Review

Review of Related Factors for Persistent Risk of Hepatitis B Virus-Associated Hepatocellular Carcinoma

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Posted Date: 26 December 2023

doi: 10.20944/preprints202312.1921.v1

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Review of Related Factors for Persistent Risk of Hepatitis B Virus-Associated Hepatocellular Carcinoma

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Simple Summary: Hepatitis B virus (HBV) affects around 300 million people worldwide and is a significant risk for the development of hepatocellular carcinoma (HCC). Nucleos(t)ide analog therapy has aided in decreasing mortality from HBV, however no cure for HBV currently exists. Despite adequate treatment based on undetectable viral load or absence of surface protein, there has been much research demonstrating persistent risk for HBV-associated HCC. The aim of this paper is to review the related factors, pathophysiology, and evidence for why this risk exists. Further clarification of the relationship and risk factors for HBV-related HCC is necessary for appropriate screening and eventual development of a cure.

Abstract: Chronic hepatitis B virus (HBV) infection is the largest global cause of hepatocellular carcinoma (HCC). Current HBV treatment options include pegylated interferon-alpha and nucleos(t)ide analogues (NAs), which have been shown to be effective in reducing HBV DNA levels to become undetectable. However, literature has shown that some patients have persistent risk of developing HCC. The mechanism in which this occurs is not fully elucidated, however it has been discovered that HBV covalently closed circular DNA (cccDNA) integrates into critical HCC driver genes in hepatocytes upon initial infection and are not targets of current NA therapy. Some studies suggest that HBV undergoes compartmentalization in peripheral blood mononuclear cells that serve as a sanctuary for replication during antiviral therapy. The aim of this review is to expand on how patients with HBV may develop HCC despite years of HBV viral suppression and carry worse prognosis than treatment-naive HBV patients who develop HCC. Furthermore, HCC recurrence after initial surgical or locoregional treatment in this setting may cause carcinogenic cells to behave more aggressively in the treatment experienced. Curative novel therapies targeting the life cycle of HBV, modulating the host immune response, and inhibiting HBV RNA translation are being investigated.

Keywords: Hepatitis B virus; hepatocellular carcinoma; nucleoside analog; antiviral therapy; hepatitis cure; cccDNA

1. Introduction

Hepatitis B virus (HBV) is a global public health problem with an estimated 350 million chronic hepatitis B carriers and causing 820,000 deaths in 2019 alone [1-3]. HBV is endemic in many parts of the world, including Southeast Asia, China, and Africa [4]. It belongs to a family of DNA viruses called hepadnaviruses which is composed of at least ten genotypes, A-J [2,5,6]. The virion is an enveloped nucleocapsid that delivers an incomplete circular DNA genome into the host cell, initiating viral replication [7]. HBV is a dynamic, hepatotropic virus and its infection has a wide spectrum of clinical manifestations [7]. Fifteen to forty percent of HBV-infected patients develop cirrhosis, liver failure, or hepatocellular carcinoma (HCC) [1,8]. In fact, HBV is the most common hepatocarcinogen, being accountable for 25% of HCC in developed countries and 60% in developing countries [9-12]. There is a limited understanding of the pathogenesis, prognosis, and treatment strategies for HBV-associated HCC which we will highlight in this review paper.

 doi:10.20944/preprints202312.1921.v1

2. HBV Pathophysiology and Hepatocarcinogenesis

HBV is transmitted by percutaneous inoculation or transmission of infectious bodily fluids. In high-prevalence areas, mother-to-child transmission is the predominant mode of transmission while unprotected sex and injection drug use are the common modes of transmission in low prevalence areas [13,14]. The incubation period of HBV is between 30 and 180 days. During infection, complete and incomplete viral particles are released into the serum of the host, facilitating viral replication [15].

HBV is a non-cytopathic virus, and the liver damage associated with HBV is caused by the host immune response [16,17]. During acute infection, host immune cells, most prominently CD8 T cells, kill infected cells, inducing hepatic inflammation [4,18]. Around 70% of patients with acute HBV have subclinical or anicteric hepatitis, while 30% have icteric hepatitis [19]. The clearance of HBV is mediated by the adaptive immune system and HBV utilizes multiple strategies to evade this line of defense [19-21]. Hypo-responsiveness of HBV-specific T cells may also contribute to persistent HBV infection [21]. While recovery commonly occurs in immunocompetent individuals, a small proportion of those infected can progress to chronic HBV, which is defined as the presence of HBsAg for greater than six months [22].

There are four phases of chronic hepatitis B (CHB) infection: immune tolerance, immune clearance, immune control, and immune escape/reactivation [23]. In the immune tolerance phase, ALT levels are still low, viral DNA levels are high (usually at least 2,000,000 IU/mL), and there is minimal or no inflammation on liver biopsy [4,18,23-26]. This phase may last for a few years to around 30 years [27,28]. In the immune clearance phase, hepatitis B e antigen (HBeAg) is positive, there is intermittent or persistent elevation of ALT levels, elevated HBV DNA levels (at least 2000 IU/mL), and some degree of inflammation or fibrosis on liver biopsy [23,28]. In this phase, HBV-specific CD8 T cells directly attack infected hepatocytes and recruit other immune cells to the liver, which further exacerbate hepatic injury [4,17,18,26,29]. During immune clearance, patients may present with flares which although are often asymptomatic, may be characterized by signs of acute hepatitis. During flares, ALT levels may be elevated to greater than 5-times the upper limit of normal [23,30]. The end of the immune clearance phase and beginning of immune control is marked by seroconversion, which is the loss of HBeAg and development of antibodies to hepatitis B e antigen (HBeAb). Intriguingly, the duration of the immune clearance phase has a critical association with the development of complications; those who seroconvert after the age of 40 have a significantly higher risk of cirrhosis, HCC, and CHB compared to those who seroconverted before the age of 30 [23,31]. In addition, HBeAg positivity is a known risk factor for HCC [26,32]. The immune control phase is characterized by lower levels of HBV DNA (usually < 2000 IU/mL) and ALT, although HBsAg remains [4,18,23]. Some patients, even after seroconversion, may continue to have moderate levels of viral replication with associated abnormal ALT, leading to eventual reactivation of the immune active phase; this phenomenon is known as immune escape [23,28,33,34]. Resolution of infection is indicated by disappearance of HBsAg [4,18].

In CHB, there are crucial changes in immune cell activity and function involving both the innate and adaptive immune systems that lead to hepatic inflammation and hepatocyte killing [17]. Chronic infection may progress to liver fibrosis, cirrhosis, and HCC [17,35]. HBV-mediated carcinogenesis is a complex process that involves viral DNA integration into the host genome, ultimately leading to viral manipulation of cell-signaling and proliferation. This leads to a cascade of events which converts normal hepatocytes into malignant cells [36-39]. Oxidative stress associated with viral hepatitis changes the cellular environment in such a way that promotes carcinogenesis. Overproduction of free radicals and reactive oxidative species leads to the upregulation of inflammatory pathways that result in hepatocyte release of cytokines and chemokines that recruit neutrophils, monocytes, and lymphocytes [18,37,40,41]. As inflammation persists, immune cells, including macrophages and myeloid-derived suppressor cells, become dysfunctional, further amplifying the pro-inflammatory environment. The chronic inflammatory state leads to compensatory hepatocyte proliferation, which leads to accumulation of mutations that promote cell growth and proliferation which predisposes the host to developing HCC (Figure 1) [40,42].

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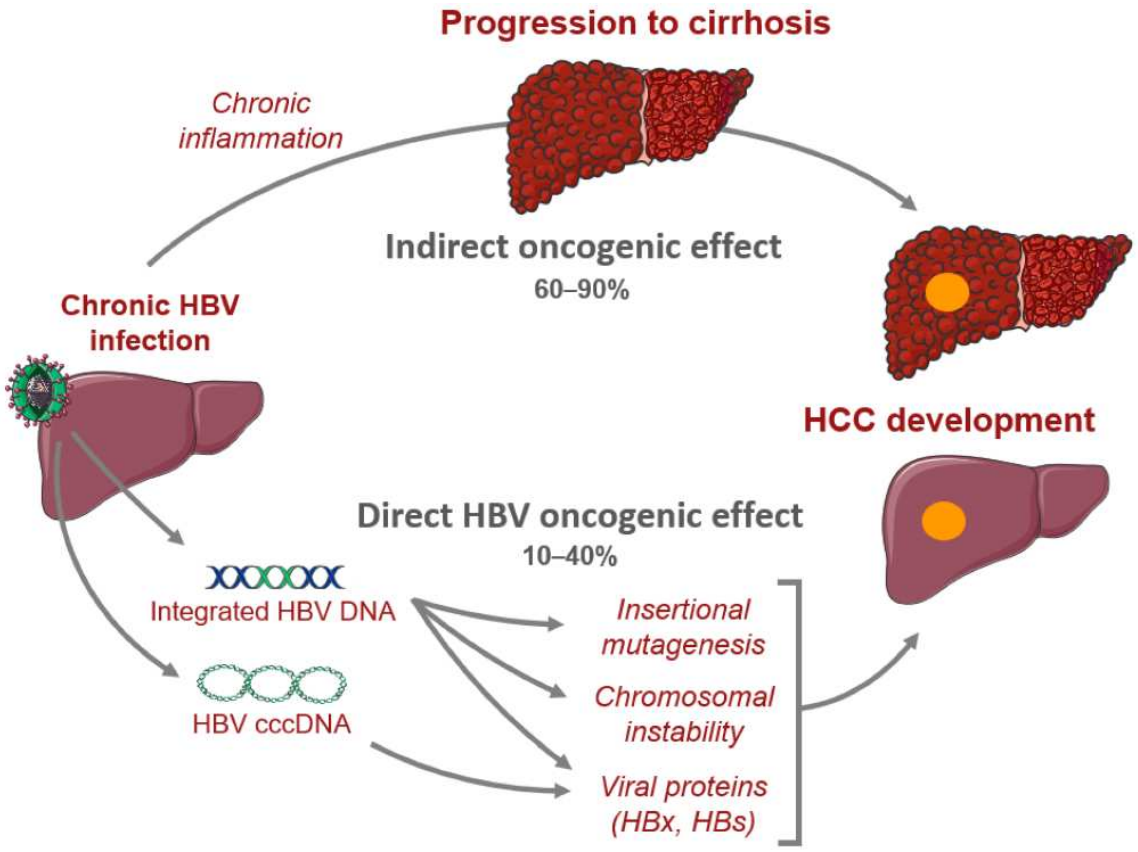

Figure 1. Mechanisms of hepatocarcinogenesis from chronic hepatitis B infection. Sourced from Péneau *et al*. [42].

Moreover, it was found that the incidence of HCC is fivefold higher among HBV-infected patients with cirrhosis compared to asymptomatic carriers, indicating that cirrhosis may be a pre-malignant condition [38]. Indeed, fibrosis of the liver disrupts the normal architecture which leads to modification of cell-cell interactions and ultimately, loss of regulation over cell proliferation [38].

Furthermore, it is important to note that numerous factors including host characteristics, HBV genotype, viral mutations, viral load and HBsAg levels all influence the clinical manifestations of HBV infection [6]. Research has shown that there are differences between the various genotypes of HBV in tendency of chronicity, primary mode of transmission, timing of seroconversion, timing of HBsAg clearance, and clinical outcomes [6]. For example, multiple studies have shown that genotype C tends to cause more severe liver disease, including cirrhosis and HCC, compared to other genotypes [6,43,44]. Genotype C also has higher serum HBV levels and was shown to cause DNA mutations more frequently than Genotype B [6,45]. Further research investigating the utility of routine HBV genotyping is needed before implementation into clinical practice.

3. HCC Risk Factors and Surveillance

HCC is one of the major malignant diseases in the world today and ranks fifth in overall frequency. Its incidence is high in Eastern Asia, sub-Saharan Africa and increasing in many parts of the Western world [46]. Unfortunately, the annual mortality from HCC is close to its incidence because of its rapid progression and poor prognosis. Additionally, the therapeutic interventions available at time of diagnosis are generally ineffective. Thus, the focus has been on early screening and treatment of known causes, including chronic hepatitis B which is the most frequent underlying cause of HCC. There are several major risk factors for development of HCC in chronic HBV infection. While having a first-degree relative with HCC, metabolic syndrome, type 2 diabetes, and central obesity are all host factors that have been linked to HCC development in HBV-infected patients or carriers, it appears that liver cirrhosis has been consistently identified to be the most significant risk

 doi:10.20944/preprints202312.1921.v1

factor for development of HCC during nucleos(t)ide therapy [47-51]. Studies indicate that the presence of male gender, increasing age, higher HBV DNA level, and core promoter mutations are also independently associated with HCC risk [52,53]. It is postulated that androgens may play a role in the observed difference in incidence of HCC based on gender [54]. While less investigated, concurrent HCV or HIV infection has also been identified to have an association with increased incidence of HCC [55,56]. Surveillance with ultrasound imaging and measurement of alpha-fetoprotein levels has been directed towards these populations to help with early detection of HCC. Guidelines suggest surveillance for HCC in high-risk groups including patients with cirrhosis, noncirrhotic patients with HBV and any of the following characteristics: active hepatitis, family history of HCC, Africans and African Americans, Asian males over 40 years of age, and Asian females over 50 years of age [57-60]. Other populations that undergo surveillance include patients with chronic hepatitis C virus and advanced liver fibrosis in absence of cirrhosis although this is not a consistent recommendation across all societies and the cost effectiveness has not been verified [58].

Currently a knowledge gap remains regarding host factors that contribute to the vagaries of HBV infection outcomes. A group of researchers attempted to explore this by investigating the diverse manifestations of CHB in three families that were observed over decades. Block *et al*. showed how only one case of HBV-related HCC occurred within every family cluster despite each having the same virus given perinatal transmission from mother to offspring [61]. Furthermore, one of the families had monozygotic twins in which only one sibling developed HBV-related HCC, while the other remained a chronic HBV carrier. The same finding is presenting in a case series by Noverati *et al*., which presented four family clusters in which patients had very variable courses, some with indolent chronic HBV infection, some requiring treatment, and others who developed HCC or cirrhosis [62]. It is postulated that inheritable immuno-genetic alleles that affect CHB differ from those that influence HBV-related HCC development, which may explain the discrepancy in manifestations in this case series [63]. The study was limited by lack of host and viral genomic analysis as previous studies have shown certain host polymorphisms and HBV mutations are associated with HBV-related HCC. Nonetheless, this highlights that there are genetic and non-genetic host factors that play a role in development of HCC.

Exogenous, non-genetic factors such as chronic stress have been implicated in the incidence and outcomes of HCC, which may also have contributed to the differing presentations in the case outlined above [18]. One of the first published studies highlighting the significance of stress was by Russ *et al*., in which the group found in a large UK meta-analysis that those with higher scores on a general health questionnaire measuring psychological distress had a higher mortality from liver disease [64]. Joung *et al*. outlined how stress can increase inflammatory, oxidative reactions including hypoxia-reoxygenation, overactivation of Kupffer cells, influx of gut-derived lipopolysaccharide and norepinephrine, and overproduction of stress hormones in the sympathetic drive, which cause hepatocellular damage and promote mutagenesis [65]. Studies have shown how the tumor milieu of existing HCC undergoes changes that make it immunosuppressive. Specifically, He *et al*. discovered how chronic stress transitions cytokines to those that are T helper 2 cell-mediated, a tumor microenvironment that carries a poorer prognosis [66]. Similar studies show how this is exacerbated by the presence of T regulatory cells that suppress pro-inflammatory cytokines [67,68].

4. Incidence of HCC in Response to Treatment of HBV

Chronic inflammation and necrosis are key factors which predispose patients to developing HCC. In HBV infection, viral load generally correlates with the severity of disease. NA therapies aimed at decreasing viral load including lamivudine, entecavir, and tenofovir have provided a breakthrough in the treatment of HBV infection as they have demonstrated a reduction in incidence of HCC [69-71] and are now standard of care. Results of prior systematic reviews demonstrate that the risk of HCC is significantly lower in CHB patients receiving oral antiviral therapy. One of the first data to demonstrate efficacy of anti-HBV therapy in HCC risk reduction came from a randomized controlled trial by Liaw *et al*. which compared lamivudine vs placebo in treatment-naive patients with cirrhosis or advanced fibrosis and active liver disease. Lamivudine treatment for 3 years reduced

 doi:10.20944/preprints202312.1921.v1

the risk of HCC by 51% compared to placebo [71]. According to Papatheodoridis *et al*., across three studies including untreated controls, HCC developed in 2.8% of treated and in 6.4% of untreated CHB patients (p=0.003). A similar study by Singal *et al*. pooled data from six other studies and showed that the incidence of HCC among lamivudine-treated patients was significantly lower than that of untreated patients (3.4% vs 9.6%, p<0.0001) [72]. This data also demonstrated that HCC occurred more frequently among CHB patients receiving therapy who were older, had cirrhosis, or had detectable HBV-DNA at the end of treatment.

Over time it was discovered that several patients on lamivudine therapy did not attain virological response in the setting of resistance. This did not come without consequence. Alarmingly, Papatheodoridis *et al*. demonstrated that the rate of HCC was significantly higher in lamivudine resistance than in nucleos(t)ide naive patients (7.1% vs 3.8%, p=0.001), even after correcting for those with cirrhosis (18% vs 11%, p=0.015). It is believed that mutations including changes at position 181 of the reverse transcriptase domain of HBV polymerase have oncologic potential. This led to the advent of newer nucleos(t)ide analog therapies such as tenofovir and entecavir, which proved to be effective. Kim *et al*. observed that the incidence of HCC in patients with CHB and cirrhosis was decreased compared to the predicted incidence for untreated CHB patients after treatment with tenofovir disoproxil fumerate (TDF) for 3 years [70]. Lim *et al*. elaborated on this finding and showed that the difference in observed versus predicted HCC risk is more pronounced with tenofovir alafenamide (TAF) than TDF treatment [73]. In a study of entecavir-treated patients, the cumulative incidences of HCC were lower with achievement of a virologic response in both non-cirrhotic and cirrhotic patients [74]. In another study which included cirrhotic CHB patients treated with entecavir, those with undetectable HBV DNA levels were associated with a lower probability of developing HCC [75]. A separate study sought to identify the long-term clinical impact of low-level viremia (<2,000 IU/mL) in patients on entecavir monotherapy. Kim *et al*. observed that among patients with cirrhosis, those with low-level viremia exhibited a significantly higher HCC risk than those with maintained virological response [76]. These findings support the current recommendation for indefinite therapy in chronic HBV patients, and that inability to achieve undetectable viral load can be consequential.

5. Persistent Risk of HCC in HBV Infection

Maintenance of viral suppression is necessary to reduce HCC risk as demonstrated above. Unfortunately, recent evidence shows that there remains a persistent risk of HCC despite successful HBV therapy resulting in viral suppression [46,77-81]. While spontaneous or treatment-induced seroclearance of HBsAg is also associated with lower risk of liver-related complications, there remains persistent risk of HCC development in these patients as well. Ahn *et al*. showed a significant reduction of necroinflammation on liver biopsy of patients before and after seroclearance, however all the patients demonstrated presence of HBV DNA without a change in the fibrosis score [82]. Additionally, Gounder *et al*. showed that seroclearance was not associated with reduced HCC risk compared to a matched control group [83]. The proposed mechanisms in which HCC develops despite adequate treatment are described here.

It has been discovered that HBV covalently closed circular DNA (cccDNA) integrates into the hepatocytes upon initial infection and can be detected even after antiviral treatment. This integration may occur at critical HCC driver genes which may give rise to malignant HBV-infected hepatocytes. Li *et al*. found that the common location of integration included the *TERT, CCNEI1,* and *MLL4* genes, and that virus-host chimera DNA circulates in 97.7% of CHB patients with HCC [84,85]. Interestingly, the group demonstrated how viral-host chimera DNA could be used as a biomarker for detecting tumor load in this patient population, and how it may aid in monitoring recurrence after tumor resection [84]. In the first detailed molecular evaluation completed by Coffin *et al*., despite undetectable HBV DNA in plasma via clinical assays, patients treated with oral anti-HBV therapy undergoing liver transplant were found to have low levels of HBV genome in the liver, circulating lymphoid cells, as well as plasma using ultrasensitive PCR/nucleic acid hybridization assay. Their data promotes the idea of HBV compartmentalization where despite adequate suppression, the liver

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supports replication and extrahepatic HBV can be detected, particularly in peripheral blood mononuclear cells (PBMC) that may serve as a sanctuary for wild type virus during antiviral therapy [86].

Other possible mechanisms that have been postulated include progression of cirrhosis, necroinflammation caused by HBV infection, and the oncogenic effect of HBV genome integration into the hepatocyte chromosomes. HBV DNA may remain detectable in the serum or liver cells, which is referred to as occult HBV infection [87]. Wong *et al*. found that 69% of 90 HBsAg-negative patients with HCC had occult infection and that of these patients, 47% had detectable cccDNA in hepatocytes and 53% had integration of HBV DNA near hepatic oncogenes [88]. In fact, the HBV genome has been detected in tumor tissue of HBsAg negative patients with HCC in a prevalence ranging from 30% to 80% [89,90]. Therefore, these patients continue to undergo surveillance per the AASLD guidelines [91]. Michalak *et al*. showed that traces of the HBV genome persist for years following recovery from hepatitis B (characterized by seronegativity) and that they can lead to HCC development in a woodchuck model [92].

HBV infection has an inhibitory effect on the innate and adaptive immune cells which may aggravate chronic inflammation and lead to carcinogenesis. These effects are thought to persist despite antiviral treatment as the microenvironment is altered upon initial infection. It has been shown that the virus may decrease the expression of STAT3 in NK cells, ultimately inhibiting their ability to clear HBV [93]. Studies demonstrate that HBV may promote polarization of cells from M1 to M2 and inhibit secretion of antiviral cytokines by macrophages [94]. Immune tolerance to HBV infection plays a key role in the acceleration of progression of HBV to HCC.

Most reported cases of HBV-HCC in literature are within the first five years of NA therapy, but data on long-term risk of HCC while on treatment is lacking. The Liver Disease Prevention Center, Division of Gastroenterology and Hepatology at Thomas Jefferson University Hospital has observed several HBV positive patients develop HCC even after 10 or more years of successful viral suppression [95]. Specifically, in our observational cohort, 17 patients developed HCC despite having noted undetectable HBV DNA for up to 12 years and on treatment for up to 19 years (Table 1) [96]. Currently this is among the longest known follow-up studies on development of HCC in patients on HBV treatment.

Table 1. Development of HCC in patients with cirrhosis on long-term antiviral therapy. Sourced from Boortalary *et al.* [96].

Pt	Date StartTx	Change in Child Class on Tx	Date HCC Dx	Yrs on anti-HBV Tx at HCC Dx	Yrs with HBV DNA(-)*	Age (yr) at HCC Dx	Tumor size at Dx	HBVDNA at HCC Dx	Anti-HBV Tx	Status
1	4/1998	B → A	7/2007	9	3	53	1.1 Junction	UD	LAM + TDF	Alive
2	1/1998	B → A	3/2008	10	8	68	2.8 Rt	UD	LAM + TDF	Dead
3	5/1998	A →A	2/2008	10	7	76	1.8 Lt	UD	LAM + TDF	Alive
4	7/2001	B → B	9/2010	9	4	54	2.8 Rt	UD	LAM + TDF	Dead
5	8/2004	B → B	11/2010	16	4	53	3.9 Rt	UD	LAM + TDF	Alive
6	7/2001	B → B	1/2011	10	5	55	2.8 Rt	UD	LAM + TDF	Dead
7	2/2004	A → A	6/2013	9	8	57	2.5 Lt med	UD	TDF	Dead
8	2/1996	A → A	7/2013	17	10	73	1.6 Rt	UD	TDF	Dead
9	8/1997	A → A	6/2014	17	6	54	2.2 Lt lat	UD	ETV	Alive
10	3/2004	B → B	6/2013	9	7	57	2.5 Lt	UD	TDF	Dead
11	7/2001	A → A	6/2014	13	7	54	2.2 Lt	UD	TDF	Alive
12	5/1996	A → A	10/2014	18	10	74	3.4 Rt	UD	LAM + TDF	Dead
13	2/2000	A → A	10/2014	14	12	62	3.8 Rt	UD	ETV + TDF	Alive
14	2/2000	A → A	4/2015	15	12	62	3.4 Rt	UD	TDF	Alive
15	2/2000	B → A	5/2015	15	12	65	3.8 Rt	UD	TDF	Alive
16	12/1998	A → A	8/2017	19	8	64	2.0 Rt	UD	LAM + TDF	Alive
17	2/2008	A → A	6/2019	11	10	57	2.2 Rt	UD	ETV + TDF	Alive

Dx: Diagnosis, ETV: Entecavir, LAM: Lamivudine, Lt: Left, Pt: Patient, Rt: Right, TDF: Tenofovir Disoproxil Fumarate, Tx: Treatment, UD: Undetectable,
*Years with HBV DNA (-) at diagnosis of HCC

Our center has observed six patients who developed new or recurrent HCC after undergoing curative therapy for HCC and achieving adequate HBV suppression for 5-11 years on antivirals [95]. Unfortunately, recent studies have shown that the patients who develop HCC after years of viral suppression, in fact, carry worse prognosis than those who develop HCC without prior treatment of HBV [97,98]. A case series of 26 patients, 13 antiviral-naive and 13 antiviral-treated, assessed clinical outcomes after HCC diagnosis. Garrido *et al*. showed that after the first HBV-HCC event, death was

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observed more frequently among the antiviral-treated cohort at 46.2% compared to 15.4% in the antiviral-naive cohort [99]. Li *et al*. compared patients who received NA therapy after hepatectomy for HCC and those who did not receive treatment and discovered that there was no difference in the recurrence rate of HCC [100]. The suggested mechanism by which this occurs is that halting HBV replication at the reverse transcription phase for a prolonged time may prime the infected hepatocytes to increasingly turnover HBV DNA integration which can cause insertional mutagenesis and genomic instability [99]. This additionally results in the accumulation of incompletely transcribed mRNAs which may lead to further integration.

It is believed that HCC inoculation in this setting of disorganized products and instability may also cause cancerous cells to be primed to behave more aggressively. Chowdhury *et al*. described two cases of HBV treatment-naive HCC where despite radiofrequency tumor ablation (RFA) and subsequent undetectable HBV viral load on NA therapy, the patients developed recurrent, more aggressive cancer [98]. The first case described an elderly female who underwent RFA and trans-arterial embolization (TACE) of a 2.1 x 5 cm mass suggestive of HCC. She started NA therapy and became HBV DNA negative, however developed recurrence 5 years later and required repeat TACE. Ten years after the recurrence, MRI demonstrated another LI-RADS 4 5 mm lesion in segment 2 which was monitored with evidence of regression. Unfortunately, this tumor had grown to 1.2 cm two years later, which marked 28 years after the development of her initial HCC. The second case described a male who was found to have cirrhosis with 1.0 cm HCC at age 68. He underwent NA therapy with undetectable HBV DNA and RFA, however at age 79, was found to have two new lesions on abdominal MRI (3 x 2.8 cm and 2 x 1.7 cm) in segment 5 adjacent to gallbladder, and segment 7/8 respectively. He received cholecystectomy and tumor ablation but four years later was found to have a new 4.8 cm lesion in segment 4 on MRI. This illustrates the complexity of the relationship between HBV and HCC, as there is accelerated progression and recurrence of tumor in patients after becoming treatment experienced.

NAs have been critical in decreasing HBV-associated mortality worldwide, however we demonstrate in this review that they do not eradicate the risk of HCC development. There is persistent risk of hepatocarcinogenesis despite adequate treatment marked by undetectable HBV DNA. Thus, there remains a public health threat as currently there is no cure for HBV infection. Achievement of a cure would require therapies which eliminate cccDNA and integrated HBV DNA as well as help restore the impaired innate and adaptive immune responses against HBV.

6. The Road to HBV Cure

The AASLD defines two different types of "cures" of HBV. First, it defines "immunological cure" as HBsAg loss and sustained HBV DNA suppression. Second, it defines "virological cure" as eradication of the virus, including cccDNA, which is not achievable with current therapies which, with current therapies, is not achievable.

The next generation of drugs in development to treat Hepatitis B can be divided into the following classes: drugs targeting the life cycle of the hepatitis B virus; drugs aiming to modulate the host's immune response to CHB infection; monoclonal antibodies aiming to directly inhibit translation of HBV RNA; and therapeutic vaccines (Figure 2) [101]. A detailed discussion of the progress of these drugs' development is beyond the scope of this paper; for further reading, the excellent review by Yardeni *et al*. is highly recommended [102].

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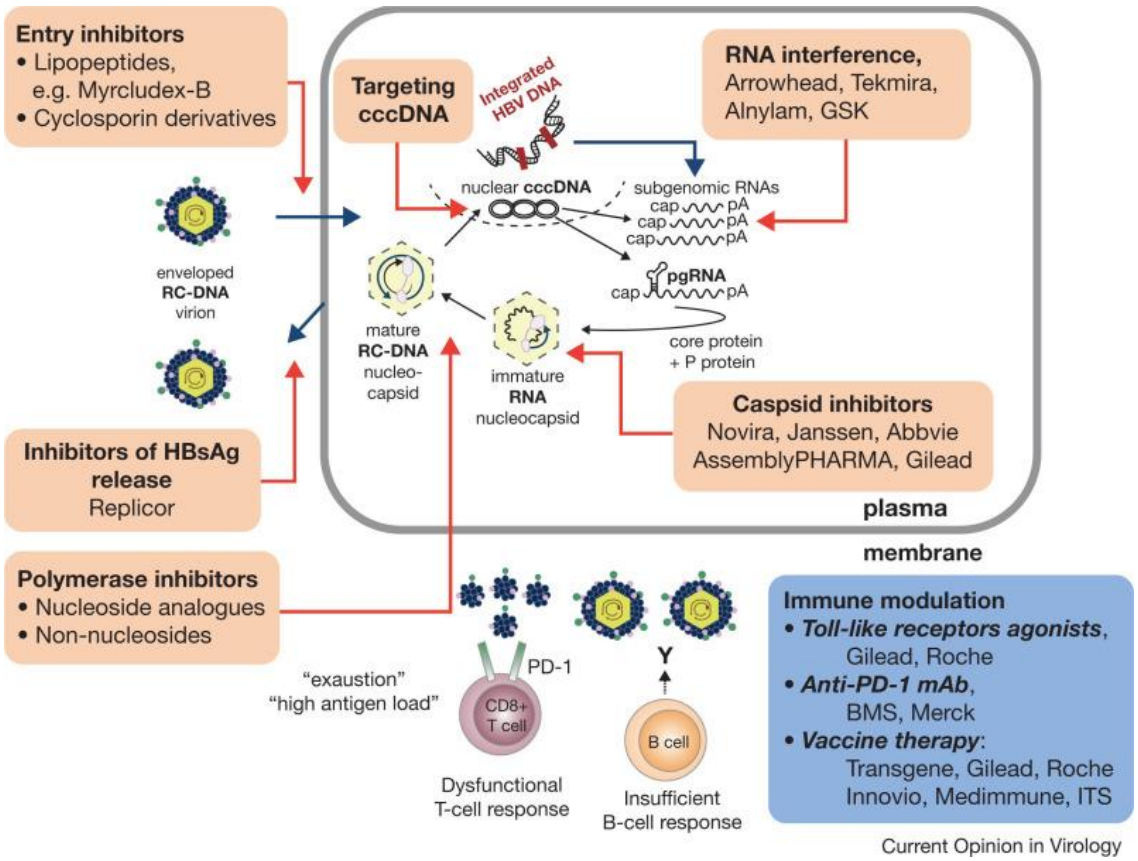

Figure 2. Therapeutic drug targets of hepatitis B virus replication. Sourced from Levrero *et al.* [101]. Used with permission from original copyright holder.

Drugs targeting the life cycle of the hepatitis B virus can be classified into entry inhibitors, capsid assembly modulators, post-transcriptional control inhibitors, and Hepatitis B surface antigen release inhibitors.

Novel drugs aiming to modulate the host's immune response to the HBV are comprised of innate immune activators – specifically, toll-like receptor (TLR) agonists and RIG-1 agonists, and checkpoint inhibitors.

Monoclonal antibodies designed to inhibit the life cycle of HBV have been investigated. One such neutralizing monoclonal antibody, VIR-3434, enhances HBsAg uptake by dendritic cells to promote its presentation to naive T cells thereby stimulating a HBV-specific immune response [103].

Finally, there has been interest in using therapeutic vaccines in effort to stimulate the host's immune response against CHB infection. However, early studies in humans showed only modest declines in HBsAg [104]. With that said, several new therapeutic vaccines are in development which may be tested in combination with drugs of other mechanisms of action, including siRNAs [105]. However, these therapies have not yet been tested in humans.

In summary, the emerging landscape for novel CHB treatments is composed of vast and varied mechanisms of action, but further clinical testing is still required to establish superiority in efficacy compared to the current standard of care.

7. Conclusion and Future Directions

HBV is a global health threat worldwide as infection can lead to HCC. Available treatment options do not fully eradicate the risk of hepatocarcinogenesis as initial integration of cccDNA into the host genome is not prevented. Continued transcription creates a milieu of genomic instability which can make recurrence of HCC even more aggressive. The phenomenon of worse prognosis in treatment-experienced HCC versus treatment-naive HCC is observed. While there remains a

persistent risk of HBV-associated HCC despite viral suppression, recent studies show promising novel approaches to HBV cure.

Author Contributions: Conceptualization, H.H.; Resources, H.H., N.V., A.M., S.N.; Writing – N.V., A.M., S.N. Original Draft Preparation, N.V., A.M., S.N.; Writing – Review & Editing, H.H., D.H., N.V., A.M., S.N., T.B.; Visualization, H.H., D.H., N.V., T.B.; Supervision, H.H., D.H., T.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: No new data was created or analyzed in this study. Data sharing does not apply to this article.

Conflicts of Interest: The authors declare no conflict of interest.

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