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Advances in Novel Investigational Agents for Functional Cure of Chronic Hepatitis B: A Comprehensive Review of Phase II and III Therapeutic Agents

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Abstract

Chronic hepatitis B virus (HBV) infection affects over 295 million people globally and an estimated 1.6 million people in the United States (U.S.). It is associated with significant morbidity and mortality due to cirrhosis, liver failure, and liver cancer. Antiviral therapy with oral nucleos(t)ide analogues is associated with high rates of virologic suppression, which in turn has been associated with a decreased risk of liver complications. However, current antiviral regimens are limited by concerns with adverse effects, adherence, resistance, long-term treatment, and ongoing risk for liver events. Novel investigational agents are currently in development and are targeted at achieving functional cure with sustained Hepatitis B surface antigen (HBsAg) loss and suppression of HBV DNA. Herein we review key evidence from phase II and III trials defining the efficacy and safety profiles for key targets for functional cure, including core/capsid inhibitors, entry inhibitors, RNA interference (siRNA/ASO), hepatitis B surface antigen (HBsAg) inhibitors, toll-like receptor agonists, checkpoint inhibitors, and therapeutic vaccines.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a global health problem with more than 295 million people infected worldwide and as many as 1.6 million people infected

in the United States[1, 2]. Developing complications of chronic HBV, including cirrhosis, hepatocellular carcinoma (HCC), and liver failure, may take years to develop and have led to more than 800,000 deaths each year[3-5].

While the ideal goal of HBV therapy would be a complete sterilizing cure, such a therapy does not exist because it is difficult to directly target the covalently closed circular DNA (cccDNA) in the hepatocyte nucleus and the integration of HBV DNA into the host genome. The best that can be achieved with current therapies is a functional cure where there is loss of HBV surface antigen (HBsAg) with undetectable HBV DNA after 6 months off therapy. This is an important endpoint given its association with reduced liver necroinflammation, reduced risk of HCC, increased liver fibrosis regression, normalization of alanine aminotransferase (ALT) levels, reduced risk of liver cirrhosis, and increased survival[6-11].

Current FDA-approved therapies include pegylated interferons (PEGIFNα) and nucleos(t)ide analogues (NA)[12, 13]. PEGIFNα is administered as subcutaneous injections on a once weekly dosing schedule for one year. They exert antiviral and immunomodulatory activities by enhancing cccDNA degradation and modifying cccDNA transcription. PEGIFNα therapy has higher rates of HBsAg loss and HBV e-antigen (HBeAg) seroconversion than nucleos(t)ide analogues, but are associated with poor tolerability and risk for depression[14, 15]. In contrast, NA are administered orally every day. They suppress HBV replication by causing chain termination when incorporated into HBV DNA undergoing reverse transcription. Early NA such as Lamivudine (LAM) and Adefovir (ADV) had high rates of antiviral resistance with only a few years of treatment[16]. NA currently used in clinical practice, namely Tenofovir disoproxil fumarate (TDF) and Entecavir (ETV), have potent antiviral activity and a high barrier to resistance[17]. Compared to PEGIFNα therapy, NA are well tolerated, but require a long term duration of maintenance therapy[18]. Both PEG-IFNα and NA therapies are unable to eliminate the HBV virus because they do not directly target

cccDNA and integrated HBV DNA. Consequently, cccDNA persists, which enables transcription of RNA and translation of viral HBV proteins, such as HBsAg, to continue[19, 20].

Rates of a functional cure with PEGIFNα and NA therapies are low. With PEGIFNα treatment, HBsAg loss has only been reported in approximately 7% of both HBeAg positive and negative patients after a year of treatment[21]. HBsAg loss is even lower in patients receiving NA with only 0.3-5% of HBeAg negative patients and 1.4% of HBeAg positive patients achieving HBsAg loss after treatment for 5-7 years[22, 23]. Given the limitations of existing HBV therapies, there is great interest in novel HBV therapeutics that can lead to the following outcomes: functional cure, improvement in quality of life, and preventing progression of chronic HBV to cirrhosis, HCC, and HBV-related mortality. Herein, this review will focus on novel HBV therapies in active phase II and III clinical trial development.

FUTURE THERAPY FOR HEPATITIS B

METHODOLOGY

This paper is a narrative review. Investigational agents for treatment of chronic HBV under active phase II and III development were identified using the National Institutes of Health Clinical Trials directory[24]. This directory includes details regarding the study design, population, treatment arms, and sponsoring pharmaceutical company for all publicly supported clinical studies. Information from this website was incorporated in the development of Table 2, which summarizes information about investigational agents in phase II and III trials without published study results. A PubMed search was conducted for each investigational agent under active phase II and II development. Data was retrieved from published original research articles and conference abstracts. The website of the sponsoring pharmaceutical company website for each investigational agent was reviewed for published presentation slides from international liver meetings.

NOVEL THERAPIES

A summary of the novel therapies in phase II and III development with study data are listed in Table 1, while therapies without study data are listed in Table 2.

Core/Capsid Inhibitors

Capsid allosteric modulators directly target the destabilization of HBV Core protein, resulting in the formation of abnormal capsids or morphologically normal capsids lacking genetic material[25]. This prevents further release and spread of HBV to other hepatocytes.

JNJ56136379 (JNJ-6379)

JNJ56136379 (also known as JNJ-6379) targets the HBV capsid assembly process needed for HBV replication. It accelerates the rate of HBV capsid assembly to form empty, morphologically intact viral capsids and has a secondary mechanism of inhibiting de novo cccDNA[26].

The JADE study was a randomized, phase II, partially blinded, placebo-controlled study evaluating the efficacy and safety of JNJ-6379 in 232 adults with non-cirrhotic, chronic HBV infection. Participants were virally suppressed or not on active treatment at the time of entry into the clinical trial. Participants were randomized to JNJ-6379 monotherapy or in combination with NA (ETV or TDF), and then compared to a control group of placebo plus NA. Dosing of JNJ-6379 at 75mg and 250mg daily was investigated. Overall, JNJ6379 did not show a clear benefit over NA monotherapy. The primary endpoint of a 1 Log IU/mL mean decrease in HBsAg from baseline to week 24

for the JNJ-6379 treatment groups and control was not achieved. Specifically, the mean change in HBsAg compared to baseline for the JNJ-6379 treatment groups ranged from -0.41 to 0.11 Log IU/mL. Among participants who were HBeAg positive at the start of the study, there was also a limited reduction of HBeAg of 0.49 and 0.70 of log IU/mL for JNJ-6379 75mg and 250mg plus NA treatment groups, respectively. Over the 24-week follow-up period, the use of JNJ6379 both as monotherapy and in combination with NA led to a marked reduction in both HBV DNA (mean JNJ-6379 75 mg or 250mg plus NA HBV DNA reduction of 5.53 and 5.58 Log IU/mL respectively, compared with 5.21 Log IU/mL in the placebo plus NA group) and HBV RNA (mean JNJ-6379 75mg and 250mg plus NA HBV RNA reduction was 2.96 and 3.15 Log IU/mL respectively, compared with 1.33 Log IU/mL in the placebo plus NA group). Both doses of JNJ6379 were safe and well tolerated[26].

ABI-H0731 (Vebicorvir)

ABI-H0731 (Vebicorvir/VBR) is an orally administered small molecule that disrupts HBV replication by inducing altered, non-functional core protein assembly[27].

One of the Phase II studies evaluated the efficacy and safety of VBR in combination with ETV for treatment naïve, non-cirrhotic, HBeAg positive study participants. As a double-blind, randomized, and placebo-controlled study, participants either received a combination of once daily VBR 300mg daily and ETV 0.5mg daily, or a combination of placebo and ETV 0.5mg daily. The study revealed that the combination of VBR and ETV was safe, well-tolerated, and augmented a reduction of HBV DNA and RNA. The primary endpoint was achieved as there was a significantly greater mean log reduction in HBV DNA from baseline with VBR plus ETV combination therapy as compared to placebo plus ETV therapy at both treatment weeks 12 (-4.45 Log IU/mL with VBR and ETV vs. -3.3 Log IU/mL with placebo and ETV) and treatment weeks 24 (-5.33 Log IU/mL with VBR and ETV vs. -4.2 Log IU/mL with placebo and ETV).

Furthermore, a greater proportion of patients had normalized ALT levels by treatment week 24 among the VBR and ETV combination therapy group (12/13 participants) as compared to placebo and ETV therapy group (5/12 participants). No resistance breakthrough occurred with the use of VBR. The study demonstrated that VBR can be combined with current NA therapy to enhance HBV anti-viral activity in treatment-naïve patients with chronic HBV[27].

Another phase II study evaluated the efficacy and safety of combination VBR and NA therapy as compared to NA monotherapy in non-cirrhotic, chronic HBV participants who were virally suppressed by NA for at least 6 months. The 73 enrolled study participants were randomized to receive VBR 300mg daily plus NA or matching placebo plus NA for 24 wk. Results showed there was no difference between both groups for the change in HBsAg or HBeAg from baseline to treatment week 24. Of note, the combination of VBR plus NA led to a more marked reduction of HBV DNA and pregenomic RNA at week 24 from baseline compared to the placebo plus NA group, irrespective of HBeAg status. Among patients with detectable HBV DNA at baseline, there were a greater proportion of patients within the VBR plus NA group (29/35 HBeAg+ patients, 16/17 HBeAg- patients) compared to the placebo plus NA group (17/59 HBeAg+ patients, 10/14 HBeAg- patients) who achieved undetectable HBV DNA levels at week 24. VBR was found to be safe and well-tolerated. This clinical study provided further support that even greater levels of viral suppression can occur with the addition of a VBR core inhibitor to existing NA therapies, although the clinical significance of this is yet to be investigated[28].

Entry Inhibitors

Entry inhibitors targets the function of HBV surface proteins or host receptors to prevent HBV entry into the host cell required for infection[29].

Bulevirtide (formerly Myrcludex)

Bulevirtide is a synthetic myristoylated peptide entry inhibitor that competitively binds and blocks a hepatocyte surface protein, sodium taurocholate cotransporting polypeptide (NTCP) receptor, such that HBsAg is unable to enter the hepatocyte[29]. Hepatitis D virus (HDV) uses the same NTCP receptor as HBV, so Bulevirtide has been also used to prevent co-infection by HDV[30]. Increases in bile acid level are expected since NTCP plays a role in bile transport[31].

The MYR-201 study was a phase Ib/IIa randomized, open-label study investigating the safety and efficacy of Bulevirtide on the HBV and HDV virologic response and tolerability. The study featured 24 participants randomized to receive either Bulevirtide for 24 wk followed by PEGIFNα-2a weekly for 48 wk (Bulevirtide cohort), 2mg Bulevirtide daily plus PEGIFNα-2a weekly for 24 wk followed by 24 wk of PEGIFNα-2a alone (Bulevirtide-IFN cohort), or PEGIFNα-2a weekly alone for 48 wk (IFN cohort). Study results revealed that HBsAg levels remained unchanged compared to baseline throughout the study in all treatment groups. ALT normalized in 6/8 patients in the Bulevirtide cohort compared to only 1/15 patient in the Bulevirtide-IFN and IFN cohorts. Notably, mean HBV DNA was significantly reduced by $10^{1.28}$ copies/mL at week 24 from baseline in the Bulevirtide-IFN cohort, with 6/7 patients showing a ≥1 Log decline. There was non-significant decline of the HBV DNA from baseline in the IFN and Bulevirtide cohorts. This was the first proof-of-concept study showing that Bulevirtide was safe, well-tolerated, and could enhance viral suppression when used in combination with PEGIFNα-2a[32].

Another phase II, multicenter, open-label study, known as MYR-202, randomized patients into 4 groups: 2mg subcutaneous Bulevirtide daily with TDF daily, 5mg subcutaneous Bulevirtide daily with TDF daily, 10mg subcutaneous Bulevirtide daily with TDF daily, or TDF alone for a total of 24 wk. Therapeutic impact on HBsAg

was investigated as a secondary endpoint. There was no significant change in HBsAg concentration from baseline in any of the treatment groups throughout the treatment and follow-up period. Like the MYR-201 study, Bulevirtide in the MYR-202 study was well tolerated. Common treatment-related adverse events included elevations in asymptomatic bile salt levels and ALT levels[33].

MYR-203 assessed the safety and efficacy of Bulevirtide alone or in combination with PEGIFNα for 48 wk. Treatment arms included PEGIFNα alone weekly, 2mg Bulevirtide daily plus PEGIFNα weekly, 5mg Bulevirtide daily plus PEGIFNα weekly, or 2mg Bulevirtide daily for a total of 48 wk. This was then followed by a treatment free period of 24 wk. By weeks 48 and 72, there was a >1 Log reduction from baseline or undetectable HBsAg levels in the Bulevirtide plus PEGIFNα combination groups, but not in the monotherapy groups. Specifically, by 72 wk, 6/15 participants in the combination arm of the 2mg Bulevirtide plus PEGIFNα group and 2/15 participants in the 5mg Bulevirtide plus PEGIFNα group achieved either >1 Log IU/mL decline or undetectable levels of HBsAg. MYR-203 study findings demonstrated a potential role of combination Bulevirtide and PEGIFNα therapy in future HBV cure given it led to a large proportion of patients achieving HBsAg loss[34].

The MYR-204 multicenter, randomized phase II trial studied the safety and efficacy of Bulevirtide administered subcutaneously at 2mg or 10mg daily dosing in combination with PEGIFNα weekly compared to Bulevirtide 10mg monotherapy over 48 wk. Interim data at the 24-week mark showed that a >1 Log IU/mL decline in HBsAg levels from baseline was achieved only in the Bulevirtide and PEGIFNα combination groups (10/100 participants) and the PEGIFNα alone group (1/24 participants). There were modest declines in HBV DNA from baseline in the groups who received Bulevirtide (mean HBV DNA change ranged from -0.3 to -0.7 Log IU/mL)[35].

The MYR-301 trial was the first phase III multicenter, randomized, parallel design study of Bulevirtide monotherapy in 2 and 10mg daily dosing compared to no active anti-HDV treatment for 48 wk, defined as delayed treatments. For HBV efficacy endpoints at week 48, no patient in any group experienced HBsAg loss and changes in HBsAg from baseline were minimal. Only small declines in HBV DNA levels were observed with Bulevirtide treatment. No severe adverse effects were observed in patients receiving Bulevirtide that led to discontinuation of the drug[36].

Small interfering RNA (SiRNA)

Small interfering RNA (siRNA) are short RNA molecules that hybridize to specific viral mRNA sequences and target bound mRNA for degradation[37]. Effectively, siRNA prevents the expression of HBV proteins needed for replication.

Bepirovirsen (GSK3228836)

Bepirovirsen is an antisense oligonucleotide that targets all HBV RNA, including mRNA and pregenomic RNA, and designates it for degradation[38].

One of the two phase II randomized controlled trials evaluated the safety, tolerability, and antiviral activity of Bepirovirsen. The study enrolled 24 treatment-naïve participants and 7 participants receiving stable NA therapy with chronic HBV infection. Patients who were treatment-naïve were randomized to placebo or Bepirovirsen at 150mg or 300mg doses. Patients on stable NA therapy were randomized to placebo or Bepirovirsen in 300mg doses. Bepirovirsen was administered twice weekly for 2 wk and then once weekly for another 2 wk, after which patients were followed for 26 wk to assess for a change in HBsAg levels from baseline. After 4 wk of 300mg Bepirovirsen for treatment-naïve patients, there was a significant decrease in HBsAg levels and HBV DNA from baseline compared to placebo; this was not observed

in the Bepirovirsen 150mg group. Specifically, among treatment naïve subjects, there was a mean 1.56 Log IU/mL reduction in HBsAg in the Bepirovirsen 300mg group from baseline to day 29, as compared to 0.5 Log IU/mL reduction in the Bepirovirsen 150mg group and <0.07 Log IU/mL reduction in the placebo group. The timing of HBsAg reduction in responders occurred rapidly after 4 wk of therapy. Bepirovirsen was found to have a favorable safety profile and treatment response, which encouraged the use in a larger study cohort[39].

The B-Clear Trial was a phase IIb, randomized controlled study investigating the efficacy and safety of Bepirovirsen in 457 enrolled participants with chronic HBV when used for 12 and 24 wk. Results revealed that 6/68 participants and 7/70 participants who received 24 wk of Bepirovirsen once weekly with and without NA therapy respectively achieved HBsAg and HBV DNA loss that persisted for 24 wk following the end of the treatment period. While there were similar results of HBsAg loss irrespective of NA therapy use or HBeAg status, HBsAg loss among patients who were HBeAg positive only occurred in those receiving NA therapy. The study also showed that levels of HBsAg at baseline can be predictive of response to therapy. Specifically, receiving-operating-characteristic analysis revealed that baseline HBsAg levels <3000 IU/mL was the cutoff level associated with functional cure when treated with Bepirovirsen. Common adverse events observed more commonly in in the study cohort receiving Bepirovirsen compared to placebo included injection site reactions, pyrexia, fatigue, and increased ALT levels. Brief increases in HBV DNA observed after stopping Bepirovirsen raised potential concerns about the durability of treatment response; however, these blips in HBV DNA levels were postulated to be due to spontaneous release of virion from the hepatic reservoir. Durability of treatment response will be investigated in future studies with longer follow-up time[40].

VIR-2218

VIR-2218 is a triantennary N-acetyl galactosamine (GalNAc) conjugated siRNA that targets the X region of the HBV genome[41]. As the X region contains overlapping HBV gene templates, the use of a single siRNA can effectively silence all HBV RNA production in this region. VIR-2218 can also suppress the X-mediated upregulation of cccDNA transcription.

VIR-2218-1001 was a two-part, phase I/II randomized, double-blind, and placebo-controlled study. The first part of the study evaluated the safety and tolerability of a single dose of VIR-2218 at six dosing levels administered to healthy adult volunteers. The second part of the study evaluated the safety and therapeutic effect across various increase doses of VIR-2218 given 4 wk apart. Study participants were non-cirrhotic adults with chronic HBV on NA therapy for at least 6 months and HBV DNA <90 IU/mL. In both parts of the study, VIR-2218 was well tolerated across all dose levels with only mild adverse events, commonly headache, injection site reactions, and mild ALT elevations. The study found dose-dependent reductions in HBsAg were observed in all VIR-2218 treatment groups compared to placebo by 48-week follow-up. A total of 12/24 participants across the VIR-2218 cohorts as compared to none in the placebo group achieved a reduction of HBsAg levels to <100 IU/mL. The greatest mean reduction of HBsAg (-1.65 Log IU/mL) occurred at week by week 20 for those receiving the 200mg VIR-2218. While no participants had serum HBsAg loss or anti-HBs seroconversion by week 48, a HBsAg level <100 IU/mL has been associated with a significantly higher chance of HBsAg loss[42]. This study demonstrated that VIR-2218 is well-tolerated with antiviral effects that could potentially lead to functional cure[43].

Another phase II trial investigated the safety and efficacy of VIR-2218 alone and in combination with PEGIFNα in non-cirrhotic participants with chronic HBV. Inclusion criteria included NA therapy for at least 2 months, HBsAg >50 IU/mL, and HBV DNA <90 IU/mL. Preliminary data revealed VIR-2218 was generally well tolerated both alone and in combination with PEGIFNα. Adverse events that occurred

were more consistent with known effects of PEGIFNα, such as mild ALT elevations and reductions in neutrophil and platelet levels. Four of 13 study participants treated with the longer duration of 48 wk combination treatment of VIR-2218 and PEGIFNα achieved HBsAg seroclearance and anti-HBs seroconversion. Patients in this longer duration combination group also had the largest mean HBsAg reduction of 2.9 Log IU/mL at the end of therapy. While the study is still ongoing with longer follow-up time, the preliminary results demonstrate that the antiviral effect of VIR-2218 may be potentiated by PEGIFNα and shows promise as a future combination therapy[44].

VIR-3314

VIR-3314 is a subcutaneously administered monoclonal antibody that targets an antigenic loop of HBsAg to block HBV cell entry[45]. In addition to clearing HBsAg, it can stimulate T cells for a vaccinal effect.

The MARCH trial was a phase II study that evaluated the safety, tolerability, and antiviral activity of VIR-2218 and VIR-3434 either as monotherapy or as combination therapy. Study participants were virally-suppressed, non-cirrhotic adults on NA therapy with chronic HBV infection. Combination of both VIR-2218 and VIR-3434 were well tolerated with mild adverse effects. The study was instrumental in showing that the combination of VIR-2218 and VIR-3434 Led to marked mean HBsAg declines >2.5 Log IU/mL in all cohorts. In fact, most participants were able to achieve a HBsAg level <10 IU/mL. VIR-3434 has an additive effect to VIR-2218 in achieving greater HBsAg reduction compared to monotherapy; this is consistent with their established complimentary mechanisms of action on HBV replication[46].

JNJ-73753989 (JNJ-3989, formerly ARO-HBV)

JNJ-3989 is composed of 2 siRNAs which target both the S gene and X gene of HBV. Consequently, it impairs the production of HBV RNA transcripts which is essential for replication[47].

A phase IIa clinical trial assessed the safety and efficacy of JNJ-3989 both with and without JNJ-6379 in 84 recruited participants with chronic HBV who were treatment naïve or on chronic NA-suppressive therapy. All participants received a NA throughout the study. JNJ-3989 was well tolerated across all doses throughout the study period. By day 112, there was HBsAg reduction ≥1 Log IU/mL from baseline in 39/40 participants who received 100 to 400mg of JNJ-3989 every 4 wk in combination with a NA daily. Also, 30/40 patients (30/40 patients) achieved HBsAg <100IU/mL by day 112. A dose-dependent relationship was seen with higher doses of JNJ-3989 achieving higher levels of HBsAg reductions. More frequent dosing intervals did not change the magnitude and rate of response compared to dosing of JNJ-3989 to every 4 wk. All 12 patients in the triple combination of JNJ-3989, JNJ-6379, and NA therapy achieved ≥1 Log IU/mL HBsAg reduction from baseline to the nadir. The HBsAg reduction was also durable – 15/19 participants maintained a ≥1 Log HBsAg reduction nearly 336 days after their last JNJ-3989 dose. This trial provided support that JNJ-3989 can be used safely in combination with a NA and that JNJ-6379 is an efficacious and durable HBV therapy[48].

The REEF-1 study was a large multicenter, double-blinded, randomized, phase IIb clinical trial studying the efficacy and safety of combination therapies of JNJ6379, JNJ-3989 and NA therapy at various doses. The study featured non-cirrhotic adults with chronic HBV who were either treatment naïve or virologically suppressed on NA therapy. The primary endpoint was the proportion of patients who met NA stopping criteria, as defined as ALT <3x upper limit of normal, HBV DNA less than the lower limit of quantitation, HBeAg negative, and HBsAg <10 IU/mL by week 48. Over the course of 48 wk, JNJ-3989 in combination with NA therapy led to a robust, dose-

dependent response for meeting NA stopping criteria as well as reducing HBsAg and HBV RNA levels. In fact, 94/96 patients in the combination JNJ3989 200mg every 4 wk and NA group had ≥1 Log IU/mL in HBsAg decline with a mean decline of 2.6 Log IU/mL. Most patients did not reach the NA stopping criteria for two reasons: due to failure to achieve HBsAg <10 IU for those who were HBeAg negative at baseline, or not achieving HBeAg seronegative status for patients who were HBeAg positive at baseline. JNJ-3989 in combination with NA was safe and well tolerated. Overall, REEF-1 showed that the combination of novel therapies, involving JNJ-3989 and/or JNJ6379, with established NA therapies is insufficient to achieve functional cure, but can achieve substantial HBsAg reductions[49].

Arbutus-729 (AB-729 or Imdusiran)

AB-729 is a subcutaneously administered, GalNAc-conjugated RNA interference agent that blocks all RNA transcripts and reduces all HBV viral antigens[50]. It has an immunostimulatory component by enhancing HBV-specific T cell responses following repeat dosing of AB-729[51].

In the AB-729-001 phase II study, healthy subjects and those with chronic HBV were subjected to single and repeat doses of AB-729 in various doses (60 or 90mg of AB-729) and frequencies (every 4, 8, or 12 wk). ABI-729 with repeat dosing was found to be safe and well tolerated. The most frequent adverse events included injection site events and asymptomatic ALT elevations which were Grade 2 in severity or lower. There were robust and persistent declines in HBsAg in most subjects across cohorts regardless of dose, dosing interval, or HBeAg status; there was a mean reduction of HBsAg by 1.5 Log IU/mL from baseline to 24 wk after the last dose. In fact, 26/34 participants achieved HBsAg < 100 IU/mL at some point in the study. As well, there was sustained reduction in HBsAg and HBV DNA in 7 of 9 patients even after discontinuation of both AB-729 and NA-therapy. Only one subject seroconverted at week 48. Study findings

demonstrated AB-729 may be considered as a potential therapy for achieving functional cure of chronic HBV[52].

The AB-729-201 trial was a randomized, open-label, multicenter phase IIa study which evaluated the safety, tolerability, and antiviral activity of AB-729 with PEGIFNα. The 43 non-cirrhotic, HBeAg negative subjects had virally suppressed chronic HBV infection and were on stable NA therapy for at least 12 months prior. Patients received 4 doses of ABI-729 60mg every 8 wk, and at week 24 randomized to either two treatment combinations (AB-729 +NA + PEGIFNα or NA + IFN) and at two treatment durations (12 wk vs. 14 wk) followed by another 24 wk of follow-up where patients were evaluated to stop NA therapy. Preliminary results showed that by week 24 of treatment there was a mean HBsAg decline of 1.6 Log IU/mL across all cohorts. As well, 38 of 41 subjects achieved HBsAg levels <100 IU/mL at some point during the treatment period. The interim data also showed that AB-729 with and without IFN was safe and well tolerated with most treatment related adverse events unrelated to AB-729 therapy[53].

HBsAg inhibition

HBsAg is a main surface protein on the envelope of the new HBV virion and subviral particles that maintains chronic infection *via* immune exhaustion[54]. HBsAg loss is one primary component required for functional cure[55]. HBsAg inhibitors disrupt the secretory processes involved in translocating HBsAg to the surface and effectively decrease HBsAg availability[56].

REP 2139/REP 2165 (Replicor)

REP2139 is a nucleic acid polymer (NAP) that stops the assembly of subviral particles in hepatocytes and blocks the release of HBsAg[57]. REP2165 is a biologically equivalent variant of REP2139 with equivalent HBV antiviral activity in vivo. However,

it has accelerated clearance which may be useful in cases requiring high frequency dosing for patients with slow rates of HBsAg clearance[58].

REP401 was an open-label phase 2 study evaluating the safety and efficacy of the combination therapy TDF, PEGIFNα, and either REP2139 or REP2165. Participants had chronic HBV infection and were HBeAg negative. Patients received 24 wk of TDF therapy, followed by 24 wk of a control backbone therapy (TDF and PEGIFNα) or combination triple therapy (TDF, PEGIFNα, and either REP2139 or REP2165). Then participants were monitored for treatment-free period of 48 wk. The addition of either REP2139 or REP2165 to TDF and PEGIFNα was safe and well tolerated. Use of REP2139/REP2165 did not affect PEGIFNα-induced thrombocytopenia and neutropenia. Notably, there was a significantly more frequent and greater increase in asymptomatic transaminase levels among patients receiving a NAP which correlated with an initial decrease in HBsAg levels. From week 25 to 48, combination triple therapy led to a rapid 4 to 6 Log IU/mL decline in HBsAg in 15/20 patients by week 35. By week 48, HBsAg was not detected in 10 of 20 patients and HBsAg seroconversion was achieved in 11/20 patients, all with HBsAg <1 Log IU/mL. In contrast, the control backbone therapy group had HBsAg declines >1 Log IU/mL in only 3 of 20 patients with no HBsAg seroconversion observed. Both the triple combination group and control group achieved similar HBV DNA declines with 18 of 40 participants achieving HBV DNA less than the lower limit of quantification by week 48. In the 48-week follow period, functional cure persisted in 14 of the 40 patients. Within the triple combination therapy group, there was no difference in response between REP2139 and REP2165 with regards to HBsAg, hepatitis B surface antibody (anti-HBs), and HBV DNA levels. REP401 showed that the addition of REP2139 or REP2165 to TDF and PEGIFNα therapy did not affect tolerability and increased rate of functional cure both during and after therapy[59].

REP301 was an open label, nonrandomized phase II trial investigating the use of REP2139 with PEGIFNα-2a in adults with chronic HBV. These participants were HBeAg positive, anti-hepatitis D antigen positive, HDV RNA positive, and had a HBsAg levels >1000 IU/mL. Study subjects received intravenous (IV) REP2139 once weekly for 15 wk, followed by a combination of IV REP2139 and subcutaneous PEGIFNα-2a once weekly for another 15 wk, and then finally, PEGIFNα-2a for 33 wk. By the end of treatment, 6/12 subjects had HBsAg < 50 IU/mL, 6/12 subjects had HBsAb > 10 mIU/mL, and 9/12 subjects had suppressed HBV DNA <10 IU/mL. The response was durable to 1 year of follow-up: 5/6 patients maintained HBsAg suppression <50 IU/mL, all 6/6 patients maintained HbsAb >10 mIU/mL, and 7/9 patients had HBV DNA<10 IU/mL. Use of both REP2139 and PEGIFNα-2a was safe and well tolerated. The most frequent adverse events in REP2139 monotherapy were pyrexia and chills, while the introduction of PEGIFNα-2a led to asymptomatic transient elevations in ALT and AST. REP301 underscored that combination REP2139 therapy with PEGIFNα-2a has robust and durable HBV and HDV antiviral effects even after completion of therapy[60].

Toll-like receptor agonists

Toll-like receptor (TLR) agonists act as immunomodulators to enhance the immune response against chronic HBV infection[61]. They induce the production of interferons, cytokines, and chemokines which upregulate antiviral effects[62].

Vesatolimod (GS-9620)

Vesatolimod selectively activates TLR-7 found in gut-associated plasmacytoid dendritic cells and B lymphocytes to upregulate T cell responses and immunoglobin B cell response[63].

The first phase II double-blind, randomized and placebo-controlled study evaluated the safety, efficacy, and pharmacodynamics of Vesatolimod in virally-suppressed, non-cirrhotic patients with chronic HBV. The 162 participants were randomized to receive weekly dosed placebo or Vesatolimod (1mg, 2mg, or 4mg) for various treatment durations (4, 8, 12, and 48 wk). Vesatolimod was safe and well tolerated at all doses with no clinically significant adverse events or lab derangements in the cohorts. Although the biological activity of Vesatolimod was verified with dose-dependent pharmacodynamic induction of biomarker ISG15, no significant HBsAg declines from baseline were observed in any of the cohorts by week 48[64].

The second phase II study evaluated the safety and efficacy of Vesatolimod on patients with non-cirrhotic, chronic HBV infection who were not on oral antiviral treatment for at least 3 months. Additionally, patients had HBV DNA ≥2000 IU/mL. In this multicenter, double blind, randomized, placebo-controlled study, patients were randomized to weekly placebo or oral Vesatolimod (1mg, 2mg, or 4 mg) for 12 wk. All subjects also received TDF of 300mg daily for 48 wk. Vesatolimod was safe and well tolerated. None of the patients achieve HBsAg loss or HbsAb seroconversion in any of the cohorts, and there was no significant difference in the decline of HBsAg among the Vesatolimod treatment groups compared to placebo. Only 3 total patients within the Vesatolimod groups had HBeAg loss and HBeAb seroconversion at week 48. There was no significant difference in HBV DNA decline among the Vesatolimod groups compared to placebo. Like the first study, a pharmacodynamic response was verified with was a consistent dose-dependent induction of ISG15 biomarker level.[65]

Selgantolimod (GS-9688)

Selgantolimod is a selective TLR-8 agonist with antiviral activity against chronic HBV infection. It leads to the production of proinflammatory cytokines, chemokines, and interferons that initiate an innate and adaptive immune response against HBV[66].

A phase II, randomized, double-blind, placebo-controlled, multicenter study investigated the safety and efficacy of Selgantolimod in virally suppressed individuals on antiviral therapy with chronic HBV. Patients were randomized to receive once weekly placebo or oral Selgantolimod dosed at 1.5mg or 3mg for a total of 24 wk while continuing oral NA agents. Only one of the 48 participants in the 1.5mg Selgantolimod group achieved the primary endpoint of a ≥1 Log IU/mL decline in HBsAg from baseline to week 24. As compared to placebo where no participants achieved HBsAg or HBeAg loss, 2 of the 39 subjects with HBeAg negative status achieved HBsAg loss and 3 of the 39 subjects had HBeAg loss in the Selgantolimod groups. The largest HBsAg reductions during the study occurred in patients who received Selgantolimod. In fact, HBsAg declines persisted even after treatment cessation. Selgantolimod was safe and generally well tolerated with the most common adverse events including nausea, vomiting, and headache[67].

Therapeutic Vaccinations

Therapeutic vaccinations present HBV vaccine antigens in a non-infective form to antigen presenting cells to stimulate a CD4 and CD8-mediated T cell response against HBV[68]. In comparison to preventative vaccines, therapeutic vaccinations are given during ongoing infection.

GS-4774

GS-4774 is a vaccination composed of heat-inactivated yeast cells expressing HBsAg, Hepatitis B core antigen (HBcAg), and HBV-encoded oncogene X protein (HBx) as a single fusion protein. Inoculation of individuals with GS-4774 as a subcutaneous injection elicits a significant T cell response[69].

A phase II study evaluated the safety, tolerability, and efficacy of GS-4774 in non-cirrhotic patients with chronic HBV infection who were virally suppressed with oral antiviral therapy for at least a year. Subjects were randomized to either oral antivirals alone or a combination therapy of oral antivirals plus GS-4772 (dosed as 2, 10, or 40 yeast units) subcutaneously every 4 wk until week 20. Subjects continued oral antiviral for the remainder of the study to week 24 and then followed to week 48. No significant difference in mean HBsAg decline was found from baseline to week 24 or week 48 between any of the GS-4772 combinations therapy groups compared to oral antivirals alone. No patient experienced loss of HBsAg. Combination therapy of GS-4774 and antivirals was found to be safe and well-tolerated – there was no virologic breakthrough or treatment discontinuations in any patient and injection sites were the most common adverse event. The study showed that GS-4774 has limited efficacy for functional cure in chronic HBV among virally suppressed patients[70].

Another phase II open-label, multicenter study evaluated the safety and efficacy of GS-4774 in combination with TDF in patients who were treatment naïve. Inclusion criteria included positive HBsAg serology for at least 6 months, HBV DNA levels ≥ 2000 IU/mL, and no use of antiviral therapy within 3 months of study screening. Subjects were randomized to oral TDF alone or GS-4774 (dosed 2, 10 or 40 yeast units) every 4 wk until week 20. GS-4774 was safe and well tolerated. There were no significant decreases in levels of HBsAg from baseline to weeks 24 and 48 among treatment groups despite a strong immune stimulatory effect on CD8+ T cells[71].

BRII-179

BRII-179 is a virus-like therapeutic vaccine expressing Pre-S1, Pre-S2 and S HBV surface antigens stimulation a HBV specific T and B cell-mediated response[72].

In a randomized, open label phase Ib/IIa study, the safety, antiviral activity, and immunogenicity of subcutaneously-administered BRII-179 at 20 mcg and 40 mcg doses with and without PEGIFNα was evaluated in subjects with non-cirrhotic, chronic HBV. Subjects did not have detectable levels of HBsAg and were on NA at least 6 months prior to the study. Results showed that both doses of BRII-179 were safe and well tolerated with no severe adverse events. Limited HBsAg reductions (<0.2 Log HBsAg IU/mL) from baseline were observed after 4 doses of BRI-179 in both dosing groups. BRII-179 was found to be immunogenic: all BRII-179 treatment groups increased HbsAb levels by at least >30%, as compared to NA therapy alone which had no detectable anti-HBs response[72].

BRII-179 was also studied in combination with VIR-2218 for treating chronic HBV infection. An ongoing phase II study with interim results compared the combination of BRII-179 and VIR-2218 to VIR-2218 alone. Subjects were virally suppressed on a NA for at least 12 months and has HBV DNA less than the lower limit of quantification. Patients were followed to week 40. Interim results showed that BRII-179 in combination with VIR-2218 was safe and well tolerated with mild adverse events, most commonly an injection site reaction. Although no significant difference in mean HBsAg reduction from baseline was found between combination therapy and VIR-2218 alone, the combination of BRII-179 and VIR-2218 Led potent increases in anti-HBs level more than 100 IU/L in more than 40% of the subjects compared to none in the VIR-2218 alone.. Final results will evaluate the long-term therapeutic and immune response of BRII-179 and VIR-2218 combination therapies[73].

Anti PD-L1

In chronic HBV infection, there is upregulation of programmed cell death ligand-1 (PD-L1) which is responsible for T-cell exhaustion and persistence of HBV

viral disease[74]. The goal of checkpoint inhibitor therapy that blocks PD-L1 therapy is to restore the function of HBV-specific T cells[75].

ASC22 (Envafolimab)

ASC22 is a subcutaneously administered immunotherapy that blocks the programmed cell death protein 1 (PD-1) /PD-L1 pathway to restore T cell function. A phase IIb, randomized, single-blind, multicenter clinical trial was conducted to assess the efficacy and safety of ASC22 in subjects with chronic HBV who were virally suppressed on NA. Included subjects had HBsAg ≤10000 IU/mL, HBV DNA <20 IU/mL, ALT/AST less than 2x upper limit of normal, and were HBeAg negative. Subjects were randomized to either 1mg/kg or 2mg/kg subcutaneously-administered ASC22 every 2 wk in combination with a NA for 24 wk or placebo with NA. Both groups then received an additional 24 wk of NA therapy. Interim results of the combination therapy group with 1mg/kg ASC22 and NA showed more significant HBsAg reduction as compared to placebo and NA therapy, especially among patients with a baseline HBsAg ≤ 100 IU/mL. This response was durably sustained – 3 of the 7 patients with baseline HBsAg ≤100 IU/mL in the ASC22 treatment group was able to sustain a HBsAg loss lower than the lower limit of quantification (0.05 IU/mL) by the end of the follow-up period. ASC22 1mg/kg combined with NA for up to 24 wk was also safe and well tolerated. Low grade ALT flares were observed in 10/48 patients from the ASC22 group compared to none in placebo; these ALT flares also tended to occur more frequently in patients with more significant HBsAg reduction. Thus, ALT flares may be a marker to monitor treatment response[76, 77].

CONCLUSION

Current antiviral therapy with PEGIFNα and NA have low rates of functional cure and have limitations with regards to adverse effects, adherence, resistance, long-term treatment, and ongoing risk for liver events. Innovative clinical trials been key in the

development of novel therapies with a diverse range of mechanism that strive to achieve the goal of functional cure (sustained HBsAg loss and undetectable HBV DNA 24 wk post-treatment). Based on available phase 2 data, it appears that single agent approaches (e.g. RNAi alone) are unlikely to result in HBsAg loss and therefore agents combining HBsAg lowering antiviral (e.g. RNAi, monoclonal antibody) +/- immunomodulator +/- NA may be required. Combination regimens with two drug (RNAi plus NA with bepirovirsen) or three drug approaches (RNAi plus immunomodulator plus NA with VIR-2218/ PEGIFNα/NA) have demonstrated proof-of-principle that functional cure can be achieved. Future randomized controlled trials in larger representative cohorts (HBeAg positive/negative, NA naïve/experienced, low vs. high HBsAg titer) are needed to further confirm the efficacy/safety profiles of functional curative regimens and predictors of virologic response.

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