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Endpoint Selection and Serum Markers in Clinical Trials of New Drugs for Hepatitis B Cure

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1. Abstract

Chronic hepatitis B virus (HBV) infection leads to a heavy disease burden globally, which is disproportionately high in Asia Pacific region. The currently available antiviral therapy can effectively suppress HBV replication but is not capable of eliminating the virus. Many new drugs aiming at HBV cure are under investigation in recent years, most of them are in preclinical or early phases of clinical studies. HBsAg loss with or without anti-HBs is now regarded as a practical endpoint for HBV cure in clinical trials and has been applied in several phase II trials. Early decline in serum HBsAg on treatment may be considered as a surrogate endpoint in studies with short duration. Eradication of intrahepatic cccDNA is a crucial step in HBV cure, therefore standardized methodology to quantify cccDNA is strongly desired. Sensitive and accurate assays for circulating HBV RNA and HBcrAg which are correlated well with intrahepatic cccDNA levels and the transcriptional activity would facilitate their widespread application in clinical trials.

2. Introduction

It is estimated that 296 million people were chronically infected with Hepatitis B Virus (HBV), leading to approximately 820,000 deaths annually by complications of cirrhosis and Hepatocellular Carcinoma (HCC) [1]. There are two types of available treatment for Chronic Hepatitis B (CHB): Interferons (IFN) or nucleoside/nucleotide analogs (NAs). Interferon can achieve sustained HBV suppression or even loss of hepatitis B surface antigen (HBsAg) in 5-7% of treated patients, whereas NAs could effectively suppression the HBV replication thereby significantly improving the clinical outcomes, but needs long-term therapy to avoid post-treatment HBV reactivation and hepatitis flares [2-4].

Better understanding of the HBV life cycle and improved experimental models facilitate the development of new therapeutic drugs aiming to HBV cure. Three definitions of HBV cure have been raised. A complete sterilizing cure is defined as loss of HBsAg, cccDNA and HBVDNA integrated into genome. A Functional cure required sustained loss of HBsAg, with or without acquisition of anti-HBs, and undetectable HBV DNA 6 months after completing treatment. A partial functional cure has been tentatively defined as a decline in HBsAg concentrations to lower levels after finite treatment. To accelerate the clinical development of therapies for HBV cure, a

workshop with key stakeholders developed a consensus on treatment endpoints in 2016 to guide the design of the clinical trials [5]. A follow-up conference was jointly held in 2019 with participants, representing patient groups, regulatory agencies, academia and industry to promote the planning and execution of new trials [6]. Both consensuses suggested that a complete sterilizing cure is an ideal endpoint but unlikely to be feasible in the near future; a functional cure is a realistic endpoint. A partial functional cure is not determined yet.

Many HBV cure drugs have entered into different phases of clinical trials. Direct antiviral drugs such as HBV entry inhibitors, targeting HBx, translation inhibitors, capsid assembly inhibitors, HBsAg secretion inhibitors, and host immune modulators including innate and adaptive immunity activators. A combination of antiviral and immune-modulatory therapies is likely a promising strategy to achieve functional HBV cure. In view of endpoint selection for HBV cure, both old and new biomarkers have been studied intensively in recent years. Besides HBVDNA and HBsAg loss, covalently closed circular DNA (cccDNA), HBV RNA and hepatitis B core-related antigen (HBcrAg) have been evaluated. We summarized their role in HBV life cycle, the dynamic change of these biomarkers in natural history, on-treatment management and application in new drug trials.

3. Nucleic Acid Biomarkers

3.1. HBVDNA

HBV is a small enveloped virus, its infectious form containing a partially double-stranded DNA genome. HBV DNA tests are important for diagnosis and management of patients with CHB. Virological response is defined as suppression of serum HBV DNA to undetectable using sensitive real-time polymerase chain reaction assays. Maintenance of complete virological response is the basis of HBV cure. Serial monitoring of HBV DNA is necessary to assure the suppression of HBV replication. Therefore, HBVDNA test is currently mandatory in new drug trials for HBV cure.

3.2. HBV RNA

Circulating HBV RNA may be a potential biomarker for evaluation of hepatic cccDNA concentration and transcriptional activity. HBV RNA is likely to be a mixture of intact, pregenomic (pg) and subgenomic, spliced, truncated, and polyA-free species [7]. Further tested in 1827 specimens confirmed that full-length pgRNA is the primary serum HBV RNA species [8]. Serum HBV RNA, particularly pgRNA, is regarded as a reliable measurement of cccDNA transcriptional activity. HBV RNA response was usually defined as HBV RNA decline of either >2 logs, or an HBV RNA decline for >1 log and below the Low Limit of Detection (LLD).

In the natural history of chronic HBV infection, serum HBV RNA levels vary greatly. The level is lower in the HBeAg-negative than those in the HBeAg-positive patients, with the lowest levels in inactive carriers [9, 10]. In a retrospective cohort study consisting of 204 outpatients with untreated CHB, the median serum HBV pgRNA level was 4.12 log10 copies/ml and 33.3% of them being under LLD (<500 copies/ml), with serum HBV pgRNA under LLD in 15.75% of HBeAg-positive and 77.59% of HBeAg-negative patients [10].

In a cohort of PEG-IFN-based therapy, the HBV RNA response was defined as HBV RNA decline of either >2 logs or an HBV RNA decline or >1 log which resulted in HBV RNA level below the LLD. The results showed that 56.4% of patients with HBV RNA response during the treatment did not experience HBsAg decline >0.5 log, resulting a low rate of HBsAg loss [11]. In a study with 388 patients, NA treatment reduced serum HBV RNA by 1.46 logs and 1.77 logs at weeks 48 and 96, respectively, with 15.8% of patients with HBsAg seroclearance having detectable HBVRNA [12]. End-of-treatment HBV RNA and off-treatment serial HBV RNA were independently associated with HBV DNA levels; patients with HBV RNA level ≥44.6 U/mL at the end of treatment had a cumulative 48-week HBV DNA >2000 IU/mL rate of 93.2%, whereas patients with serum HBV RNA undetectable (<44.6 U/mL) and HBsAg <10 IU/mL had a durable off -treatment response [13].

The role HBV RNA as an endpoint has also been investigated in trials on novel therapy. During the phase I study in HBeAg-positive non-cirrhotic CHB patients, NVR 3-778 (the first capsid assembly modulator, CpAM) plus PEG-IFN reduced HBV RNA further by 2.09 log10 copies/mL compared with monotherapy of NVR 3-778 (by 1.42 log10 copies/mL) or PEG-IFN (0.89 log10 copies/mL), respectively [14]. Similarly, dose-dependent decreases in serum RNA levels are also observed consistently with HBV DNA declines in phase I trial of ABI-H0731 (oral CpAM) [15].

for a longtime there had been no standardized assay available, although serum HBV RNA has been quantified in many studies by high-throughput HBV-RNA tests. Recently, an automated assay for the quantification of serum HBV pgRNA has been improved to be six-fold more sensitive over in-house assays [16]. In a study, four assays to quantify serum HBV RNA levels were developed and proved to be capable of predicting HBeAg loss in ETV treated patients [17]. Further studies to establish a standardized sensitive quantification detection of serum HBV RNA would be expected to facilitate the widespread application in clinical trials.

3.3. Covalently Closed Circular DNA (cccDNA)

Covalently closed circular DNA in the nucleus of hepatocytes provides a transcriptional template for pregenomic and subgenomic RNAs. With a highly sensitive PCR assay, it is observed that the median intrahepatic cccDNA levels were 1.4, 0.01, 0.02, 0.002 copies/cell in HBeAg positive patients, HBeAg negative patients, inactive carriers, and patients with HBsAg clearance, respectively [18]. Decay of cccDNA is slow with a half-life of about 40 days and 35-57 days in vitro and in vivo models [19, 20]. Recently, it is estimated with a mathematical model that cccDNA lifespan is 61 (36–236) days in HBeAg positive patients and is only 26 (16–81) days in HBeAg negative patients [21]. The new genesis of cccDNA via intra-cellular amplification contributed to the maintenance/replenishment of

cccDNA pools, which is responsible for the persistence of chronic HBV infection.

Approved antiviral therapy does not directly target the cccDNA reservoir but has an indirect effect in reducing cccDNA. Forty-eight weeks of PEG-IFN therapy resulted in a decrease of cccDNA from median 33.8 copies/cell to 2.53 copies/cell [22]. In CHB patients treatment with NAs (ETV, LAM, or TDF) for one year could also slightly decrease the HBVcccDNA [18, 23].

New approaches to target HBV cccDNA are under investigation. Neddylation inhibitor MLN4924, which is currently in several phase II clinical trials for anti-cancer application, can effectively inhibit the production of cccDNA and HBV antigen [24]. Cheng et al found that dicoumarol, an inhibitor of NAD(P)H: quinone oxidoreductase 1 (NQO1) could dose-dependently blocks cccDNA transcription through promoting degradation of HBx [25].

Though eradication of intrahepatic cccDNA is considered to be a crucial step for HBV cure, there are two obstacles for cccDNA to act as an endpoint of trials. First, there are still no sensitive, specific, and quantitative commercial assays available for the detection of cccDNA. Second, a liver biopsy specimen is required for cccDNA detection. Therefore, a surrogate non-invasive markers for cccDNA may need to be developed.

4. Protein Biomarkers

4.1. Hepatitis B Surface Antigen (HBsAg)

HBsAg was first reported as a "new" antigen in leukemia serum by Dr. Blumberg in 1965, it is also termed the “Australian antigen” because it was first found in an Australian aborigine [26]. All three HBV surface proteins isoforms, namely Small (SHBs), Medium (MHBs), and Large (LHBs) are encoded within one open reading frame of HBV (S-ORF), but are translated from different mRNAs by alternate use of three translational start codons [27]. The SHBs composites the majority (~80%) of the HBsAg, MHBs and LHBs form the minority. The proportion of MHBs and LHBs vary in Subviral Particles (SVP), with MHBs accounts for a small amount (~10%), while LHBs accounts for about 25% in SVP filaments and virions [28]. The half-life of circulating HBsAg is about seven days in patients with HBV infection, with the SHBs having a shorter half-life than MHBs and LHBs [29]. HBsAg can be expressed from both HBV cccDNA which are the major reservoir and template for HBV DNA replication and transcription and integrated viral DNA sequences [30, 31]. HBsAg forms the envelope of virions, but the incomplete HBV viral particles such as empty virions and RNA virions exit in 1000–100,000-fold excess relative to the number of virions [32]. HBsAg turnover in the blood during chronic infection is rapid, which may be driven by antibody-mediated or antibody-independent mechanisms.

In the natural history of untreated CHB, serum HBsAg levels vary in different phases of chronic HBV infection. In general, HBsAg levels are higher in the hepatitis B e antigen (HBeAg) positive phase compared to the HBeAg-negative phase [33]. It takes a long time for HBsAg levels to decline even after transitioning to the HBeAg-negative phase. A meta-analysis showed that spontaneous HBsAg loss occurred in 3489 (7.6%) of 45,975 patients with 341,862 person-years of follow-up, resulting a pooled annual incidence rate of HBsAg loss of 1.13%, which is higher in HBeAg negative (1·44%) than that in HBeAg positive patients (0·74%) [34].

HBsAg loss during antiviral therapy requires effective inhibition of transcription from both cccDNA and integrated HBV DNA. HBsAg loss with or without anti-HBs confirmed on two occasions at least six months apart has been considered a valid surrogate endpoint for functional cure of HBV infection [5].

HBsAg loss would be a practical endpoint because there are commercialized assays that can quantitate HBsAg at a relatively low cost. Standardized assays are available for quantification of HBsAg with a Lower Limit of Detection (LLOD) of around 0.05 IU/ml and even 0.005 IU/ml. the upper limit of detection to over 50,000 IU/ml as the systems have automatic onboard dilution. However, the assays are not capable of discriminating between the three different HBs proteins or transcription sources of HBsAg [35, 36].

Therefore, HBsAg loss is currently used as the primary efficacy endpoint in most treatment trials of novel therapeutic agents irrespective of treatment targets [6] (Table 1 and Table 2). Myrcludex B (MyrB) is the first approved drug for hepatitis D by the European Medicines Agency. MyrB prevents the entry of HBV into hepatocytes by competing with HBV for sodium taurocholate co-transporting polypeptide (NTCP). Administration of MyrB combined with injection of pegylated interferon α (Peg-IFN α) for 48 weeks was safe and effective in phase II studies in patients co-infected with HBV and HDV, yielding a HBsAg loss rate of 13% [37].

As a translation inhibitor, siRNA agent JNJ 3989 combined with NAs resulted in sustained declines of HBsAg, HBeAg, HBcrAg, and HBV RNA [38]. By treatment day 168, the average HBsAg level fell from baseline by 1.93 log10 IU/mL, with 39 of the 40 participants (98%) having at least a 1 log10 (10-fold) drop at the lowest point of the decline [39]. Similarly, in Phase II trial of ARB-1467 which comprises three RNAi triggers that target all four HBV transcripts, seven of the 12 patients met the predefined response criteria (at least 1 log10 reduction in serum HBsAg level and a serum HBsAg level below 1000 IU/mL) at or before day71. GSK3389404 (an antisense oligonucleotide inhibits synthesis of HBsAg and other HBV proteins) dosing has been tested up to 120 mg for 4 weeks with an acceptable safety and pharmacokinetic profile, supporting further clinical investigation in patients with chronic hepatitis B [38].

Nucleic Acid Polymers (NAPs) inhibit the assembly and secretion of Hepatitis B Virus (HBV) Subviral Particles (SVP). In an open-label, phase 2 study, the addition of NAPs REP 2139 or REP 2165 to Tenofovir Disoproxil Fumarate (TDF) plus PEG-IFN did not alter tolerability and significantly increased rates of HBsAg loss and HBsAg seroconversion during and after the therapy [40].

Studies showed that early decline in serum HBsAg on treatment at least 1 log by week 12 or 24 are associated with HBsAg loss during or after treatment, with a positive predictive value of up to 45% and a high negative prediction value of up to 97% [5]. A systematic review suggested that patients with decreasing HBsAg levels or lower HBsAg levels (<100 IU/ml) are capable of maintaining control of HBV after discontinuation of long-term NA therapy [41]. However, the ability of HBsAg decline to predict HBsAg loss might differ with HBeAg status, HBV genotype, and the treatment. Therefore, it is suggested that a decline in HBsAg levels in phase II clinical trials should currently only be considered as exploratory [6], whether it can be used as a surrogate endpoint is still an issue at discussion.

Table 1: Direct antiviral drugs of HBV cure

Drug name (Sponsor)	Mechanism of action	Stage	Primary Endpoint	Secondary Endpoints
Entry Inhibitor				
Myrcludex B (MYR)	NTCP blocker	2	Proportion of patients with HBsAg response at week 12	Proportion of patients with HBsAg response at week 24
HH003 (Huahui)	NTCP blocker	1		
Hepalatide (Shanghai HEP)	NTCP blocker	2	Virological response at week 24: HBV DNA<20 IU/ml	HBV DNA down from baseline log10
VIR-3434 (Vir)	HBV-neutralizing antibody	1		
GST-HG131 (Fujian)	S-antigen inhibitor	1		
ALG-010133 (Aligos)	S-antigen transport inhibitor	1		
Targeting HBx				
CRV431 (Contravir)	Blocks NTCP and protein folding	1		
Nitazoxanide (Romark)		2	Mean change in quantified HBsAg from baseline to week 12	
Translation inhibitors				
JNJ3989 (Janssen)	siRNA	2	Percentage of patients with HBsAg sero-clearance up to week 96 without restarting NA treatment	
AB-1467 (Arbutus)	siRNA	2	Reductions in serum HBsAg levels	
GSK3389404 (GSK)	ASO	2	Safety	Change from baseline in log10 HBV DNA and HBsAg levels in plasma at baseline, Day 1, 3, 8, 15, 22, 30 and 60
GSK3228836 (GSK)	ASO	2	Percentage of patients who achieve SVR for 24 weeks after the end of treatment; percentage of participants achieving HBsAg and HBV DNA < LLOQ from off-treatment week 1 to off-treatment week 24	
BRII-835 (VIR-2218) (Brii)	siRNA	2	Percentage of participants with sustained HBsAg loss during the 48-week follow-up period after NA withdrawal	
Capsid assembly inhibitors				
ABI-H0731+AB729 (Assembly)	Small molecule	2	Safety	Change from Baseline in mean log10 HBsAg, number of participants with HBV RNA <LLOQ at week 48, change from baseline in mean log10 HBcrAg
JNJ6379 (Janssen)	Small molecule	2	Percentage of participants with HBsAg seroclearance up to week 96 without restarting NA treatment	
JNJ0440 (Janssen)	Small molecule	1		
GLS4 (HEC Pharma)	Small molecule	2	The value of serum HBsAg decreased from baseline to week 48 weeks after dosing	The value of serum HBeAg decreased from baseline

RO7049389 (Roche)	Small molecule	2	Percentage of participants with HBsAg loss at 24 weeks post-EOT (up to 72 weeks)	Percentage of participants with HBsAg loss up to 96 weeks; Percentage of participants with HBsAg seroconversion up to 96 weeks.
AB-506 (Arbutus)	Small molecule	1		
ABI-H2158 (Assembly)	Small molecule	2a	Safety, change from baseline in mean log10 HBV DNA	
QL-007 (Qilu)	Small molecule	2	The change of HBV DNA level at week 24 of treatment compared to baseline	The changes of HBsAg and HBeAg level from baseline at week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72, 84, 96
EDP-514 (Enanta)	Small molecule	1		
ALG-000184 (Aligos)	Small molecule	1		
AB-836 (Arbutus)	Small molecule	1		
GST-HG141 (Fujian)	Small molecule	1		
HBsAg secretion inhibitors				
REP 2139, 2165 (Replicor)	NAP	2	Safety	The primary action of NAPs is to lower serum HBsAg. This effect is monitored throughout treatment and for 48 weeks following treatment.

Abbreviations: NTCP, sodium taurocholate cotransporting polypeptide; HBsAg; Hepatitis B surface Antigen; HBV: Hepatitis B Virus; SVR: Sustained Virologic Response; siRNA: small Interfering RNA; ASO: Antisense Oligonucleotide; LLOQ: Lower Limit of Quantification; NA: Nucleos(t)ide Analogs; EOT: End of Treatment; NAP: Nucleic Acid Polymer.

Table 2: Immune modulators of HBV cure

Drug name	Mechanism of action	Stage	Primary Endpoint	Secondary Endpoints
Innate immunity activators				
RO7020531 (Roche)	TLR7 agonist	1		
RO6864018 (Roche)	TLR7 agonist	2	Safety	Pharmacodynamics: peripheral blood levels of interferon (IFN)-alpha in QOD dosing cohorts
GS-9620 (Gilead)	TLR7	2	Mean change (log10 IU/mL) in HBsAg from baseline at week 24	Percentage of participants with HBeAg loss and seroconversion at week 24
GS-9688 (Gilead)	TLR8	1		
APG-1387 (Ascentage)	IAP inhibitor	2	Safety, decline in HBsAg	
TQ-A3334 (Chia Tai Tianqing)	TLR7 agonist		pharmacokinetics/pharmacodynamics	HBV biomarker, including HBsAg, HBsAb, HBeAb, anti-HBc, HBV-DNA, HBV RNA and HBcrAg at day 1 pre-dose, day 84, day 168, day 336 post-dose
Adaptive immunity activators				
TG-1050 (T101) (Transgene/ Talsy)	Ad5 delivery Vaccine	1		
GS-4774	therapeutic vaccine	2	Mean change in serum HBsAg from baseline to week 24	Mean change in serum HBsAg from baseline to week 12
HepTcell (Altimmune)	Peptide plus IC31 (adjuvant) Vaccine	2	The proportion of patients achieving virologic response	The proportion of patients achieving clearance of HBsAg
BRII-179 (Brii)	immunotherapeutic vaccine	2	Percentage of participants with sustained HBsAg loss during the 48-week follow-up period after NrtI withdrawal	
CVI-HBV-002 (CHA)	Therapeutic vaccine	2b	The proportion of patients achieving clearance of HBsAg	
SLGN+nivolumab (GS)	CTLA-4 inhibitor+PD-1 inhibitor	2a	Proportion of participants with (HBsAg loss and HBV DNA < 20 IU/mL at follow-up (FU) week 24	
ASC22 (Ascletis)	anti-PD-1 antibody	2	The decreased HBsAg levels at 12 or 24 weeks of treatment or at 4, 12, or 24 weeks of follow-up visits compared with baseline, the number of patients with ≥0.5 log reduction in HBsAg log10IU/ mL at 12 or 24 weeks of treatment, or at 4, 12, or 24 weeks of follow-up visits compared with baseline	

HLX10 (Henlix)	anti-PD-1 antibody	2	The proportion of the subjects who achieve 0.5 log decline in HBsAg log10 IU/mL from baseline	
Cemiplimab (Regeneron)	anti-PD-1 antibody	2-Jan	Safety	Change in quantitative HBsAg from pre-treatment to weeks 6, 8, 12, 14, 18, 24, 36, 54, 72, 90
Inarigivir Soproxil (GS)	NOD agonist	2	Proportion of participants With ≥ 0.5 log10 IU/mL decline in HBsAg from baseline at week 12	

4.2. Hepatitis B Core-Related Antigen (HBcrAg)

Serum HBcrAg test recognizes denatured HBcAg (10%), HBeAg (80%) and pre-core protein (10%) [42]. In untreated patients, HBcrAg levels were significantly higher in HBeAg positive than in HBeAg negative patients and correlated with intrahepatic cccDNA levels, and transcriptional activity [43]. HBcrAg significantly declined after 2-year first-line oral antiviral therapies (ETV, TDF, and TAF), all of them with a similar magnitude of reduction [44]. The presence of HBcrAg seems to predict viral relapse after discontinuation of NAs therapy.

HBcrAg could be useful in the evaluation of novel therapies aiming at a functional cure either by directly or indirectly targeting the intrahepatic cccDNA pool. A high-sensitivity assay for HBcrAg (iTACT-HBcrAg) has been developed, which is about 10-fold more sensitive than the conventional HBcrAg assay [45]. This new assay is timely for the ongoing pursuit of an HBV cure and may be applied as an exploratory endpoint in clinical trials.

5. Conclusions

New agents aiming at HBV cure are developing rapidly in recent years. HBsAg loss is now the practical endpoint for HBV cure in clinical trials. Early decline in serum HBsAg may be an exploratory or surrogate endpoint. Standardization for direct quantification of cccDNA or indirect measurement of intrahepatic cccDNA levels as well as its transcriptional activity by HBV RNA and HBcrAg, are in urgent need to facilitate their widespread application in clinical trials.

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