New Viral and Immunological Targets for Hepatitis B Treatment and Cure: A Review

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ABSTRACT

Although current therapies can be successful at suppressing hepatitis B viral load, long-term viral cure is not within reach. Subsequent strategies combining pegylated interferon alfa with nucleoside/nucleotide analogues have not resulted in any major paradigm shift. An improved understanding of the hepatitis B virus (HBV) lifecycle and virus-induced immune dysregulation has, however, revealed many potential therapeutic targets, and there are hopes that treatment of hepatitis B could soon be revolutionized. This review summarizes the current developments in HBV therapeutics—both virus directed and host directed.

Keywords: Antiviral therapy; Hepatitis B virus; Immunomodulators; Nonnucleoside antivirals; Nucleoside analogues

INTRODUCTION

Chronic infection with hepatitis B virus (HBV) remains a major healthcare problem, with an estimated 240 million persons infected worldwide [1]. In patients with untreated HBV infection the 5-year incidence of evolution to cirrhosis is 8–20%, and among those with cirrhosis the annual risk of developing hepatocellular carcinoma is 2–5% [2–4]. Although the implementation of hepatitis B vaccines have been effective in reducing the incidence of HBV in vaccine recipients, significant declines in end-stage liver disease rates have not yet been seen.

Sustained virological response [i.e., hepatitis B surface antigen (HBsAg) seroconversion] is only seldom observed with currently approved HBV antiviral drugs [pegylated interferon (PEG-IFN) and nucleoside/nucleotide analogues (NUCs)] because of the persistence of covalently circular closed DNA (cccDNA) in the nucleus of hepatocytes [5, 6]. Moreover, the widely used NUCs most likely have to be taken lifelong to prevent rebound [7], and while treatment with PEG-IFN does have a finite duration, the responses are suboptimal at best [7]. Novel therapeutic strategies are therefore needed.
With the recent revolutionary advances in the treatment of viral diseases such as hepatitis C virus (HCV) infection (with direct-acting antivirals) and the development of multiple classes of anti-human immunodeficiency virus (HIV) agents, the way may now be paved for similar advances in HBV therapeutics—with direct-acting antivirals and host-directed therapeutic strategies [8–10]. Improved understanding of the HBV life cycle has illustrated multiple potential antiviral targets, and there are many agents in preclinical and early clinical investigation, and other promising strategies for HBV clearance include immune modulators, whose potential is strongly supported by the fact that natural immune responses are capable of effectively preventing chronic HBV infection in 90% of infected adults.

This review aims to give a comprehensive overview of the major new drug developments in the treatment of HBV infection, specifically focusing on novel direct-acting antivirals and host-directed anti-HBV therapeutics. This article is based on previously conducted studies and does not involve any new studies of human or animal subjects performed by any of the authors.

CURRENT TREATMENT OPTIONS FOR HBV INFECTION

The presently available antiviral treatments for HBV infection include two classes of therapeutic agents: PEF-IFN-α and NUCs. NUCs, which target HBV through inhibition of viral polymerase, are the most commonly used and have an excellent safety profile and tolerability. Most patients, however, require indefinite NUC therapy because of frequent relapse or reactivation of HBV infection after cessation of treatment, which is a hindrance for patient treatment adherence [9]. In contrast, PEG-IFN-α has predominant immunoregulatory effects along with limited direct antiviral properties [11, 12], and has a finite duration of therapy and a slightly higher occurrence of attaining anti-hepatitis B e antigen (HBeAg) and anti-HBsAg seroconversion than with NUCs [13, 14]. However, interferon (IFN) sensitivity differs among the different HBV genotypes, and the main drawback of PEG-IFN-α is poor tolerability with frequent severe side effects, which considerably limits its use. Neither NUCs nor PEG-IFN-α is therefore optimal or results in long-term control or cure except in a minority of HBeAg-negative noncirrhotic individuals who experience durable HBsAg seroconversion, in which case therapy can be stopped under close monitoring.

There has therefore been interest in whether increased efficacy could be obtained by combination of these classes. Conceptually, combining PEG-IFN-α and NUC therapy could result in improved HBV control due to potential synergistic effects of their different mechanisms of actions, and there has been interest in simultaneous use (i.e., commencing both a NUC and PEG-IFN-α together) or sequentially or add-on administration [15, 16]. Earlier studies into simultaneous use of lamivudine with PEG-IFN-α showed more pronounced on-treatment virological response although without clear long-term benefit [14, 17]. Some newer studies investigating PEG-IFN-α combined with tenofovir disoproxil fumarate (TDF) describe a significantly larger proportion of participants attaining HBsAg loss (9.1% vs 0% and 2.8%, respectively) and conversion to anti-HBsAg among patients receiving TDF plus PEG-IFN-α than in those receiving either TDF or PEG-IFN-α alone [18]. Two recent studies analyzing PEG-IFN-α therapy as an add-on to long-term entecavir (ETV) therapy demonstrated higher HBsAg seroconversion rates in the combination arm than in the ETV monotherapy arm [15, 16]. Nonetheless, a third trial examining PEG-IFN-α add-on therapy in patients receiving ETV did not establish superiority in comparison with the PEG-IFN group or patients allocated to ETV add-on treatment when receiving PEG-IFN-α [19]. A summary of recent published articles on this subject is depicted in Table 1. Overall, recent evidence suggests that in patients receiving long-term NUC therapy, both an add-on approach and sequential administration of PEG-IFN-α may have some minor advantages [15, 16, 20]. However, such combination therapy appears unlikely to lead to a paradigm shift or significant improvement for most HBV-infected individuals.
Table 1 Summary of recent main randomized controlled trials demonstrating hepatitis B virus (HBV) virological responses in pegylated interferon (PEG-IFN) and nucleoside/nucleotide analogue treatment strategies

| Study            | Sample size (n) | Patient status | HBV genotype | Treatment regimen                                                                 | Follow-up (weeks) | Virological response |
|------------------|----------------|----------------|--------------|----------------------------------------------------------------------------------|------------------|----------------------|
|                  |                |                |              |                                                                                  |                  | HBsAg conversion (%) | HBsAg suppression (%) |
| Add-on strategy  |                |                |              |                                                                                  |                  |                      |                       |
| Brouwer et al.   | 175            | HBeAg positive | A 7%         | 1. ETV for 48 weeks + PEG-IFN-α in weeks 24–48                                  | 96               | 26<sup>a</sup>        | 77 (<20<sup>2</sup> IU/ml) |
| [15] (ARES study)|                |                | B 20%        | 2. ETV for 48 weeks                                                              |                  | 13                   | 0                     |
|                  |                |                | C 42%        | 3. ETV for 24 weeks + PEG-IFN-α in weeks 13–36                                  |                  | 26<sup>a</sup>        | 31.5                  |
|                  |                |                | D 31%        | 4. PEG-IFN-α for 48 weeks                                                         |                  | 1.4                  | 40.3 (<10<sup>3</sup> copies/ml) |
|                  |                |                |              |                                                                                  |                  |                      | 37.0                  |
| Xie et al. [19]  | 218            | HBeAg positive | –            | 1. PEG-IFN-α for 48 weeks                                                        | 72–92            | 31                   | 4.1                   |
|                  |                |                |              | 2. PEG-IFN-α for 48 weeks + ETV in weeks 13–36                                  |                  | 26<sup>a</sup>        | 31.5                  |
|                  |                |                |              | 3. ETV for 24 weeks + PEG-IFN-α in weeks 21–68                                   |                  | 1.4                  | 37.0                  |
| Sequential strategy |           |                |              |                                                                                  |                  | 0                    | 72.0 (<10<sup>3</sup> copies/ml) |
| Ning et al. [16] | 200            | HBeAg positive | –            | 1. PEG-IFN-α for 48 weeks                                                         | 48               | 14.8<sup>a</sup>      | 72.0 (<10<sup>3</sup> copies/ml) |
|                  |                |                |              | 2. ETV for 48 weeks                                                              |                  | 6.1                  | 0                     |
|                  |                |                | ETV for 9–36 months |                                                  |                  |                      | 97.8<sup>a</sup>      |
|                  |                |                | HBV DNA < 10<sup>4</sup> copies/ml |                                                  |                  |                      |                       |
| Piccolo et al. [81] | 30              | HBeAg negative | D 87%        | 1. PEG-IFN-α for 24 weeks + LbT in weeks 25–48                                  | 72               | –                    | 0                     |
|                  |                |                |              | 2. LbT for 24 weeks + PEG-IFN-α in weeks 25–48                                   |                  | 13.3 (<20<sup>3</sup> IU/ml) | 46.7<sup>a</sup>    |
| Study                        | Sample size (n) | Patient status | HBV genotype | Treatment regimen | Follow-up (weeks) | Virological response |
|------------------------------|-----------------|----------------|---------------|-------------------|-------------------|----------------------|
|                              |                 |                |               |                   |                   |                      |
|                              |                 |                |               |                   |                   | hbv DNA suppression  |
|                              |                 |                |               |                   |                   | (%)                  |
|                              |                 |                |               |                   |                   | HBsAg conversion     |
|                              |                 |                |               |                   |                   | (%)                  |
|                              |                 |                |               |                   |                   | HBcAg conversion (%) |
|                             | 61              | HBeAg positive | –             | 1 PEG-IFN-α + ADF  | 52                | 36.7                 |
|                              |                 |                |               | for 52 weeks       |                   | 3.3                  |
|                              |                 |                |               | 2 PEG-IFN-α for 52 |                   | 76.7 (undetectable)  |
|                              |                 |                |               | weeks              |                   |                      |
|                              | 125             | HBeAg negative | B 19.8%       | 1. PEG-IFN-α + ETV | 96                | –                    |
| Tangkijvanich et al. [83]    |                 |                | C 78.6%       | for 48 weeks       |                   | 3.2                  |
|                              |                 |                | Other 1.6%    | 2. PEG-IFN-α for 48|                   | 7.9                  |
|                              |                 |                |               | weeks              |                   | 38.1 (< 20^3 IU/ml)  |
|                              | 159             | HBeAg positive | A 17%         | 1. PEG-IFN-α + LbT | 24                | 8                    |
| Marcellin et al. [84]        |                 |                | B 24%         | for 24 weeks       |                   | 0                    |
|                              |                 |                | C 19%         | 2. LbT for 24 weeks|                   | 0                    |
|                              |                 |                | D 28%         | 3. PEG-IFN-α for 24|                   | 12                   |
|                              |                 |                | Other 4%      | weeks              |                   | 0                    |
|                              |                 |                | Unknown 9%    |                    |                   | 35                   |

**Concomitant strategy**
| Study          | Sample size (n) | Patient status                        | HBV genotype | Treatment regimen          | Follow-up (weeks) | Virological response |
|---------------|-----------------|---------------------------------------|--------------|----------------------------|-------------------|----------------------|
| Marcellin et al. [18] | 740             | HBeAg positive or negative Treatment naïve HBV DNA ≥ 20^d^ copies/ml (HBeAg positive) or ≥ 20^d^ copies/ml (HBeAg negative) | A 8%         | 1. PEG-IFN-α + TDF for weeks | 72                | 25.0^b^ 8.1          |
|               |                 |                                       | B 27%        |                            |                   | 23.8 0.6            |
|               |                 |                                       | C 42%        | 2. PEG-IFN-α for 16 weeks + TDF for 48 weeks |                   | 12.8 0          |
|               |                 |                                       | D 21%        |                            |                   | 24.5 2.9          |
|               |                 |                                       | E–H 1%       | 3. TDF for 120 weeks       |                   | 71.9^d^           |
|               |                 |                                       |              | 4. PEG-IFN-α for 48 weeks  |                   | 9.2                |

PEG-IFN-α was administered at 180 mg/week or 1.5 μg/kg/week.

*ADF* adefovir 10 mg/day, *ETV* entecavir 0.5 mg/day, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *LdT* telbivudine 600 mg/day, *TDF* tenofovir disoproxil fumarate 300 mg/day

^a* P < 0.05.

^b* P < 0.05 versus arm 3.

^c* P < 0.05 versus arms 2 and 3.

^d* P < 0.05 versus arms 1 and 2.
FOR WHAT SHOULD WE BE AIMING?

The presently available agents can suppress plasma viremia and may occasionally change the plasma antigen profiles (HBeAg or HBsAg loss/seroconversion). They, however, have no (in the case of NUCs) or little (in the case of PEG-IFN) impact on hepatocyte HBV DNA levels, let alone in any way influence cccDNA levels intrahepatically. Therefore, these agents can control active disease, but do not lead to a radical virological cure [where all viral nucleic acid (i.e., cccDNA) is removed from the patient] or long-term control when the patient is not receiving treatment in most individuals. Even those few individuals who currently naturally or pharmacologically clear circulating HBV infection remain prone to reactivation/relapse of HBV infection if they become significantly immunosuppressed.

Therefore, paradigm shifts are required to lead to either long-term off-treatment suppression in most individuals or to complete radical virological cure. Can the newer treatments being investigated potentially lead to these end points as monotherapies or in combination?

NOVEL DIRECT-ACTING ANTIVIRALS FOR HBV

Very significant progress has been made in elucidating the life cycle of HBV and thereby identifying potential targets and agents for therapy. We will examine the major steps in the life cycle, illustrating the main potential drug classes and some of the leading contenders for direct-acting antivirals (Fig. 1).

Binding and Attachment of HBV to the Hepatocyte

HBV attaches initially to heparan sulfate proteoglycans on the hepatocyte cell surface, and then with high affinity binds its specific receptor—sodium–taurocholate cotransporting polypeptide (NTCP) [21]. This latter step can be targeted with a specific synthetic lipopeptide, myrcludex B, which has been shown to significantly inhibit viral NTCP binding and thereby reduce HBV viremia and serum surface antigen levels in humans [22, 23]. To date little toxicity has been demonstrated, although as the NTCP receptor's natural activity is bile acid transportation, it will be of interest to determine if there will be some pharmacokinetic interactions with drugs metabolized via this route. Although seemingly potent at inhibiting the infection of uninfected hepatocytes, as a monotherapy it would not be expected to significantly influence hepatocyte HBV DNA levels (as these are replenished via pathways within the cell not requiring entry via NTCP binding; see later). It may well have an interesting role in combination, however, with other novel HBV agents and also in protecting the graft from infection in the setting of liver transplant in HBV infection.

Entry into the Cytoplasm, Nucleocapsid Release, and Entry of DNA into the Nucleus and Conversion to cccDNA

After fusion with the NTCP receptor, the virus enters the cytoplasm of the hepatocyte, is uncoated, and the HBV nucleic acid, in the form of relaxed circular DNA (rcDNA), leaves the nucleocapsid to subsequently enter the nucleus. Within the nucleus the rcDNA is converted into cccDNA. This is a vital step for the chronic nature of HBV infection as this cccDNA is highly stable and acts as a long-lived template for subsequent transcription of HBV RNA and proteins. It is the cccDNA (which effectively persists as an HBV minichromosome not integrated into the host DNA) that allows reactivation of apparently cleared HBV on significant immunosuppression, and is the major obstacle to radical cure of HBV infection.

Multiple agents are in preclinical development to target this cccDNA moiety: from zinc finger nucleases to disubstituted sulfonamide compounds, CRISPR–Cas9 technologies, and lymphotoxin beta receptor agonists [24–29]. However, to date, it is RNA interference methods that have entered human studies [30].
Mixtures of small interfering RNA molecules have shown activity in humans in decreasing serum surface antigen levels for prolonged periods after single dosing [31]. They are being developed to be pangenotypic, and some compounds are being targeted specifically at hepatocytes by combination with ligands such as N-acetylgalactosamine, which promotes uptake via asialoglycoprotein receptor [32, 33]. However, issues remain with the development of stable delivery systems and, to date, studies have been small.

Transcription by cccDNA, Production of Viral Proteins, and Capsid Assembly

The cccDNA serves as a template for the transcription of viral RNA of two types. The first is the pregenomic RNA (pgRNA) that will produce the core and capsid proteins and also the nucleic acid template that ultimately enters the new nucleocapsid and is converted into DNA, creating a new infectious virion. The second is messenger RNA that is translated into the viral proteins—predominately the surface proteins (HBsAg and HBeAg) and X protein.

The capsid is created by multiple copies of the core protein combining. This step is one of the main targets of new HBV therapies, and

Table 2 Overview of ongoing clinical trials for new hepatitis B virus therapeutics

| Compound | Phase |
|----------|-------|
| Entry inhibitors | |
| Myrcludex B | Phase I |
| RNA interference | |
| ALN-HBV | Phase I–II |
| ARC-520 | Phase II |
| ARB-1467 | Phase II |
| Lunar-HBV | Preclinical |
| BB-HB-331 | Preclinical |
| Ionis HBVRx (GSK3228836) | Phase I |
| IONIS-HBVLRx (GSK33389404) | Phase I |
| Capsid assembly modulators/core inhibitors | |
| GLS-4 (morphothiadine mesilate) | Phase II |
| NVR 3-778 | Phase Ia |
| BAY41-4109 | Phase I |
| JNJ56136379 | Phase I |
| Core protein allosteric modifier | Phase I |
| Nucleoside/nucleotide analogues | |
| AGX-1009 (prodrug of tenofovir) | Phase III |
| LB80380 (besifovir) | Phase III |
| CMX-157 (prodrug of tenofovir) | Phase Ia |
| Surface antigen/release inhibitors | |
| REP 2139 and REP 2165 | Phase II |
| RO7020322 (RG7834) | Phase I |
| GC 1102 | Phase II |
| Therapeutic vaccines | |
| GS-4774 (recombinant antigen containing X, Env, core epitopes) | Phase II |
| ABX-203 (recombinant antigen containing HBsAg and HBeAg) | Phase II |
| TG-1050 (nonreplicative adenovirus encoding a large fusion protein (truncated core, modified Pol, and 2 Env domains) | Phase I |

Table 2 continued

| Compound | Phase |
|----------|-------|
| INO-1800 (DNA plasmids encoding HBsAg and HBcAg) | Phase I |
| FP-02.2 (Hep’Tcell) (peptide encoding CD4+ and CD8+ epitopes) | Phase I |
| Innate immune defense pathway | |
| GS 9620 | Phase II |
| RO6864018 (RG7795, ANA773) | Phase II |
| SB9200 | Phase II |

This table provides a current overview of compounds in clinical development to the best of our knowledge. HBcAg hepatitis B core antigen, HBsAg hepatitis B surface antigen.
current capsid assembly modulators/core inhibitors generally form one of two main classes. The first class contains compounds that promote capsid assembly but inhibit the entry of pgRNA into the immature nucleocapsid. This results in nucleocapsids that appear to be normal in geometry and size but are empty of nucleic acid and therefore noninfectious. Such compounds include phenylpropenamides and sulfamoylbenzamide derivatives [34, 35]. The other class contains compounds that directly inhibit the correct formation of the nucleocapsid itself, resulting in virus particles that are deformed with abnormal structure and size and appear noninfectious. Examples include the heteroaryldihydropyrimidines (e.g., BAY41-4109) [36] and NVR 3-778 [37, 38].

The activities of a further viral protein, the X protein, remain poorly defined. It appears to have a role in inhibiting the SMC5–6 complex and thereby promoting productive HBV gene expression [39]. Some preclinical work is ongoing at targeting this protein.

**Nucleocapsid Coating and Conversion of pgRNA to rcDNA**

HBV surface proteins (which have been processed within the host Golgi apparatus) now coat the nucleocapsid, and within this structure the pgRNA is converted by HBV polymerase to rcDNA. This latter step is the point of action of the currently available NUCs. There are new NUCs in development (e.g., besifovir, CMX 157, AGX-1009, and MIV-210) but it is currently unclear what advantages they will have compared with the existing agents [31, 40, 41].

**Surface Protein Secretion from the Hepatocyte**

It has long been recognized that large quantities of viral proteins, especially HBsAg, are secreted from the hepatocyte unassociated with virions. It is thought that this extra protein acts to absorb potentially neutralizing antibodies in the host plasma and also to induce a state of
immune exhaustion and tolerance to the virus. HBsAg release inhibitors have been developed (e.g., REP 2139 and REP 2165) that appear potent in preventing the release of HBsAg in humans and thereby decreasing serum HBsAg levels and also potentially promoting surface antibody seroconversion [42, 43]. Whether these compounds may cause detrimental intrahepatocyte accumulation of HBsAg is still to be determined.

Release of Virions and Intrahepatocyte cccDNA Replenishment

Once coated in surface protein, the virions are released from the hepatocyte to infect new cells. But not all nucleocapsids are released, with some diverted to replenish the nuclear cccDNA compartment. Some agents that affect the formation of nucleocapsids, such as the capsid assembly inhibitor JNJ-379, have been shown to ultimately decrease cccDNA levels. Berke et al. [35] suggested that this is presumably by preventing the cccDNA replenishment cycle through blocking of pgRNA synthesis. Therefore, multiple potential therapeutic targets have become apparent as a result of improved understanding of the cellular life cycle of HBV (Table 2). It is quite probable, however, that none of these agents as monotherapies will result in radical cure of HBV infection, and combination direct-acting antivirals may be required. Even with such combinations it may well be that host-directed therapies will also be required to reverse immune exhaustion and tolerance, and therefore promote viral clearance and cure.

NOVEL IMMUNOLOGICAL TARGETS FOR HBV THERAPY

Cellular Immune Response

The human cellular immune response plays a key role in attaining immune control and viral clearance after HBV infection [44, 45]. Cytotoxic T lymphocytes (CTLs) have been identified to be the major contributing factor for HBV clearance [46]. Two signal types are mandatory to activate T cells: interaction between T-cell receptors and antigens presented by major histocompatibility complex molecules on antigen-presenting cells (APCs) on the one hand, and interaction between co-stimulatory or co-inhibitory receptors on T cells with their ligands on APCs on the other [47, 48]. The latter molecules can amplify or inhibit active immune responses and are called “immune checkpoints” [49]. Immune checkpoints are physiologically necessary for maintaining self-tolerance and minimizing collateral host tissue destruction [50].

As a consequence of persistently high antigen levels in chronic viral infections, CTLs and CD4+ cells have been observed to become functionally exhausted [51–53]. This was first discovered in mice infected with lymphocytic choriomeningitis virus infected that exhibited CTLs with reduced capability to kill infected cells and reduced cytokine secretion despite persisting indefinitely [54, 55]. To date, T-cell exhaustion, classified by the overexpression of inhibitory receptors such as programmed death 1 (PD1), CTL-associated antigen 4, and lymphocyte activation gene 3 protein, is a hallmark of chronic viral infection and has been observed in infections with HIV, HCV, human T-cell lymphotropic virus, and HBV [56, 57].

In HBV, enhanced PD1 expression by CTLs and CD4+ cells has been demonstrated in mouse models of persistent HBV infection [54] and on human HBV-specific exhausted CTLs in chronic HBV infection [58]. Therefore, targeting these inhibitory receptors and thereby reversing CTL responses (i.e., rescuing exhausted T cells) is one of the therapeutic strategies currently being explored (Table 3). For example, in chronic infection with woodchuck hepatitis virus (WHV; a virus closely related to HBV), infected woodchucks were treated with a combination of ETV, therapeutic DNA vaccination, and PD1 ligand 1 (PD-L1) antibodies, which led to sustained immune control of the infection and even viral clearance in some woodchucks [57]. In addition, an ex vivo study using intrahepatic and peripheral T cells from patients with chronic HBV or HCV infection observed increased...
cytokine secretion of HBV-specific T cells in the presence of APCs treated with anti-PD-L1 in combination with stimulation of the co-stimulatory receptor CD137 [59].

However, some important points should be taken into account. First, sufficient presence of HBV-specific exhausted T cells may be crucial to the effectiveness of these drugs [38]. As such it may prove to be difficult to determine which patients should be treated with immune checkpoint inhibitors. Second, longer infection duration with excessive antigen exposure may lead to the irreversible exhaustion of T cells [52, 60]. Combined treatment with other antiviral drugs may thus be necessary to bring down antigen levels before initiation of immunotherapy [39]. Lastly, because of the mechanisms of action of immune checkpoint inhibitors, important immune-related adverse events have been observed in trials with CTL-associated antigen 4, PD1, and PD-L1 antibodies, affecting several organ systems, including the liver [51, 61–63].

### Toll-like Receptor 7 and Toll-like Receptor 8 Agonists

Another HBV strategy, targeting the innate immune system, is to activate Toll-like receptors (TLRs) as activation of virus-specific TLRs leads to production of type I IFNs (mainly IFN-α and IFN-β) [54]. IFN-β in particular is capable of inhibiting HBV replication through destabilization of pgRNA capsids and interfering with their assembly. TLR7—present mainly in the endolysosomal compartment of plasmacytoid dendritic cells and B cells—can induce intrahepatic type I IFN responses without causing systemic harmful symptoms. As an activator of the innate immune response in the liver, it became a major focus in TLR agonist trials focusing on viral clearance of HBV [64].

| Table 3 | Summary of immune checkpoint inhibitors for targets of potential interest for hepatitis B virus immunotherapy |
|---------|---------------------------------------------------------------|
| **Target** | **Target function** | **Binding partner** | **Drug** | **Indication** | **Phase** |
| CTLA4 | Inhibitory receptor | CD80, CD86 | Ipilimumab | Melanoma | FDA approved |
| | | | | Multiple malignancies | Phase II/phase III |
| | | | Tremelimumab | Malignant mesothelioma | FDA approved |
| PD1 | Inhibitory receptor | PD-L1, PD-L2 | Pembrolizumab | Melanoma | FDA approved |
| | | | Nivolumab | Melanoma | FDA approved |
| | | | Pidilizumab | Diffuse large B-cell lymphoma | Phase II |
| | | | AMP-224 | Colorectal cancer | Phase I |
| | | | MDX-1106 | Hepatitis C virus | Phase I |
| | | | | Multiple malignancies |
| PD-L1 | PD1 ligand | PD-1 | Avelumab | Multiple malignancies | Phase II |
| | | | BMS-936559 | HIV-1, multiple malignancies | Phase I |
| | | | MPDL33280A | Multiple malignancies | Phase I |
| | | | MEDI4736 | Multiple malignancies | Phase I |
| CD137 | Stimulatory receptor | CD137L | BMS-663513 | Solid tumors | Phase I/II |
| | | | PF-05082566 | Lymphoma | Phase I |

*CD137L, CD137 ligand, CTLA4 cytotoxic T lymphocyte associated antigen 4, HIV-1 human immunodeficiency virus, PD1 programmed death 1, PD-L1 programmed death 1 ligand 1, PD-L2 programmed death 1 ligand 2*
GS-9620, an oral TLR7 agonist, was demonstrated in animal studies to activate expression of IFN-stimulating genes, while early human studies showed transient increases in the levels of IFN-γ-induced protein 10 (also known as IP-10) 8 h after GS-9620 administration, suggesting an IFN-γ response [65, 66]. In a recent phase II trial, three different lengths of GS-9620 dosing (4, 8, and 12 weeks) were investigated in 156 chronic virally suppressed HBV infected patients [67]. Within each cohort, patients were randomized to receive three different doses (1, 2, or 4 mg). Although GS-9620 administration was safe and well tolerated, there was no significant decline in HBsAg levels. Additional in-depth analysis of HBV-specific T-cell and natural killer cell responses showed improved interplay between the two cell types, together with a transient improvement of T-cell response [68]. This was most notable for the highest dose of GS-9620 (4 mg).

These somewhat disappointing results are in contrast to the results of earlier animal studies in both chimpanzees and woodchucks in which GS-9620 was demonstrated to induce rapid sustained reduction in serum and liver HBV DNA levels together with loss of woodchuck hepatitis surface antigen (WHsAg) in woodchucks with chronic WHV infection [69, 70]. One possible explanation could be the difference in dosing—from 1 to 2 mg/kg in animal trials to 1–4 mg per patient in human studies. However, further research into this TLR7 agonist was subsequently discontinued.

TLR8-mediated recognition has been associated with viral infections. A first demonstration of its possible role in HBV infection came from an in vitro study by Jo et al. [71] demonstrating detectable intrahepatic IFN-γ production on stimulation of mononuclear cells by a TLR8 agonist [71]. Moreover, TLR8 expression and function was impaired in peripheral blood mononuclear cells from patients with chronic HBV infection compared with healthy controls [72]. When these HBeAg-negative patients were treated with a 48-week course of PEG-IFN-α, TLR8 messenger RNA level could discriminate between those who achieved a complete response and those who did not. This has led to a number of drugs in development targeting TLR8.

**HBV Therapeutic Vaccines**

The concept behind therapeutic vaccines is the generation of new HBV-specific T cells capable of controlling chronic HBV infection. HBV therapeutic vaccines have been shown to restore both T-cell responses and IFN-γ production [73]. In patients with high levels of virus in the blood, HBV-specific CD8 T cells have been shown to be dysfunctional although they are necessary for future viral control [58, 74, 75]. Therefore, in the setting of effective oral anti-HBV therapy with NUCs, therapeutic vaccines may have a role in the restoration of T-cell control.

The first HBV therapeutic vaccine to be studied in HBV-infected humans was GS-4774, a yeast-based T-cell vaccine containing HBV core, surface, and X proteins that has been shown to be immunogenic in mouse models and healthy volunteers. In a small phase II study the effects of GS-4774 were investigated on HBV-specific T-cell responses in 12 naïve HBeAg-negative genotype D patients with chronic HBV infection [76]. They received six consecutive monthly doses of the vaccine in combination with TDF. Although T-cell function improved, mostly an effect on CD8 cells, it was insufficient to induce a substantial decline of HBsAg levels. Multiple other vaccines are currently in development (early phase). These vaccines include vaccines targeting the preS1 domain [77] and the immune-stimulant vaccine ABX203 [78].

It might be that a combination of the aforementioned strategies (directed at reversal of T-cell exhaustion and generation of new T-cell responses) in patients with HBV infection stably suppressed with NUCs might be a way forward. A recent study in WHV-infected woodchucks investigated such a triple therapy combination, consisting of therapeutic vaccine (i.e., DNA plasmids expressing woodchuck hepatitis core antigen and WHsAg), the immune checkpoint inhibitor anti-PD-L1, and ETV. Sustained immunological control with the development of antibodies against WHsAg and
even viral clearance in some woodchucks was achieved [79]. Recently, a phase I trial evaluating the efficacy of anti-PD1 treatment with or without GS-4774 in HBeAg-negative chronic hepatitis B patients was published [80]. A single dose of anti-PD1 resulted in a significant decline in HBsAg levels, but with no apparent added benefit of GS-4774.

CONCLUSIONS

Although treatment with the current nucleoside/nucleotide inhibitors is very successful in suppressing HBV DNA to undetectable levels, functional let alone sterilizing cures are not within reach for the vast majority of HBV-infected patients. New exciting advances have led to new compounds targeting multiple steps in the viral life cycle as well as approaches to attenuate virus-induced immune dysregulation. Further research into these agents remains problematic however, with key issues needing to be resolved. First, firm therapy-related end points of cure need to be establish. Second, it is likely that combination therapies will be required, and it is currently hard to envisage how the most promising combination of drug targets can be determined. Thereafter, however, the future looks bright for patients with chronic HBV infection.

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