breakthrough and resistance mutations) to LdT occurred in 3% of patients with HBeAg(+) and 2% of patients with HBeAg(-). After 104 weeks of

therapy, HBV DNA rebound in 17.8–21.6% of HBeAg(+) and 7.3–8.6% of HBeAg(–) LdT-treated patients associated with breakthrough and resistance mutations [155]. LdT is not active against lamivudine-resistant HBV. In the GLOBE study, patients who failed LAM therapy showed cross-resistance to LdT. LdT was relegated to second-line status in the management of chronic HBV infection due to increasing resistance over time.

In AASLD and EASL guidelines LdT is not recommended as first-line drug for monotherapy. When necessary the combined ADV or TDF is recommended. The “Roadmap concept” was derived primarily from a phase III global registration study of LdT. An optimized strategy based on the Roadmap concept is supposed to improve the clinical outcomes of patients with suboptimal antiviral response. Sun et al. [156] conducted the Efficacy Optimization of Response to Telbivudine (EFFORT) study to investigate the efficacy and safety of the Roadmap strategy by adding ADV to LdT for suboptimal responders. In all, 606 HBeAg(+), NA naive patients with CHB were randomized to the Mono or Optimize group. Patients in the optimize group were treated with LdT for 24 weeks. Subsequently, patients with HBV DNA ≥ 300 copies/mL at 24 weeks were added ADV to 104 weeks, while those with HBV DNA < 300 copies/mL continued monotherapy. Mono group received LdT monotherapy and added ADV if development of viral breakthrough. Due to suboptimal response 68% patients in the Optimize group had been added ADV. In the Optimize group, more patients at 104 weeks achieved HBV DNA < 300 copies/mL versus the Mono group (76.7% vs 61.2%, $P < 0.001$), and with less genotypic resistance (2.7% vs 25.8%, $P < 0.001$). Combination therapy showed an additive antiviral and low resistance potency. In two groups all patients were well tolerated. Patients with LdT which are suboptimal virological responders at 24 weeks are recommended to add ADV. These patients with LdT monotherapy can be benefited from combination therapy without increased side effects.

Chan et al. [139] studied the safety and efficacy of LdT and LAM in HBV-related decompensated cirrhosis patients. In this double-blind trial, 232 treatment-naive patients with decompensated cirrhosis in 80 academic hospitals were randomized (1:1) to receive LdT or LAM for 104 weeks. A modified endpoint was used HBV DNA < 300 copies/mL and ALT normalization. In intent-to-treat analysis (missing = failure) response rates were 56.3% vs 38.0% after 76 weeks ($P = 0.018$) and 45.6% vs 32.9% after 104 weeks ($P = 0.093$) for LdT vs LAM. Cumulative death and HCC rates were 16% and 15% in patients with LdT, and 22% and 16% compared to LAM, respectively. Cumulative genotypic resistance rate were 27% in patients with LdT, and 36% compared to LAM during a 2-year period. Comparable to LAM, LdT can effectively stabilize liver function and is well tolerated. However, these two drugs are not recommended as first-line drugs due to high virological breakthrough rates.

Entecavir (ETV)

International clinical trials [133, 157] and independent registrational studies in China have shown that ETV achieved statistically superior virological and biochemical responses compared with LAM. Shim et al. [158] evaluated ETV as

first-line monotherapy in 70 patients with HBV-related decompensated cirrhosis primarily treated with 0.5 mg/day ETV. There was no significant differences between groups in mean HBV DNA changes, rates of HBeAg seroconversion or HBeAg loss, the proportion of patients with ALT normalization after treatment. After 12 months of treatment, the MELD score and the CTP score in decompensated patients improved significantly. In these patients, 66% achieved CTP class A status and 49% showing a decrease of CTP score P2 points. The cumulative death rates and HCC rates in 2 years were 17% and 6.9%, respectively. Zoutendijk et al. [159] conducted a study to investigate the effect of ETV on disease progression in patients treated with ETV. All 372 patients were divided into three groups, chronic hepatitis B without cirrhosis group (n = 274), decompensated cirrhosis group (n = 9) and cirrhosis group (n = 89). The virological response (VR) was not influenced by the severity of liver disease (p = 0.62). In cirrhosis group, the probability of developing clinical events was higher (HR 15.41 (95% CI 3.42–69.54), p < 0.001) compared to two other groups during a median follow-up of 20 months. VR was associated with a lower probability of disease progression (HR 0.29 (95% CI 0.08–1.00), p = 0.05). Patients with compensated and decompensated cirrhosis who have achieved VR can achieve significant clinical benefits (HR 0.22 (95% CI 0.05–0.99), p = 0.04). In patients with cirrhosis, virological response to ETV treatment lead to a lower probability of disease progression. The association between disease progression and viral replication was reduced with a threshold of 2000 IU/mL. It suggested that complete viral suppression was essential for patients with NA treatment, especially in cirrhosis patients.

Tenofovir (TDF)

Tenofovir disoproxil fumarate (TDF) is an acyclic adenine nucleotide analogue. TDF as a potent, oral antiviral with low resistance rates, has been recommend first-line drug for the treatment of CHB in a number of international guidelines. A study [160] evaluated the effects on CHB patients with fibrosis and cirrhosis treated with TDF of at least 5 years. 489 (76%) patients of 641 completed 240 weeks treatment. 54% patients (348/641) had biopsy results at both baseline and 240 weeks. 87% patients (304/348) had histological improvement, and 51% (176/348) had regression of fibrosis at 240 weeks (p < 0.0001). 28% (96/348) cirrhosis patients (Ishak score 5 or 6) at baseline, 74% (71/96) no longer had cirrhosis (≥ 1 unit decrease in score). The histological response and regression of fibrosis seen in this study are probably due to the potent viral suppression achieved with long-term use of TDF.

Liaw et al. [161] conducted a clinical trial to observe 112 CHB patients with decompensated liver disease received either ETV (n = 22), emtricitabine (FTC)/TDF (n = 45), or TDF (n = 45). After 48 weeks treatment, HBV DNA was <69 IU/mL (400 copies/mL) occurred in 72.7% (ETV), 87.8% (FTC/TDF), and 70.5% (TDF) of patients. ALT normalization occurred in 55% (ETV), 76% (FTC/TDF), and 57% (TDF). HBeAg loss/seroconversion rate were: 0/0% (ETV), 27/13% (FTC/TDF), and 21/21% (TDF). In three groups MELD scores and CTP scores both improved. This study demonstrated that all NAs were well tolerated in CHB patients

with decompensated liver disease and can effectively improve virologic and biochemical parameters.

5.3.1.4 Response-Guided Therapy and Viral Resistance Management in Patients with HBV-Related Liver Cirrhosis

Response-Guided Therapy

4006 study [126] suggested continuous treatment with LAM (10 years) delayed clinical progression in patients with chronic hepatitis and advanced fibrosis by significantly reducing the incidence of the risk of hepatocellular carcinoma and hepatic decompensation. The biggest risk is the occurrence of viral resistance in the long-term antiviral therapy. Although rescue therapy can save some patients regain virologic response, but improper treatment or less often sicker or even canceling the original clinical benefit. Although treatment can save some patients regain virologic response, but improper or untimely treatment often leads to deterioration or even canceling the existing clinical benefit. Therefore, in the long-term antiviral therapy in patients with cirrhosis, how to overcome and prevent resistance, maximize the clinical benefit of antiviral therapy (including histological improvement and prevent and delay disease progression) is required by clinicians to consider.

Response-guided therapy refers to select appropriate medication according to the baseline characteristics of patients, and based on the patient's response to treatment, especially for those who did not achieve an early virological response, timely to adjust treatment to achieve a better long-term results. Response-guided therapy is a hot point in the current study of antiviral treatment for CHB, it is also an important strategy and important measure for the prevention of viral resistance. Some experts even believe that all of antiviral therapy will need to be optimized.

Response-guided therapy means optimization based on baseline characteristics and early virologic response. Numerous studies have demonstrated that baseline parameters such as low viral load, high serum ALT level, high inflammation activity score prompted by liver biopsy and early virological response predict better long-term effect. In 2007 Keeffe's [80] "road map concept", Response-guided therapy according to virologic response at 24 weeks has been recommended.

Combination therapy is an important part of Response-guided therapy. Combination therapy includes an initial stage combination, the combination in the course of treatment (poor response or resistance), the combination treatment for treated patients with relapse. In 2009 EASL guideline it was referred that ADV or TDF with LAM combination treatment need to consider in patients with liver cirrhosis. In 2009 AASLD guideline LAM or ADV was recommend initial treatment for patients with decompensated cirrhosis, but their combination is recommended to reduce the risk of resistance and rapid inhibition of virus.

The combined treatment for LAM+ADV is the most clinically used and studied. In first-generation NA LAM, YMDD mutation (rtM204V/I) developed in up to 65% of patients in 5 years [162]. The combination of ADV dipivoxil and LAM was found to lower the rates of resistance to LAM and serum HBV DNA levels, and fasten the

rates of ALT normalization in HBeAg(+) patients, with similar rates of HBeAg seroconversion [163]. In China a prospective cohort study [164] from eight medical centers was conducted to observe the effect of response-guided combination therapy with LAM and ADV in patients with CHB. According to HBV DNA level at 24 weeks, a total of 100 patients with CHB and cirrhosis treated LAM were divided into three groups: complete response group (Arm A, n = 49, HBV DNA ≤ 60 IU/mL), partial response group (Arm B, n = 31, HBV DNA: 60–2000 IU/mL) and inadequate response group (Arm C, n = 20, HBV DNA >2000 IU/mL). Patients was added ADV at week 24 in Arm C, but at week 48 in Arms A and B. At 144 weeks undetectable rate of HBV DNA and YMDD mutation rate in three arms was 95.96%, 66.67%, 35.29% (P = 0.000) and 0%, 3.23%, 15% (P = 0.015), respectively. The data showed that early complete virologic response at 24 weeks was associated with maintained viral suppression.

HBV DNA level of these patients without complete virological response at week 24, adding ADV therapy further decreased by 1 \log_{10} IU/mL. All patients achieved biochemical improvement including ALT/AST decline and ALB elevation. In patients with HBV DNA breakthrough due to YMDD mutations, ADV and LAM combination therapy did not lead to further multiple drug resistance. In CHB patients with compensated liver cirrhosis, continuous HBV suppression for long-term and liver function improvement could be obtained by optimized response-guided add-on therapy of LAM and ADV.

In recent years, some new antiviral drugs have gradually entered people's field of vision. Truvada is a fixed-dose combination of two antiretroviral drugs (emtricitabine and TDF) approved by the FDA for anti HIV therapy. The molecular structure of FTC was similar to that of LAM, and the drug resistance was also similar to LAM. In 2005 a double-blind study [165] evaluated 48 weeks treatment of 25, 100 or 200 mg once daily doses of emtricitabine in patients with chronic hepatitis B. Then these patients were followed 200 mg emtricitabine treatment for an additional 48 weeks. After 2 years, 85% of the patients had normal ALT, 33% seroconverted to anti-HBe and 53% had serum HBV DNA ≤ 4700 copies/mL. Eighteen percent of the patients treated with 200 mg emtricitabine developed resistance mutations after 2 years. Emtricitabine 200 mg once daily was chosen as the optimal dose for CHB based on these data. Emtricitabine was well tolerated and confirmed a potent antiviral response for up to 2 years. In a randomized double-blind, 96-week trial [166], patients were divided (1:1) to groups given a combination of emtricitabine (FTC, 200 mg; n = 139) and TDF (300 mg, FTC/TDF) or monotherapy of TDF (300 mg, n = 141). Patients were HBeAg(+) or HBeAg(-), with levels of HBV DNA $\geq 3 \log_{10}$ IU/mL and LAM resistance mutations (rtM204I/V \pm rtL180M). After 96 weeks of treatment, 86.3% in the FTC/TDF group and 89.4% of patients in the TDF group had levels of HBV DNA <69 IU/mL (P = 0.43). HBeAg loss and seroconversion was not significant difference between groups; only 1 patient (0.7%) in the FTC/TDF group lost HBsAg. No additional benefit was observed with the combination therapy of emtricitabine and TDF vs TDF monotherapy.

5.3.2 Resistance Management in the Long Term of Nucleos(t)ide Treatment

Prolonged therapy with an oral nucleoside or nucleotide can lead to the development of antiviral resistance. Loss of initial response and HBV DNA rebound induce the development of resistance. These patients with resistance may develop biochemical breakthrough and histologic deterioration. Sometimes severe exacerbations occur due to virus resistance in patients with cirrhosis. There are many risk factors for resistance development, such as potency of the antiviral agent, pretreatment HBV DNA titer, period of treatment, nucleotide antiviral therapy or oral nucleoside history, and the degree of genetic barrier to resistance to the individual drug. Thus, either ETV or TDF, which possess the lowest genotypic resistance, should be used as the initial therapy. Patients should be evaluated in the course of treatment, and these patients with poor response should be treated combination therapy early.

Managing resistance recommendations vary but generally involve either adding a drug in a separate class or switching to a more potent drug within the same class. In clinical practice, most members of the panel generally avoid monotherapy in patients with resistance and either use add-on therapy with TDF or ETV or switch to tenofovir/emtricitabine. However, in patients with LAM resistance, there are data providing compelling reassurance that TDF monotherapy is sufficient [166]. Data suggest that TDF may also be sufficient for patients with adefovir resistance [167] was limited. However, with newer anti-HBV agents such as ETV and TDF, viral resistance in previously treatment-naïve patients is very rare and the vast majority of cases of virologic breakthrough in clinical practice are due to nonadherence [168, 169]. To see treatment measures in Sect. 5.1.

5.3.3 Adverse Events of Nucleos(t)ide Analogues in HBV-Related Liver Cirrhosis

Clinical trials and cohorts from clinical practice have shown that NAs are generally well-tolerated and safe [170]. Rare serious adverse reactions includes renal insufficiency, myositis, rhabdomyolysis, lactic acidosis, etc. Some of these drugs can induce impairment of mitochondrial replication with mitochondrial dysfunction or loss due to a low level of activity against the human mitochondrial DNA polymerase gamma. LAM has been well-tolerated and effective in patients with HBV related decompensated cirrhosis [127, 171]. Adefovir dipivoxil and TDF are associated with a dose-dependent renal toxicity, except for LdT, a drug that may even improve creatinine clearance [172]. 1.7% patients had elevation of serum creatinine ≥ 0.5 mg/dL above baseline have been reported after 7 years of TDF therapy in CHB patients [173]. Hadziyannis et al. [132] conducted a cohort study to investigate the efficacy, safety, and resistance profile of adefovir dipivoxil treatment for up to 240 weeks in patients with HBeAg(-) CHB that was lost when 48 weeks ADV treatment was discontinued. A total of 125 HBeAg(-) CHB patients treated with ADV for 5 years. Serum creatinine elevations (0.5 mg/dL above baseline) occurs in 3% of these

patients. Similarly, 8% of the 65 HBeAg(+) CHB patients treated ADV for 5 years had reversible creatinine elevations, 3% developed hypophosphatemia, and 5% had albuminuria [174]. There were a growing number of studies of LAM and ADV combination therapy in both treatment-experienced and treatment-naïve patients with lamivudine-resistant HBV. Lampertico et al. [175] conducted a study to investigate the risk of resistance in the long term of LAM and ADV combination therapy in lamivudine-experienced CHB patients. The results showed that 8% of 145 patients with lamivudine resistance developed mild nephrotoxicity. After increasing the ADV-dosing interval, all these patients were able to continue combination therapy. Before treatment estimated creatinine clearance and serum creatinine levels should be tested in all CHB patients treated with NAs. In patients with creatinine clearance <50 mL dosing adjustments are needed, regardless of the type of NAs.

ETV preclinical research data showed that a higher incidence of solid tumors in animals was associated with prolonged administration of high-dose compared to placebo. However, in clinical trials prolonged administration of ETV was not associated with an increased incidence of malignancy. In a clinical trials conducted by Lai et al. [134] the severity and frequency of laboratory and clinical adverse events were similar among LAM-treated and ETV-treated patients. Furthermore no evidence of serious adverse events and mitochondrial were observed in patients with ETV treatment for up to 5 years [176]. In recent years, in patients with liver cirrhosis in high MELD score (>20) taking ETV, lactic acidosis, and even death cases were reported. Although rare happening, it still has to be pay more attention by the clinician, and needs for future research.

LdT may cause myopathy and peripheral neuropathy. In treatment of LdT combined with PEG-IFN-2a, moderate–severe peripheral neuropathy may occur in patients. Therefore LdT was forbidden in combination with PEG-IFN. In these studies [155, 177], patients treated with LdT at 2 years had a significantly higher incidence of severe elevations of serum creatine phosphokinase (i.e., seven times upper limit of normal) compared to patients treated with LAM, 12.9% and 4.1%, respectively. Although most of them are asymptomatic, there are still two cases of patients with LdT-induced symptomatic myopathy had to be terminated treatment.

In 2010 Chinese guideline, it has been stated that careful medical history investigation before treatment was needed to reduce the risk. In the course of treatment, patients with serum creatinine, CK or lactate dehydrogenase significantly increased, and accompanied by myalgia or weakness, should be immediately tested. Once diagnosed with uremia, myositis, rhabdomyolysis or lactic acidosis, patients should be promptly discontinued treatment or switched to other drugs, and given the appropriate treatment.

5.3.4 Summary and Perspective

Antiviral therapy with nucleos(t)ide analogue is an important means to delay or reverse progression of liver fibrosis and cirrhosis. Cirrhotic patients need long-term or even lifelong antiviral treatment. All of the five antiviral agents could effectively

inhibit virus replication, improve biochemical and pathological parameters in CHB cirrhotic patients with good tolerance. Patients should be fully assessed baseline characteristics before treatment, and closely monitored therapeutic response and adverse reactions during treatment, then to optimized treatment. According to both drug resistance and efficacy profile, ETV and TDF are superior to LdT, ADV, and LAM, and can be recommended as the first-line drug for Nuc-naïve patients with HBV related decompensated cirrhosis. Finally, HBV related cirrhosis patients treated oral Nuc(s) must be frequently laboratory and clinical assessed to insure medication compliance and surveillance for clinical and virological response as well as drug resistance, drug side effects, and hepatocellular carcinoma.

5.4 Antiviral Treatment for HBV Related Hepatocellular Carcinoma

Ke Ma and Qin Ning

In the world hepatocellular carcinoma is one of the most frequent malignancies: estimated 782,000 new cancer cases worldwide occurred in 2012 (50% in China alone). It is the fifth most common cancer in men (554,000 cases, 7.5% of the total) and the ninth in women (228,000 cases, 3.4%) [178]. Hepatocellular carcinoma is the second most common cause of cancer death worldwide, estimated to be responsible for nearly 745,000 deaths in 2012 (9.1% of the total). Given the very poor prognosis for liver cancer (the ratio of mortality to incidence is 0.95), the geographical patterns in incidence and mortality are quite similar [179]. Chronic infections with hepatitis C virus (HCV) and hepatitis B virus (HBV) are the major recognized risk factors for HCC worldwide [180], HBV being most common in Eastern Asia and HCV in Mediterranean countries [181]. Current HCC treatment is the comprehensive treatment based on resection, liver transplantation, or percutaneous local ablative treatment. More and more studies indicated that after resection antiviral therapy effectively inhibit HBV replication and sequentially decrease the rate recurrence of HCC.

5.4.1 HBV and HCC

5.4.1.1 Epidemiology

Chronic hepatitis B is the most frequent etiology of HCC. Chen [182] conducted a prospective cohort study to evaluate the relationship between mortality and past HBV DNA level for 11 years of follow-up. HBV DNA level had been measured on stored samples in 2763 HBsAg(+) adults from cohort entry (1992–1993). There was a significant increase in mortality in patients with HCC across viral load categories ($P < 0.001$). Compared to the HBV undetected category, the relative risk for HCC mortality in the high viral load group was 11.2 (95% CI 3.6–35.0) and 1.7 (95% CI 0.5–5.7) in the low viral load group. The relative risk for chronic liver disease

mortality were 15.2 (95% CI 2.1–109.8) and 1.5 (95% CI 0.2–12.1), respectively ($P < 0.001$). With increased follow-up time, the RR associated with high viral load did not change. In surviving cohort patients evaluated for liver disease in 2003, the disease significantly associated with viral load. The data showed that increased mortality from HCC and CLD was associated with viral load in patients infected HBV. HBV DNA level may be a useful prognostic indicator in CHB patients, and treatment interventions to inhibition of virus replication should be explored.

The REVEAL-HBV study of Chen [148] assessed the relationship between risk of serum HBV DNA level and HCC. From 1991 to 1992, this prospective cohort study in Taiwan enrolled 3653 participants who were HBsAg(+) and 30–65 years from a community-based cancer screening program. During 41,779 person-years of follow-up and a mean follow-up of 11.4 years, there were 164 incident cases of liver cancer and 346 deaths. The incidence of liver cancer grew in patients with HBV DNA level at baseline in a dose-response relationship ranging from 0.108% person-years for an HBV DNA <300 copies/mL to 1.152% person-years for an HBV DNA 1×10^6 copies/mL or greater. The cumulative incidence rates of liver cancer in these patients were 1.3% and 14.9%, respectively. After adjustment for age, sex, alcohol consumption, cigarette smoking, serum ALT level, HBeAg, and liver cirrhosis at baseline, the biological gradient of liver cancer by HBV DNA levels were significant different ($P < 0.001$). The dose-response relationship was most prominent for HBeAg(–) patients without liver cirrhosis and with normal serum ALT levels at baseline. CHB patients with persistent elevation of viral load had the highest liver cancer risk during follow-up. These data proved that high level of HBV DNA ($\geq 10,000$ copies/mL) was a strong risk factor of liver cancer independent of liver cirrhosis, HBeAg, and serum ALT level.

These data showed that the correlation between HCC and HBV DNA level was more closely than ALT. The current guidelines [183–185] for management of CHB are of the view that: For patients with chronic hepatitis B the primary goal of treatment is to permanently suppress or eliminate hepatitis B virus replication. Thus hepatic infectivity and pathogenicity could be decreased, and thereby necroinflammation could be stopped or reduced. The short-term goal in clinical terms is to reduce hepatic inflammation, to prevent the development of hepatic fibrosis and decompensation, to ensure a sustained loss of HBV-DNA and ALT normalization. The long-term goal is to prevent progression to cirrhosis and HCC, to prevent ALT flares that may lead to hepatic decompensation, and finally prolong the survival time. Therefore, the ideal treatment for patients with CHB should be able to effectively reduce the HBV DNA level, thereby inhibit or stop the deterioration of liver disease, reduce the incidence of severe exacerbation and HCC.

5.4.1.2 Pathogenic Role of HBV in HCC

HBV chronic infection is a major risk predictor for the development of hepatocellular carcinoma. The hepatocarcinogenesis in CHB patients has been extensively investigated, and a number of predictors relate to occurrence of hepatocellular carcinoma. The most significant predictors associated with hepatocellular carcinoma include chronic HCV and HBV infection, aflatoxin B1, chronic alcohol

consumption and virtually all cirrhosis-inducing conditions [186]. For hepatocellular carcinoma in human, chronic infection of HBV was considered as the major environmental etiological factor [187]. HBV-induced hepatocarcinogenesis can involve an array of processes, including host–viral interactions, sustained cycles of necrosis–inflammation–regeneration, viral–endoplasmic-reticulum interactions (induction of oxidative stress), viral integration into the host genome (and associated host DNA deletions) and the targeted activation of oncogenic pathways by various viral proteins.

A predominant risk factor for HCC is chronic active hepatitis. The mechanisms of chronic active hepatitis consist of a combination of complementary effects, several involved in liver cell inflammation, and necrosis thus fibrosis and cytokine synthesis. The underlying chronic active hepatitis inflammatory is a major risk factor for the higher HCC occurrence in patients with progressive cirrhosis. Fundamentally important information on these issues have been provided in animal models for Hepadna virus infection [188].

Integration of HBV DNA results continuous replication of the virus, which induces occurrence of genetic alterations. The HBV-DNA sequences are integrated into cellular DNA in most (approximately 90%) liver-tumor samples from HBsAg-positive patients [189]. HBV genome integration should be viewed as a “driver” of liver carcinogenesis. In the impact of genes, some of the other genes involved, and play an important role in the carcinogenesis process. In addition, HBV DNA integration effects on host cells including cell gene deletion, chromosomal rearrangements, genomic DNA copy number variation, loss of chromosomal heterozygosity, etc. [190, 191]. In the host cells, the integration of HBV damage mechanisms to protect the integrity of the chromosome.

Hepatitis B virus X protein (HBx) exhibits pleiotropic effects on different pathways involved in intracellular signaling and transcriptional activation that modulate cell responses to protein degradation, genotoxic stress, apoptosis and cell division; these biological effects might contribute to the potential transforming activities of HBx. HBx has been confirmed to interact with p53, accordingly inactivating several essential p53-dependent activities, including p53-mediated apoptosis transactivation properties of p53, regulation of cell cycle DNA repair genes and tumor suppressor genes. HBx may also play a role in tumorigenesis of hepatocellular carcinoma through modulation of angiogenesis pathway. Indeed, HBx expression induces up-regulation of the vascular endothelial growth factor (VEGF) transcription and stabilizes hypoxia inducible factor (HIF)-1 [192, 193]. Moreover, HBx causes multipolar spindle formation, chromosome segregation defects, and appearance of multinucleate cells by inducing aberrant centrosome duplication; these biological actions might be due to sequestration of the nuclear transport receptor Crm1 in the cytoplasm [194]. It has been frequently reported that HBx with mutations in amino acids 130 and 131 may be associated with the severity of CHB. Studies indicated that these mutations had also been detected in HCC tissue and arose before the development of HCC [195].

Other studies [196] have pointed out that many signaling pathways have been outlined as common targets deregulated during hepatocarcinogenesis, including the

Wnt/ β -catenin, TGF- β pathway, Ras/MAPK pathway, PI3K/Akt/mTOR pathway, Jak/STAT pathway, PKC pathway, etc. In addition, fibrinogen-like protein 2 (fgl2), HGF, IGFs and other coagulation factors, growth factors and other angiogenesis gene may also be involved in the occurrence and development of HCC [197].

1. Wnt/ β -catenin pathway: The Wnt signaling pathway is an evolutionary highly conserved pathway and involved in the regulation of proliferation, motility, cell/cell interaction, organogenesis and axis formation. The accumulation and expression of β -catenin in the nucleus were decreased, and cell proliferation was suppressed followed by up-regulated GSK-3 β activity due to HBx induction [198]. HBx mutants may participate in the development and progression of HCC, at least in part through the Wnt-5a pathway [199].
2. TGF- β pathway: TGF- β is a central regulatory factor in control of hepatocyte proliferation and death. Paradoxically, either under- or overexpression seem to have deleterious consequences resulting in an increased turnover of liver cells and thereby predisposing to cancer progression [200]. In both cases the escape from the antiproliferative, proapoptotic action of TGF- β would be a prerequisite for tumor progression. At the stage of tumor occurrence, TGF- β can promote tumor cell invasion, metastasis, but suppresses tumor growth in liver damage stage. TGF- β receptor I kinase inhibitors, blocking the TGF- β signaling pathway, show anti-tumor effect [201].
3. Ras/MAPK pathway: Ras/MAPK signaling pathway is a signal cascade waterfall reaction caused by external signal activated receptor in the cytoplasm, involving a variety of connectors, nucleotide exchange factor, small GTP binding protein. HBx retains the ability to overcome RAS-induced senescence in human cells immortalized by hTERT, although HBx alone could neither immortalize nor transform human cells. The ability of HBx to collaborate with active RAS in cell transformation may explain its role in HCC [202].
4. PI3K/Akt/mTOR pathway: The PI3K/Akt/mTOR protein cascade is one of major signaling pathways associated with receptor tyrosine kinases (RTKs) [203]. In nontransformed cells, a tumor suppressor PTEN (phosphatase and tensin homolog deleted from chromosome 10), which inhibits this pathway by blocking Akt activation and reversing the PI3K reaction, control the PI3K/AKT/mTOR pathway. In almost half of the studied HCCs, PTEN expression was reduced or absent, and hepatocyte-specific abrogation of PTEN expression in mice results in the development of HCC [204].
5. Jak/STAT pathway: The Jak/Stat pathway is activated by more than 40 cytokines and growth factors and involves in multiple cell functions such as differentiation, proliferation, and apoptosis [205]. In this pathway, the cytokines induce phosphorylation of the Janus tyrosine kinases (Jak1, 2 and 3, Tyk2), followed by activation of Stat1–6 [206]. Both HBV and HCV are able to induce Jak/Stat pathways [207]. In HCC, phosphorylation of Jak1, Jak2, and Tyk2 tyrosine kinases was not detected in normal livers but increased significantly from surrounding non-neoplastic livers to HCCs. Activation of Stat1, Stat3, and Stat5 was statistically higher in tumors than in respective surrounding livers, with

- pStat3 being higher in HCC with poor prognosis than HCC with better prognosis. The levels of Jak/Stat targets, including Bcl-x1, Mcl-1, cyclin D1, and c-Myc were markedly increased in the majority of HCCs [208].
6. PKC pathway: PKC isozymes have a central role in cellular signaling transduction involved in cell proliferation, differentiation, apoptosis and angiogenesis [209]. PKC- α , PKC- δ , and PKC- ι have been found to be over-expressed in human HCC cell lines. The focus of PKC research in HCC has predominantly been on PKC- α . Its expression is significantly increased in cancerous tissue and is correlated with tumor size and TNM stage. In addition, over-expression of the mRNA of this isozyme has been associated with a shorter survival rate, and thus may be a marker for disease prognosis in cancer patients [210].
 7. Fgl2: Fgl2 could directly generate thrombin from prothrombin without activation of the conventional coagulation cascade. It was confirmed to be overexpressed in various human malignant tumors [211]. The hfg12 was associated to the hypercoagulability in cancer and may induce tumor metastasis and angiogenesis via cytokine induction [212]. Fgl2 was overexpressed in HCC tissues and co-localized with fibrin deposition. Fgl2 contributed to HCC tumor angiogenesis and growth in a thrombin-dependent manner, and down regulation of its expression might be of therapeutic significance in HCC [211].

5.4.2 The Prevention of HBV Related HCC

5.4.2.1 HBV Vaccine for Prevention of HBV-HCC

To investigate whether prevention of HCC by the HBV vaccine and to identify the predictors of HCC for vaccinated birth cohorts, a population-based study [213] in Taiwan has been conducted. Between 1983 and 2004, 1958 HCC patients aged 6–29 years in Taiwan were collected. HCC incidence was significantly higher in unvaccinated birth cohorts among children aged 6–19 years compared with vaccinated birth cohorts (444 cases in unvaccinated subjects with 78,496,406 person-years vs 64 HCC cases among vaccinated subjects with 37,709,304 person-years, RR = 0.31, $P < 0.001$, for persons vaccinated at birth). For vaccinated cohorts incomplete HBV vaccination who received fewer than three doses of HBV vaccine was significantly associated with the risk of developing HCC (OR = 4.32, 95% CI = 2.34–7.91); with prenatal maternal HBsAg(+) (OR = 29.50, 95% CI = 13.98–62.60); with prenatal maternal HBeAg seropositivity (with administration of HBIG at birth, OR = 5.13, 95% CI = 2.24–11.71; and without administration of HBIG at birth, OR = 9.43, 95% CI = 3.54–25.11). From childhood to early adulthood this HBV vaccine can prevent the development of HCC in these patients. The reason of failure to prevent HCC was mostly because of unsuccessful control of HBV infection by highly infectious mothers.

The study shows strong evidence that the HBV vaccine reduce the incidence of HCC. Those who received incomplete HBV vaccination (i.e., less than three administrations of the vaccine) during infancy and infants of HBeAg- and HBsAg-seropositive mothers without HBIG injection at birth had higher risk of developing

HCC. Approximately 30% of children with HCC born to HBeAg and HBsAg carrier mothers did not receive HBIG at birth. Improvements of the HBIG injection rate within 24 h after birth in infants of high-risk mothers should be implemented. This study has limitations in that the role of host factors, such as genetic polymorphisms, HBV genotype, virus mutation, were not studied, which could influence the interpretation of the data.

5.4.2.2 Antiviral Therapy for Prevention of HBV-HCC

A number of studies on the long-term treatment of interferon (IFN) or NA for patients with HBV showed the prevention of HCC.

A meta-analysis [214] compared risk of HCC in CHB patients who received IFN or NA. A total of 17 studies were included in this review. IFN treatment (12 studies; $n = 2742$) showed a significantly reduced risk of HCC for patients treated by IFN compared to controls (RR 0.66, 95% CI 0.48–0.89; 12 studies) and for compensated cirrhotic patients (RR 0.53, 95% CI 0.36–0.78; 6 studies). There was no statistical heterogeneity for these comparisons. NA treatment (5 studies; $n = 2289$) showed a significantly reduced risk of HCC for patients treated by NAs compared with controls (RR 0.22, 95% CI 0.10–0.50; 5 studies). NA treatment demonstrated a more profound reduction in HCC risk of 78% compared to IFN which produced only a modest effect of 34%. This is perhaps not a surprising finding, as the viral load is found to be the most important factor leading to cirrhosis and cancer development in the liver. The more effective reduction in HCC risk may be related to the more profound effects of viral suppression of oral anti-viral agents than IFN [215].

Across subgroups there was a significantly reduced risk of HCC: HBeAg(+) patients (RR 0.21, 95% CI 0.10–0.44; five studies); compensated cirrhotic patients (RR 0.17, 95% CI 0.04–0.79; three studies); non-cirrhotic patients (RR 0.21, 95% CI 0.10–0.47; two studies); patients with drug resistance (RR 0.52, 95% CI 0.28–0.97; three studies); and those without drug resistance (RR 0.37, 95% CI 0.17–0.77; three studies). In subgroup analysis of IFN studies, a more significant reduction in HCC risk among those with early cirrhosis was found. The effects of IFN could be beyond its viral suppressive activities. Previous studies have shown that at least IFN- α 2b has inhibitory activities on hepatic stellate cells (HSCs) activation by suppressing the effects of IL-1 β , TNF- α and probably inducing apoptosis of HSC [216, 217]. As HSCs play a central role in fibrogenesis, the effects of IFN- α on HSCs are worthy of further investigation. On the other hand, the anti-cancer effect of NAs, and probably IFN, was more prominent among HBeAg-positive than among HBeAg-negative patients. This discrepant results based on HBeAg status is consistent with the fact that while HBeAg(+) patients usually have a higher HBV DNA level, treatment of HBeAg(-) patients is more difficult and sustained virological responses are uncommon [216, 217].

5.4.2.3 Indications for Antiviral Therapy of High Risk Population of HBV-HCC

High risk population HBV-HCC refers to patients who are the middle-aged men with high HBV load, with HBV and HCV co-infection, with family history of liver

cancer, alcoholics, and with diabetes mellitus. The long-term effect of antiviral therapy for patients with HBV showed the prevention of HCC. However, according to current national management guidelines for CHB, there are still some patients without antiviral treatment. Thus, some of the high risk population of HBV-HCC may lose the opportunity of early interventional treatment.

Current guidelines recommend liver biopsy to assess the degree of necroinflammation and liver fibrosis prior to treatment initiation in patients with increased HBV DNA and/or mild elevated ALT levels (1–2 times the ULN). For patients older than 40 years, liver biopsy should be considered. In those with “high normal” ALT levels liver biopsy is strongly recommended [183]. Although liver biopsy remains the gold standard for assessing hepatic fibrosis, its use has several limitations including sampling error and intra- or inter-observer sampling variability [4]. Inadequate liver biopsy may further pose misleading histological information that precludes cirrhotic patients from antiviral treatment [218]. In the report by Tong et al. [219], 5 of 9 patients who developed HCC were diagnosed as having chronic hepatitis by histology and therefore did not fulfill the recommended treatment criteria. These patients probably had normal ALT and/or intermediate HBV DNA levels (between 10,000 and 100,000 copies/mL). In this report, 7 of 15 patients with cirrhosis who developed HCC could not be identified by the treatment recommendations. In other words, patients with cirrhosis have a significant risk of developing HCC even when their HBV DNA levels are not high.

Patients with elevated ALT between 0.5 and 1 times the ULN also was a strong risk predictor of HCC or complications [220, 221], a claim supported by a Korean population study.

The REVEAL study suggested that high HBV DNA level significantly increased risk of HCC independent of liver cirrhosis, HBeAg, and serum ALT level. HBV DNA consistently replicates and is integrated into the host genome, adding to the coexistence of metabolic disorders, inflammatory responses and oxidative injuries, which induce genetic instability and an imbalance of cell growth and apoptotic tolerance signals. These are all biologic driving forces for HCC development in CHB patients. Therefore, we must pay more attention to the effect of continuous HBV replication on the prognosis of patients. Any antiviral drugs, if not completely clear the virus but can reduce the viral load, it may reduce the risk of patients with HCC.

The risk factors for HCC include progression to cirrhosis, longer duration of HBV infection, higher serum viral load ($\geq 10^5$ copies/mL), males, age >40 years, alcohol, ethnic groups native to regions of East Asia and Sub-Saharan Africa, the virus genotype (genotype A in African population or genotype C in Asian population), co-infection with human immunodeficiency viruses (HIV) or hepatitis C, D, decompensated liver cirrhosis, persistent inflammation of the liver, continuous HBeAg positive, and a family history of liver cancer [222]. Cirrhosis is the most important independent risk factor for HCC. Up to 70–90% of HCC occur in patients with cirrhosis. Effective antiviral therapy inhibits HBV replication, reduces serum viral load and accelerates serum conversion of HBeAg, which may therefore

alleviate liver damage and reduce the development of cirrhosis. All the patients with the above risk factors should be received antiviral therapy.

5.4.2.4 Anti-HBV Drugs

IFN- α is an immune modulator inducing antiviral, immune regulation, anti-tumor and anti-fibrosis effects. Its antiviral mechanism is a complex mode of action including the destabilization of viral nucleocapsid, inhibition of viral genome transcription, activation of natural killer (NK)/NKT cells. However, disadvantages of IFN shortcomings are prominent, such as the high cost of PEG-IFN, intolerance to IFN therapy in patients with cirrhosis. Compared with IFN, NA is safer, better tolerance for these patients.

Current guidelines from APASL, EASL and AASLD, do not provide treatment recommendations for patients with HBV-HCC. The Chinese expert consensus [223] on antiviral therapy to treat HBV/HCV-related HCC has been published in 2014. This expert consensus indicated that promptly initiation of antiviral therapy is not only important for preventing the incidence of HCC in patients CHB, but also essential for reducing HBV reactivation, improving liver function, delaying or reducing recurrence of HCC, and prolonging survival of patients with HBV-HCC after palliative and curative therapies. It puts forward the overall principle and target of antiviral therapy of HBV-HCC, and emphasizes the antiviral therapy is a part of comprehensive treatment.

5.4.3 Effects of Antiviral Therapy on Patients with HBV-HCC

5.4.3.1 Treatment of HCC

At present, suitable treatment for HBV-HCC is multidisciplinary comprehensive treatment. A large number of evidence-based medical evidence suggested, standard anti-HBV treatment for these patients help to improve the overall curative effect, prevent the recurrence of the tumor, and improve the OS. Therefore, anti-HBV therapy should be taken as a very important part of comprehensive treatment of HBV-HCC (Fig. 5.4).

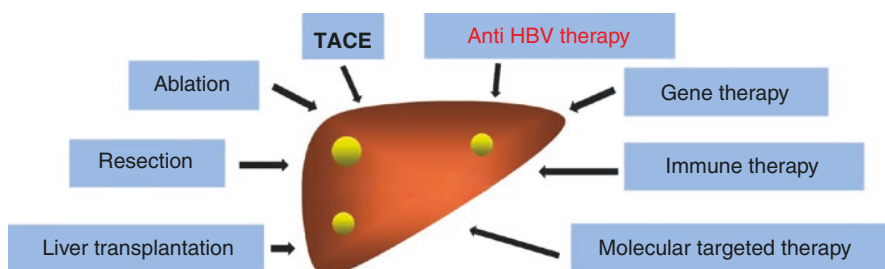


Fig. 5.4 Comprehensive treatment of HBV-HCC

5.4.3.2 High HBV DNA Level is Independent Risk Factor of Recurrence of HBV-HCC After Resection

Following curative liver resection for HCC, 50–90% of postoperative death is caused by recurrent disease [224]. High serum HBV DNA levels were a strong predictor of HCC. Effective control of HBV replication with antiviral therapy may lower its recurrence after liver resection. In 2000, Kubo et al. [225] first reported the relationship between recurrence of HBV-HCC after resection and HBV DNA level. Later in another study [226] he pointed out that patients with high HBV DNA levels (more than 5 mEq/mL) have high risk with recurrence and poor prognosis. On the contrary, Kim et al. [227] included 230 consecutive patients undergoing curative resection and found that, sustained HBV viremia (>5 log copies/mL) increased recurrence, but did not have a marked effect on survival.

An et al. [228] investigated the HBV DNA changing patterns and their effects on outcome in HBV-HCC patients with resection. All 188 patients were followed up. Data from 115 alive patients without recurrence at 12 months were collected. The mean period of follow-up was 48.5 months and the mean age was 53 years. For the entire population with multivariate analysis, tumor size >5 cm ($P = 0.047$), HBV DNA $>10^4$ copies/mL, Child-Pugh class B ($P = 0.017$) at the time of resection ($P = 0.003$), and vascular invasion ($P = 0.028$) were independently risk factors of HCC recurrence. On multivariate analysis for 115 patients, sustained HBV DNA level $<10^4$ copies/mL was the only risk predictor for both longer survival (OR 3.76; 95% CI 1.61–8.78; $P = 0.002$) and low recurrence (OR 3.13; 95% CI 1.55–6.35; $P = 0.002$). That clarified that a sustained high HBV DNA is among the most important risk factors of adverse outcome after liver resection of HBV related HCC. The sustained suppression of HBV DNA $<10^4$ copies/mL strongly benefit to long-term survival and recurrence-free.

Kim et al. failed to show the difference in survival between the sustained viremia (>5 log copies/mL) and non-viremia groups despite the high recurrence rate in the sustained viremia group. The reason may be that researchers have used a higher HBV DNA cut-off value ($>10^5$ copies/mL) to differentiate between patients with high and low viremia. In An's results, they found that a lower HBV DNA level cut-off value of 10^4 copies/mL is superior to $>10^5$ copies/mL in predicting outcomes after resection. It is therefore needed to suppress further HBV DNA to a lesser level in order to obtain better clinical outcomes after surgery.

5.4.3.3 Positive HBeAg is a Risk Factor for Recurrence of HBV-HCC After Resection

Studies have shown that positive HBeAg was a risk predictor for recurrence of patients after resection of HCC. Sun et al. [229] evaluated the impact of HBeAg on patients' survival and tumor recurrence after curative resection of HBV-HCC. All 203 patients undergone curative resection with small HCC (≤ 3 cm) were divided into HBeAg(+) and HBeAg(−) groups. Postoperative outcomes and clinicopathological factors of two groups were compared, and risk predictors for recurrence and survival were investigated. HBeAg(−) Patients had higher 5-year disease free survival rates (52.9% vs 37.4%, $P = 0.046$) and 5-year overall survival rates (76% vs

53.9%, $P = 0.002$). There was no significant difference in tumor factors and operative morbidity between HBeAg(+) and HBeAg(-) groups, but more macronodular cirrhosis, higher serum alanine aminotransferase levels, and younger age were found in the HBeAg(+) group. In patients for multivariate analysis, macronodular cirrhosis, HBeAg(+) and age >50 years were independent risk predictors for overall survival, and multiple tumor nodules and HBeAg(+) were independent predictors for disease free survival. In patients with small HCC after curative resection, HBeAg(+) was a significant risk factor of early recurrence (within 1 year).

5.4.3.4 IFN Prevents HCC Recurrence and Prolongs OS of HBV-HCC Patients After Resection

Because of the adverse effects, the impact of IFN- α therapy after curative resection on recurrence of HCC and the OS among patients with HBV infection are controversial. Theoretically, the effect of postoperative IFN- α therapy on recurrence might be related with the direct suppression of tumor growth and metastasis, the inhibition of HBV replication, down-regulating expression of vascular endothelial growth factor (VEGF), antiangiogenesis effect, and modulating some factors in tumor microenvironment. However the results of clinical trials are not the same. In recent years, more and more studies show that reasonable application of IFN- α can prevent the recurrence of the tumor and prolong the survival time of the patients.

Qu et al. [230] conducted a retrospective study to investigate the impact of IFN- α therapy on survival and recurrence after curative resection in patients with HBV-HCC. Of 568 HBV-HCC patients with curative resection, 101 patients were treated postoperative by IFN- α therapy (5 million units three times every week for 18 months). Patients with postoperative IFN- α therapy had higher OS rates ($P=0.010$, HR: 0.612, 95% CI: 0.422–0.889). There was no significant difference in DFS rates between the two groups ($P=0.086$, HR: 0.786, 95% CI: 0.597–1.035). On multivariate analysis, postoperative IFN- α therapy was an independent factor for OS ($P=0.010$, HR: 0.611, 95% CI: 0.421–0.887) and significantly reduced early recurrence ($P=0.005$, HR: 0.562, 95% CI: 0.375–0.840). However, patients with or without postoperative IFN- α therapy had similar cumulative late recurrence rates (HR: 1.205, 95% CI: 0.781–1.858, $P=0.399$).

Sun et al. [231] evaluated the effects of postoperative IFN- α treatment on survival and recurrence in patients with HBV related HCC. All 236 patients were randomized after curative resection into IFN- α treatment ($n = 118$, 5 μg i.m. tiw for 18 months) and control groups ($n = 118$). If recurrence was diagnosed, treatment was terminated, these recurrence patients was managed in the same way in both groups. All clinicopathological parameters in two groups were analyzed. The median OS was 63.8 months in the treatment group and 38.8 months in the control group ($P = 0.0003$); the median DFS period was 17.7 versus 31.2 months ($P = 0.142$). That concluded that IFN- α therapy improved the OS of HBV-HCC patients after curative resection, probably by postponing recurrence.

Someya et al. [232] investigated 80 consecutive patients with HBV cirrhosis and HCC who underwent potentially curative ablation for HCC. Eleven patients received long-term IFN therapy. Initial DNA was high (>6.0 log copies/mL) in 41 patients

and low (<6.0 log copies/m) in 39. HCC recurrence rates in the high DNA group and low DNA group were 82.6% and 46.9% at the fifth year, and 91.3% and 73.5% at the tenth year, respectively ($P = 0.0103$). Similarly, recurrence rates after treatment of HCC in the abnormal AST group ($n = 38$) and normal AST group (<38 IU/L, $n = 42$) were 84.0% and 50.6% at the fifth year, and 100% and 71.3% at the tenth year, respectively ($P = 0.0003$). Five of 42 patients with normal AST, and 6 of the 38 patients with abnormal AST, received IFN- α after confirmation of tumor ablation. In the subgroup of abnormal AST, tumor recurrence rates in the untreated and IFN- α groups were 37.9% and 16.7% at the end of the first year, 60.1% and 16.7% at the second year, and 83.4% and 16.7% at the third year, respectively ($P = 0.0139$). On multivariate analysis, IFN- α significantly reduced the recurrence rate ($P = 0.037$, HR = 0.21) even after adjusting for background characteristics.

Pathogenic mechanism of HBV-HCC mainly related with the integration of HBV DNA into host hepatocytes. Therefore, inhibition of inflammation and viral replication can reduce the HBV DNA level and the risk of HCC. After the resection the residual liver is still cirrhosis, still have a high risk of new cancer. HBV-HCC occurrence seems to have the relationship with the HBV greater than the liver repeatedly inflammation [233]. Tang et al. [234] reported that high HBV DNA levels is associated with increased risk for development of HCC.

IFN has a dual role of antiviral and immune regulation. IFN as immune regulator can not only activate or mediated macrophages, NK cells and cytotoxic T lymphocyte, but also adjust the antibody. Its antiviral activity includes induction of 2,5 oligonucleotide adenosine monophosphate synthetase and protein kinase. Moreover, IFN also has the anti-fibrosis, anti-proliferation and anti-tumor effects. The experimental study [235] confirmed that IFN exerts potent growth inhibitory effects on the HCC cell line PLC/PRF/5 both in vitro and in vivo and its mode of action in this animal model system appears to be predominantly mediated by a direct antiproliferative effect on tumor cells.

Breitenstein et al. [11] searched 7 cochrane central register of controlled trials between January 1998 to October 2007 and evaluated the effects of IFN- α or - β in patients after surgical resection or ablation of HBV-HCC. Seven RCTs ($n = 620$ patients) were included in a meta-analysis review. Patients treated with IFN had a significantly decreased mortality rate than control group (RR 0.65, 95% CI 0.52–0.80, $P < 0.001$) and significantly reduced risk of tumor recurrence (RR 0.86, 95% CI 0.76–0.97, $P = 0.013$). As 6 of the 7 trials used IFN- α , it is interesting that the only study [236] on IFN- β showed the largest beneficial effect on tumor recurrence. Due to the small number of cases in this study, further clinical evaluation of IFN- β in the adjuvant setting of HCC treatment seems to be indicated. The rate of treatment discontinuation ranged from 8% to 20% because of the side-effects of IFN which were dose dependent and often serious. Severe adverse effects of the adjuvant IFN treatment leading either to treatment disruption or dose reduction occurred in up to a quarter of the patients. In particular, work is needed to optimize the type and dosage of IFN to minimize side-effects, and to study the combination of IFN treatment with other (neo)adjuvant agents.

5.4.3.5 NAs Prevent HCC Recurrence and Prolong OS of HBV-HCC Patients After Resection

Reasonable application of NAs can prevent the recurrence of HCC and prolong the survival time of the patients. A comparative nonrandomized study [224] of postoperative antiviral treatment was conducted on 71 HCC patients who underwent curative hepatectomy. The authors assessed the impact of antiviral therapy for patients who underwent partial hepatectomy for HBV-HCC in the immune-active phase of HBV infection. All 43 patients in the treatment group treated by lamivudine (LAM) with or without adefovir dipivoxil (ADV), while 36 patients in control group received no antiviral treatment. At 6-month postoperation, the treatment group also had a significantly greater increase in residual liver volume per unit surface area following hepatectomy ($78.0 \pm 40.1 \text{ cm}^3/\text{m}^2$ vs. $35.8 \pm 56.0 \text{ cm}^3/\text{m}^2$). The OS rate was a significant difference between two groups. The OS rates of 1- and 2-year were 33.3% and 0%, respectively, for the control group, and 41.9% and 7.0%, respectively, for the treatment group. These results revealed that antiviral therapy with NAs effectively relieved HBV-induced liver damage, improved liver function, promoted hepatocyte regeneration, and increased volume of residual liver, thus enhancing tolerance to subsequent therapy. There were no serious adverse effects to LAM therapy in this study. However, the most significant problem associated with long-term therapy with LAM is emergence of resistance. In this study, the emergence of YMDD mutants was observed in 6 of 43 patients in the LAM group. Administration of ADV successfully controlled the virus.

In a meta-analysis, Wong et al. [237] assessed whether anti-viral therapy with NAs could prevent HBV-HCC patients from tumor recurrence after curative treatment. A total number of 551 patients from 9 cohort studies were included: 347 patients without antiviral treatment (control group) and 204 patients with antiviral treatment group. LAM was the primary antiviral therapy in the majority of patients. Patients with LAM resistance was treated by either switching to entecavir (ETV) or adding ADV therapy. Thirteen patients received ETV as primary antiviral therapy. Most of the antiviral therapies were started after the curative treatment of HCC. The recurrence rate of HCC in the antiviral treatment group was significant lower compared to control group (55% and 58%; $P = 0.04$). In the antiviral treatment group the risk of HCC was reduced by 41%. There were also significant differences in favour of antiviral treatment group in terms of overall mortality (38% vs. 42%; $P < 0.001$) and liver-related mortality (0% vs. 8%; $P = 0.02$). HCC patients with anti-viral therapy after curative treatment may be reduced the risk of HCC recurrence for 41%. Besides, antiviral therapy significantly improved OS, as overall mortality was reduced by 78%. After curative treatment of HCC, patients should be monitored regularly concerning their viral status for consideration of antiviral therapy. Antiviral therapy was beneficial as it not only might reduce HCC recurrence and liver failure secondary to reactivation of HBV due to viral suppression (90% reduction in the mortality secondary to liver failure in the antiviral therapy group).

5.4.3.6 Antiviral Therapy Prevents HCC Recurrence and Prolongs OS of HBV-HCC Patients After Ablation

After ablation, the use of IFN or NAs can reduce the recurrence of HCC, improve liver function, thus enhancing tolerance to subsequent therapy and prolong the survival time of the patients. Recurrence in patients with HBV-HCC after ablation was common. Chung et al. [238] assessed the correlation between viral load and intrahepatic recurrence and predictors of intrahepatic recurrence after percutaneous ablation. HBV-HCC patients undergoing percutaneous ethanol injection (PEI) or radiofrequency ablation (RFA), between 2004.10 and 2008.12 were prospectively enrolled. A total of 145 patients (mean age, 55.3 years; male, 81.4%) were included. Ninety patients (62.1%) had serum HBV DNA ≥ 2000 IU/mL. The median follow-up duration was 28.9 months (range, 12.0–57.0) and 43.4% patients ($n = 63$) experienced intrahepatic tumor recurrence. On multivariate analysis, HBeAg(+) was an independent risk predictor of late recurrence (≥ 1 year) ($P = 0.012$; HR 0.288) and intrahepatic recurrence ($P = 0.026$; HR 0.473). The AFP level also significantly predicted late recurrence ($P = 0.005$; HR, 1.001). However, neither serum HBV DNA titers nor the ablation method were correlated with intrahepatic recurrence.

Xia et al. [239] conducted a study to investigate the risk factors of recurrence in patients with HBV-HCC after RFA. All 152 patients with small HCC enrolled in this study. In 67 patients the intrahepatic recurrence rate was 44.1% by median follow-up of 35 months. On univariate analysis, MELD score, AFP, HBV DNA, precollagen III, and hyaluronic acid were independent risk factors for recurrence. On multivariate analysis, hyaluronic acid and HBV DNA were independent risk factors for recurrence. The cumulative 1-, 3-, and 5-year DFS rates were 86.8%, 41.2%, and 22.8% in the high HBV DNA group ($>1 \times 10^5$ copies/mL) and 96.4%, 65.8%, and 36.7% in the low HBV DNA group ($\leq 1 \times 10^5$ copies/mL), respectively. That showed significant difference between the two groups ($P = 0.003$). Goto et al. [240] reported the similar results that serum HBV DNA load ($>4.0 \log_{10}$ copies/mL) were associated with the recurrence. Thus reasonable antiviral therapy can improve liver function and prevent the recurrence of the tumor.

Lin et al. [241] assessed the impact of IFN- α in preventing HCC recurrence. Thirty eligible patients were randomized into three groups: IFN- α -continuous group ($n = 11$, IFN- α 3 MU tiw for 24 months), IFN- α -intermittent group ($n = 9$, IFN- α 3 MU daily for 10 days every month for 6 months followed by 3 MU daily for 10 days every 3 months for a further 18 months), and control group ($n = 10$, no IFN- α therapy). The three groups were comparable in terms of demographics, laboratory data, and etiology at entry and HCC characteristics. After a median follow-up of 27 months, 90% patients ($n = 9$) in the control group and 45% patients ($n = 9$) in 2 treatment groups (3 patients in the IFN- α -intermittent group and 6 patients in the IFN- α -continuous group) developed an HCC recurrence ($P = 0.021$). Cumulative HCC recurrence rates in the control groups IFN- α -intermittent, IFN- α -continuous, and were 40%, 22.2%, and 27.3% at the end of 1 year and 90%, 33.3%, and 54.6% at the end of 4 years ($P = 0.0375$), respectively (control vs. IFN- α -continuous group, $P = 0.0822$; vs. IFN- α -intermittent group, $P = 0.0123$). The cumulative HCC recurrence rate of the patients treated with IFN- α and the control group was 25% and

40% at the end of 1 year and 47% and 90% at the end of 4 years, respectively ($P = 0.0135$) if both IFN- α groups were combined. The conclusion was that HCC recurrence may be reduced by IFN- α therapy after medical ablation therapy for primary tumors.

Antiviral, anti-tumor and anti-angiogenesis effect of INF can effectively resist the risk factors of recurrence after ablation. Some patients do not tolerate the adverse reaction of IFN, still should be treated with NAs to inhibit viral replication, relieve the liver inflammation, improve liver function, enhancing tolerance to subsequent repeated ablation.

Yoshida et al. [242] evaluated the efficacy of LAM in HBV-HCC patients who were treated with RFA. Complete ablation of HCC was achieved in 104 patients in this study. After RFA, 33 patients was treated by LAM. There were 24 (73%) patients with serum HBV-DNA negative conversion. Four patients showed ALT elevation and redetection of HBV-DNA. There was no difference in recurrence-free survival and overall survival between the two groups. In the LAM group no specific adverse effect was observed. The conclusion was that LAM for patients with HBV-HCC after RFA was safe and liver function was improved.

Kuzuya et al. [13] evaluated the impact of antiviral therapy with LAM on patients after initial treatment for HBV-HCC. Comparison was made between 33 patients who did not received LAM therapy after treatment for HCC (control group) and 16 patients who received at a dose of 100 mg/day (LAM group) in terms of changes in survival, HCC recurrence, and remnant liver function. There was no significant difference in cumulative recurrence rates of HCC between the two groups ($P = 0.622$). However, median CTP score at the time of HCC recurrence was significantly different; 7 (range 5–12) in the control group versus 5 (range 5–6) in the LAM group ($P = 0.005$). In the LAM group, all patients were able to receive curative treatment for recurrent HCC. In contrast in the control group, 10 of 15 patients were unable to receive curative optimal therapy for recurrent HCC due to deterioration of remnant liver function. In the LAM group, the cumulative survival rates of patients tended to be higher than those of patients in the control group ($P = 0.063$). The conclusion is that LAM therapy is beneficial for HBV-HCC patients after initial treatment because it contributes to improving remnant liver function, accordingly decreasing the probability of liver failure and increasing the possibility to receive available treatment for recurrent HCC.

5.4.3.7 Antiviral Therapy Prevents HCC Recurrence in HBV-HCC Patients After Transcatheter Arterial Chemoembolization (TACE)

Clinical evidence showed HBV reactivation may occur in chronic HBV carriers with tumor during chemotherapy, then followed by hepatic decompensation and various complications. HBV reactivation occurs in nearly between 19% and 44% [243–245]. Similarly, HBV reactivation may occur in patients with HBV-HCC after TACE. Some of these patients even treated with LAM still occur hepatic decompensation or liver failure and eventually death, because of the delay of LAM antiviral therapy, suggesting that these patients need to be treated by

antiviral drugs before TACE. Moreover, more studies [246–248] also suggested that before chemotherapy early antiviral therapy can significantly reduce the chemotherapy-induced reactivation of HBV. TACE is local treatment, different from systemic chemotherapy. Therefore, early antiviral treatment before TACE in patients with HBV-HCC can reduce the occurrence of postoperative virus reactivation, reduce the hepatitis flare caused by HBV, and reduce the mortality of acute exacerbation of CHB.

In 2006, a study about reactivation of HBV in patients with HBV-HCC undergoing TACE of Jang et al. [249] was published in *Hepatology*. In a prospective and randomized study, 73 consecutive patients with HBV-HCC undergoing TACE (cisplatin 60 mg/m² and epirubicin 50 mg/m²) at monthly intervals were assigned to receive LAM 100 mg daily from the start of TACE (preemptive group) or not (control group). During the study, 2.8% patients (1/36) in the preemptive group and 29.7% patients (11/37) in the control group developed hepatitis due to HBV reactivation ($P = 0.002$). In addition, there were significantly more incidences of severe grade of hepatitis ($P = 0.035$) and overall hepatitis ($P = 0.021$) in the control group. On multivariate analysis, HBV DNA level $>10^4$ copies/mL in baseline was the only independent predictor of hepatitis due to HBV reactivation during chemo-lipiodolization ($P = 0.046$). These data demonstrated preemptive LAM therapy effectively reduced hepatitis due to HBV reactivation and hepatic morbidity during TACE. Preemptive therapy should be considered in HCC patients with an HBV DNA level $>10^4$ copies/mL. Preemptive antiviral therapy would effectively reduce liver-related morbidity attributable to HBV reactivation and would allow more prolonged chemotherapy. This study also suggested that preemptive LAM therapy decreases the severity of clinical hepatitis if it develops during TACE.

Zhu et al. [250] investigated the efficacy of adjuvant TACE with or without antiviral therapy for HBV-HCC patients after radical hepatectomy. This study enrolled 176 patients, 58 of whom were treated using TACE combined with antiviral therapy and 118 using TACE alone. Analysis of all patients suggested that overall survival of the combination therapy group was better compared to the TACE-only group ($P=0.048$), while disease free survival was similar between the two groups ($P=0.322$). Analysis of only propensity score-matched pairs proved 5-year overall survival in the combination therapy group was significantly better (64.6% vs. 37.5%, $P=0.033$) and also suggested better 5-year disease free survival (37.9% vs. 14.6%, $P=0.048$). For patients after HCC recurrence, radical hepatectomy was the treatment choice for a significantly larger proportion of patients from the combination therapy group than from the TACE-only group ($P=0.018$). These data suggested that combining TACE with antiviral therapy significantly improved overall survival and potentially disease free survival relative to TACE alone in HBV-HCC patients. Combination TACE with antiviral therapy also improves remnant liver function, increasing the chance of curative resection in case of tumor recurrence. Combination TACE with antiviral therapy may benefit to prevent recurrence of HCC after radical hepatectomy.

5.4.3.8 Antiviral Therapy in HBV-HCC Patients Undergoing Radiotherapy

It has been observed that HBV reactivation occurs after the end of radiotherapy in a way similar to that after cytotoxic chemotherapy [251]. Radiotherapy to HCC can damage immune system, and cause leukocytes decreased, following by HBV reactivation. So antiviral therapy before radiotherapy for HBV DNA positive patients is necessary. Kim et al. [251] evaluated the impact of three-dimensional conformal radiotherapy (3D-CRT) on HBV reactivation and hepatitis exacerbation in HBV-HCC patients. This study enrolled 48 HBV-HCC patients who underwent 3D-CRT to the liver. Group 1 ($n = 16$) treated LAM before and during 3D-CRT and Group 2 ($n = 32$) did not treat with NAs before 3D-CRT. To investigate spontaneous HBV reactivation, 43 HCC patients received no specific treatment for CHB or HCC were included as a control group. The cumulative rate of radiation-induced liver disease in Groups 2 was higher than Group 1 (12.5% vs. 21.8%, $P > 0.05$). The cumulative rate of HBV reactivation was significantly higher in Group 2 (21.8%) than in Group 1 (0%) or the control group (2.3%) ($P < 0.05$ each). There was no significant difference in cumulative rate of CHB exacerbation between Groups 1 (0%) and 2 (12.5%) or the control group (2.3%) ($P > 0.05$ each). That demonstrated that HBV reactivation and consequent CHB exacerbation should be considered in HBV-HCC patients after 3D-CRT and antiviral therapy should be recommended to prevent liver function after RT. In study of Huang et al. [252], the rate of HBV reactivation and CHB exacerbation was 24.6% (17/69) and 21.7% (15/69), respectively. There was a relatively high rate of HBV reactivation in those patients and whose prognosis was unfavorable. The serum HBV DNA level and some dosimetric parameters (normal liver volume, V20, and Dmean) were the prognosis factors for HBV reactivation and should be considered carefully before CRT.

5.4.4 Summary

The goal of anti-HBV therapy is to effectively reduce the HBV DNA level, thereby reduce the incidence of cirrhosis and HCC. Although antiviral therapy is recommended in guidelines from APASL, EASL and AASLD, the specific procedures are not the same. And these guidelines do not give treatment recommendations for patients with HBV-HCC. Current clinical studies have confirmed that early antiviral therapy is necessary for the prevention of liver function and reduce the integration of the viral DNA. Although antiviral therapy inhibits viral replication, the integration of viral DNA continued. There are two distinct types of HCC recurrence: tumors grown from dissemination of the primary tumor and de novo tumors arising from the “field effect” in diseased liver [253, 254]. This may argue for an earlier antiviral intervention, as adjuvant therapy after the resection for the HCC patients with a high HBV DNA level to prevent recurrence.

Anti-HBV therapy were performed in the light of the recently updated HBV treatment guidelines, on the recurrence and prognosis of HCC. To substantiate the

beneficial effects of antiviral therapies, future randomized clinical trials (RCTs) with longer follow-up, larger sample size, and regular HBV DNA level monitoring will be needed to conduct. The molecular mechanisms of preventing tumor recurrence also need to be further studied.

5.5 Antiviral Therapy for Liver Transplant Patients

Jun-Ying Qi and Ming Ni

Abstract

Liver transplant is an effective treatment for HBV-related end-stage liver disease. The risk of HBV reinfection after liver transplant is the main limiting factor for long-term survival rate. Combination therapy of lamivudine and hepatitis B immunoglobulin (HBIG) reduced the rate of recurrence. However, considering the disadvantages of high dose HBIG and high rate of lamivudine resistance, other therapies that composed by entecavir, tenofovir, or lamivudine plus adefovir, with or without HBIG have been used in several liver transplant centers. Other researchers have used posttransplant HBV vaccination for achieving a lasting endogenous anti-HBs antibody, yet the efficacy is still controversial. The combination HBIG/nucleotide (acid) prophylaxis should be converted to oral prophylaxis within 1 or 2 years after liver transplantation. Recently, the discovery of sodium taurocholate co-transporting polypeptide (NTCP) as the cellular receptor for HBV entry has opened up many channels of investigation, which indicate the possibility of using NTCP inhibitor in the prophylaxis of hepatitis B recurrence post LT.

5.5.1 The Current Situation of Antiviral Therapy for Liver Transplant Patients

Liver transplant (LT) is an effective treatment for hepatitis B virus (HBV)-related end-stage liver disease (such as acute or chronic liver failure, cirrhosis, hepatocellular carcinoma and so on). China has become the world's second-largest LT country as there are nearly 3000 operations annually. Until the end of 2010, there were 19,330 cases of liver transplant had been completed in China, 80% of which were due to hepatitis B-related liver disease, and nearly 40% recipients with detectable HBV DNA. The risk of HBV reinfection after LT is the main limiting factor for long-term survival rate. The rate of HBV reinfection is as high as 80% without antiviral prophylaxis [255]. LT recipients with recurrent hepatitis B develop an aggressive form of fibrosing cholestatic hepatitis, cirrhosis or graft failure within 2 years [256, 257], which lead to death or re-LT. Combined treatment of hepatitis B immunoglobulin (HBIG) and nucleos(t)ide analogues (NAs) reduced the HBV recurrence rate to 5–10% after 2 years of liver transplantation [258–262].

5.5.2 Options of Antiviral Therapy for Liver Transplant Patients

Pathogenic mechanisms of graft reinfection with hepatitis B post LT have been known. The source of the graft's infection is either presence of HBV in the circulation of the patient or replicating extrahepatic sites. Degertekin et al. analyzed the data from the NIH HBV-OLT database. Between 2001 and 2007, a total of 183 liver transplant patients received a study with a median of 42 months after LT. Multivariate analysis showed that the HBV DNA level at liver transplant is the major risk factor associated with the recurrence of hepatitis B [257]. Samuel et al. also showed the highest risk of hepatitis B recurrence 3 years after LT in patients who had HBeAg in serum and detectable HBV DNA [263]. Another problem in patients with HBV disease post LT is the presence of extrahepatic reservoirs of the virus that are continuous latent source of HBV recurrence [264]. On the other hand, late recurrence is related to low anti-HBs titer or the development of HBs viral escape mutations or YMDD mutations [265].

The strategies to prevent HBV reinfection after LT involve three stages: pre-, and post-transplant. Currently, the strategies include passive immunization (HBIG), antiviral therapy (NAs) and active immunization (hepatitis B vaccine).

HBIG was the first drug to effectively prevent HBV recurrence. Limited duration of HBIG therapy (<12 months) [10,000 IU IV at LT, 10,000 IU IV daily for 8 days after LT, then IV at different intervals to maintain anti-HBs titers >100 IU/L] delayed but did not prevent HBV reinfection [266]. The efficacy of this treatment seemed to be dependent on the viral load pretransplant. There was 96% developed recurrent hepatitis B 2 years after transplant in patients with detectable HBV DNA in serum. The recurrent rate were 29% in patients who were HBV DNA negative pretransplant [267]. This problem was partially resolved by using higher doses of HBIG. Monthly fixed doses of 10,000 IU of HBIG (to keep anti-HBs levels >500 IU/L) or different HBIG doses adjusted to maintain anti-HBs >500 IU/L for the first 6 months after liver transplant significantly reduced the rate of recurrence in patients with detectable HBV DNA pretransplant [268–270]. However, using high doses of HBIG was very expensive.

Lamivudine had apparent effect on HBV DNA replication, decreasing the positive rate of HBV DNA in patients undergoing or waiting for liver transplantation. Data from 20 North American transplant centers showed that treatment with lamivudine improved pre-liver transplantation and liver transplantation-free survival of patients awaiting liver transplantation for HBV-related cirrhosis [271]. The early results of monotherapy of using lamivudine to prevent HBV recurrence post-LT were promising. In nine of ten survivors, HBsAg and HBV DNA were negative, and liver biopsy showed no evidence of recurrent by week 24 [272]. However, 50% patients re-infected with lamivudine-resistant HBV by 8–15 months post-transplant [273].

HBIG and lamivudine are different in action mechanisms. HBIG is a specific passive immune agents prepared from individual plasma who has been infected by HBV or injected hepatitis B vaccine. High concentration of HBIG can neutralize HBV and block its infection of hepatocytes. Lamivudine is a potent inhibitor of

HBV-associated DNA polymerase to block HBV replication. Therefore HBIG and lamivudine play a complementary role to each other. Combined treatment of high dose IV HBIG and lamivudine had been the accepted standard prophylaxis for post-LT HBV recurrence. Lamivudine was used pre-LT for reducing the viral load in the peri-LT period in most centers. HBIG was used at a dose of 10,000 IU daily for the first week post-operative and then either at a fixed dose of 10,000 IU/month or with different doses to keep anti-HBs titers >100 IU/L [260, 261, 274–276]. Some centers had targeted anti-HBs titers >500 IU/L in HBV DNA positive patients for 3–6 months post-LT. Compared to the monotherapy of HBIG or lamivudine, these combined treatments are highly effective [261, 277]. However, the long-term use of HBIG has many disadvantages, such as high cost, the need for injection, headache, flushing, and chest pain [270, 278]. Moreover, the long-term use of lamivudine induces viral resistance, which leads to a high rate of recurrence post-LT [162].

A number of studies have shown that IM HBIG has similar kinetics and produces roughly equivalent trough concentrations of anti-HBs compared to IV equivalent doses of HBIG but less expensive [279]. The largest reported data of prophylaxis with using of IM HBIG comes from investigators in Australia and New Zealand [280]. IM 400 or 800 IU HBIG daily for 1 week from transplantation and monthly thereafter. Lamivudine, 100 mg/day, was administered to candidates waiting for transplantation with hepatitis B surface antigen (HBsAg)-positive and continued posttransplantation. Thirty-seven patients with positive HBsAg (34 patients had hepatitis B, 2 patients had hepatitis B and C, and 1 patient had hepatitis B, C, and D) underwent liver transplantation using this protocol. Thirty-six patients were HBV DNA positive. The therapy had no significant adverse events and was well tolerated. All patients were HBV DNA negative and 31 patients were HBsAg negative at latest follow-up. This investigation suggested that low-dose HBIG combined with Lamivudine prevented recurrence of hepatitis B posttransplantation is more cost-effective. Long-term results of this protocol showed that the actual rate of HBV recurrence at 5 years was 4% in 147 HBsAg-positive patients underwent liver transplantation [281].

Recently, entecavir and tenofovir have been approved as the first-line regimen for the treatment of chronic hepatitis B. These new NAs have replaced lamivudine as the prophylaxis of HBV recurrence post LT. According to EASL Clinical Practice Guidelines, to achieve the lowest level of HBV DNA pre-LT, NAs with high barrier to resistance is recommended as pre-transplant antiviral therapy for all HBsAg positive patients undergoing liver transplantation [4]. Hu et al [282] reported a lower hepatitis B recurrence rate in patients received entecavir than those received lamivudine. A total of 145 patients were administered entecavir combined with low-dose HBIG, and 171 patients received lamivudine plus low-dose HBIG in the control group. Two patients in the entecavir group developed HBV recurrence with no evidence of viral resistance in the median 36 months follow-up time. Eleven patients in the lamivudine group developed HBV recurrence, three of whom were proved HBV resistance in the median 77 months follow-up period. Further analysis demonstrated that HCC at the time of liver transplantation and low anti-HBs titer post-liver transplantation were independent risk factors for HBV recurrence. Perrillo

et al. [20] investigated the efficacy of entecavir combined with various HBIG regimens after liver transplantation. Sixty-one patients with HBV-related liver disease took 1.0 mg/day of entecavir plus various HBIG regimens. In the median 72 weeks follow-up period, only 2 patients showed positivity HBsAg but HBV DNA remained undetected. Na et al. [283] showed that 4 of 262 recipients who received entecavir plus HBIG experienced HBV recurrence after liver transplantation in the median 49 months follow-up time. Among these 4 patients, 3 had received lamivudine followed by entecavir. Studies concerning the efficacy of tenofovir in the prophylaxis of HBV reinfection post-LT are limited. Jiménez-Pérez et al. [284] reported that four patients received tenofovir plus HBIG with or without entecavir for the prophylaxis of hepatitis B recurrence. No hepatitis B recurrence was observed in these four patients at 12 months.

Several researchers have investigated if it was possible to stop HBIG after an initial successful prophylaxis with combined HBIG/lamivudine. In one largest prospective study, 29 patients who were HBVDNA negative before liver transplantation received prophylaxis with HBIG/lamivudine for 1 month after transplantation, then they were randomized to continue combination prophylaxis or lamivudine monotherapy [285]. The early results showed that there was no recurrence case in either group by 18 months. However, 15–20% of the patients who were converted to lamivudine monotherapy had viral breakthrough because of lamivudine resistance in longer follow-up [286]. An alternative choice was to change from HBIG/lamivudine to a combination of antiviral drugs had a higher barrier of resistance than lamivudine. Several studies indicated that this method may be more effective [287, 288]. In a prospective study, 16 of 34 patients receiving prophylaxis with low-dose IM HBIG/lamivudine 12 months post-LT were changed to adefovir/lamivudine combination therapy, whereas the other patients continued previous prophylaxis [288]. At a median of 21 months post-switch, patients in both group had no recurrence. One A low titer of HBsAg in serum was detected in 1 patient in the adefovir/lamivudine group but HBV DNA was negative. This change in therapy improved the life quality of patients and significantly saved the cost. More recently, a multicenter, prospective study demonstrated the results of HBIG-sparing regimen combined with lamivudine plus adefovir dipivoxil initiated at the time of waiting for liver transplantation and continued post-transplantation [289]. Twenty-six patients were recruited into this study at the time of listing for transplantation. Twelve patients had LAM exposure before the study, but none had lamivudine resistance. To the 20 patients who underwent transplantation, 800 IU/day of intramuscular HBIG was given immediately after transplantation for 7 days. All transplanted patients remained alive without HBV recurrence at a median of 57 months after transplantation. After the completion of this prospective study, the regimen was modified that no perioperative HBIG was administered if the pretransplant HBV DNA level $<3 \log_{10}$ IU/mL. Another 28 patients with HBV-related liver disease underwent transplantation (18 without HBIG). All the patients remained alive without HBV recurrence at a median of 22 months post-transplantation. This study indicated that combination of lamivudine and adefovir initiated at the time of listing for transplantation was safe and effective prevention of HBV recurrent without high costs and long-term HBIG therapy.

Other researchers used posttransplant HBV vaccination for achieving a durable endogenous anti-HBs antibody. Two studies reported that 60–80% of patients achieved an anti-HBs titer >10 IU/L following cessation of HBIG and immunization with 1–3 courses of recombinant IM HBV vaccine [290, 291]. However, other investigations using the same protocol have failed to replicate these results [292, 293]. Moreover, the low response (16–62%) was reported in cirrhotic patients awaiting for LT [294]. More recently, Di Paolo et al investigated the efficacy of 1 year, monthly vaccination together with HBIG plus lamivudine in LT patients. One year after vaccination, 44.4% patients maintained anti-HBs titers more than 100 IU/L [295]. These results suggested that HBIG can be considered as an additional strategy in the prophylaxis against HBV recurrence post LT: (1) vaccine administration should be long-lasting (e.g. 1 year); (2) passive prophylaxis with HBIG should preferably be maintained during the initial phase of vaccination and NAs should be maintained during the entire vaccination period.

5.5.3 Management for Hepatitis B Recurrence and Drug Resistance After Liver Transplant

Lamivudine is the most widely used NA to prevent hepatitis B recurrence. However, lamivudine resistance can result in hepatic decompensation and increases the rate of post-transplant recurrence. Newer NAs with lower resistance rates should therefore replace lamivudine in hepatitis B prophylaxis. Schiff et al [296] investigated the effect of adefovir dipivoxil as the rescue therapy in listing or post-LT patients with chronic hepatitis B and lamivudine-resistance. Among listing patients, the percentage of HBV DNA levels undetectable at weeks 48 and 96 was 59% and 65%, respectively. After 48 weeks, liver function normalized in 77% and 76% of these patients respectively. And coagulation function normalized in 60% and 84% of these patients respectively. Among post-transplantation patients, the percentage of serum HBV DNA levels undetectable at weeks 48 and 96 was 40% and 65%, respectively. After 48 weeks, liver function and coagulation function normalized in 51%, 81%, 76%, and 56% of these patients, respectively. Among listing patients who underwent liver transplantation, prevention of graft reinfection over a median of 35 weeks was similar among patients who did or did not receive HBIG. HBsAg was detected on the first test only in 6% and 9% of patients who did or did not receive HBIG, respectively. Serum HBV DNA was detected on follow-up in 6% and 0% of patients who did or did not receive HBIG, respectively. Adefovir dipivoxil-related adverse events occurred in 4% of patients and led to drug withdrawal. Cumulative resistance rate were 0%, 2%, and 2% at 48, 96, and 144 weeks, respectively. In conclusion, adefovir dipivoxil is safe and effective in prevention of graft reinfection with or without HBIG for listing or post-transplant CHB patients with lamivudine-resistance. More recently, one study indicated that late HBIG replaced by adefovir dipivoxil (at least 12 months post-transplant) can prevent late HBV recurrence at less cost [288]. In a prospective open-labeled study, lamivudine plus adefovir dipivoxil given from the time of listing was well tolerated, prevented

lamivudine resistance pre-transplantation and post-transplantation, regardless of the baseline HBV-DNA level [289]. The rescue therapy for patients with lamivudine or telbivudine resistance is to add adefovir or tenofovir, or change to tenofovir + emtricitabine. For patients with adefovir resistance, the approach is to add lamivudine or entecavir, or switch to tenofovir + emtricitabine. For patients with entecavir resistance, the approach is to add adefovir or tenofovir. Combination therapy is still effective for some patients with multi-antiviral drugs-resistance according to evidence based medicine and clinical practice [297].

Regular monitoring and follow-up for patients post LT is also very important. Items include liver function, hepatitis B markers, HBV DNA quantitative, mutant, blood concentration of immunosuppressive drug and ultrasound examination. For hepatitis B recurrence patients, therapy include: support treatment, hepatocyte protection, anti HBV therapy, immunosuppressant regimen adjustment (withdrawal, reduction or change immunosuppressive agents) and liver retransplant.

5.5.4 Prospects of Antiviral Therapy for Liver Transplant Patients

Hepatitis B is a major cause of liver failure in Asia, although the use of HBIG plus lamivudine can effectively prevent HBV reinfection in liver transplantation, but the cost is high. Active immunization approach is still controversial. Combined HBIG/nucleos(t)ide prophylaxis should be considered to switch to oral prophylaxis at 1 or 2 years post-LT, particularly in patients with low HBV DNA loads before antiviral therapy or HBV DNA negative at LT, and in patients with liver failure due to HBV or HDV coinfection, since these patients are at lower risk of recurrence once HBIG is ceased. Recently, the seminal discovery of sodium taurocholate co-transporting polypeptide (NTCP) as the cellular receptor for HBV entry has opened up many channels of investigation, making HBV entry amenable to therapeutic intervention. Several FDA approved drugs with NTCP inhibiting activity were tested for their ability to inhibit HBV infection of the cell line [298–300]. These investigations indicate the possibility of using NTCP inhibitor in the prophylaxis of hepatitis B recurrence post LT.

5.6 Novel Antiviral Therapies for Hepatitis B

Di Wu and Qin Ning

5.6.1 Definition of “Cure” for HBV Infection

Both host and viral factors are associated with the chronicity of HBV infection. HBV has a capability of escaping the host immune responses. More importantly,

The HBV genome forms a stable minichromosome, namely covalently closed circular DNA (cccDNA), in the nuclei of infected hepatocytes, enabling HBV to persist its infection [301]. The goal of anti-HBV therapy is to prevent the progression of HBV-related liver disease, which may be achieved initially through sustained immunologic control over HBV, and ultimately, by completely eliminating the virus [4, 302, 303]. However, due to the fact that HBV cccDNA persists stably at a very low level even after the loss of HBsAg in chronic infected patients, elimination of HBV (complete cure) is rarely achieved. It is suggested that serum HBsAg could represent a surrogate marker of intrahepatic cccDNA and a marker of host immune control of HBV infection. Seroclearance of HBsAg is found to be associated with functional remission and improved long-term clinical outcomes in patients with chronic hepatitis B, under this circumstance, even though HBV genome cannot be cleared, the host immune system is in general sufficient to control the few persisting infected hepatocytes [304–306]. Therefore, HBsAg seroclearance with or without the appearance of HBsAb (functional cure) is considered the ideal endpoint for anti-HBV therapy, representing durable immunologic control over the virus and complete suppression of HBV replication, which is the critical step towards complete cure for hepatitis B [4, 302, 303].

NUC and IFN or its PEGylated form, Peg-IFN, are the only two types of approved antiviral therapeutics. As the ideal endpoint for anti-HBV treatment, HBsAg loss is achieved in very few patients after long-term NUC or 48-week courses of Peg-IFN therapy [307–309]. These current standard antiviral therapies can only suppress the HBV replication and viral protein production, but cannot eliminate HBV cccDNA and cure chronic HBV infection. Therefore, new treatment approaches such as optimal combination therapy with the approved antivirals or emerging novel therapeutics are needed to improve rates of HBsAg loss and, ideally, HBsAg seroconversion.

5.6.2 Interferon-Based Combination Therapy

Different characteristics, mechanisms of action and antiviral activities of NUC and IFN provide the possibility of combining these two types of agents for improving chances of sustained post-treatment response, thereby allowing the discontinuation of NUC without virus relapse, through harnessing both immunomodulatory and direct antiviral mechanisms [310, 311]. According to the updated Chinese Guidelines, Asian-Pacific guidelines, as well as European guidelines for the treatment of chronic hepatitis B, sequential therapy with additional Peg-IFN or switching to Peg-IFN can be considered in CHB patients who have achieved virological remission by long-term NUC treatment [312], though clinical trials evaluating either simultaneous or sequential combination therapy with NUC and IFN for CHB patients drew different conclusions.

Several previous studies exploring the efficacy of simultaneous combination with Peg-IFN and LAM or ADV have demonstrated that the therapeutic strategy led to higher rates of virological response during treatment, but did not improve durable

post-treatment responses [308, 309, 313, 314]. An exploratory study showed that combination treatment with Peg-IFN plus ADV for 48 weeks led to remarkable decline in serum HBV DNA level and intrahepatic cccDNA, which was significantly correlated with reduced serum HBsAg [315]. A recent study evaluating the efficacy of combination therapy with LdT and Peg-IFN in HBeAg-positive CHB patients have demonstrated that the combination therapy led to greater reductions in HBV DNA and HBsAg levels, however, it may contribute to an increased risk of unexpected severe peripheral neuropathy, combination therapy with LdT and Peg-IFN should not be used [316]. In a prospective, active-controlled randomized trial evaluated loss of HBsAg in patients receiving the combination of TDF and Peg-IFN for a finite duration, CHB patients were randomly assigned to receive combination therapy for 48 weeks, combination therapy for 16 weeks followed by TDF for 32 weeks, TDF for 120 weeks, or Peg-IFN for 48 weeks. The study demonstrated that, 9.1% of patients receiving 48-week course of combination therapy with TDF and Peg-IFN had HBsAg loss, which was significantly higher than those receiving TDF or Peg-IFN given alone [317]. However, it is worth noting that a prolonged follow-up of these subgroups of patients is required to determine the durability of treatment response and long-term benefits. Although simultaneous combination of Peg-IFN and NUC other than TDF may not improve sustained response rate, the optimal approach for combination treatment remains to be determine and should take into consideration the time schedule of drug administration.

Late breaking clinical trials have demonstrated that sequential combination therapy with NUC and IFN, either “switch” or “add-on”, showed more promising results, with higher probabilities of HBeAg seroconversion and HBsAg loss than NUC monotherapy. An observation study has shown that the add-on of Peg-IFN to a stable NUC therapy in CHB patients with suppression of HBV DNA, induced HBsAg seroconversion in 2 out of 12 patients [318]. A prospective study demonstrated that additional of Peg-IFN to a long-term NUC treatment in HBeAg-negative patients with undetectable HBV DNA, led to a durable HBsAg loss in 6 out of 10 patients [319]. A global multicentered, randomized controlled trial (ARES study) assessed the effectiveness of add-on Peg-IFN to ETV therapy in HBeAg positive patients, compared to ETV monotherapy, 24 weeks of Peg-IFN add-on therapy did not improve response rates (defined as HBeAg loss with HBV DNA <200 IU/mL at week 48), but led to greater viral decline and appeared to prevent relapse after stopping ETV, which may facilitate the discontinuation of NUC treatment [320]. Another randomized controlled trial has shown that neither ETV pretreatment nor ETV add-on to Peg-IFN demonstrated superiority in sustained posttreatment response compared with 48 weeks of peg-IFN alfa-2a monotherapy in treatment-naïve HBeAg-positive patients [321].

A prospective, randomized controlled trial (OSST study) reported that switching to 48-week course of Peg-IFN in HBeAg positive CHB patients who achieved virologic remission after long-term ETV treatment led to significantly increased rates of HBeAg seroconversion and HBsAg loss (8.5%) [322]. Data from 1-year follow-up of these patients who received sequential therapy showed that rates of HBeAg seroconversion increased from 17.7% at the end of treatment to 38.7% at 1-year

post-treatment, besides, HBsAg loss was durable in 6 of 7 patients [323]. These results are consistent with findings from earlier studies exploring sequential combination therapy with NUC and IFN but only tested in a small number of patients [324, 325]. An exploratory study assessed the efficacy of sequential therapy in genotypes A, B, C and E CHB patients with high HBV viremia, patients receiving ETV alone for 12 weeks, followed by ETV plus Peg-IFN for 12 weeks, lastly only Peg-IFN for 36 weeks achieved significantly higher rates of HBeAg and HBsAg seroconversion than those receiving Peg-IFN monotherapy for 48 weeks [326]. An interim analysis from NEW SWITCH study demonstrated that sequential combination therapy with ETV and Peg-IFN for 48 weeks in NUC-experienced HBeAg positive CHB patients who achieved partial responses, with HBV DNA suppression and HBeAg loss, led to a high rate of HBsAg loss (17.3%) [327].

Accumulating evidences suggest that quantitative HBsAg (qHBsAg) is a useful marker for guiding treatment decision, e.g. individualizing the treatment, implementing stopping rules for ending or extending IFN treatment [328]. Recently, several studies identified that HBsAg loss occurred in patients with a low baseline qHBsAg and high on-treatment reduction, therefore, a baseline or response-guided approach based on HBsAg kinetics may help identify CHB patients with the greatest chance of benefit. The OSST study has demonstrated that patients undergoing long-term ETV treatment with low HBsAg titers (<1500 IU/mL) and HBeAg loss were suitable for sequential therapy with Peg-IFN as they had a good chance of both HBsAg loss (22.2%) and HBeAg seroconversion (33.3%). Patients whose HBsAg levels declined to 200 IU/mL at week 12 of sequential therapy have the greatest chance of achieving HBsAg loss. While, patients whose HBsAg levels were >1500 IU/mL at week 12 might consider stopping Peg-IFN treatment as they had a minimal chance of achieving HBeAg seroconversion and HBsAg loss [322]. These findings are consistent with results from previous studies and interim analyses from the NEW SWITCH study, suggesting that qHBsAg identifies NUC-treated patients who are the best candidates for sequential therapy, and allows response-guided treatment [327–329]. However, given the small number of patients included in the exploratory analyses, these results need to be interpreted cautiously and warrant further investigation and validation.

Differences in study designs and characteristics of patients make it difficult to determine the optimal combination therapy with NUC and Peg-IFN for CHB patients at this stage. Nevertheless, we could speculate that once suppression of HBV viremia has been achieved by pretreatment with NUC, the additional use of Peg-IFN would be more beneficial. These new therapeutic strategies require further investigation before being introduced into routine clinical practice.

5.6.3 Emerging Novel Antiviral Approaches Toward a Cure of HBV Infection

Complete cure of HBV infection depends not only on the deep suppression of HBV replication, but also on the induction of durable antiviral immune response [330].

Besides the approved therapeutics, several novel therapeutic approaches including direct acting antivirals (DAA) targeting different stages of the life cycle of HBV (including HBV entry, HBV genome processing, virus protein assembly, etc.) as well as immunological approaches are currently under early stage of preclinical or clinical investigation, these promising therapeutics may act in a synergistic way with currently available antiviral agents and have the potential to achieve a cure of HBV infection.

Specific inhibition of HBV entry may be a promising therapeutic concept to control HBV infection. A currently identified cellular receptor for HBV entry, the sodium taurocholate co-transporting polypeptide (NTCP), is an emerging target providing new research possibilities and allowing the development of HBV entry inhibitors [331]. Cyclosporine A (CsA) can interfere with the binding between large envelope protein and NTCP, and thus prevent HBV entry into cultured hepatocytes [298, 332]. Myrcludex-B also can inhibit the binding of the HBV envelope proteins to NTCP, blocking the HBV/hepatitis D virus (HDV)'s entry, which is now under clinical development [333]. However, these entry inhibitors can prevent new HBV infection [334, 335], but do not directly target on cccDNA or eliminate the pre-existing HBV infection. Therefore, antiviral strategies combining entry inhibitors with anti-HBV agents might be superior to their use as monotherapy by taking advantage of synergy.

Therapeutic approaches targeting cccDNA for HBV cure aim to directly degrade or alternatively block cccDNA formation, or silence cccDNA transcription. RNA-guided nucleases, such as the clustered regularly interspaced short palindromic repeats (CRISPR)/CAS9 [336, 337], might be the most promising strategy to target cccDNA. However, the risk of undesired off-target mutagenesis and delivery constitute the major limits. Histone modifications, e.g. inhibitors of histone acetyltransferase, offer great potential as therapeutic candidates for CHB patients through transcriptional silencing of cccDNA [301, 338]. Activation of IFN- α and lymphotoxin beta receptor (LT β R) has been shown to induce cytidine deaminases of the APOBEC3 family, triggering degradation of cccDNA in HBV cell culture model systems. These novel strategies will make elimination of HBV a real possibility [339].

Several attempts have been made to develop capsid assembly modulators/core inhibitors, which can be divided into two main classes. The first class, including phenylpropanamides (PPAs) and sulfamoylbenzamide derivatives, e.g. AT-61 and AT-130, can inhibit the entry of pregenomic RNA (pgRNA) into the immature nucleocapsid [340, 341] resulting in nucleocapsid with normal size and geometry but empty of nucleic acid. The other class, including heteroaryldihydropyrimidines (HAPs) antiviral compounds, e.g. BAY41-4109 [342] and NVR 3-778, can directly inhibit the nucleocapsid formation, resulting in virus particles with abnormal size and structure. PPAs and HAPs show synergy *in vitro* with nucleoside reverse transcriptase inhibitors (NRTIs) and overcome resistance to NRTI [340, 343], highlighting the value for developing combination therapy.

Post-transcriptional gene silencing by RNA interference (RNAi), is a new therapeutic approach to hepatitis B [344, 345]. Inhibiting protein production by targeting

HBV messenger RNA (mRNA) for translational repression or degradation can impair virus replication and augment the HBV-specific immune response. Several RNAi-based drug candidates have currently entered early-phase clinical development for the treatment of CHB, including ALN-HBV and TKM-HBV [346, 347], showing clinical efficacy significant declines in HBV DNA, HBsAg, and cccDNA levels. Despite lingering concerns about delivery, the risk of resistance and safety, the RNAi-based therapeutics might be promising when combined with other antiviral agents. Future trials with RNAi-based therapeutics in combination with IFN or other antivirals are required to determine whether these agents would act synergistically to reduce viral antigen production, activate and restore the host immune responses, and subsequently eliminate HBV infection.

HBsAg production and secretion is capable of altering the host immune response by inducing T cell exhaustion and tolerance to HBV, which partially mediate HBV persistence. Control of HBsAg secretion may help restore T cell function, suggesting the possibility of developing anti-HBV treatments targeting HBsAg production and release. Nucleic acid polymer (NAP) could prevent HBsAg release from infected hepatocytes, leading to a restoration of the immune response. Newly developed HBsAg release inhibitors, e.g., REP 2139 and REP 2165 [348–350], appear potent in preventing the release of HBsAg in humans and thereby reducing serum HBsAg levels and also potentially promoting surface antibody seroconversion. However, it remains to be seen whether these compounds may cause detrimental intrahepatocyte accumulation of HBsAg.

Emerging exciting advances have also led to new promising approaches to attenuate HBV-induced immune impairment, such as toll like receptor (TLR) agonists [351, 352], pleiotropic cytokines [353–355], programmed cell death-1 (PD-1) and its ligand PD-L1 blockages [356], therapeutic vaccines [357, 358], etc. TLR agonists can activate intracellular innate pathways and stimulate both innate and adaptive immune responses. A recently developed oral active agonist of TLR-7, GS-9620, has been shown to enhance IFN- α and ISG expression and activate NK cells, T cells and B cells in animal studies [351, 352], however, early human studies have shown limited efficacy of the TLR-7 agonist at tolerated doses, and further research into this TLR7 agonist was subsequently discontinued. Therapeutic cytokines play critical roles in the control of HBV infection and mediate a non-cytolytic clearance of the virus [353–355]. Several studies investigated the antiviral activities and therapeutic potential of cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-2, IL-7, etc. A previous study has shown that HBsAg vaccine in combination with LAM or IL-2 could induce antiviral immune response and consequently elimination of HBV may be achieved in CHB patients [353]. A prospective study investigated whether additional GM-CSF could enhance the immunomodulatory effect of IFN, demonstrating that the combination treatment with GM-CSF and IFN was effective in patients who had previously failed IFN monotherapy [354]. A recent prospective, randomized controlled trial (Anchor study) evaluated whether sequential combination therapy with NUC, Peg-IFN and GM-CSF could induce HBsAg loss in CHB patients treated with long-term NUC and demonstrated that for patients who achieved virological suppression with NUC, this

sequential combination therapy significantly increases rates of HBsAg loss and HBsAb appearance [359].

The difficulties in eliminating cccDNA and breaking the immune tolerance constitute the major obstacles for a cure of HBV infection. Combination of potent DAA with immunotherapeutic approach may help overcome both persistence of cccDNA and immune escape, creating synergies, reducing resistance and creating the potential for durable and sustained post-treatment virological and serologic responses.

5.6.4 Conclusions

Theoretically, NUC treatment may suppress viral replication, partially restore function in exhausted T cells and allow HBV-specific T cells to be more receptive to Peg-IFN treatment. Although at this stage which agents or which combination may be preferable remains to be determined, clinical trials involving sequential combination therapy with NUC and Peg-IFN, either “switch” or “add-on”, have shown statistically significant decline in HBsAg levels on treatment and high rates of sustained post-treatment serologic response [360, 361]. Combination therapy with novel DAA and immunotherapeutic approach may hold promise to overcome both cccDNA persistence and immune escape, representing a critical step towards HBV cure. Several large prospective trials (e.g. COST and OCEAN study) investigating the effectiveness and long-term benefit of sequential combination treatment with NUC and Peg-IFN are currently being carried out.

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