Novel therapies and potential therapeutic targets in the management of chronic hepatitis B

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Chronic hepatitis B is a persistent and progressive inflammatory liver disease caused by infection with the hepatitis B virus (HBV). More than 240 million individuals are infected with HBV worldwide and hepatitis B accounts for an estimated 650 000 deaths annually. Approximately up to 30% of chronically infected patients will develop complications of HBV infection including, but not limited to, liver cirrhosis, end-stage liver disease, and hepatocellular carcinoma. Currently approved therapies have improved clinical outcomes, but have a considerable side-effect profile, elevated cost, and a finite course of treatment. This has led to a growing interest in research for new therapies. As the mechanisms for HBV replication are becoming better understood, new potential targets have been discovered, leading to the development of new therapies. In this article, we describe the promising therapies that are under evaluation, showing their mechanisms of action, effects, and stage of development. Eur J Gastroenterol Hepatol 00:000–000

Introduction

Chronic hepatitis B is a potentially progressive infectious liver disease. It is distinguished from acute infection by the persistence of hepatitis B surface antigen (HBsAg) for 6 months or more, with or without evidence of viral replication and/or hepatocellular damage. More than 240 million individuals are infected with hepatitis B virus (HBV) worldwide and hepatitis B accounts for an estimated 650 000 deaths annually. Up to 30% of chronically infected patients will develop complications of HBV infection including but not limited to liver cirrhosis, end-stage liver disease, and hepatocellular carcinoma (HCC) [1].

HBV belongs to the hepadnavirus family. It is characterized by a small, enveloped, double-stranded DNA virus. The viral genome encodes HBsAg, hepatitis B core antigen (HBcAg), a viral polymerase, and the HBx protein [2]. The understanding of the life cycle of HBV has improved in recent years (Fig. 1). The initiation of viral infection is characterized by viral attachment and entry of the virion into the hepatocyte. This first step is mediated by protein receptors on the cellular membrane and on the viral envelope [2–4]. Recent studies suggest that a peptide derived from the pre-S1 region of the viral envelope protein may play an important role in viral entry [5]. Inside the hepatocyte, the virion loses its envelope and the nucleocapsid disassembles in a process called uncoating, which allows entry of the genomic DNA in to the nucleus [6].

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Synthesis of covalently closed circular DNA (cccDNA) then occurs inside the nucleus. This step requires normal activity of the HBV reverse transcriptase or cellular DNA repair enzymes [7]. The persistence of a chronic infection in certain patients after exposure to HBV is explained by the fact that cccDNA can remain dormant inside the hepatocytes and serve as a template for the formation of new viral mRNA [2]. The cccDNA subsequently becomes transcribed into six unspliced mRNAs that encode all viral proteins. The mRNA is transported to the ribosomes, where translation occurs with the production of core and polymerase proteins, precore, middle, and small envelope proteins, large envelope, and HBx protein [8].

These final products of DNA replication will self-assemble in the cytoplasm and reform as a nucleocapsid in the endoplasmatic reticulum or Golgi apparatus. They will eventually acquire an HBsAg-containing envelope before being released with similar characteristics as the initial virion. The currently available nucleoside analogs work by interrupting the formation of new DNA strands through early prohibition of the viral DNA polymerase.

Current anti-HBV therapies

The current therapies against chronic hepatitis B have been shown to suppress HBV replication, decrease the rate of progression to cirrhosis, and reduce the incidence of HCC; however, the long-term management of HBV-infected patient remains a challenge, given the low rates of durable response and the high costs associated with life-long therapy [9]. The currently available therapies include peginterferon-α, lamivudine, adefovir, entecavir, telbivudine, tenofovir, and most recently tenofovir alafenamide. When patients are started on therapy, the end points are usually complete viral suppression in all patients and seroconversion into HBeAg negative in patients with positive HBeAg. An ideal, although not often achieved, end point is sustained HBsAg loss, with or without HBs antibody (Ab) seroconversion [10,11].

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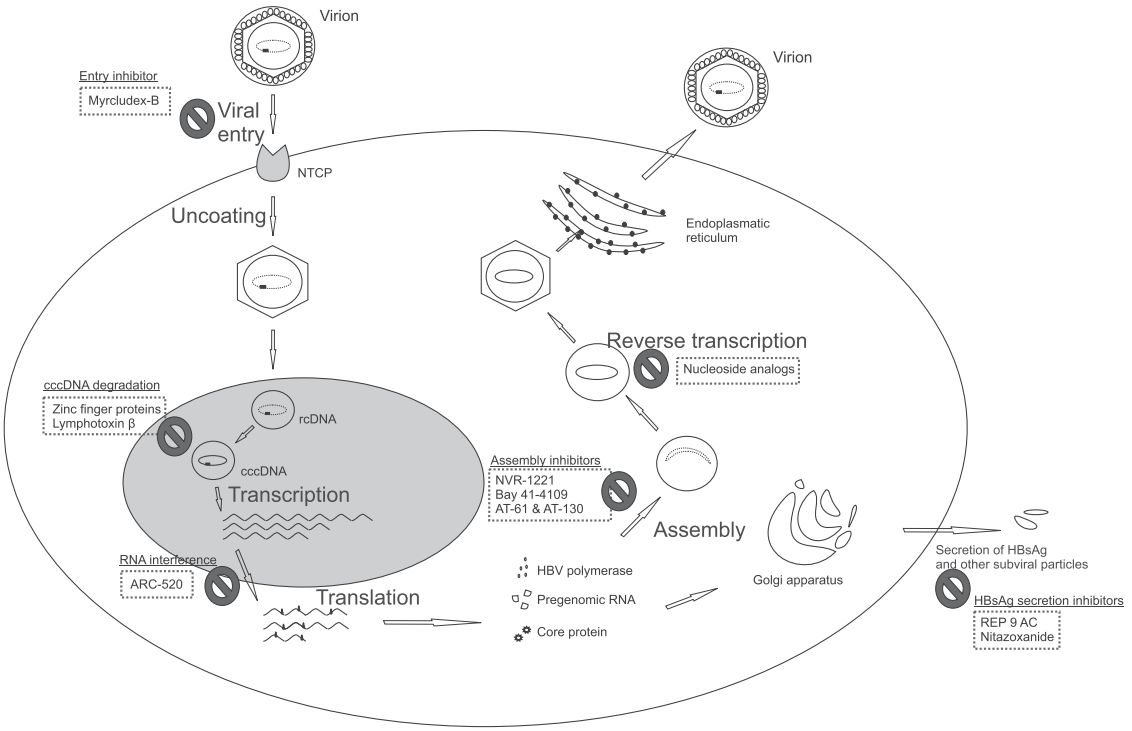

Fig. 1. Hepatitis B virus (HBV) lifecycle and potential new therapeutic agents. The virion enters the hepatocyte in a process mediated by NTCP and its ligand pre-S1. Inside the hepatocyte, the virion loses its envelope in a process called uncoating, allowing entry of the viral DNA into the nucleus. cccDNA is synthesized and then transcribed into mRNA. In the cytoplasm, the products of mRNA translation then assemble to form a new virion that will be secreted either through the Golgi apparatus or the endoplasmatic reticulum. cccDNA, covalently closed circular DNA; HBsAg, hepatitis B surface antigen; NTCP, sodium taurocholate cotransporting polypeptide; rcDNA, relaxed circular DNA.

This article aims to review novel investigational therapies for chronic HBV as well as the potential new targets in the HBV lifecycle that can ultimately help in the quest for the cure of chronic hepatitis B.

New therapies and therapeutic targets

Viral entry

Myrcludex-B

The characterization of several HBV attachment sites in the hepatocyte enabled the development of a new potential target in the treatment of HBV entry (Fig. 1). Initial studies in animal hepatocytes showed that the pre-S1 sequence 2–48 on the viral membrane mediates attachment of the virus to hepatocytes [5,12]. Subsequent studies further defined the mechanism of viral entry by identifying the receptor for the pre-S1 sequence polypeptide, the sodium taurocholate cotransporting polypeptide, a sodium-dependent transporter that had been implicated in bile acid uptake within the hepatocyte [13–17].

The results of the aforementioned investigations led to the development of myrcludex-B, a synthetic lipopetptide derived from the pre-S1 domain.

In a preliminary study, mice preinfected with HBV virus received subcutaneous Myrcludex at 3 days, 3 weeks, or 8 weeks after infection to assess the efficacy of Myrcludex in the prevention of cell-to-cell dissemination. Viral loads were then quantified by PCR and immunohistochemistry and cccDNA was measured. Results from this preclinical trial showed that myrcludex could prevent initial infection of the hepatocytes in addition to de-novo infection as shown by decreased viral loads in mice that were only treated for 3 or 8 weeks. Surprisingly, cccDNA levels were also decreased in all three controls [18].

Subsequently, a phase 1 clinical trial in healthy volunteers was initiated, with preliminary results indicating a favorable safety profile and pharmacokinetics [19].

A recent phase 2 trial was conducted in patients with hepatitis D virus showing good viral suppression with normalization of liver tests [20].

Further studies on its use in patients mono-infected with chronic hepatitis B are necessary (Table 1).

Viral assembly

NVR-1221 (NVR 3-778)

NVR-1221 works through inhibition of assembly of the nucleocapsid. Preclinical studies using in-vitro developed hepatocyte (HepaRG) showed good antiviral activity against several HBV genotypes with a favorable safety profile.

In a subsequent phase 1a clinical trial, oral doses of NVR-1221 ranging from 50 to 1200 mg were administered in healthy volunteers for 14 days. No major side effects were noted and potentially effective plasma concentrations were achieved at doses of 200 mg daily [21].

Preliminary results from a phase 1b trial combining NVR-1221 with pegylated interferon showed that NVR-1221 had increased reduction in HBD DNA compared with pegylated interferon alone [22].

Table 1. Summary of future potential hepatitis B virus therapies

Therapeutic targets	Drug name	Mechanism	Phase of clinical trial
Viral entry	Myrcludex-B	Target NTCP receptor and blocks viral entry	Phase 2
Viral assembly	NVR-1221	Inhibit viral replication by inducing incorrect capsid formation	Phase 1a [21]
	BAY 41-4109	Inhibit viral replication by inducing incorrect capsid formation	Preclinical
	AT-61 and AT-130	Initiation of capsid assembly too early, leading to nonfunctional virions	Preclinical
HBsAg secretion	REP 9AC	Interfere with secretion of noninfectious subviral particles	Phase 2 [40]
	Nitazoxanide	Inhibit intracellular HBV replication	Preclinical
cccDNA degradation	Lymphotoxin-β	Inhibit transcription of cccDNA and increase in cccDNA degradation	Preclinical
	CRISPR	Nucleases that lead to disruption of cccDNA production	Preclinical
RNA interference	ARC-520	Post-transcriptional gene inactivation	Phase 1 [60]
PD-L1 inhibition		Immunemodulator	Phase 1 [69]
Therapeutic vaccines	DV-601	Immunemodulator	Phase 1b [72]
Nucleoside analogues	CMX157		Phase 2 [84]
	AGX-1009		Preclinical
	Lagociclovir		Preclinical
	Besifovir		Phase 3 [94]

cccDNA, covalently closed circular DNA; CRISPR, clustered regularly interspaced short palindromic; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NTCP, sodium taurocholate cotransporting polypeptide; PD-L1, programmed death ligand-1.

Heteroaryldihydropyrimidine compound (BAY 41-4109 and GLS4)

One of the hallmarks of chronic hepatitis B is the replication of the HBV inside the hepatocyte, allowing for the persistence and transmission of the virus to other cells. This important step takes place within the cytoplasm of the hepatocyte and is dependent on the correct formation of several core proteins subunits [23,24]. Inhibition or deregulation of those core proteins triggered the emergence of new agents.

Originally identified by Weber *et al.* [25], heteroaryldihydropyrimidine compounds were found to activate and accelerate assembly of capsid subunits, leading to improper, misfolded, and nonfunctional capsid structures. The most extensively investigated of these compounds is BAY 41-4109, which has resulted in a significant reduction in cytoplasmatic HBcAg and HBV viral load in studies using a hepatoma cell line developed in-vitro (HepG2.2.15) [26–28] and in-vivo studies, an HBV mouse model [25].

Currently, only preclinical data are available.

Phenylpropenamides (AT-61/AT-130)

Phenylpropenamides are another class of compounds that were found to have inhibitory properties on viral encapsidation. Despite being identified in 1998 [29], the mechanism of action of AT-61 and AT-130, derivatives of phenylpropenamides, was only fully understood in 2007 by Feld *et al.* [30] using immune electron microscopy on selected cell lines pretreated with AT-130. These investigators could show that these agents interact with chaperone proteins, which in turn stabilize pregenomic RNA polymerase, an important enzyme in viral encapsidation [2,31]. By disrupting the pregenomic RNA polymerase, AT-61 and AT-130 induce early initiation of capsid assembly, creating normal capsids without infectious properties [32,33].

These agents are currently in the preclinical stage of development.

HBsAg secretion

REP 9AC

REP 9AC is a member of a new class of therapeutic agents called amphipathic DNA polymers. The class was initially discovered as investigational agents for the treatment of HIV-1 [34] and these agents were found to be effective against several other viruses [35,36], including HCV [37,38].

The mechanism of action of amphipathic DNA polymers in the case of HBV is not yet fully understood, but on the basis of studies carried out in other viruses, it is believed that these agents block the secretion of HBsAg by interfering with secretion of noninfectious subviral particles [39].

In an initial phase 1/2 clinical trial, patients with chronic hepatitis B received REP 9AC parenterally and had levels of HBV DNA, HBsAg, and anti-HBs assessed weekly. Interim results showed that five of the eight patients had clearance of HBsAg as early as 7 days after treatment and no later than week 32. There were no major side effects at therapeutic doses [40].

In another phase 2 clinical trial, 12 HBeAg-positive patients received a similar polymer (REP 2139-Ca), in conjunction with pegylated interferon or thymosin-α. Results are promising, with 2–7 log reductions of serum HBsAg, 3–9 log reductions in serum HBV DNA, and appearance of anti-HBsAg in all nine patients [41].

Nitazoxanide

Although nitazoxanide (NTZ) is currently being evaluated in three ongoing phase 2 trials for the treatment of hepatitis C [42,43], its use is also being investigated for the treatment of chronic hepatitis B. Initially developed for the treatment of protozoal infections, the antiviral properties of NTZ were incidentally observed in HIV patients, prompting the investigation of NTZ for the treatment of other viruses including HBV. Its antiviral mechanism is yet to be fully understood.

In an initial in-vitro study, NTZ was found to decrease the levels of HBsAg and HBeAg, as well as the level of intracellular HBcAg. Notably, the action of NTZ was found to be synergistic with the use of lamivudine or adefovir [44].

In preliminary open-label studies, NTZ showed evidence of efficacy in the treatment of HBV, resulting in a decrease in HBV DNA in all four patients studied and undetectable levels in two of four [45,46].

Additional clinical trials are still required to assess the safety and tolerability of this agent.

cccDNA

The use of nucleoside analogs enables control of HBV infection by targeting the dsDNA genome when the virus is in its virion form. Unfortunately, when the virus is inside the hepatocyte, its genome changes conformation to a cccDNA, which has been resistant to therapeutic targeting [2].

Initial attempts to target the cccDNA were made using zinc finger proteins (ZFP) that were designed to target the enhancer region in viral DNA. After being inserted into a plasmid, ZFPs were then injected into duck hepatitis B virus. The results of these efforts showed that ZFPs could interfere with viral transcription, resulting in decreased expression of viral RNA and protein expression [47]. Although a promising treatment modality, this therapy is dependent on vectors to deliver ZFPs to the hepatocyte, and the optimal way to produce adequate vectors is still under investigation [48].

Also under investigation is lymphotoxin-β, a physiologic anti-inflammatory agent that induces apoptosis [49]. Activation of the lymphotoxin-β receptor leads to upregulation APOBEC3A and APOBEC3B, two deaminases that lead to cccDNA degradation [50]. Only preclinical data are currently available.

Another approach under investigation to target the cccDNA is the clustered regularly interspaced short palindromic (CRISPR)/CRISPR-associated protein-9 (Cas9) system. These nucleases target specific DNA sequences in the HBV genome, leading to disruption of cccDNA production [51–53]. This agent is currently under preclinical phase of investigation.

RNA

mRNA is another potential target in the battle against HBV. Initial experiments using RNA interference (RNAi) were conducted in 1998 and showed that RNAi, by hybridization with endogenous mRNA, led to post-transcriptional gene inactivation [54]. Further studies could distinguish two subtypes of RNAi; microRNA (miRNA) and small interfering RNA (siRNA) [55]. Of these, siRNA showed promising results, leading to a reduction in HBeAg, HBsAg, and cccDNA levels both *in vitro* and *in vivo* [56–58].

ARC-520 is a therapeutic agent derived from siRNA. In an initial preclinical study, the injection of ARC-520 was well tolerated and led to a decrease in the levels of HBeAg, HBsAg, and HBV DNA [59]. A phase 1 clinical trial was initiated in Australia, showing promising preliminary results with respect to the tolerability, safety, and pharmacokinetics of this agent [60].

A phase 2 trial is ongoing to evaluate degree of HBsAg reduction in response to one dose of ARC-520 in combination with entecavir [61].

Programmed death protein-1/programmed death ligand-1

Programmed cell death protein-1 (PD-1) and its ligand (PD-L1) play an important role in autoimmunity [62], the regulation of host defenses against infectious agents [63], and control of tumor propagation [64]. This regulatory pathway plays several roles in the regulation of the immune system and several promising agents targeting PD-1/PD-L1 are in development.

In the case of chronic hepatitis B, persistent exposure to viral antigens leads to both quantitative and qualitative impairments on the T-cell linage, specifically of CD8 + cells [65–67]. These dysfunctional T cells overexpress PD-1, which, after conjugation with its ligand, will induce further depletion of both CD4 + and CD8 + lymphocytes. In a follow-up study, the inhibition of this pathway enhanced CD8 + lymphocyte re-expansion and led to normalization of cytokine production [65]. This initial hypothesis was then tested *in vivo*, with similar results suggesting that this agent can be valuable in viral suppression [68].

A phase 1 clinical trial is currently ongoing [69].

Vaccines

Vaccines may be another potential mode of defense against HBV. As opposed to preventive vaccines, therapeutic vaccines are designed to overcome the deficiency in both the quantity and the quality of host T cells [70].

The therapeutic vaccines that are currently under development are vaccines based on recombinant viral proteins or the viral envelope, naked DNA, or on T-cell peptide epitopes derived from HBV proteins [71].

DV-601 is an example of a recombinant vaccine derived from HBsAg and HBcAg. A phase 1b clinical trial has been completed. In this trial, six injections of DV-601 were administered over a period of 12 weeks, with a primary goal to evaluate the safety and tolerability of the drug, in addition to assessing improvement in CD8 + T-cell number and function. Preliminary results from this trial showed a favorable safety profile and repletion of CD8 + cells [72] (ClinicalTrials.gov, Identifier: NCT01023230).

DNA vaccines work by induction of the cellular immune response compared with protein-based vaccines that work by induction of humoral immunity. DNA vaccines have proven to be effective therapeutic agents in other viral infections and virally induced malignancies [73]. However, in chronic hepatitis B, initial results have been disappointing. A phase 1 trial evaluating the efficacy and safety profile of plasmid DNA (pSG2.HBs) vaccine, followed by recombinant modified vaccinia virus Ankara (MVA.HBs), showed that none of the participants lost HBsAg, and no sustainable effect on HBV viral load was observed [74].

A second group proposed to investigate the effect of DNA vaccines in the rates of relapse after discontinuation of nucleoside analogues. In a phase 1/2 trial, 70 patients effectively treated with nucleosides analogues received an HBV envelope-expressing DNA vaccine. After a median of 28 days, reactivation occurred in 97% of the patients, with only two of the 70 patients maintaining an undetectable HBV DNA, rates comparable with the control group [75].

Epigenetic therapies

Epigenetic changes are alterations to gene expression that do not involve mutations in the DNA sequence. The DNA of the HBV virus can be modulated by a variety of

mechanisms including DNA methylation of viral and host genomes, post-translational modification of histone proteins associated with cccDNA, and ATP-dependent chromatin remodeling [76,77].

With the recent advances made in the field of epigenetics, some therapeutic targets have been identified. Belloni *et al.* [78] showed that treatment with peginterferon-α induces cccDNA-bound histone hypoacetylation, one of the possible mechanisms by which peginterferon-α leads to a decrease in HBC cccDNA transcriptional activity. Chen *et al.* [79] showed a decrease binding of transcription factors to active cccDNA.

Another potential target are DNA methyltransferases. These enzymes have been shown to regulate viral genome methylation, which in turn affects protein production and viral replication [80].

DNA methylation has also been shown to be involved in the development of HCC [81].

Although no drugs targeting these mechanisms are currently under development, epigenetics could play an important role in the treatment of HBV.

Nucleoside analogues

CMX157

A prodrug of Tenofovir, CMX157 consists of tenofovir conjugated to a lipid terminal (1-0-hexadecycloxypropyl). With a development history similar to TAF, CMX157 was also initially developed for the treatment of HIV-1 patients and showed promising results in both phase 1 and 2 trials [82].

A phase 1 clinical trial of CMX157 in chronic hepatitis B patients was completed with good drug tolerability and a phase 2 trial is being started [83].

A subsequent phase 2a clinical trial yielded promising results, with a 99% reduction in viral load. This antiviral activity was achieved at smaller doses compared with tenofovir, possibly indicating a safer medication profile [84].

Lagociclovir (MIV-210)

MIV-210 is a prodrug of the nucleoside analogue 2′,3′-dideoxy-3′-fluoroguanosine originally developed for the treatment of HIV-1. As the DNA polymerase of the HIV virus shares some similarities with the HBV polymerase, research groups have been attempting to evaluate this agent as a potential therapy for chronic hepatitis B.

Initial in-vitro studies in duck hepatocytes showed that 2′,3′-dideoxy-3′-fluoroguanosine resulted in decreased levels of HBV DNA [85] by inhibition of viral replication. Interestingly, lagociclovir showed a broader spectrum of activity compared with other agents of its class, with activity against mutant HBV polymerases [86].

Subsequent studies could confirm these results and showed a favorable safety and tolerability profile [87]. More recently, Michalak *et al.* [88] characterized the antiviral efficacy and safety of MIV-210 using different test doses in a woodchuck HBV model.

Besifovir (LB80380)

Besifovir is an acyclic nucleotide phosphonate with a structure similar to adefovir and tenofovir. The active metabolite, LB80317, has been shown to induce potent HBV viral suppression [89]. A phase 1 clinical trial enrolled treatment-naive HBeAg-positive chronic hepatitis B patients and showed that at a dose of 60 mg daily, HBV DNA suppression was achieved over a follow-up period of 28 days [90]. A similar trial enrolling patients with lamivudine-resistant HBV strains showed similarly promising results [91].

In a phase 2b multicenter randomized trial, besifovir was compared with entecavir in treatment-naive patients with chronic hepatitis B, who were HBeAg positive and HBeAg negative, for a period of 48 weeks. In this study, besifovir and entecavir achieved similar decreases in HBV DNA levels, alanine aminotransferase levels, and HBeAg seroconversion, showing the noninferiority for besifovir and supporting its use as a potential alternative agent for the treatment of HBV [92]. A parallel study by Yuen *et al.* [93] showed similar results.

A phase 3 trial is ongoing [94].

Conclusion

Chronic hepatitis B remains a global health problem that is highly endemic in certain parts of the world. Despite the available treatments, a functional cure has not been achieved as yet. There are currently more than 240 million chronic carries of this virus of whom 20–30% might develop HBV-related sequelae including, but not limited to, cirrhosis, end-stage liver disease, and HCC.

This review shows that there are multiple promising anti-hepatitis B agents that are in the process of development. Some of the therapies reviewed attack different targets in the life cycle of this challenging virus, including viral entry (myrcludex-B), viral assembly (NVR-1221, BAY 41-4109, AT-61, AT-130), HbsAg secretion (REP 9AC), cccDNA degradation (lymphotoxin-β, CRISPR), and RNA interference (ARC-520). We also identified potential therapeutic vaccines (DV-601) and new nucleoside analogues (CMX157, AGX-1009, lagociclovir, besifovir). The addition of these new agents to the currently available regimens could markedly improve the outcomes in this patient population. With promising new agents currently under investigation and a growing knowledge of viral mechanisms, the future might carry an end to the quest for curing hepatitis B.

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Conflicts of interest

There are no conflicts of interest.

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