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TITLE: **The impact of currently licensed therapies on viral and immune responses in Chronic Hepatitis B: considerations for future novel therapeutics**

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Abstract

Despite the availability of a preventative vaccine, chronic hepatitis B (CHB) remains a global healthcare challenge with the risk of disease progression due to cirrhosis and hepatocellular carcinoma. Although current treatment strategies, Interferon and nucleos(t)ide analogues have contributed to reducing morbidity and mortality related to CHB, these therapies are limited in providing functional cure. The treatment paradigm in CHB is rapidly evolving with a number of new agents in the developmental pipeline. However, until novel agents with functional cure capability are available in the clinical setting, there is a pressing need to optimise currently licensed therapies. Here we discuss current agents used alone and/or in combination strategies along with the impact of these therapies on viral and immune responses. Novel treatment strategies are outlined and the potential role of current therapies in the employment of pipeline agents is discussed.

KEYWORDS: Hepatitis B surface antigen; Nucleos(t)ide analogues; Pegylated Interferon; T cells, NK cells

Introduction

The introduction of a preventative vaccine for Hepatitis B virus (HBV) has led to the overall reduction in the incidence of chronic infection. This unfortunately is not the case for many middle and low income countries, thus chronic hepatitis B (CHB) remains a major global healthcare challenge. In line with this, the viral hepatitides are the only communicable disease whereby there has been an increase in related morbidity and mortality over the last 20 years[1]. Both HBV and hepatitis C virus (HCV), contribute to end-stage liver disease and hepatocellular carcinoma (HCC). HCV remains prevalent in North America and Europe, although novel direct-acting antivirals (DAAs) now provide cure rates of >90% for chronic HCV[2]. CHB remains prevalent in Asia and sub-Saharan Africa[1], but recent migration patterns have lead to an increase in the prevalence of CHB in the western world[3]. An estimated 250 million people are chronically infected, hepatitis B surface antigen (HBsAg) seroprevalence is approximately 4% globally, with much higher prevalence in Africa approaching 10% and 5% in the Western Pacific[4,5]. HBV is transmitted haematogenously and sexually; in high prevalence regions, the majority of HBV infections are transmitted vertically (or perinatally) [6].

HBV infection acquired at birth or in early childhood, will result in chronicity in >95% of subjects. On the contrary, only 5-10% of those who acquire the virus in adulthood will progress to chronic infection. Despite the use of passive-active immunoprophylaxis with HBV immunoglobulin and HBV vaccine, babies born to highly viraemic mothers are at risk of acquiring the infection. However, the risk of vertical or perinatal transmission can be significantly reduced by the administration of antiviral therapy to highly viraemic Hepatitis B envelope Antigen (HBeAg) positive mothers in the last trimester of pregnancy, which is in line with current national and international treatment guidelines [7-9]. Given the rise in UK prevalence of HBV, secondary to migration from endemic areas, hepatitis B

has now been added to the infant vaccination schedule[10]. It is predicted that the incidence of HBV-related HCC will increase in the coming years[11] and thus it is vital for investment into new HBV therapeutics to prevent the complications of chronic infection and HCC.

Previous national and international guidelines suggested that HBeAg positive patients in the immune active (HBeAg positive chronic hepatitis; raised ALT with HBV DNA) or those in the HBeAg negative immune escape phase (HBeAg negative chronic hepatitis; raised ALT and/or HBV DNA) are deemed treatment candidates. More recent guidelines have lowered the threshold for treatment candidacy to HBV DNA levels >2,000 IU/ml [12-14]. Despite evidence that high viral load, regardless of liver inflammation correlates with HCC[15], those patients previously considered immune tolerant (HBeAg positive chronic infection) are excluded from therapy[16]. Recent data have challenged the concept of immune tolerant disease[17,18], providing weight to the argument for earlier treatment in young CHB patients [19], where blocking viral replication and reducing oncogenic progression in earlier stages of CHB may be a more effective strategy. When treatment is withheld until there is elevation in serum ALT or until HBeAg seroconversion occurs, it is likely that patients will already experience significant cumulative hepatocyte turnover and be at increased risk of clonal hepatocyte expansion[20]. The recent European Association for The Study of the Liver international guidelines (EASL 2017) proposed new nomenclature for disease phase in CHB with potential implications for the early treatment of patients[12]. The timing of treatment initiation in patients is discussed in detail elsewhere[16,21].

With novel CHB therapies in the developmental pipeline, the potential to increase the treatment candidacy pool also exists. Although existing therapies are limited in providing a functional cure, defined as HBsAg loss, they may still be employed in combination or sequential therapy strategies, whilst new drugs are in early phase clinical trials. Currently licensed therapies are discussed here along with their impact on the immune response in conjunction with modifications in viral response. In addition, we briefly outline novel pipeline therapies and how existing therapies may retain a role in combination approaches with new therapies in the future.

Treatment paradigm in HBV

Therapy options with curative intent for CHB are unlikely to be available for several years, thus patients with chronic infection remain at risk of developing liver cirrhosis and HCC. Current treatments for HBV include Pegylated Interferon (Peg-IFNα) and Nucleos(t)ide analogues (NAs), but neither are efficient in delivering functional cure[22].

Interferon

Conventional interferons (IFN) (Intron A) were first licensed in 1991 for use in the treatment of CHB. In 2005, a pegylated interferon (Peg-IFNα; Pegasys) version, with the attachment of polyethylene glycol, replaced standard IFN due to improved pharmacokinetic properties, providing continuous drug exposure over the entire dosing interval, thus offering a less demanding injection schedule with comparable efficacy[23]. IFN provides a dual mode of action; antiviral via inhibition of viral replication, and immunomodulatory via enhancement of the host immune response against the virus. Peg-IFNα's direct antiviral activity induces epigenetic modifications in the histones binding cccDNA causing an accelerated decay of replication-competent HBV nucleocapsids[24]. These direct antiviral effects, however, have limited potency in HBV infection. This is confirmed by the slow kinetic of HBV DNA inhibition observed in IFNα treated CHB patients, in comparison with the sharp decline of HCV RNA observed in patients with chronic HCV infection[25]. The ability of IFNα therapy to suppress HBV replication is more likely associated with its immunomodulatory effects[26,27].

Peg-IFNα therapy offers a finite treatment course, is primarily more effective in those of younger age, with moderate viraemia and has the advantage of no antiviral resistance. Its overall success is limited to a small proportion of patients; approximately 10% of those treated achieve functional cure, defined as sustained serum aviraemia and loss of HBsAg. However, approximately 30% of HBeAg-positive patients have a favourable response to Peg-IFNα with sustained HBeAg seroconversion with a proportion of these patients going on to achieve HBsAg loss[28]. Importantly, Peg-IFNα also has a role in HBeAg-negative disease, where a sustained virologic response (HBV DNA <2000 IU/ml) is seen in up to 40% of patients and HBsAg loss reported in approximately 12% at 5 years post-treatment[29]. The use of 'early stopping rules' based primarily on HBsAg decline at week 12 (or 24) of therapy can guide physicians in determining a sub-optimal response; thus avoiding the potential undesirable systemic effects associated with a full treatment course. This strategy would allow an early switch to NA therapy, providing an individualised approach to CHB treatment[28-30].

Nucleos(t)ide analogues

NAs sufficiently suppress the production of new virions, reducing HBV DNA to undetectable levels in the serum and normalising transaminases, but HBsAg loss is rarely achieved[31]. Lamivudine (LAM) was the first nucleoside analogue approved for use in 1998 and although it has now been replaced by agents with higher genetic barriers to resistance, it played a major role in the transition of CHB management allowing dramatic reductions of HBV DNA with the potential to improve disease outcomes[32]. In 2002 Adefovir (ADV), the first nucleotide analogue was licensed, however, although it had adequate viral potency, this was outweighed by problems associated with resistance and renal toxicity[33]. Entecavir (ETV) was introduced in 2005 as a potent inhibitor of HBV polymerase and still has a role in the HBV treatment arena today. Generally, it has a high genetic barrier to resistance, which is decreased in patients with previous LAM resistance. Another nucleoside analogue, Telbivudine (LdT) was introduced in 2006 and although this agent was efficacious in reducing HBV DNA and having a role in the prevention of mother-to-child-transmission, it is no longer recommended as first-line therapy, due to viral resistance and its side-effect profile[34]. In 2008 Tenofovir Disporoxil Fumarate (TDF) was approved for use in CHB. It is structurally similar to ADV, but has excellent durability of response. Thus, older agents such as LAM and ADV have now been

superseded by TDF and ETV (3rd generation NAs) *(Figure 1),* characterised by a high genetic barrier to resistance, these drugs now represent first-line therapy or are employed following sub-optimal response to Peg-IFNα[30].

Recent studies have demonstrated histological improvement (reversal of fibrosis) and reduced development of cirrhosis with long-term NAs[35]. Importantly there may also be a reduction in HCC development, but this needs to be substantiated in large clinical trials[36]. The REVEAL study demonstrated elevated HBV DNA to be strongly associated with cirrhosis and the development of HCC and thus NAs may have an impact on limiting disease progression[37]. NAs directly target HBV DNA synthesis and are ineffective in their ability to eradicate the cccDNA, the episomal form of HBV from infected cells[31]. Treatment with NAs is considered long-term with limited data on treatment discontinuation, which results in reactivation of HBV in the majority[31]. Recent data, however, demonstrates that certain patient cohorts may be able to stop NA therapy, with declines in HBsAg [38,39] and immune markers to identify such patients are emerging[40]. Although the side-effect profile of 3rd generation NAs is favourable, potential drug toxicity with long-term use of TDF may occur, with a negative impact on bone mineral density[41]. For this reason, newer agents namely Tenofovir Alfenamide (TAF), although equally efficacious as 3rd generation TDF, has been shown to have a more favourable side-effect profile[42].

Viral and Immune aspects of therapy

The drug development pipeline in HBV is rapidly advancing and thus we are on the cusp of major change in the treatment of CHB. It is likely that many of these strategies may require combination therapy with NAs and/or Peg-IFNα, and therefore current therapies may constitute a central component of any future treatment regimen[43]. In this regard, the optimisation of currently licensed therapies still remains important. Both NAs and Peg-IFNα have shown some ability to restore immune function in CHB. A number of studies, albeit limited, have investigated the role of current therapies on viral and immune responses, to determine if these can be harnessed to deliver better treatment outcomes.

Interferon

Peg-IFNα can offer sustained immune control in a proportion of CHB patients, lead to HBsAg loss and seroconversion at higher rates than that seen with NAs. IFN is an innate immune cytokine; it induces ISG's encoding antiviral proteins and activates immune cells. A recent study from the woodchuck model showed the induction of a T/NK cell signature in the liver correlating with treatment outcome, highlighting that it may have a more predominant role in immune modulation, rather than an antiviral mechanism[44]. Although the decline in HBV DNA may be slow with Peg-IFNα, circulating virus decreases as does HBsAg in a cohort of patients. These markers, however, may not be ideal surrogates for the viral kinetics of the intrahepatic compartment. More recently, hepatitis B core related antigen (HBcrAg), which can be measured in the blood has been proposed as a more accurate surrogate of the intrahepatic milieu (cccDNA and intrahepatic viral replication) than HBsAg.

HBcrAg was recently demonstrated to reflect cccDNA in HBeAg negative disease and thus may be a better determinant of the viral dynamics in patients treated with Peg-IFNα. In line with this, hepatitis B-core antibody levels have also been shown to correlate with HBV DNA and HBsAg seroclearance[45]. In addition, HBV RNA can also be measured in the serum and levels are thought to reflect intrahepatic cccDNA[46]. Validation of these markers along with their correlation with immune responses in patients treated with Peg-IFNα, or other novel immune modulators, requires further study.

IFN is known to activate the innate immune response. Micco et al., demonstrated with a 48-week course of Peg-IFNα that there was potent expansion of activated (HLA-DR+, Ki67+ and TRAIL+) $CD56^{bright}$ NK cells and recovery of their antiviral potential, (IFNγ production), in HBeAg negative disease,[26] findings subsequently confirmed in a HBeAg positive cohort[27]. Peg-IFNα, following administration, induces a rapid upregulation of the IFN signalling pathway, as marked by increases in serum cytokines, IL-16, IL-6, CXCL10. Therapy with Peg-IFNα alone, however, does not result in a rapid decline of viral load, thus highlighting its predominant immune modulatory action[47]. In a recent study, patients treated with IFN; NKp30+ NK cells were found to be associated with HBV control, with IL-15 contributing to the upregulation of functional antiviral NK cells. Interestingly, in non-responders to IFN, NKp30+ NK cells were found to be dysfunctional with an expansion of the inhibitory receptor NKG2A[48]. In the same cohort of patients, an expansion of $CD3^{bright}$CD56+ T cells (innate-like T cells) expressing high levels of NKG2A and low CD8 were associated with non-response to IFN. These non-responders had increased levels of TIM3+ $CD3^{bright}$CD56+ T cells, which negatively correlated with IFNγ production contributing to the dysfunction of these cells and potentially contributing to poor responses to IFN[49]. Further evaluation of innate-like T cell populations, such as MAIT cells, during IFN therapy is required to determine if their function can be recovered by reduced expression of inhibitory molecules PD-1 and CTLA-4, which contribute to the dysfunctional immune response in CHB[50]. KIR genotyping has also been studied where the combination of genes encoding KIR3DS1 and HLA-B Bw4-80Ile synergistically predicted sustained responders to Peg-IFNα[51]. Toll-like-receptors (TLRs) have been studied in Peg-IFNα treated patients where a favourable response is associated with elevated levels of TLR2 and TLR2 associated IL-6 production at baseline, indicating that an inflammatory phenotype is more likely to be associated with a favourable treatment response[52]. Such markers and genotyping might facilitate patient selection for treatment with Peg-IFNα, which would be relatively simple to undertake.

Although only proven in small studies, IFNα therapy may have a more negative effect on HBV-specific adaptive immunity. Despite the fact that the cytokine can increase T cell survival, boost viral antigen presentation and trigger IL-12 production, which might directly rescue the function of exhausted T cells[53], HBV-specific T cell responses in treated patients are inhibited by Peg-IFNα therapy[26,54]. A recovery of HBV-specific T cell function is only observed after Peg-IFNα therapy cessation in treatment responders[55] and these patients also exhibit increased HBV-specific T-helper cell proliferation [56]. Of note, the expression of inhibitory check-point markers (PD-1, Lag-3 and CTLA-4) does not change on T cells during Peg-IFNα therapy[26,47]. Peg-IFNα leads to an expansion of IL-10 producing T-regs in non-responders, which may contribute to HBV persistence. In addition, γδ T cells

have been shown to decrease during Peg-IFNα treatment, although the effector phenotype (CD27-CD45RA+) and production of cytokines from these cells is enhanced in patients with sustained response, but not in non-responders[57]. The negative effects of IFNα on virus-specific T cell responses have been detected in studies of LCMV infected mice, where T cell responses were restored by inhibiting the effects of virus-induced IFNα rather than by treating the infection with it[58,59]. As NK cells can negatively regulate HBV-specific T cells, the mechanisms of HBV-specific T cell inhibition during IFNα therapy might be mediated by NK cells[60]. However, this is controversial since recent studies in animal models demonstrated that type-I IFN may protect T cells from NK cell mediated attack[61,62] and thus merits further investigation. As Peg-IFNα and NAs act differentially on the immune response, the rationale for re-evaluating combination or sequential treatment is required for future therapeutic approaches, which are discussed further here.

Nucleos(t)ide analogues

The 3rd generation NAs have excellent rates of viral suppression, but have little impact on HBsAg levels. Detailed evaluation of the intrahepatic viral repertoire during NA therapy is limited. Studies have shown that serum HBV DNA correlates with intrahepatic HBV DNA mainly in treatment naïve patients, but this is less clear in patients undergoing NA therapy. For example, after many years of therapy, serum HBV DNA may be undetectable, but the intracellular/intrahepatic viral HBV DNA only appears to reduce by 1-2 logs[63]. Thus, a lack of serum viraemia does not reflect the intrahepatic viral DNA. The decline in HBsAg levels during NA therapy is slow, and any correlation between HBsAg and cccDNA is unlikely to be statistically significant as the regulation of HBsAg expression is complex. It is now recognised that factors other than the quantity of cccDNA in infected hepatocytes, its transcriptional regulation, and the possibility that envelope proteins could be expressed from viral sequences integrated into the host genome also contribute to HBsAg levels[64]. As HBcrAg may correlate with serum HBV DNA and intrahepatic cccDNA[65],as shown in patients treated with NA with a decline in intrahepatic cccDNA, it is possible that HBcrAg may better reflect the intrahepatic compartment than HBsAg[66].

The rapid decline of HBV DNA secondary to NAs, and the inhibition of HBV DNA polymerase function, may allow for the restoration of IFN signalling, however, the data to support this are lacking. The increment of ISGs has only been demonstrated in the peripheral compartment of HBeAg positive patients treated with TDF[47]. The effect of NAs on T cell responses has shown a recovery of CD4 and CD8 T cell function[67-71]. During the initial phases of NA treatment CD4 T cell responses are stronger than those from CD8 T cells, and the presence of new and expanded clonotypes inversely associate with the decline of viral antigen, demonstrating that a broad T cell expansion is critical in HBeAg control[72]. It is important to note that following NA administration, T-cell recovery is often partial and not uniform in all treated subjects[69]. The functional recovery of antiviral immunity is likely to be dependent on the ability of NAs to reduce liver inflammation, marked by a reduction in serum transaminase levels. These events are linked with the reduction of a number of immunological suppressive factors (e.g. IL-10, arginase and T-reg frequencies)[73], impacting T cell recovery. Importantly studies have demonstrated a restoration of the balance of Th17/T-regs with reductions of IL-10 and TGF-β upon viral suppression[74,75].

In addition to a robust HBV-specific T cell response, critical in eliminating HBV infected hepatocytes, NK cells have also shown importance in HBV pathogenesis. A number of studies describe NK cell dysfunction in CHB patients, compared with healthy controls[27,76,77]. NA monotherapy does not appear to restore antiviral NK cell function and further adjunct therapy is likely required for this innate boosting[27,76]. Such findings have also been confirmed in the intrahepatic compartment, where viral suppression resulted in limited changes in NK cell function[78]. However, during early NA treatment, in patients with raised transaminases, LdT demonstrated an expansion of $CD56^{bright}$ NK cells via upregulation of IL-15 and NKG2D, which may be important in viral control[79], however, larger studies are required to evaluate NK cell phenotype and function along with KIR genotyping. NAs have been shown to improve T cell function, although the data regarding non-conventional T cells is sparse. One study reported on the presence of activated MAIT cells in CHB, with functional recovery upon viral suppression[80]. With regards to antigen specific adaptive immune responses, Boni et al., reported on the recovery of HBV-specific T cells, following expansion, in patients on long-term NA therapy, revealing that T cell function may be improved with HBV DNA suppression. This recovery is more pronounced in those who clear HBsAg during NA therapy. Regardless, these data reveal that T cell function may be improved with reduction of viral load[69]. This reduction of viraemia by NAs provides an ideal window for reconstitution of the antigen specific immune response, which may be important in future therapeutic strategies for HBV.

Antigen specific T cell recovery, albeit partial, is possible with NAs, but the impact on antiviral NK function is still inferior[76]. In HBV, the role of NK cells has generated some controversy; described as a 'double edged sword', whether they provide a pathogenic or protective role continues to be debated[60]. A robust antiviral NK cell response is important for cytolysis of HBV infected hepatocytes[81], however, NK cells also have a regulatory role, causing deletion of HBV-specific T cells when in close contact[82]. The interaction of TRAIL+ and NKG2D+ NK cells with T cells expressing the receptor, TRAIL-R2 and/or NKG2D ligands leads to T cell apoptosis, which, in vitro, can be partially prevented by blockade of these pathways[82,83]. The data on whether the phenotype of NK cells is altered, with viral load reduction, are limited. A recent report demonstrated an inverse correlation with an 'activatory' NK cell phenotype (HLA-DR+, CD38+, Ki67+, TRAIL+, NKG2D+) and the proportion of HBV-specific T cells in patients undergoing NA therapy[84]. The regulatory role of NK cells and the interaction with T cells, may of course be a protective homeostatic mechanism for the liver microenvironment, where NK cells govern T cell mediated immune pathology. However, in an attempt to curb liver damage by down-regulating bystander T cells, HBV-specific T cells are also dampened. These concepts, however, require further elucidation, ideally with focused 'on-treatment' studies of the liver compartment and to determine the impact of therapy on tissue-resident immunity [85-88].

Combination/sequential/add-on therapies

With their differential action on the immune response; NAs and Peg-IFNα used in combination, 'add-on' or sequentially may generate additive or synergistic effects and could be important in future therapeutic strategies. Recent clinical studies have shown the combination or addition of Peg-IFNα to NAs results in greater HBeAg seroconversion rates in HBeAg positive patients along with greater

declines and loss of HBsAg[89-91]. Thus their use in the clinical setting requires further consideration, while the outcomes of such studies performed to date, have been extensively reviewed elsewhere[92].

Addition of Peg-IFNα, in a cohort of patients virally suppressed on NAs, induced the activation of DC's, expansion of CD56^{bright} NK cells and increased the frequency of Th1/Th17-orientated HBV-specific T cells[93]. Notably, these effects were not associated with improved clinical outcomes. Viral load reduction is able to maintain the immune stimulatory effects of Peg-IFNα, when administered in combination or sequence, compared to Peg-IFNα alone, which implies there may be beneficial outcomes with add-on or combination therapies[47]. This has been further demonstrated where Peg-IFNα add-on was employed in patients virally suppressed with ETV, resulting in a reduction in T-reg frequencies with an increase in NKG2C+ NK cells and TLR-2+ CD14 monocytes, which was associated with treatment response[94]. In the same cohort the expansion of CD56^{bright} NK cells expressing activatory receptors NKp30 and NKp46 along with TRAIL and IFNγ correlated with HBsAg decline with potential cccDNA clearance through TRAIL induced cytolysis, demonstrating the importance of Peg-IFNα for immune modulation and HBV clearance[95]. Similarly, in patients primed with Peg-IFNα prior to viral suppression the maintenance of expanded functional CD56^{bright} NK cells has been shown to correlate with treatment response[27]. It is noteworthy that the recovery of non-conventional T cells (iNKT and γδ T) was limited despite significant declines in HBsAg in a cohort of patients undergoing combination therapy[96].

In a study of combination Peg-IFNα with ADV, those patients achieving HBsAg loss demonstrated increased frequency of TRAIL+, IFNγ+ NK cells at the end of treatment. This indicates that NK cells may play a role in the clearance of HBsAg with this therapeutic approach[97]. In the same patients with HBsAg loss, T cells, with broad antiviral capacity could be expanded[98]. Interestingly, in this study, baseline levels of HBsAg/anti-HBs immune complexes were higher in patients that went on to lose HBsAg, which may also be a factor in selecting patients for such combination therapy approaches[99]. Peg-IFNα add-on therapy has also been shown to increase TNFα-monofunctional HBV-S and core-specific CD4 T cell numbers, which may contribute to viral control[100]. Along with the direct analysis of immune cells microRNAs (miRNA) have been reported to be regulated in liver disease. miRNA-155 is a key regulator of innate and adaptive immunity and the higher expression of miRNA-155 at baseline was associated with improved treatment response and NK cell function[101]. Further studies of miRNA's and their role in HBV and the immune response is warranted and important in future therapeutic strategies. The type-III interferon Peg-IFNλ, in combination with NA therapy has also been shown to induce robust innate and adaptive immune responses, where NK cell polyfunctionality along with recovery of HBV-specific T cells was observed in patients with enhanced HBV DNA and HBsAg decline[102]. This highlights the importance of immune modulation with viral suppression as key elements for HBV therapy.

The schedule of therapies remains crucial and this may need revisiting to optimise patients for future trials, especially if the treatment pool is widened. The concept of combination or IFN based therapies still needs further investigation in larger studies with parallel analysis of both the innate

and adaptive immune response. Adjusting the sequence in which therapies are combined is potentially important in altering treatment outcome, but may also prime patients for future clinical trials, which is a key avenue of exploration.

Novel pipeline therapies for HBV

Multiple therapeutic approaches for HBV, targeting steps of HBV replication and restoring the host immune response are in development. Even with the advent of new therapies, currently licensed therapies are likely to remain a backbone of HBV management in the short to medium term, especially the employment of NAs for viral suppression. Novel drug targets are entering clinical trials to determine efficacy and are discussed at length elsewhere[103-106]. Here, we outline a selection of novel agents and their potential role in combination with currently licensed therapies.

Viral Targets

The identification of the cellular receptor for HBV entry, NTCP, along with an improved understanding of cccDNA formation, degradation and its epigenetic control and targets for viral entry have provided significant impetus to the field. The entry inhibitor Myrcludex B, has shown promise in pre-clinical trials of HBV and hepatitis delta virus (HDV) and is being tested in clinical trials with and without Peg-IFNα[107]. Targets against cccDNA include antiviral cytokines (IFNα, IFNγ, TNFα, lymphotoxin-β receptor agonists)[108] and technologies such as CRISP-R/Cas9 are being utilised to eliminate cccDNA along with the use of histone deacetylase (HDAC) inhibitors[109,110]. It will be important to establish if these agents are best deployed in combination with NA's and/or immune modulating *agents (Figure 2; potential effects of therapies).* Secretion Inhibitors*,* such as nucleic acid polymers (NAPs) have shown promise in inhibiting HBsAg release. Clinical trials of molecules REP-2044 and REP-2139 used as monotherapy or in combination with Peg-IFNα induced rapid declines of HBsAg. In addition, TDF with Peg-IFNα in combination with REP-2139 and REP-2165 have also generated promising results[111]. The core/HBc/Cp proteins have emerged as promising DAA targets [Core Allosteric Modulators (CpAM)]. These agents allow for inhibition of nucleocapsid assembly leading to the inability of pgRNA enscapsidation or capsid formation with arrest of the neo-synthesis of viral rcDNA.[112] Whether these will show increased potency in combination with current NAs and/or Peg-IFNα remains to be seen. Silencing RNA using RNA interference (RNAi) to prevent HBV replication is also being investigated. Preliminary results of a phase II trial showed that a single dose of ARC-520 in combination with ETV resulted in rapid decreases in HBV DNA in HBeAg positive and negative patients, but only showed a decline in HBsAg in HBeAg positive patients[113]. It will also be interesting to see if viral targets also induce beneficial effects on the host immune response as has been shown with DAAs in HCV [114]

Immune Targets

Immune therapies include molecules directing innate responses within HBV infected hepatocytes triggering antiviral mechanisms (cytokine production, direct killing) of liver non-parenchymal cells. Check-point modulators, therapeutic vaccines and targeted T cell therapies are also being investigated.

Agents for immune stimulation include pathogen recognition receptor (PRR) agonists, the TLR-7 agonist GS-9620, which was shown to induce strong anti-HBV activity[115], but a trial in CHB patients did not show any effect on viral replication/HBsAg levels. However, GS-9620 used as add-on therapy in patients virally suppressed with NAs demonstrated increased levels of T-cell effector cytokines compared to NAs alone. NK cell activation and function increased after the addition of GS-9620, which signalled via the IFN-type I pathway, while the ability of NK cells to delete T cells was diminished, indicating the importance of type-I IFN signalling[116]. The use of TLR-8 and TLR-9 agonists may provide more promise, potentially in combination with NAs. Other potential targets for immune stimulation include TLR-1/2, RIG-I and stimulator of interferon genes (STING)[117]. These agents induce direct HBV inhibition in infected hepatocytes and SB9200, an oral molecule activating RIG-I is able to decrease HBV DNA and HBsAg levels in the WHV, with early clinical trials showing promise in humans[118]. Innate immune therapies have been designed which activate intrahepatic NK/NK-T cell responses, with antibody-blocking inhibitory NK cell receptors[119] or via NK cell triggering cytokines such as IL-12, IL-18[120] or with classical IFNα, utilised alone or conjugated with antibodies for selective delivery[121]. Modulation of innate-adaptive interactions could also hold therapeutic promise, for example targeting the regulatory role of NK cells and MDSC's to improve HBV-specific T cell immunity[82,86]. Cytokines such as TNF-α, IL-2 and IL-12 have been shown to inhibit HBV replication in vitro and thus could potentially be used with NAs[122] However, these molecules have not yet been successfully used in clinical trials[103].

HBV-specific T cells are exhausted, overexpressing inhibitory check-point molecules such as PD-1 and CTLA-4[123-125]. Blockade of these molecules has shown potential in vitro, with promising data emerging in HBV-related HCC with the anti-PD1 agent, Nivolumab[126]. Anti-PD1/PDL-1 blockade can partially restore exhausted HBV-specific T cells in CHB patients[125,127] and it will be important to determine if these agents are more efficacious in combination with NAs. Therapeutic vaccines, such as GS-4774 and TG-1050 are being investigated in clinical trials[128,129]. These have been designed to boost quantity and function of antiviral T cells through HBV-specific stimulation[130]. Initial trials of vaccines showed suboptimal results[131,132], but new formulations or combination therapies with NAs have demonstrated some effect in selected trials[133]. Increasing the number of HBV-specific T cells by autologous infusion of T cells expressing chimeric antigen receptors (CARs) or by engineering T cells to over express HLA-restricted HBV-specific TCRs have been used in human studies and show some promise[134,135]. Design and expansion of engineered HBV-specific T cells for adoptive transfer is feasible[136,137] and data in animal models[138] or selected clinical situations[135] have been encouraging, but further investigation is required and how best to employ these options in patients remains to be seen.

Summary

In order to achieve functional cure in HBV, suppression of HBV replication and a robust host immune response are paramount. Many strategies are currently being exploited both as viral and immune targets. How best to employ these therapies, whether they should be used in isolation or in combination with currently licensed therapies remains to be seen. NAs and Peg-IFNα used either in combination, sequentially or as 'add-on' therapies have shown encouraging results in terms of clinical outcome along with changes in viral immune responses. However, these studies are limited in number, thus comprehensive analyses of the innate and adaptive immune responses along with viral parameters, performed in parallel with large clinical trials are mandated to better understand clinical outcomes of combination strategies. These results will be critical in determining how best to employ novel therapeutics and whether these novel agents should be used in combination with currently licensed therapies to maximise treatment response in a broad range of patients.

Figure Legends

Figure 1: Timeline indicating the licensing and development of therapies for CHB as indicated by national and international guidelines.

Figure 2: Diagram depicting the viral and immune responses with NA and Peg-IFN based therapies, and potential viral and immune outcome with novel therapies indicated (used in isolation or in combinations) with a view to achieving functional cure in HBV.

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Figure 1

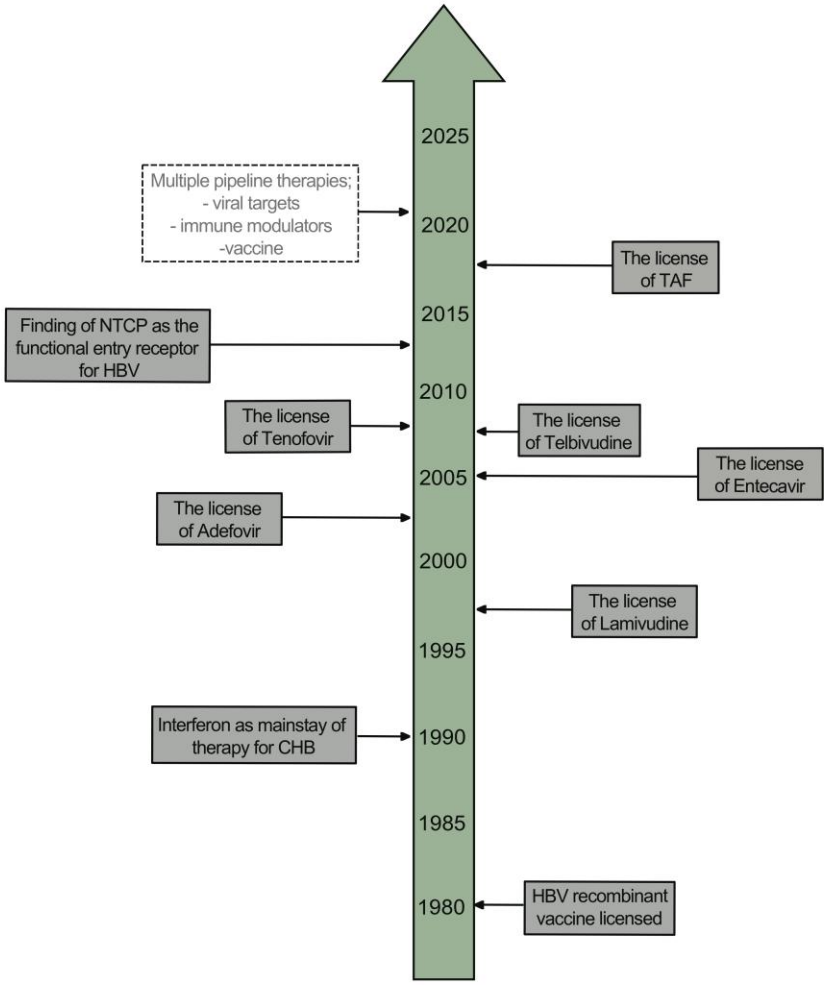

Figure 2