Active site polymerase inhibitor nucleotides (ASPINs): Potential agents for chronic HBV cure regimens

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Abstract
Chronic hepatitis B virus (HBV) infection affects 240 to 300 million people worldwide. In the nucleus of infected hepatocytes, the HBV genome is converted to covalently closed circular DNA (cccDNA), which persists and serves as a transcriptional template for viral progeny. Therefore, a long-term cure for chronic HBV infection will require elimination of cccDNA. Although currently available nucleos(t)ide analogues (eg, tenofovir disoproxil fumarate, tenofovir alafenamide, entecavir) effectively control HBV replication, they are seldom curative (functional cure rate \(\sim 10\%\)) and require lifelong treatment for most patients. As such, antiviral agents with novel mechanisms of action are needed. Active site polymerase inhibitor nucleotides (ASPINs) noncompetitively distort the HBV polymerase active site to completely inhibit all polymerase functions, unlike traditional chain-terminating nucleos(t)ide analogues, which only target select polymerase functions and are consumed in the process. Clevudine, a first-generation ASPIN, demonstrated potent and prolonged HBV suppression in phase 2 and 3 clinical studies, but long-term treatment was associated with reversible myopathy in a small number of patients. ATI-2173, a novel next-generation ASPIN, is structurally similar to clevudine but targets the liver and demonstrates potent anti-HBV activity on and off treatment, and may ultimately demonstrate an improved pharmacokinetic and safety profile by significantly reducing systemic clevudine exposure. Thus, ATI-2173 is currently in clinical development as an agent for HBV cure. Here, we review the mechanism of action and preclinical and clinical profiles of clevudine and ATI-2173 to support the role of ASPINs as part of curative regimens for chronic HBV infection.

Keywords
ATI-2173, clevudine, nucleos(t)ide analogues, chronic hepatitis B, cccDNA

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Background
Hepatitis B virus (HBV) is a global public health concern that may lead to serious complications, including cirrhosis, liver failure, and hepatocellular carcinoma.¹,² Although most healthy adults are able to clear an acute HBV infection, approximately 5% to 10% of adults and 90% of infants with acute HBV will develop a chronic infection.¹,³,⁴ Globally, 296 million people are living with chronic HBV infection, with the highest infection burden observed in Africa and the Western Pacific region.⁵ Chronic HBV and related complications, including hepatocellular carcinoma, contribute to >800,000 deaths each year worldwide.³ In the United States, an estimated 1.9 to 2.4 million people have chronic HBV infection, ~1.5 million of whom were foreign born, including individuals from Asia, the Caribbean, and Africa.²,⁶ Because clinical symptoms may take decades to develop, many individuals with chronic HBV infection are unaware of their disease unless they are tested and diagnosed.⁷ In 2015, the World Health Organization estimated that only 9% of individuals living with chronic HBV infection worldwide were aware of...
their diagnosis, only 8% of whom were receiving treatment. Thus, chronic HBV infection is underdiagnosed and undertreated globally, presenting substantial risk for viral transmission and subsequent disease-related morbidity and mortality.

Currently available treatments for chronic HBV infection include pegylated interferon-α and nucleos(t)ide analogues.8–10 Although recommended as first-line therapy in certain patients, pegylated interferon-α is associated with frequent adverse reactions that lead to treatment discontinuation. Nucleos(t)ide analogues currently recommended as first-line therapy include entecavir, tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide, all of which have a relatively high barrier to HBV resistance. Other approved nucleos(t)ide analogues, such as adefovir, lamivudine, and telbivudine, are no longer preferred as first-line chronic HBV treatment because of their low barrier to HBV resistance. Hepatitis B surface antigen (HBsAg) seroclearance and seroconversion is the ultimate goal of HBV treatment.11 Unfortunately, this is rarely achieved with current treatments. Chronic HBV infection is a currently incurable disease typically requiring lifelong treatment with nucleos(t)ide analogues to maintain viral suppression.12

The infectious HBV virion is composed of an outer envelope that exposes small, middle, and large HBsAg proteins surrounding an inner nucleocapsid that contains the DNA genome in a partially double-stranded, relaxed circular conformation.13 After attachment and entry into host hepatocytes via the sodium taurocholate cotransporting peptide receptor, the nucleocapsid is delivered to the nucleus and the relaxed circular DNA genome is converted into covalently closed circular DNA (cccDNA) by host cell factors. The cccDNA persists in the nucleus and serves as a template for transcription of all viral RNAs, including pregenomic RNA (pgRNA).14 In association with HBV polymerase, pgRNA is packaged into capsid particles consisting of hepatitis B core proteins. The pgRNA is reverse transcribed into the relaxed circular DNA genome by HBV polymerase, which serves as a protein primer to initiate minus-strand DNA synthesis and is responsible for DNA chain elongation. Traditional chain-terminating nucleos(t)ide analogues function by incorporating into the DNA, thereby inhibiting protein priming and/or DNA chain elongation.15 Nucleocapsids containing the newly synthesized relaxed circular DNA genome are either recycled in the nucleus to maintain the cccDNA pool or assembled with mature envelope proteins and secreted.14

Because cccDNA persists in the nucleus, a cure for chronic HBV infection will require elimination of cccDNA, which does not occur with current nucleos(t)ide analogues.13,16 To avoid the need for invasive liver biopsy, presence and transcriptional activity of intrahepatic cccDNA can be measured using surrogate biomarkers, including serum HBV RNA and hepatitis B core-related antigen (HBcrAg).13,17–20 The HBV RNA and components of HBcrAg are synthesized by transcription of cccDNA.13,16 Serum HBV RNA and HBcrAg both strongly correlate with intrahepatic cccDNA levels, making them useful serologic biomarkers in clinical research.17–20 On-treatment declines in serum HBV RNA and HBcrAg levels have been reported with nucleos(t)ide analogues or pegylated interferon-α, but detectable levels remain in most patients, indicating that cccDNA is functional and also not being cleared or eliminated.21–2 Anti-HBV agents with novel mechanisms of action (MOAs) that can complement the antiviral activity of traditional nucleos(t)ide analogues are needed to achieve a curative regimen for chronic HBV infection.9 Many HBV therapies from several drug classes are currently in development that have novel MOAs and target either host systems (ie, host-targeting antivirals) or viral systems (ie, direct-acting antivirals; Figure 1).24,25 Key areas of drug development include RNA interference gene silencers and antisense oligonucleotides, which target viral RNA; release inhibitors, which disrupt HBsAg production; and core protein allosteric modulators, which may cause aberrant capsid assembly, create empty capsids, or interfere with the delivery of relaxed circular DNA to the nucleus for cccDNA establishment or replenishment.26,27 Anti-HBV drugs with alternative targets or MOAs are also being explored, such as host system disruption (apoptosis induction), nuclear targets (CRISPR/Cas9), and immune modulation (toll-like receptor agonists). Combining complementary antiviral agents that target multiple HBV DNA replication mechanisms may ultimately lead to HBV curative regimens. Similar combination treatment strategies have been employed for other viruses, including hepatitis C virus and HIV-1.28,29

Unlike traditional chain-terminating nucleos(t)ide analogues, active site polymerase inhibitor nucleotides (ASPINs) function by noncompetitively distorting the HBV polymerase active site, completely inhibiting all polymerase functions.30 The novel ASPIN ATI-2173 is the only ASPIN in development as part of a potential curative regimen for chronic HBV infection. Here, we review the MOA and preclinical and clinical profiles of ASPINs, including ATI-2173 and clevudine.

**Mechanism of action of nucleos(t)ide analogues versus ASPINs**

After being phosphorylated into their active metabolites, anti-HBV nucleos(t)ide analogues compete with endogenous deoxynucleoside triphosphates for incorporation into the growing HBV DNA chain (Figure 2).15,31 Upon incorporation, lack of a 3′-hydroxyl group on the ribose of nucleos(t)ide analogues, except for entecavir, prevents binding of additional nucleotides, immediately terminating HBV DNA chain elongation (Figure 3). Entecavir, which
has a 3′-hydroxyl group, inhibits HBV DNA elongation a few nucleotides downstream of incorporation by increasing steric hinderance.\textsuperscript{15,32} Unique among nucleos(t)ide analogues, entecavir also inhibits protein priming by HBV polymerase through competition with deoxyguanosine triphosphate, the native nucleoside triphosphate that initiates protein priming.\textsuperscript{33,34}

Differentiated from nucleos(t)ide analogues by their unique MOA, ASPINs include clevudine, an unnatural L-nucleoside, and ATI-2173, a phosphoramidate nucleotide that delivers the same active metabolite as that of clevudine to the liver (Figure 2).\textsuperscript{30,36} Both clevudine and ATI-2173 are phosphorylated and function through the active metabolite clevudine-5′-triphosphate. Unlike chain-terminating

\textbf{Figure 1}. Pillars of HBV treatment.\textsuperscript{25} CpAM, core protein allosteric modulator; NAP, nucleic acid polymer; NTCP, sodium taurocholate cotransporting polypeptide; NUC, nucleos(t)ide analogue; TLR, toll-like receptor.

\begin{itemize}
  \item **NUCs and ASPINs**
    \begin{itemize}
      \item a. Tenofovir alafenamide
      \item b. Tenofovir disoproxil fumarate
      \item c. Entecavir
      \item d. ATI-2173
      \item e. Other
    \end{itemize}
  \item **Viral RNA targets**
    \begin{itemize}
      \item a. JNJ-3989 (J&J/Arrowhead)
      \item b. VIR-2218 (Vir)
      \item c. GSK3228836 (GSK)
      \item d. AB-729 (Arbutus)
      \item e. RG8345 (Roche)
      \item f. RNA destabilizers
    \end{itemize}
  \item **Release inhibitors**
    \begin{itemize}
      \item a. NAPs: REP 2139/REP 2165 (Replicor)
    \end{itemize}
  \item **CpAMs**
    \begin{itemize}
      \item a. Type 1:
        \begin{itemize}
          \item GLS4 (HEC Pharma)
          \item RO9389 (RG7907) (Roche)
        \end{itemize}
      \item b. Type 2:
        \begin{itemize}
          \item ABI-H0731 (Assembly)
          \item ABI-H3733 (Assembly)
          \item ABI-4334 (Assembly)
          \item JNJ-6379 (J&J)
          \item ALG-184 (Aligos)
          \item EDF-514 (Enanta)
        \end{itemize}
    \end{itemize}
  \item **Host systems**
    \begin{itemize}
      \item a. Farnesoid X receptor
      \item b. NTCP
      \item c. Apoptosis induction
    \end{itemize}
  \item **Nuclear targets**
    \begin{itemize}
      \item a. CRISPR/Cas9
      \item b. cccDNA histone modifier
      \item c. Endonuclease
    \end{itemize}
  \item **Immune modulators**
    \begin{itemize}
      \item a. Therapeutic vaccine
      \item b. Engineered T cells
      \item c. TLR agonist
      \item d. Interferon
      \item e. Checkpoint
    \end{itemize}
  \item **Extracellular targets**
    \begin{itemize}
      \item a. Anti-HBV antibodies:
        \begin{itemize}
          \item Monoclonal
          \item Bifunctional
        \end{itemize}
      \item b. Therapeutic vaccine
      \item c. Engineered T cells
    \end{itemize}
\end{itemize}
nucleos(t)ide analogues, clevudine-5′-triphosphate binds to
the HBV polymerase active site and induces conformational changes that prevent HBV DNA chain elongation noncompeti-
tively (Figure 3). Clevudine-5′-triphosphate also inhibits
HBV protein priming, but does so independently of the initiating
nucleotide and without being used as a substrate for initiation,
unlike entecavir. In addition, clevudine-5′-triphosphate inhi-
bits primer elongation, the second step of protein priming.
Third, clevudine noncompetitively inhibits general DNA syn-
thesis without being incorporated into the DNA, unlike tenofo-
vir. Thus, ASPINs can completely inhibit all HBV polymerase
functions, including protein priming, primer elongation, and
DNA synthesis, unlike traditional chain-terminating nucleos(t)
ide analogues, despite having generally similar structures;
however, ASPINs are L-nucleosides, unlike many nucleoside
analogues, which are D-nucleosides. Although there are
other L-nucleosides, they do not function as noncompetitive
inhibitors of HBV polymerase, and thus may point to a unique
structure-based explanation beyond solely the L-conformation
for clevudine-5′-triphosphate’s unique noncompetitive nature.

**Summary of clevudine preclinical and clinical program**

**Preclinical studies**

Clevudine demonstrated potent antiviral activity in cell-
based in vitro assays, with a 50% effective concentration
(EC\text{50}) of 0.1 \mu M in HepG2.2.15 and HepAD38 human
hepatoma cell lines. Clevudine has also demonstrated
potent antiviral activity against Epstein-Barr virus
(90% effective concentration, 5 \mu M) but has shown no
inhibition of HIV-1 or herpes simplex virus type 1 or 2 rep-
lication in vitro. Clevudine treatment in vitro resulted in
no cellular toxicity in multiple cell types, including
HepG2.2.15, MT-2, CEM, and H1 lymphoma cell lines
and bone marrow precursor cells. Clevudine also
had no adverse effect on mitochondrial function or mito-
chondrial DNA content in HepG2.2.15 cells in vitro. The
triphasphate did not inhibit mitochondrial DNA poly-
merase gamma. In HepG2 cells, clevudine demonstrated
in vitro potency that was similar to lamivudine and
greater than adefovir. In HepG2 cells, clevudine demonstrated
a synergistic effect on potency with lamivudine, adefovir, tenofovir,
and entecavir, whereas the effect was antagonistic when combined
with telbivudine, another L-thymidine analogue.

The antiviral activity of clevudine in vitro and in vivo
has been evaluated in an acute duck HBV (DHBV) infection
model. Similar to results in human hepatoma cell
lines, clevudine demonstrated a 50% inhibitory concentra-
tion (IC\text{50}) against DHBV of 0.1 \mu M, with no signs of cyto-
toxicity in primary duck hepatocytes. In ducklings treated
3 days following acute HBV infection, slightly decreased
viremia was observed with once-daily clevudine 40 and 80 mg/kg for 7 days (mean [standard deviation] DHBV, 3.90 [0.76] and 4.08 [0.78] log10 pg/mL, respectively) compared with control-treated ducklings (mean [standard deviation] DHBV, 4.10 [0.81] log10 pg/mL); viral replication relapsed in all groups following treatment cessation.44 In a similarly designed study, once-daily clevudine 40 mg/kg for 5 and 8 days resulted in 55% and 72% inhibition in peak DHBV viremia, respectively, relative to control-treated ducklings.43 Transient viral rebound was observed following treatment in ducklings that received clevudine for 5 days, whereas those treated for 8 days maintained viral suppression throughout the 2-week follow-up period.

A woodchuck model of chronic HBV infection has been used to assess the antiviral activity of clevudine against woodchuck hepatitis virus (WHV).45,46 Once-daily clevudine for 4 weeks resulted in a dose-dependent decline in serum WHV DNA across a dose range of 0.1 to 10 mg/kg, with mean decreases of 1.1 to 8.2 WHV genome equivalents/mL observed after 4 weeks.45 Following treatment cessation, viremia rebounded in a generally dose-dependent manner, returning to pretreatment levels by 2 weeks off treatment with clevudine 0.3 mg/kg and by 8 to 10 weeks off treatment with clevudine 1.0 or 3.0 mg/kg. Remarkably, all 4 woodchucks treated with clevudine 10 mg/kg maintained viral suppression through 6 weeks off treatment, with 2 animals not returning to pretreatment HBV DNA levels during the 12-week follow-up period. Clevudine treatment also resulted in a dose-dependent reduction in serum WHV surface antigen (WHsAg) levels, with a 4-fold average reduction at the end of treatment, 20-fold reduction at 4 weeks off treatment, and >5-fold reduction at 12 weeks off treatment in the 10-mg/kg group. After 4 weeks of clevudine 10 mg/kg, reductions from pretreatment levels were observed in many intrahepatic markers, including WHV DNA replication intermediates, intracellular WHV RNA, WHV core antigen, and cccDNA. At 12 weeks following treatment discontinuation, WHV replication intermediates remained below pretreatment levels in the clevudine 10-mg/kg group. The timing and dose-dependency of WHsAg declines, intrahepatic cccDNA, and prolonged off-treatment reductions in viral

Figure 3. Mechanism of action of (a) nucleos(t)ide analogues and (b) ASPINs. DR1, direct repeat 1; NRTI, nucleos(t)ide reverse transcriptase inhibitor; RNase H, ribonuclease H; RT, reverse transcription domain; TP, terminal protein domain.
replication markers at the highest clevudine dose suggest that clevudine may have reduced WHV polymerase activity to below the level needed to maintain steady-state hepatic WHV cccDNA levels. Long-term treatment with clevudine 10 mg/kg also demonstrated antiviral activity against chronic WHV, with >6-log_{10} declines in serum HBV DNA observed in all treated animals after 32 weeks of treatment. After treatment discontinuation, viral suppression was maintained in 6 of 8 woodchucks throughout the 72-week follow-up period. Overall, clevudine demonstrated potent antiviral activity in woodchucks with chronic WHV infection, with sustained viral suppression observed for several months off treatment in many animals, likely because of cccDNA reductions.

A study performed in 2001 evaluated the kinetics of cccDNA loss in primary woodchuck hepatocyte cultures and liver biopsies from woodchucks with chronic WHV infection receiving clevudine 10 mg/kg once daily. After 6 weeks of treatment, intrahepatic cccDNA declined to between 13% and 44% of pretreatment levels; however, cccDNA decreased much more slowly than WHV DNA replication intermediates, which were between 1.8% and 6% of pretreatment levels at the same time point. At 6 to 15 weeks of treatment, intrahepatic cccDNA levels were 13% to 46% of pretreatment levels in 2 of 3 woodchucks and 9% in the remaining woodchuck. By 30 weeks of treatment, intrahepatic cccDNA declined in all 3 woodchucks to between 1.2% and 5.4% of pretreatment levels. Before treatment, the average cccDNA copy number across all 3 woodchucks was 19 to 63 copies/cell, which decreased to <1 copy/cell in each woodchuck by 30 weeks of treatment, supporting a mechanism of hepatocyte turnover. Intrahepatic cccDNA was estimated to have a first-order decay half-life of 33 to 50 days, consistent with the observed 32-day half-life for cccDNA loss in primary woodchuck hepatocyte cultures. Another study of woodchucks with chronic HBV infection also reported declines in average cccDNA copy number from 20 copies/cell before treatment to 2 copies/cell after 4 weeks of treatment, with estimated half-lives that were comparable between cccDNA and WHV-infected hepatocytes. The prolonged antiviral effect following clevudine treatment may be related to potent suppression of viral replication, allowing for natural clearance of cccDNA with endonucleases or via turnover of hepatocytes that contain inactive cccDNA, rather than via a direct mechanism acting directly on cccDNA reduction. Overall, these results suggest that the observed cccDNA decrease and potential loss with clevudine treatment may be because of the replacement of infected hepatocytes.

The activity of clevudine against resistance-associated mutations of other anti-HBV agents was evaluated to potentially predict the clinical resistance profile of clevudine. In a preclinical study, clevudine showed cross-resistance in vitro to lamivudine resistance–associated mutations L180M and L180M + M204V (IC_{50}, >100 μM for each) but not M204V alone (IC_{50}, 1.5 μM). Clevudine also demonstrated in vitro cross-resistance to mutations that confer resistance to adeovir, including N236T (mean EC_{50} fold resistance, 7.4-fold change) as well as A181V/T alone and combined with N236T (mean EC_{50} fold resistance, 117- to >191-fold change).

Clinical program

A summary of phase 2 and 3 clinical trials evaluating once-daily clevudine monotherapy in nucleos(t)ide therapy-naive subjects with chronic HBV infection is presented in the Table 1. An initial phase 2 study evaluated the antiviral efficacy of once-daily clevudine for 4 weeks at doses of 10, 50, 100, and 200 mg in subjects with chronic HBV infection with or without hepatitis B e antigen (HBeAg). After 4 weeks of treatment, each clevudine dose decreased serum HBV DNA, with median declines from baseline of 2.5 to 3.0 log_{10} copies/mL. Reductions in HBV DNA persisted through 24 weeks off treatment in each group (median change from baseline, −1.2 to −2.7 log_{10} copies/mL). Median alanine aminotransferase (ALT) levels were decreased from baseline in the 10-, 50-, and 200-mg groups after 4 weeks of treatment and reduced in all groups after 24 weeks off treatment, with 50% to 100% of subjects across all doses achieving ALT normalization at 24 weeks off treatment. Through the off-treatment follow-up period, HBeAg loss and seroconversion occurred in 22% and 11% of subjects, respectively, across all clevudine doses.

The extent and durability of the antiviral response following 12 weeks of clevudine treatment were assessed in a subsequent phase 2 study in HBeAg-positive subjects (Table 1). After 12 weeks of treatment, median serum HBV DNA decreased ≥4.4 log_{10} copies/mL from baseline with clevudine 30 or 50 mg and 0.2 log_{10} copies/mL with placebo. Sustained HBV DNA reductions were observed through 24 weeks after stopping clevudine treatment, with median decreases of 2.3 and 1.4 log_{10} copies/mL in the 30- and 50-mg groups, respectively. Median serum ALT levels in both groups were substantially reduced from baseline by the end of treatment; ≥63% of subjects receiving clevudine had normalized ALT values at the end of the off-treatment follow-up period, with only 1 subject receiving clevudine experiencing an off-treatment ALT flare. In both clevudine groups, rates of HBeAg loss and seroconversion were 19% and 16%, respectively, similar to the rates observed in the placebo group (20% and 16%, respectively).

The potential role of drug accumulation in the extended viral suppression of clevudine was investigated in a pharmacokinetics (PK) study in subjects with or without HBeAg treated with clevudine 10, 30, or 50 mg for 12 weeks (Table 1). Similar to the previous study, 12
| Study     | Clinical Trials.gov identifier | Inclusion criteria | Dose, mg | n | Treatment duration, weeks | Normal ALT, % | Median change in HBV DNA, log_{10} copies/mL | Follow-up duration, weeks | Normal ALT, % | Median change in HBV DNA, log_{10} copies/mL |
|-----------|-------------------------------|--------------------|----------|---|--------------------------|---------------|---------------------------------------------|--------------------------|---------------|---------------------------------------------|
| **Phase 2** |                               |                    |          |   |                          |               |                                             |                          |               |                                             |
| Marcellin 2004 | —                             | HBeAg +/− | 10      | 5 | 4                        | NR            | −2.5                                        | 24                       | 50            | −1.2                                        |
|            |                               | HBV DNA >3 × 10^6 copies/mL | 50      | 10 |                        | NR            | −2.7                                        | 60                       | 70            | −1.4                                        |
|            |                               | HBV DNA >3 × 10^6 copies/mL | 100     | 10 |                        | NR            | −3.0                                        | 70                       | 70            | −2.7                                        |
|            |                               |                    | 200     | 7  |                        | NR            | −2.6                                        | 100                      | 100           | −1.7                                        |
| Lee 2006 | NCT00305019                   | HBeAg + | Placebo | 32 | 12                       | 7             | −0.2                                        | 24                       | 12            | −1.0                                        |
|            |                               | HBV DNA ≥3 × 10^6 copies/mL | 30      | 32 |                       | 53            | −4.5                                        | 71                       | 71            | −2.3                                        |
|            |                               | HBV DNA ≥3 × 10^6 copies/mL | 50      | 32 |                       | 55            | −4.5                                        | 63                       | 63            | −1.4                                        |
| Lim 2008 | NCT00044135                   | HBeAg +/− |                  | 10  | 10                       | 40            | −3.2                                        | 24                       | NR^a         | −0.8                                        |
|           |                               | HBV DNA ≥3 × 10^6 copies/mL | 30      | 11 |                       | 60            | −3.7                                        | 71                       | NR^a         | −1.4                                        |
|           |                               | HBV DNA ≥3 × 10^6 copies/mL | 50      | 10 |                       | 20            | −4.2                                        | 33                       | 33            | −0.9                                        |
| **Phase 3** |                               |                    |          |   |                          |               |                                             |                          |               |                                             |
| Yoo 2007a | NCT00313287                   | HBeAg + | Placebo | 30  | 61                       | 18            | −0.3                                        | 24                       | 28            | −0.7                                        |
|           |                               | HBV DNA >6 log_{10} copies/mL | 182     | 68 |                       | −5.1          |                                             | 61                       | 61            | −2.0                                        |
| Yoo 2007b | NCT00313274                   | HBeAg + | Placebo | 30  | 23                       | 33            | −0.5                                        | 24                       | 29            | −0.7                                        |
|           |                               | HBV DNA >1 × 10^5 copies/mL | 63      | 75 |                       | −4.3          |                                             | 71                       | 71            | −3.1                                        |

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NR, not reported.

^aPercentages were not reported for the 10- and 30-mg groups. Median ALT values were within the normal range at the end of follow-up in both groups.
weeks of clevudine treatment resulted in dose-dependent decreases from baseline in median HBV DNA of 3.2 to 4.2 $\log_{10}$ copies/mL, with sustained antiviral response observed through the 24-week off-treatment follow-up period. The proportion of subjects with normal ALT levels increased over time in the 30-mg group and decreased or remained stable in the 10- and 50-mg groups. At steady state at week 12, clevudine demonstrated dose-proportional PK, no evidence of accumulation, and a mean plasma half-life of $\sim 70$ h. Dose-response analysis showed minimal benefit of increasing the clevudine dose from 30 to 50 mg, supporting the selection of clevudine 30 mg for further clinical development.

Two phase 3 studies further assessed the efficacy and sustained antiviral effect of clevudine 30 mg compared with placebo following 24 weeks of treatment with a 24-week off-treatment follow-up period in HBeAg-positive and HBeAg-negative subjects with chronic HBV infection (Table 1). After 24 weeks of treatment, median HBV DNA decreases from baseline of 5.1 and 4.3 $\log_{10}$ copies/mL were observed in clevudine-treated HBeAg-positive and HBeAg-negative subjects, respectively, compared with $<0.5$-$\log_{10}$ copies/mL declines in either placebo group. The antiviral response was sustained off treatment in both studies, with HBV DNA declines from baseline of 2.0 and 3.1 $\log_{10}$ copies/mL, respectively, after 24 weeks of follow-up. In both trials, ALT normalization rates were $\geq 68\%$ at the end of treatment and $\geq 61\%$ after the 24-week follow-up period. After both the treatment and follow-up periods, rates of HBeAg loss and seroconversion in HBeAg-positive subjects were similar between the clevudine and placebo groups. Overall, these pivotal studies demonstrated that clevudine was a potent antiviral agent with sustained off-treatment viral suppression in both HBeAg-positive and HBeAg-negative subjects with chronic HBV infection.

Viral breakthrough and clevudine resistance were not detected in either phase 3 clinical trial of clevudine monotherapy. However, viral breakthrough with resistance mutations has been detected in subjects after $>8$ months of clevudine treatment, with the reverse transcriptase mutation M204I being the most common. The M204I mutation emerged alone and in combination with additional reverse transcriptase mutations in viral isolates, including L229V + N238H + K333N, V207I + N238K + L269I, and L129M + V173L + H337N. Each of these viral isolates demonstrated in vitro resistance to clevudine (average IC$_{50}$, $>100$ µM for each) relative to wild-type isolates (average IC$_{50}$, 0.9 µM). In addition, each M204I-containing isolate demonstrated in vitro resistance to lamivudine but remained susceptible to adefovir and TDF. One isolate containing M204I + V207I + N238H + Q267L also demonstrated in vitro resistance to entecavir.

Clevudine was generally safe and well tolerated at all doses in each phase 2 and 3 study. Drug-related serious adverse events (AEs) were rare overall, with no clevudine dose-related safety concerns emerging in the phase 2 studies. In the phase 3 studies, overall AE profiles were generally similar between the clevudine and placebo groups, with ALT elevations $\geq 5$ times the upper limit of normal and exacerbations of HBV during treatment occurring in more subjects in the placebo vs clevudine groups.

No muscle-related AEs were noted in subjects who received clevudine for 6 months in either phase 3 study. However, after approval in South Korea, a small number of subjects treated with clevudine 30 mg for 9 to 13 months developed myopathic symptoms, resulting in cessation of further clevudine clinical development outside of South Korea and the Philippines in 2009.

Subjects presenting with myopathy reported impairment in completion of daily physical activities and experienced generalized muscle weakness, difficulty walking and/or climbing stairs, and difficulty standing from a supine position. Later characterization of myopathy associated with clevudine use in 36 Korean subjects revealed high ratios of aspartate aminotransferase to ALT and elevated mean serum levels of creatine kinase, lactate dehydrogenase, and lactic acid. Muscle biopsies of 23 subjects diagnosed with myopathy during clevudine therapy revealed histologic characteristics including degeneration of muscle fibers, type II fiber atrophy, lymphocyte and fat infiltration, and ragged red fibers in 91% of cases. Electron microscopy of muscle tissue biopsies confirmed enlarged mitochondria with abnormal inclusion bodies in those subjects. The incidence of myopathy after clevudine therapy reported in the literature generally ranges from 1.7% to 3.9%; however, in all studies examining clevudine-associated myopathy, myopathic symptoms and clinical features reversed upon termination of clevudine therapy.

Clevudine-associated mitochondrial toxicity was unexpected, as early characterization showed that mitochondrial DNA polymerase was not a target of clevudine and no mitochondrial toxicity was observed with clevudine treatment in vitro. While the initial description of clevudine-induced myopathy did not find any evidence of mitochondrial damage, subsequent reports linked myopathy to mitochondrial abnormalities. The mitochondrial isof orm of thymidine kinase (TK2) is capable of catalyzing clevudine phosphorylation to its $'S'$-monophosphate form. The TK2 enzyme is critically involved in mitochondrial DNA synthesis; deficiencies of TK2 are associated with mitochondrial DNA depletion and subsequent myopathy because of the combination of a high requirement for mitochondrial-encoded proteins, such as cytochrome c oxidase, and low basal TK2 activity in muscle tissue, making it sensitive to inhibition. As previously suggested, competition between thymidine antiviral nucleos(t)ide analogues and deoxynucleoside substrates for TK2 may deplete mitochondrial DNA, resulting in
mitochondrial toxicity. Although an unconfirmed hypothesis, the rare skeletal myopathy cases observed with long-term clevudine treatment may occur via a mitochondrial TK2-mediated mechanism resulting from systemic circulation of unphosphorylated clevudine, similar to other thymidine antiviral nucleos(t)ides, such as zidovudine.

**ATI-2173: a next-generation ASPIN**

**Preclinical characterization**

The novel next-generation ASPIN ATI-2173 is structurally similar to clevudine, with the addition of a phosphoramide modification at the 5’ hydroxyl group, and generates the same active metabolite as that of clevudine. ATI-2173 is proposed to undergo an initial hydrolysis step catalyzed by human cathepsin A and/or carboxylesterase-1 before non-enzymatic nucleophilic attack and hydrolysis reactions yield the stable metabolite M1. The M1 metabolite is then cleaved by histidine triad nucleotide-binding protein-1 to generate clevudine-5’-monophosphate, which is then further phosphorylated to yield the active clevudine-5’-triphosphate. Thus, the phosphoramide modification allows ATI-2173 metabolism to bypass the first phosphorylation step of clevudine, targeting clevudine-5’-monophosphate directly to the liver and reducing systemic exposure to clevudine. In addition, avoidance of the first phosphorylation step, which may use the TK2 enzyme in some tissues, could reduce the risk of mitochondrial DNA depletion and injury that was associated with skeletal myopathy in some clevudine-treated subjects.

ATI-2173 showed potent anti-HBV activity in vitro, with EC50 values of 0.26 μM in HepG2.2.15 cells and 1.31 nM in primary human hepatocytes. The antiviral activity of ATI-2173 in HepG2.2.15 cells was similar to that of both clevudine and entecavir (EC50, 0.1 μM and 0.6 nM, respectively). ATI-2173 specifically inhibited HBV replication across genotypes A through H (EC50, 212–718 nM), with no efficacy observed against hepatitis C virus, HIV-1, influenza virus, respiratory syncytial virus, or herpes simplex virus type 1. In addition, ATI-2173 did not induce cytotoxicity or apparent mitochondrial toxicity in any cell type evaluated, including mitochondrial DNA content, mtDNA copy number, oxygen consumption, or glucose utilization/lactate acid production (data not published). ATI-2173 demonstrated in vitro cross-resistance with mutations associated with resistance to lamivudine (M204I, V173L + M204I, and L180M + M204V), entecavir (S202G + M204I and S202G + M204I + M250V), and adefovir (A181V); no cross-resistance was observed between ATI-2173 and the capsid inhibitors GLS4 or AT-130. Similar to results observed with clevudine, the lamivudine resistance-associated mutation M204V was susceptible to ATI-2173.

In primary human hepatocytes, ATI-2173 exhibited additive anti-HBV activity when combined with lamivudine, TDF, or the capsid inhibitor GLS4, and synergistic activity when combined with entecavir or adefovir. The efficacy observed between ATI-2173 and other anti-HBV agents suggests that they could be successfully paired in the clinic. Combining ATI-2173 and a nucleotide with a high barrier to resistance, such as TDF, is a reasonable strategy based on these data. The high barrier to resistance of TDF may benefit the regimen by protecting against the known resistance mutations for ATI-2173 and clevudine while targeting a different replication MOA.

To determine liver targeting of ATI-2173, PK analyses were conducted in Sprague-Dawley rats administered a single equimolar oral dose of ATI-2173 50 mg/kg or clevudine 25 mg/kg. ATI-2173 administration resulted in ~5-fold reductions in maximum plasma clevudine concentrations while maintaining similar concentrations of active clevudine-5’-triphosphate in the liver compared with clevudine dosing, confirming liver targeting of ATI-2173. In addition, ATI-2173 dosing resulted in approximately 10- and 4-fold reductions in maximum skeletal muscle concentrations of clevudine and clevudine-5’-triphosphate, respectively, compared with clevudine dosing. Portal vein–cannulated cynomolgus monkeys administered a single oral dose of ATI-2173 20 mg/kg demonstrated an 82% hepatic extraction ratio, further supporting liver targeting of ATI-2173.

**Early clinical program**

Because of its potent anti-HBV activity and favorable PK profile in preclinical studies, ATI-2173 is currently in clinical development as a potential component of a curative regimen for chronic HBV infection. The safety, tolerability, and PK of ATI-2173 in healthy adults were initially assessed in a phase 1a trial as part of the ANTT101 study (ClinicalTrials.gov identifier: NCT04248426), which enrolled healthy adults to receive ATI-2173 doses of 10, 25, 50, or 100 mg or placebo once daily for 14 days. Of 24 subjects who received ATI-2173, the most common treatment-emergent AE was headache (n = 8; 33%); no serious AEs or dose-limiting toxicities were observed. Following single and repeated dosing, maximum plasma ATI-2173 concentrations were achieved at <1 h post-dose and then rapidly declined. ATI-2173 exposure at the 10- and 25-mg doses was approximately dose proportional and approached saturation at the 50- and 100-mg doses. Of note, mean maximum plasma clevudine concentrations with all ATI-2173 doses were lower than the steady-state minimum plasma clevudine concentration following dosing with the 30-mg marketed dose of clevudine. Overall, ATI-2173 demonstrated dose-proportional PK and significantly reduced systemic clevudine exposure,
with no safety or tolerability concerns in healthy subjects,\textsuperscript{67} supporting its continued clinical development in subjects with chronic HBV infection.

The antiviral efficacy of ATI-2173 was initially evaluated in the phase 1b portion of the ANTT101 study, in which treatment-naive adults with chronic HBV infection received once-daily ATI-2173 10, 25, or 50 mg or placebo for 28 days (Figure 4).\textsuperscript{68} After 28 days of treatment, mean HBV DNA decreased from baseline by 2.7 to 2.8 log\textsubscript{10} IU/mL with all ATI-2173 doses and increased by 0.2 log\textsubscript{10} IU/mL with placebo, similar to the antiviral effect observed after 4 weeks of clevudine treatment (2.5–3.0 log\textsubscript{10} IU/mL reduction).\textsuperscript{59} Surrogate cccDNA biomarkers were decreased from baseline with ATI-2173 25 or 50 mg, with mean decreases of 0.6 log\textsubscript{10} copies/mL in HBV RNA and 0.2 log\textsubscript{10} U/mL in HBcrAg at the end of treatment. Sustained off-treatment viral load responses were observed in the ATI-2173 25- and 50-mg groups, with 1 of 9 subjects (11%) who achieved undetectable HBV DNA at the end of treatment maintaining undetectable HBV DNA after 24 weeks of follow-up. Levels of the cccDNA biomarker HBV RNA also demonstrated sustained suppression through 24 weeks off treatment in the ATI-2173 25- and 50-mg groups. The safety and PK of ATI-2173 were consistent with results in healthy subjects without HBV infection, with no dose-related AEs reported and significantly reduced systemic clevudine exposure observed. Mean plasma clevudine 24-h area under the concentration-time curve after daily dosing with ATI-2173 10, 25, and 50 mg for 28 days was 5%, 13%, and 34%, respectively, of the plasma exposure observed at steady state with clevudine 30 mg.\textsuperscript{51} Overall, ATI-2173 monotherapy demonstrated potent anti-HBV activity with declines in HBV RNA and HBcrAg levels and prolonged off-treatment viral load responses in subjects with chronic HBV infection, which may be suggestive that ATI-2173 is capable of affecting cccDNA.

Based on the efficacy and safety demonstrated in phase 1 studies, clinical development of ATI-2173 is ongoing. The 25- and 50-mg doses of ATI-2173 have been advanced into a phase 2a study in combination with TDF to evaluate safety and efficacy in subjects with chronic HBV infection (ClinicalTrials.gov identifier: NCT04847440; Figure 5). In this randomized, double-blind, placebo-controlled study, subjects will receive ATI-2173 + TDF or placebo + TDF for 12 weeks, with continued off-treatment follow-up for 24 weeks. Results from this study will inform the design of larger phase 2 trials including combination therapy studies evaluating potential curative regimens containing ATI-2173 + TDF and possibly agent(s) from other drug classes.

**Conclusions**

The ASPINs ATI-2173 and clevudine disrupt HBV replication by completely and noncompetitively inhibiting all HBV polymerase functions,\textsuperscript{30,34,37} a unique feature compared with traditional chain-terminating nucleos(t)ides, exemplified by the reduction in cccDNA biomarkers and prolonged off-treatment HBV suppression in clinical studies.\textsuperscript{49–53,68} In preclinical animal models and phase 1a/b clinical trials, the liver-targeted, next-generation ASPIN ATI-2173 significantly decreased systemic exposure to unphosphorylated clevudine, potentially reducing the risk of myopathy observed with long-term clevudine treatment.\textsuperscript{30,67,68} In addition, ATI-2173 demonstrated a favorable safety profile in phase 1a/b clinical trials, with no dose-related AEs reported.\textsuperscript{67,68} Therefore, the sustained off-treatment responses and favorable PK profile of ATI-2173 make this next-generation ASPIN a promising agent as a component of treatment for chronic HBV infection.

**Figure 4.** Summary of results from a phase 1b clinical trial evaluating once-daily ATI-2173 monotherapy in treatment-naive subjects with chronic HBV infection.
of potential curative treatment regimens for chronic HBV infection.

A curative regimen for chronic HBV infection will require elimination of cccDNA. Although cccDNA persists in infected cells following treatment with current nucleos(t)ide analogues, ASPINs have demonstrated the potential ability to reduce cccDNA biomarkers. In a woodchuck HBV model, cccDNA reductions were observed after 4 to 6 weeks of clevudine treatment, with one study demonstrating 95% to 99% cccDNA loss after 30 weeks of clevudine treatment. In a phase 1b study in subjects with chronic HBV infection, serum HBV RNA and HBcrAg, both serologic biomarkers for cccDNA, were decreased from baseline after 28 days of ATI-2173 treatment. A 2021 retrospective study in subjects with chronic HBV infection estimated the half-life of cccDNA to be 5.6 to 21.7 weeks, a substantially faster turnover rate than the previous prediction of 10 to 20 years, and further confirmatory studies are needed. The reduction of cccDNA biomarker levels, which suggests a reduction in either cccDNA copy number or transcriptional activity, after 28 days of ATI-2173 treatment is promising for its continued development. Overall, these results suggest that ASPINs may potently suppress viral replication, allowing for productive clearance of HBV-infected cells and effectively leading to reduction or possible elimination of intrahepatic cccDNA, and would be of value as part of a curative combination treatment regimen. Importantly, while traditional chain-terminating nucleos(t)ides are incorporated into and consumed in the process of replication of de novo genome, ASPINs bind to and distort HBV polymerase, which may lead to more efficient inhibition.

Combining complementary antiviral agents to target multiple HBV DNA replication mechanisms may lead to a treatment regimen for HBV cure. In primary human hepatocytes, ATI-2173 demonstrated additive-to-synergistic activity with nucleos(t)ide analogues, including TDF and entecavir, and additive activity with the capsid inhibitor GLS-4. Thus, combination regimens of ATI-2173 and chain-terminating nucleos(t)ides will target HBV polymerase via multiple mechanisms, potentially resulting in increased anti-HBV potency, which may further improve cccDNA reductions, in addition to preventing viral breakthrough and emergence of drug resistance. Supporting this approach, combination therapy of clevudine 20 mg and adefovir effectively maintained HBV suppression in the absence of drug resistance during a 96-week treatment period. Whether the potential benefits of combining an ASPIN, such as ATI-2173, and a chain-terminating nucleotide with a high barrier to resistance, such as TDF, extends to HBV treatment in humans is currently being evaluated in a phase 2a study. Overall, the potent HBV polymerase inhibition, prolonged HBV suppression, and cccDNA biomarker reductions associated with ASPIN monotherapy make ASPINs a promising agent for inclusion in a finite combination regimen to achieve HBV cure.

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KS is an employee of Antios Therapeutics and may hold company stock or stock options.

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References
1. Hepatitis B Foundation. Hepatitis B fast facts, https://www.hepb.org/assets/Uploads/hep-b-fast-facts.pdf (accessed 30 June 2021).
2. Hepatitis B Foundation. Hepatitis B facts and figures, https://www.hepb.org/what-is-hepatitis-b/what-is-hepb/facts-and-figures/ (accessed 9 July 2021).
3. Hyams KC. Risks of chronicity following acute hepatitis B virus infection: a review. Clin Infect Dis 1995; 20: 992–1000.
4. Tang LSY, Covert E, Wilson E, et al. Chronic hepatitis B infection: a review. JAMA 2018; 319: 1802–1813.
5. World Health Organization. Global progress report on HIV, viral hepatitis and sexually transmitted infections. 2021. https://www.who.int/publications/i/item/9789240027077 (2021, accessed 22 September 2021).
6. Wong RJ, Brosgrl CL, Welch S, et al. An updated assessment of chronic hepatitis B prevalence among foreign-born persons living in the United States. Hepatology 2021; 72: 607–626.
7. World Health Organization. Global hepatitis report. 2017. https://www.who.int/publications/i/item/global-hepatitis-report-2017 (2017, accessed 29 June 2021).
8. EASL. 2017 Clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol 2017; 67: 370–398.
9. Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 2016; 10: 1–98.
10. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 2018; 67: 1560–1599.
11. Tout I, Loureiro D, Mansouri A, et al. Hepatitis B surface antigen seroc clearance: immune mechanisms, clinical impact, importance for drug development. J Hepatol 2020; 73: 409–422.
12. Seto WK, Lo YR, Pawlotsky JM, et al. Chronic hepatitis B virus infection. Lancet 2018; 392: 2313–2324.
13. Asselah T, Loureiro D, Boyer N, et al. Targets and future direct-acting antiviral approaches to achieve hepatitis B virus cure. Lancet Gastroenterol Hepatol 2019; 4: 883–892.
14. Tsukuda S and Watashi K. Hepatitis B virus biology and life cycle. Antiviral Res 2020; 182: 104925.
15. Gupta N, Goyal M, Wu CH, et al. The molecular and structural basis of HBV-resistance to nucleos(t)ide analogs. J Clin Transl Hepatol 2014; 2: 202–211.
16. Nassal M. HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. Gut 2015; 64: 1972–1984.
17. Giersch K, Allweiss L, Volz T, et al. Serum HBV pgRNA as a clinical marker for cccDNA activity. J Hepatol 2017; 66: 460–462.
18. Huang Q, Zhou B, Cai D, et al. Rapid turnover of hepatitis B virus covalently closed circular DNA indicated by monitoring emergence and reversion of signature-mutation in treated chronic hepatitis B patients. Hepatology 2021; 73: 41–52.
19. Wong DK, Tanaka Y, Lai CL, et al. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. J Clin Microbiol 2007; 45: 3942–3947.
20. Suzuki F, Miyakoshi H, Kobayashi M, et al. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. J Med Virol 2009; 81: 27–33.
21. Beudeker BJ, Groothuismink ZM, de Man RA, et al. Hepatitis B core-related antigen levels predict pegylated interferon-α therapy response in HBeAg-positive chronic hepatitis B. Antivir Ther 2020; 25: 217–222.
22. Vachon A and Osiowy C. Novel biomarkers of hepatitis B virus and their use in chronic hepatitis B patient management. Viruses 2021; 13: 951.
23. Zhang H, Wang F, Zhu X, et al. Antiviral activity and pharmacokinetics of the hepatitis B virus (HBV) capsid assembly modulator GLS4 in patients with chronic HBV infection. Clin Infect Dis 2021; 73: 175–182.
24. Lopatin U. Drugs in the pipeline for HBV. Clin Liver Dis 2019; 23: 535–555.
25. Hepatitis B Foundation. Drug watch: compounds in development for chronic hepatitis B, https://www.hepb.org/treatment-and-management/drug-watch/ (updated 2022, accessed 9 July 2021).
26. Guo F, Zhao Q, Sheraz M, et al. HBV Core protein allosteric modulators differentially alter cccDNA biosynthesis from de novo infection and intracellular amplification pathways. PLoS Pathog 2017; 13: e1006658.
27. Mak LY, Seto WK and Yuen MF. Novel antivirals in clinical development for chronic hepatitis B infection. Viruses 2021; 13: 1169. DOI: 10.3390/v13061169
28. The American Association for the Study of Liver Diseases and the Infectious Diseases Society of America. HCV guidance: recommendations for testing, managing, and treating hepatitis C. http://hcvguidelines.org (2020, accessed 9 August 2021).

29. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in adults and adolescents with HIV, https://clinicalinfo.hiv.gov/sites/default/files/guidelines/archive/AdultandAdolescentGL_2021_08_16.pdf (2021, accessed 9 August 2021).

30. Squires KE, Mayers DL, Bluemling GR, et al. ATI-2173, a novel liver-targeted non-chain-terminating nucleotide for hepatitis B virus cure regimens. Antimicrob Agents Chemother 2020; 64: e00836–20.

31. Pastuch-Gawołek G, Gillner D, Król E, et al. Selected nucleos(t)ide-based prescribed drugs and their multi-target activity. Eur J Pharmacol 2019; 865: 172747.

32. Langley DR, Walsh AW, Baldick CJ, et al. Inhibition of hepatitis B virus polymerase by entecavir. J Virol 2007; 81: 3992–4001.

33. Seifer M, Hamatake RK, Colombo RJ, et al. In vitro inhibition of hepadnavirus polymerases by the triphosphates of BMS-200475 and lobicavir. Antimicrob Agents Chemother 1998; 42: 3200–3208.

34. Jones SA, Murakami E, Delaney W, et al. Noncompetitive inhibition of hepatitis B virus reverse transcriptase protein priming and DNA synthesis by the nucleoside analog clevudine. Antimicrob Agents Chemother 2013; 57: 4181–4189.

35. Kim S, Chen J, Cheng T, et al. Pubchem in 2021: new data content and improved web interfaces. Nucleic Acids Res 2021; 49: D1388–d1395.

36. Balakrishna Pai S, Liu SH, Zhu YL, et al. Inhibition of hepatitis B virus by a novel L-nucleoside, 2’-fluoro-5-methyl-beta-L-arabinofuranosyl uracil. Antimicrob Agents Chemother 1996; 40: 380–386.

37. Chong Y and Chu CK. Understanding the unique mechanism of L-FMAU (clevudine) against hepatitis B virus: molecular dynamics studies. Bioorg Med Chem Lett 2002; 12: 3459–3462.

38. Chu CK, Ma T, Shanmunathan K, et al. Use of 2’-fluoro-5-methyl-beta-L-arabinofuranosyluracil as a novel antiviral agent for hepatitis B virus and Epstein-Barr virus. Antimicrob Agents Chemother 1995; 39: 979–981.

39. Niu C, Bao H, Tolstykh T, et al. Evaluation of the in vitro anti-HBV activity of clevudine in combination with other nucleoside/nucleotide inhibitors. Antivir Ther 2010; 15: 401–412.

40. Yao GQ, Liu SH, Chou E, et al. Inhibition of Epstein-Barr virus replication by a novel L-nucleoside, 2’-fluoro-5-methyl-beta-L-arabinofuranosyluracil. Biochem Pharmacol 1996; 51: 941–947.

41. Painter GR, Trost LC, Blum MR, et al. Chapter 24 – clevudine: a novel 1-beta-L nucleoside analogue in clinical development for the treatment of HBV infection. In: Schinazi RF, Sommadossi J-P and Rice CM (eds) Frontiers in viral hepatitis. Amsterdam: Elsevier, 2003, pp. 281–300.

42. Chin R, Shaw T, Torresi J, et al. In vitro susceptibilities of wild-type or drug-resistant hepatitis B virus to (-)-beta-D-2,6-diaminopurine dioxolane and 2’-fluoro-5-methyl-beta-L-arabinofuranosyluracil. Antimicrob Agents Chemother 2001; 45: 2495–2501.

43. Aguesse-Germon S, Liu SH, Chevallier M, et al. Inhibitory effect of 2’-fluoro-5-methyl-beta-L-arabinofuranosyl-uracil on duck hepatitis B virus replication. Antimicrob Agents Chemother 1998; 42: 369–376.

44. Seignères B, Martin P, Werle B, et al. Effects of pyrimidine and purine analog combinations in the duck hepatitis B virus infection model. Antimicrob Agents Chemother 2003; 47: 1842–1852.

45. Peek SF, Cote PJ, Jacob JR, et al. Antiviral activity of clevudine [L-FMAU, (1-(2-fluoro-5-methyl-beta, L-arabinofuranosyl) uracil)] against woodchuck hepatitis virus replication and gene expression in chronically infected woodchucks (marmota monax). Hepatology 2001; 33: 254–266.

46. Korba BE, Cote PJ, Menne S, et al. Clevudine therapy with vaccine inhibits progression of chronic hepatitis and delays onset of hepatocellular carcinoma in chronic woodchuck hepatitis virus infection. Antivir Ther 2004; 9: 937–952.

47. Zhu Y, Yamamoto T, Cullen J, et al. Kinetics of hepadnavirus loss from the liver during inhibition of viral DNA synthesis. J Virol 2001; 75: 311–322.

48. Qi X, Xiong S, Yang H, et al. In vitro susceptibility of Adefovir-associated hepatitis B virus polymerase mutations to other antiviral agents. Antivir Ther 2007; 12: 355–362.

49. Marcellin P, Mommeja-Marin H, Sacks SL, et al. A phase II dose-escalating trial of clevudine in patients with chronic hepatitis B. Hepatology 2004; 40: 140–148.

50. Lee HS, Chung YH, Lee K, et al. A 12-week clevudine therapy showed potent and durable antiviral activity in HBVeAg-positive chronic hepatitis B. Hepatology 2006; 43: 982–988.

51. Lim SG, Leung N, Hann HW, et al. Clinical trial: a phase II, randomized study evaluating the safety, pharmacokinetics and anti-viral activity of clevudine for 12 weeks in patients with chronic hepatitis B. Aliment Pharmacol Ther 2008; 27: 1282–1292.

52. Yoo BC, Kim JH, Chung YH, et al. Twenty-four-week clevudine therapy showed potent and sustained antiviral activity in HBVBeAg-positive chronic hepatitis B. Hepatology 2007; 45: 1172–1178.

53. Yoo BC, Kim JH, Kim TH, et al. Clevudine is highly efficacious in hepatitis B e antigen-negative chronic hepatitis B with durable off-therapy viral suppression. Hepatology 2007; 46: 1041–1048.

54. Ko SY, Kwon SY, Choe WH, et al. Clinical and virological responses to clevudine therapy in chronic hepatitis B patients: results at 1 year of an open-labelled prospective study. Antivir Ther 2009; 14: 585–590.

55. Kwon SY, Park YK, Ahn SH, et al. Identification and characterization of clevudine-resistant mutants of hepatitis B virus isolated from chronic hepatitis B patients. J Virol 2010; 84: 4494–4503.

56. Seok JI, Lee DK, Lee CH, et al. Long-term therapy with clevudine for chronic hepatitis B can be associated with myopathy characterized by depletion of mitochondrial DNA. Hepatology 2009; 49: 2080–2086.
57. Kim BK, Oh J, Kwon SY, et al. Clevudine myopathy in patients with chronic hepatitis B. *J Hepatol* 2009; 51: 829–834.
58. Kim JY, Yoon YS, Park KD, et al. Myopathy due to chronic clevudine therapy: A case report. *Korean J Pathol* 2009; 43: 575.
59. Tak WY, Park SY, Jung MK, et al. Mitochondrial myopathy caused by clevudine therapy in chronic hepatitis B patients. *Hepatol Res* 2009; 39: 944–947.
60. Meyers C. Pharmasset voluntarily halts clinical studies with clevudine in hepatitis B infected patients. https://www. fiercebiotech.com/biotech/pharmasset-voluntarily-halts-clinical-studies-clevudine-hepatitis-b-infected-patients (2009, accessed 9 August 2021).
61. Tak WY, Park SY, Cho CM, et al. Clinical, biochemical, and pathological characteristics of clevudine-associated myopathy. *J Hepatol* 2010; 53: 261–266.
62. Jang JH, Kim JW, Jeong SH, et al. Clevudine for chronic hepatitis B: antiviral response, predictors of response, and development of myopathy. *J Viral Hepat* 2011; 18: 84–90.
63. Park SH, Park KS, Kim NH, et al. Clevudine induced mitochondrial myopathy. *J Korean Med Sci* 2017; 32: 1857–1860.
64. Liu SH, Grove KL and Cheng YC. Unique metabolism of a novel antiviral L-nucleoside analog, 2′-fluoro-5-methyl-beta-L-arabinofuranosyluracil: a substrate for both thymidine kinase and deoxyxycytidine kinase. *Antimicrob Agents Chemother* 1998; 42: 833–839.
65. Saada A, Shaag A and Elpeleg O. mtDNA depletion myopathy: elucidation of the tissue specificity in the mitochondrial thymidine kinase (TK2) deficiency. *Mol Genet Metab* 2003; 79: –5.
66. Wang L, Sun R and Eriksson S. The kinetic effects on thymidine kinase 2 by enzyme-bound dTTP may explain the mitochondrial side effects of antiviral thymidine analogs. *Antimicrob Agents Chemother* 2011; 55: 2552–2558.
67. Mayers D, Squires K, Ogilvie L, et al. ATI-2173, a novel active site polymerase inhibitor nucleotide (ASPIN), for HBV cure regimens Is well tolerated and has favorable pharmacokinetics in healthy volunteers. Presented at HBV-TAG 2021; June 11-12, 2021; Virtual.
68. Squires K, Ogilvie L, Jucov A, et al. A randomized phase 1b trial of the active site polymerase inhibitor nucleotide ATI-2173 in patients with chronic hepatitis B virus infection. 2022 (Unpublished report).
69. Mayers DL, Squires KE, Rush R, et al. ATI-2173, a novel HBV nucleoside phosphoramidate for HBV cure regimens. Presented at: The Digital International Liver Congress; August 27–29, 2020. Poster SAT-422.
70. Tak WY, Yang JM, Kim BI, et al. A randomized, open-label study comparing low-dose clevudine plus Adefovir combination therapy with clevudine monotherapy in naïve chronic hepatitis B patients. *Hepatol Int* 2014; 8: 375–381.