Despite the significant burden it places on communities across the globe, hepatitis had been mainly ignored as a health and clinical development priority until recently. Hepatitis B infections are a leading cause of disability and death worldwide. In fact, hepatitis causes as many deaths annually as tuberculosis, AIDS or malaria. Unlike many other infectious diseases, the absolute burden and relative risk of developing viral hepatitis has actually increased in recent years. Despite the availability of vaccines to help prevent hepatitis B, the coverage rate remains poor in highly endemic areas.

The estimated number of individuals chronically infected with HBV is 350 million. The World Health Organisation estimates that more than 686,000 people die every year as a result of complications of hepatitis B, including cirrhosis and liver cancer. HBV can be transmitted through exposure to infective blood, semen and other bodily fluids. In endemic areas, the virus is typically spread by close person-to-person contact, for example: from mother to child at birth, through exposure to infected blood or by sexual contact. Other common routes for transmission include contaminated needles and syringes.

Goals of therapy
There are several approved treatments for hepatitis B, but they are not ideal. The efficacy of these agents is often lacking and viral resistance has emerged. Considerable research efforts are being expended by several companies, both large and small, to identify and test new agents for the treatment of chronic hepatitis B. The goal of therapy is improvement in quality of life and survival by preventing or significantly delaying progression of the disease toward cirrhosis, decompensated cirrhosis, end-stage liver disease and hepatocellular carcinoma (HCC). This goal can only be achieved if HBV replication remains suppressed in a sustained manner. Block and colleagues have proposed definitions for three types of a cure:

**Absolute cure:** The patient is virus-free and returned to his state of health prior to illness, including his age- and gender-appropriate likelihood of developing cirrhosis or HCC.

**Functional cure:** The patient has returned to a state of health equivalent to that of a person who has recovered spontaneously from HBV infection, and has similar likelihood of developing cirrhosis or HCC.

**Apparent virologic cure:** Defined as a sustained off-drug suppression of virologic markers and the normalisation of liver function.

As the understanding of biology of HBV infection has improved, it has become evident that both viral factors and determinants of the host immune

Hepatitis B (HBV) is the cause of a silent epidemic that has resulted in a worldwide healthcare crisis.
response play roles in viral persistence. Novel strategies are therefore needed to produce a shift in the paradigm for chronic hepatitis B treatment.

**Hepatitis B treatments**

No specific treatments are available for acute hepatitis B infections. Care focuses on maintaining comfort and nutritional balance with fluid replacement to compensate for losses due to vomiting and diarrhoea.

There are two main categories of therapies for chronic hepatitis B: direct-acting antivirals (DAA), which target the lifecycle of the virus and host-targeting antivirals (HTA) which target host factors. The first drug to be approved for managing chronic infection was interferon-α2b, in 1991. Since that time, seven drugs have been approved.

The first drug to be approved for managing chronic infection was interferon-α2b, in 1991. At the time, seven drugs have been approved. To date, the only approved HTAs are the interferons, and there are two approved for use in the United States: interferon-α2b (Intron A®, Merck) and peginterferon-α2a (Pegasys®, Genentech). Interferon-α is an immune modulator that induces the expression of interferon-stimulated genes (ISGs) encoding intracellular or secreted proteins with antiviral properties affecting both infected and non-infected cells. Interferons also promote the activation and differentiation of immune cells in the host, which drive down the levels of circulating HBsAg. There are currently six globally approved DAAs for chronic hepatitis B, all of which are nucleos(t)ide analogues: lamivudine (Epivir®-HBV, GlaxoSmithKline), adefovir dipivoxil (Hepsera®-Gilead Sciences), entecavir (Baraclude®-Bristol-Myers Squibb), telbivudine (Tyzeka®-Novartis), clevudine (Leovir®, Korea and Philippines, Bukwang Pharmaceutical Co) and tenofovir disoproxil fumarate (Viread®-Gilead Sciences). All of these agents inhibit the reverse transcriptase/polymerase activity, resulting in a decrease in viral replication as measured by reductions in serum HBV DNA.

**Limitations of current therapies**

In addition to the shortcomings described below, available therapies for HBV often require lifelong treatment and surveillance, as reactivation frequently occurs following medication cessation. A remaining serious problem is the occurrence of HCC, which is decreased, but not eliminated, even after years of successful viral suppression.

**DAAs**

Nucleos(t)ide analogues: Older nucleoside analogues have been associated with high rates of antiviral resistance. Lamivudine has shown rates of up to 76% in patients treated for five or more years. In some instances, the appearance of antiviral resistance can be accompanied by a transient but occasionally severe exacerbation of the underlying liver disease, which can lead to acute liver failure.

**HTAs**

Interferons: Because interferons induce a non-specific generalised immune response, they result in poorly-tolerated side-effects and can aggravate fatal or life-threatening neuropsychiatric, autoimmune, ischaemic and infectious disorders.

cccDNA is the stored copy of HBV genetic material. It is hard to reach in the nucleus of infected hepatocytes. A cure for hepatitis B should ultimately either eliminate cccDNA, or prevent transcriptional activity of cccDNA. However, this is rarely achieved in clinical practice even with the use of both nucleos(t)ide analogues and interferon. While viral suppression can be achieved in the majority of patients with the high barrier-to-resistance new-generation of nucleos(t)ide analogues (NUC) (e.g. entecavir and tenofovir), HBsAg loss was achieved by PegIFN-α and/or NUC in only 10% of patients, after a five-year follow-up.

Nucleos(t)ide analogues interfere late in the viral lifecycle, preventing DNA replication. However, they do not affect the transcriptional activity of cccDNA or viral protein production, and only rarely induce immune control. HBsAg
loss is therefore rare, and, with the current therapies, lifelong treatment is often required in order to prevent viral rebound after treatment is halted.

**Combination therapy**

The limitations of current therapy have prompted a search for new therapeutic strategies. Combinations with anti Hep C drugs have proven to give way to a cure. Over the past decade there has been substantial interest in combining nucleoside analogues and peginterferon therapy with the thought that these drugs might act synergistically. However, the results are disappointing.

The cure of chronic HBV infection will be more difficult and complicated than it was for HCV. HBV persists in the liver as viral cccDNA. The infected person is unable to eliminate this highly persistent form of the virus despite available therapies.

This underscores the need for combination therapy with new classes of agents to eradicate HBV. New mechanisms of action are being identified to attack the virus at different parts of its lifecycle. Combinations of agents with complementary mechanisms of action that attack the virus at different parts of its lifecycle will be needed to achieve a cure.

First-line highly potent nucleoside analogues are able to suppress viral loads to undetectable, however there are serious limitations. When therapy is stopped there is viral rebound in the vast majority of patients. Since therapy must be continued long term, there are also toxicities that must be managed. With one mechanism action of the virus blocked, replication of circulating HBV DNA, the addition of new agents, bring the field closer to a cure.

There is a rapidly growing portfolio of antiviral agents that are being explored (Table 1). This includes DAAs and HTAs and encompasses a wide variety of mechanisms of action. Two compounds in development from ContraVir: CMX157, a novel prodrug of tenofovir (polymerase inhibitor) and CRV431 (cyclophilin inhibitor) have complementary and synergistic mechanisms of action. These agents could be used as part of a combination therapy moving towards the functional cure.

**Direct-acting antivirals**

Nucleoside analogues inhibit the HBV polymerase, stopping the replication of HBV DNA for new virions. Some currently in development include besifovir (LBO80380/ANA380, Idong Pharma) and elvucitabine (Achillion). CMX157 (ContraVir), AGX1009 (Agenix), and TAF (GS-7340, Gilead) are all prodrugs of tenofovir. These prodrugs are designed to increase the bioavailability of tenofovir, while decreasing circulating tenofovir levels, reducing potential renal and bone side-effects that are characteristic of treatment with tenofovir. For example, CMX157 (ContraVir) is a lipid conjugate of tenofovir designed to utilise lipid uptake pathways in the liver in order to concentrate tenofovir at the site of HBV replication.

**RNA Interference**

siRNA drugs target HBV DNA transcripts and interfere with HBV RNA production. cccDNA formed from HBV RNA can therefore be repressed by preventing its formation. ARC-520 (Arrowhead) is a siRNA, which is a lipid conjugate that utilises a nanoparticle-assisted delivery system. ALN-HBV (Alylam) and ARB-1467 (Arbutus) also use lipid nanoparticle technology for delivering their siRNA. shRNA (short hairpin RNA) is produced inside the target cell from DNA that has been delivered to the nucleus where it interferes with the HBV

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**Table 1:** From Block et al 2015 and Durantel et al 2016

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**References**


Genome, inhibiting viral replication and protein production. BB-HB-331 (Benitec Biopharma) is delivered using an AAV vector that targets the liver and expresses three shRNAs that target highly conserved regions on the HBV genome.

Problems to overcome with this technology include the unpredictable efficiency of the system, parenteral administration and potential for off-target genomic effects.

Cyclophilin inhibitors
Cyclophilin inhibitors are showing promising results against HBV. CRV431 (ContraVir) is a next-generation cyclophilin inhibitor that interrupts the HBV lifecycle at multiple points. The compound is designed to reduce liver toxicity through an antifibrotic mechanism. Several other agents in this class are being evaluated for efficacy against hepatitis B (eg, Alisporivir [formerly Debio 025, Debiopharm Group]; NIM811 [Novartis]; and cyclosporin A).

Capsid inhibitors
Capsid formation is an essential viral process that does not occur in the uninfected cell, and therefore would be expected to provide a virus-selective target. BAY4109 (AiCuris) is highly specific for HBV. NV1221 (Novira) prevents HBV capsid formation in a way analogous, but not identical, to BAY4109. GLS 4 was developed as a structural optimisation of BAY4109. The compound has a unique mechanism of action by which it causes abnormal capsid protein formation. NVR 3-778 is another capsid inhibitor from Novira.

RNase H Inhibitors
Unlike other DNA viruses, HBV replication depends upon the RNase H activity of HBV polymerase to degrade pregenomic RNA. RNase H inhibition is therefore another viable antiviral target. A group at St Louis University has reported identifying several compounds in this class, some of which are based on validated HIV drugs.

Host-targeting agents
Viral entry inhibitors
Entry inhibitors bind to HBV to block entry into hepatocytes. Myrcludex B (Hepatera/Myr-GBM) is a lipopeptide derived from the cell receptor for the HBV large envelope protein. This agent neutralises virions to prevent their association with target cells by neutralising antibodies. Cyclophilin inhibitors have also demonstrated viral entry inhibition, in addition to the characteristics mentioned above.

Immune enhancers
The immune system is made up of two pathways, innate and adaptive. The innate system is the first line of general defence when the body recognises foreign molecules. The adaptive immune system is much more specific both for cell and antibody mediated responses to clear invading pathogens. Chronic hepatitis B infection is a result of the inability of the entire immune system to clear the
virus from infected hepatocytes. Immune enhancers are therefore expected to be beneficial.

The limited success of interferons drives research for better ways to stimulate the immune system. The toll-like receptor (TLR)-7 is a ‘pathogen recognition receptor’ in the innate immune system. GS9620 (Gilead) is a small molecule that is a TLR-7 agonist\(^{14}\). SB 9200 (Spring Bank) is another small molecule that stimulates the host’s innate immune system.

It is expected that vaccines will broadly stimulate an exhausted immune system. New therapeutic vaccines are being evaluated in clinical trials. GS4774 (Gilead) is engineered to express a fusion protein containing HBsAg sequences. The vaccine TG1050 (Transgene) is based on a viral vector expressing three HBV antigens. Altravax is testing a DNA vaccine that is a chimera of wild-type and xenogenic HBs surface peptides\(^{11}\). INO-1800 (Inovio) is a recombinant DNA vaccine that encodes the consensus sequence of HBV core antigen. Virus-like particle (VLP), from VLP Biotech, is based on the HBV core antigen. Chimigen HBV/NU500 (Akshaya Bio) is a chimeric polypeptide consisting of regions of the core and surface antigen proteins and the Fc-binding domain of IgG. It is hypothesised the Fc component should have the capacity to direct the chimeric protein to dendritic antigen presenting cells and enhance presentation of HBV protein.

The dimethylxanthenone Stimulator of Interferon Gene (STING) acts as an adaptor polypeptide for several cytoplasmic DNA-sensing receptors and an intracellular modulator of innate immune responses. STING agonists (Arbutus Biopharma in collaboration with the Blumberg Institute) have been demonstrated to suppress HBV replication in preclinical studies.

There is a high level of interest in finding molecules that stimulate the adaptive immune system, which is suppressed by chronic HBV infection. The programmed cell death (PD-1)/PD-1 lig-and pathway plays an important role in the antigen-mediated exhaustion of T-cells in several chronic infections\(^{11}\). PD-1/PD-L1 pathway inhibition with monoclonal antibodies has been demonstrated to reverse immune dysfunction and HBV viral persistence.

Epigenetic modifiers
Epigenetic modifiers, such as small-molecule histone deacetylase (HDAC) inhibitors, have been shown to suppress cccDNA transcription\(^{11}\). A potential issue, associated with the use of these modifiers in oncology, is their lack of specificity for
viral genome sequences, which could lead to serious adverse effects\textsuperscript{14}. The identification of viral mechanisms involved in epigenetic regulation of cccDNA, therefore, will be critical to the success of these agents.

Other HTAs

Birinapant (Tetralogic Corp) is a small molecule that mimics the second mitochondrial activator of caspases\textsuperscript{11}. With this drug, infected cells are selectively eliminated, assuming that they are more sensitive to apoptotic (programmed cell death) stimuli, compared to the uninfected cells.

Conclusion

Although it has been more than 50 years since the discovery of the Australia antigen, there are still no therapies that provide a cure for chronic hepatitis B\textsuperscript{11}. To date there are still only two families of drugs to treat chronic hepatitis B, the interferons and the polymerase inhibitors\textsuperscript{13}. There is a high level of renewed interest and an increasing momentum in terms of innovation for hepatitis B therapeutic strategies. It is clear that an explosion of innovation has occurred in the treatment of HBV. Investigational prodrugs, including CMX157 (ContraVir) and TAF (Gilead), which are in clinical development, have the potential to improve upon these existing therapies by increasing efficacy and decreasing the incidence of side-effects. There are several agents with new mechanisms of action that are currently being tested in clinical trials including siRNA, capsid inhibitors (eg BAY4109 from AiCuris and NV1221 from Novira), HBsAg inhibitors (eg REP2139 from Replicor), and cyclophilin inhibitors (eg CRV431 from ContraVir). New delivery technologies such as lipid nanoparticles have revived the siRNA approach. Advanced technologies for discovery, screening and profiling, have also allowed for the development of many investigational agents in the early phases of development that utilise areas of HBV biology that were previously unexplored (eg novel chimeric epitopes, entry inhibitors, selective killing of infected hepatocytes and novel therapeutic vaccines).

Combinations of these investigational agents with their novel and complementary mechanisms for inhibiting HBV replication should lead to new drugs for chronic hepatitis B management in the near term. This could result in a functional cure within the next few years\textsuperscript{11}.

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