Optimizing Antiviral Therapy for Chronic Hepatitis B

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A controlled shift towards cure

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Colophon

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Optimizing Antiviral Therapy for Chronic Hepatitis B
A controlled shift towards cure

Antivirale therapie optimaliseren voor chronische hepatitis B
Gecontroleerd verschuiven naar genezen

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CHAPTER 1

GENERAL INTRODUCTION

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HEPATITIS B VIRUS INFECTION

The hepatitis B virus (HBV) is a hepatotropic virus that may cause acute and chronic liver disease, which may ultimately progress to hepatic decompensation or hepatocellular carcinoma (HCC). HBV was serendipitously discovered in 1967 when Dr. Blumberg and Dr. Alter identified the Australia antigen in an infected aborigine.[1] This antigen, nowadays known as the hepatitis B surface antigen (HBsAg), is both the hallmark of a chronic hepatitis B (CHB) infection and the antigenic compound in the HBV vaccine. Fifty years later, HBV infection remains one of the most substantial and prevalent public health problems, ranking seventh among the principal causes of death worldwide.[2]

Epidemiology

Nearly a quarter of the global population has been in contact with HBV and approximately 257 million people are chronic HBV carriers.[3–5] CHB accounts for 786,000 annual deaths from HBV-related liver disease.[2] The global prevalence of HBV is 3.6%, which is higher than that of human immunodeficiency syndrome (HIV), malaria or tuberculosis.[6,7] The HBsAg seroprevalence varies substantially per region, from as low as 0.01% in the United Kingdom to 20% or higher in resource limited regions such as sub-Saharan Africa and several Western Pacific countries. Over 85% of people reside in regions with intermediate to high HBV endemicity.[8] In the European Union and European Economic Area, approximately 0.9% of the population have CHB.[9] In the Netherlands, the prevalence of people living with CHB was estimated at 0.34% in 2016, with the highest prevalence among first-generation migrants from Turkey, Somalia and China.[10] Globally, the number of infected people has decreased in recent years, according to a systematic review, possibly due to preventative strategies, improved socio-economic factors and effects of antiviral treatment.[11] However, the incidence of HBV infection has increased in low-endemic countries impacted by the opioid crisis (USA, Canada) [12,13] or by migration between high and low-endemic regions (Europe).[14–16] Furthermore, the current seroprevalence rates possibly underestimate the true number of chronic carriers, since many studies excluded studies focusing solely on at-risk populations, such as immigrants, incarcerated, institutionalized or homeless people.

Impact of hepatitis B virus infection on Public Health and counter measures

Disease burden

CHB has a high disease burden[3,17] and accounts for 786,000 annual deaths from HBV-related liver disease, as estimated in the Global Burden of Disease Study 2010[2]. Combined with hepatitis

C virus (HCV) infection, an estimated 1.29 million viral hepatitis-related deaths occurred in 2010. To put these numbers in perspective, the number of deaths attributable to HIV/AIDS was 1.34 million, 1.29 million for tuberculosis and 850,000 for malaria[18]. Furthermore, an estimated 42.5 million disability-adjusted life-years and 41.6 million years of life lost are related to viral hepatitis. Strikingly, while the global burden for other communicable diseases has diminished, the absolute burden of HBV infection continues to increase, primarily due to changing demographics: population growth and changing age structures.[19]

Apart from the clinical burden, HBV infection severely impacts the economic burden [20,21] in both low- and high-income countries.[20–22] The burden is especially high among risk groups, such as incarcerated people[23] or immigrants[22,24], indicated by 1.5 years loss of quality-adjusted life years and substantially increased health care costs, as modelled with Markov cohort models.[25] These findings emphasize that better public health interventions are needed to reduce the substantial HBV-related burden.

Vaccination

Among strategies to counter the disease burden, the HBV vaccine has been the most effective and safe intervention[26], and was the first vaccine to be effective against a human cancer. Nowadays, universal vaccination of new-borns has been adopted in 94% of countries worldwide.[27] The long-term experience of Taiwan with a nationwide HBV immunization program has revealed >90% reduction in HBsAg carriage rates.[28] Similarly, HBV-related end-stage liver disease, mortality and the incidence of HCC caused by CHB have decreased continuously and significantly in all age and sex groups during 30 years of implemented HBV vaccination.[29,30]

Screening and linkage to care

Undiagnosed viral hepatitis poses a large problem to elimination.[31,32] A cross-sectional screening among Asian-Americans in San Francisco showed that 65% of HBsAg positive tested people were unaware of the present CHB infection.[33] Screening of at-risk populations could decrease this discrepancy. Examples of risk groups are patients receiving immunosuppressive therapy, people who inject drugs, incarcerated or health care workers. To be effective, linkage to care networks should be established. Examples of large screening projects are the Hepatitis Outreach Network (HONE) in New York City[34] and the Toronto Viral Hepatitis Care Network (VIRCAN).[35] The efficacy of screening for viral hepatitis is based on identification of people at risk and subsequent intervention that prevents complications. The former has been confirmed by large observational studies that showed that a significant burden of advanced liver disease was detected by screening.[36–38]

Public Health interventions

Recognizing the persistent viral hepatitis burden, the World Health Organisation (WHO) adopted a resolution that acknowledges the global viral hepatitis burden, aims to eliminate viral hepatitis as a public health problem by 2030 and helps countries establish national action plans.[39] These five target areas focus on prevention (increasing infant vaccination coverage to 90%; preventing mother-to-child transmission; improving blood and injection safety; harm reduction); diagnosis and treatment (90% diagnosis coverage and 80% eligible treated), with the ultimate goal of elimination (90% incidence reduction in chronic viral hepatitis infections, 65% decrease in mortality). Thus far, only 13.1% of patients has been diagnosed with CHB and 2.4% of those eligible has received treatment.[40]

A comprehensive simulation study that modelled the current requirements to achieve global HBV elimination supported the areas of need specified by the WHO: increased vaccination coverage, scale-up of preventative measures for mother-to-child transmission, and implementing wide-scale population-based screening and therapy.[41] Other Public Health interventions to be addressed include improving patient and public knowledge of the disease, coordinate policies between the affected population, governments and other stakeholders, mobilize resources and collect data on quantifiable objectives that enable measurement of progress.[42] Reaching these goals facilitates achieving the Sustainable Development Goal to combat viral hepatitis.

Viral structure and lifecycle

HBV is one of the smallest (3.2 kb) enveloped DNA viruses and a family member of the *Hepadnaviridae*. The virion consists of a surface envelope (protein S [HBsAg], pre-S1 and pre-S2), nucleocapsid consisting of core protein (HBcAg), partially double-stranded HBV DNA and viral polymerase. The main virologic features of HBV are the specificity to liver tissue, formation of the stable minichromosome covalently closed circular (ccc)DNA that causes persistent infection, and the unique mechanism of viral replication.[43,44]

HBV virions, or 'Dane particles', are enveloped by an outer lipid membrane (HBsAg) which contain a spherical nucleocapsid core protein (HBcAg) of HBV virions. The core contains a polymerase protein and encapsidated HBV genome, both of which are essential for viral replication. Since the viral genome consists of relaxed circular DNA (rcDNA) that is only partially double-stranded, HBV requires the viral DNA polymerase to complete the (+) strand of DNA, which can then be converted to cccDNA.[45–47]

The minichromosome cccDNA is an episome within the hepatocyte nucleus that serves as the sole transcription template for all four viral mRNAs. CccDNA therefore constitutes a major barrier

to cure CHB infection. cccDNA is continuously replenished by recycling of nucleocapsids. Both cell division and treatment with entry inhibitor Myrcludex can deplete the nuclear cccDNA *in vivo*.[48] A better understanding is needed of the production, maintenance, and monitoring of cccDNA. An important downside to measuring cccDNA is that a liver biopsy would be needed and that standardizing the test remains difficult. Serum HBV RNA and pgRNA are promising biomarkers of cccDNA level and its transcriptional activity during NA and PEG-IFN treatment.[49,50]

Understanding the complex lifecycle of HBV gains important insight into antiviral treatment targets. The viral lifecycle of HBV starts with circulating virions binding to heparan sulphate proteoglycans (HSPGs) which act as hepatocyte 'homing beacons' for several viruses, at the basolateral membrane of the hepatocyte. The binding is followed by attachment to the Pre-S1 domain of the large component of HBsAg to the sodium taurocholate co-transporting polypeptide (NTCP or SLC10A1) receptor enabling entering of the hepatocyte. As most enveloped viruses, indirect transport within the hepatocyte of HBV depends on clathrin-mediated endocytosis. Direct intracellular transport to the nucleus uses a microtubule network and dynein motor proteins, which usually regulate macromolecules and organelle transport.[51] The endosome migrates to the nucleus where it complexes with a nuclear core complex situated in the nuclear membrane and disassembles the HBV nucleocapsid into the cytoplasm. Interaction with a domain of the HBV core protein is important for this step. The disassembled nucleocapsid then initiates nucleus entry of HBV DNA and HBV polymerase.[52]

Within the hepatocyte nucleus, virions release the HBV genome and form a completely double-stranded DNA. Subsequently, hepatocyte polymerase II converts the DNA into the minichromosome cccDNA. The cccDNA is an episome within the hepatocyte nucleus that serves as the sole transcription template for all four viral mRNAs. Here, rcDNA converts into the highly-stable minichromosome cccDNA, thereby enabling viral transcription (and synthesis of pgRNA by host RNA polymerase II) and replication.[51] These proteins are used for viral replication (pregenomic RNA [pgRNA]) and translation of viral proteins (mRNA). The viral mRNAs are transcribed by host RNA polymerase II into: the envelope protein comprising three surface antigens (large, middle and small); DNA polymerase with reverse transcriptase ability; core protein (HBcAg); hepatitis B x protein; and hepatitis B e antigen (HBeAg).[46] Since nucleos(t)ide analogue (NA) therapy does not directly target cccDNA, prevention of infection and complete eradication of HBV remains hard to reach.[49]

After transportation to the cytoplasm, pgRNA is used as template for HBcAg and HBV DNA polymerase. The polymerase reverse transcribes pgRNA to single-stranded and relaxed circular DNA (rcDNA). New rcDNA-containing virions are then packaged in nucleocapsids and enveloped

and then secreted in the bloodstream (Dane particles) or recycled back into the nucleus to replenish the cccDNA pool.[46] The process from HBV entry until detection of HBV genomes takes less than 15 minutes. Infected hepatocytes also produce a large amount of non-infectious sub-viral particles that possibly overwhelm the immune response. Apart from cccDNA, integrated viral genome in the host genome can produce truncated HBsAg.

The discovery in 2012 of the NTCP receptor has enabled researchers to develop cell culture and animal models to study antiviral drugs for HBV.[53] *In vivo* studies indicate that apart from the NTCP receptor and HSPGs, other (yet to be identified) receptors are required for HBV entry into the hepatocyte.[54]

Viral genotypes

Since the reverse transcriptase function of HBV polymerase lacks proof-reading, transcription may lead to random changes in the genetic sequence. Based on a genetic sequence difference of at least 8%, HBV is categorized in nine major genotypes (A-I).[47] The distribution of HBV genotypes around the world is very distinct: HBV genotypes A and D are most common in Europe, Africa and India, whereas genotype B is more prevalent in Asia and genotype C in East and Southeast- Asia.[55] Viral genotypes are related to differences in disease progression, severity of disease and response to interferon treatment.[56] Moreover, differences in HBV genotypes may account for suboptimal efficacy of the HBV vaccine through mismatch between the vaccine strain (serotype *adw*) and the receiving population[57,58], arguing for the need to adjust vaccines for some regions.

Natural history of hepatitis B virus infection

Transmission

HBV is predominantly transmitted through contact with infected blood or semen. Other bodily fluids, such as saliva[59], breast milk[60], urine, sweat and tears[61], may be additional sources of HBV infectivity. HBV DNA could be detected in these bodily secretions by polymerase chain reaction (PCR), but only blood, semen and saliva were demonstrably infectious in animal models and humans.[62,63] The most common routes of transmission are mother-to-child transmission at birth (vertical transmission), sexual contact and drug injection use.[64–66] Mother-to-child transmission occurs more often in high-endemic regions such as South-East Asia[67] where the large proportion of HBeAg positive status and high viremia among childbearing women results in high infectivity.[68] In comparison, horizontal transmission occurs more frequently in Africa, possibly due to a lower proportion of HBeAg positivity and therefore a presumably lower perinatal infectious risk.[69] Horizontal transmission occurs through non-sexual close contact, such as between family members in the same household.[70] Injection or transfusion of contaminated blood (products)

may lead to HBV transmission, although preventative measures have decreased this mode of transmission. HBV is more infectious than hepatitis C virus (HCV) or HIV[71] and has a longer incubation period (8-20 weeks) than HIV (3-10 weeks).[72]

Acute hepatitis B virus infection

An acute HBV infection in adults has an average lifespan of 6 weeks and is often self-limiting. A quiescent phase is followed by an exponential phase during which serum HBV DNA levels can become very high and infect nearly all hepatocytes, although patients often remain asymptomatic. The age at time of infection strongly influences the risk of CHB development. While an acute HBV infection progresses to chronic disease in <5% of adults, infection below 6 years of age progresses in 30-50% of children and infection at birth or perinatally in 80-90% of infants.[73,74] The WHO therefore recommends that all new-borns receive the HBV vaccine at birth, preferably within the first 24 hours. The underlying immunological processes have been understudied, but could be due to a preserved immune responsiveness. *In vivo* studies showed that T cell function was conserved (marginal PD1 expression and larger number of T cells) in children in the IT phase, which allows a better response to manage HBV infection.[75] Similarly, *in utero* exposure to HBV can lead to Th-1 mediated sustained immunity.[76]

Signs and symptoms

Most patients with acute HBV infection remain asymptomatic, especially if infection occurred at a young age. The spectrum of signs and symptoms ranges from mild to severe and appears on average 1-4 months after infection. The clinical manifestations of HBV infection may include weakness, fatigue, loss of appetite, nausea and vomiting, abdominal pain, fever, jaundice.[77] Fulminant hepatic failure occurs rarely (0.1-0.5% of patients) and is related to immune-mediated liver cell death[78]

Immunological processes during a resolving hepatitis B virus infection

A better understanding of the immune response to HBV infection may provide knowledge to improve the antiviral treatment response, with the ultimate goal of restoring the immune response of treated patients to a state similar to that of healthy subjects.

Innate immunity

HBV is often described as a 'stealth' virus for several reasons. In the early phase of infection, the innate immune system often fails to detect the viral particles, which primarily comprise

HBsAg.[79] In the case of a self-limited infection, non-cytolytic factors and interferon (IFN)-gamma presumably control the virus in the early stages. HBV can avoid the innate detection system by blocking the production of IFN-gamma and reducing Toll-like receptor (TLR) expression with HBV proteins HBsAg and HBeAg. *In vivo* data suggest that a lack of IFN-inducible genes increases viral entry and replication, suggesting a compromised innate immunity including reduced activation and effector function. HBV is thus able to circumvent the innate immune response. Other animal and cell culture studies claim that infection with HBV leads to an intrahepatic innate immune response, but only if high inoculum concentrations were used. Type-I (IFN-alpha and beta) and type-III IFN (IFN-gamma) activate IFN-stimulated-genes (ISGs) expression. However, ISG expression did not occur during HBV infection.

Liver-resident NK-cells represent another important component of the cellular immune system, comprising 30-40% of lymphocytes in the liver. On the one hand, the induction of NK-cells appeared to be delayed as the highest number and activity of NK-cells occurred after the peak of viremia had passed and after adaptive immunity had started. Alternatively, other groups reported that NK-cell activation and function were observed during the early phase of infection, even before T-cell expansion, contrasting previous results.[80]

Adaptive immunity

T-cell exhaustion and lack of T-cell memory maturation characterize the delayed adaptive immune response, which might also aid the initial evasion of the immune system by HBV.[81] CD8+ T-cells are timely recruited to the liver upon platelet activation and produce cytokines that can clear HBV without killing off hepatocytes. In the acute phase the HBV-specific T-cell response is poly-clonal and strong. Although functionally fully activated, T-cells are unable to secrete cytokines or perform effector functions otherwise due to T-cell inhibitory molecules (arginine from necrotic hepatocytes) that presumably dampen disproportionate liver cell damage.[79] In the event of spontaneous HBV infection resolution, the number of activated T-cells decreases and protective memory T-cells mature.

Chronic hepatitis B virus infection

Disease phases in the natural history of chronic hepatitis B

Infection with HBV becomes chronic if patients remain HBsAg positive for at least 6 months. The natural history of CHB is very heterogeneous and is generally categorized into five disease phases, based on HBeAg status, HBV DNA, alanine aminotransferase (ALT) and presence of liver

inflammation.[64–66] Each of these phases is associated with a different rate of disease progression. Patients do not necessarily go through all phases, nor can each patient be categorized accordingly. The nomenclature for the various phases has changed over time, but definitions have essentially remained the same.

(1) HBeAg-positive chronic infection (formerly known as immune tolerant phase) is defined by the presence of HBeAg and high HBV DNA levels, normal ALT (conventionally defined as ≤40 IU/mL) and minimal liver inflammation. Due to the high viral load in this phase patients are very infectious. Integration of viral DNA in the host genome occurs frequently, which might initiate hepatocarcinogenesis[82] and HBsAg levels are high. This phase might last for 1-3 decades, depending on the age of infection, mode of transmission, ethnicity and HBV genotype. Patients with HBeAg-positive chronic infection have a good prognosis as observational studies have reported no HCC occurrence during 10.5 years of follow-up.[83]

(2) The HBeAg-positive chronic hepatitis phase (immune active HBeAg-positive phase) is marked by HBeAg positive status, high HBV DNA levels and ALT elevation. The rise in ALT reflects underlying liver necroinflammation from the host immune response, and fibrogenesis. Patients may become symptomatic. Serum ALT levels are an important factor in starting antiviral treatment. HBeAg seroconversion and virological suppression occur frequently, which may be preceded by ALT flares. Recurrent flares are associated with an increased risk of cirrhosis and HCC.[84]

(3) HBeAg-negative chronic infection (inactive carrier phase) is categorized by the presence of anti-HBe, undetectable to low (<2,000 IU/mL) viral load and ALT normalisation. Disease progression to cirrhosis or HCC is low. Some patients (1-3%) may spontaneously lose HBsAg in this phase. Although patients in this phase have a normal ALT, biopsy studies revealed a higher-than-expected rate of liver injury.[85]

(4) HBeAg-negative chronic hepatitis (immune active HBeAg-negative hepatitis phase) displays absence of HBeAg with or without detectable anti-HBe, a high viral load and fluctuating ALT levels. Evidence of fibrosis and necroinflammation was found in liver biopsies.

(5) Patients in the HBsAg-negative phase (occult infection) are anti-HBc positive. Anti-HBs may be detectable.

Immunology during chronic hepatitis B virus infection

During a chronic HBV infection, hallmarks of the deficient host immune system comprise functionally exhausted T-cells and a defective B-cell response.

T-cell exhaustion

One of the hallmarks of an impaired adaptive immunity to HBV infection is T-cell exhaustion, both in quality as in quantity. Various factors contribute directly to the reduced T-cell responsive state. Persistent exposure to a high viral load, tolerogenic properties of liver cells, and several cytokines (interleukin [IL]-10, tissue growth factor [TGF]-beta, arginase) contribute to reduced T-cell activation.[86] Recent experimental work revealed that functional T-cell exhaustion is also evident from the low metabolic and anergic state of T-cells.[87] Targeting the dysfunctional mitochondria improved antiviral activity of exhausted CD8 cells.[88]

Less direct mechanisms may also lead to T-cell exhaustion. Various co-inhibitory molecules (T-cell Immunoglobulin and Mucin-domain containing-3 [TIM3], Cytotoxic T-Lymphocyte–associated Antigen 4 [CTLA4], 2B4, Programmed Death 1 [PD1]) that are present on exhausted T-cells indirectly inactivate other T-cells.[89] The effect of anti-PD/PD1 antibodies is considerably stronger intrahepatically than peripherally, suggesting a liver-specific immune dysfunction. Furthermore, hyperactivity of CD4+ CD25+ FOXP3+ regulatory T-cells and impaired T-cell signalling amplify the T-cell exhaustion. NK-cells rapidly delete HBV-specific T-cells upon up-regulation of death receptor tissue necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL).[90–92] To what extent the exhaustion of T-cells can be overcome remains unclear.

A closer look at HBV-associated T-cell impairment reveals a progressive loss of function over time. The gradual dysfunction of T-cells starts with lower cytotoxicity, followed by reduced cytokine production and lastly, the inability to expand. Attrition of HBsAg-specific T-cell is directly related to HBsAg levels and changes to the HBV-specific T-cell repertoire persist after HBsAg seroclearance, as observed with global immune profiling (CyTOF).[93]

Clinical studies described that reducing the viral load with NA therapy was associated with restored T-cell function, which could be further improved *in vivo* by blocking co-inhibitory molecules.[94–96] Dysfunctional T-cell signalling can be restored with arginine suppletion.[86]

The role of B-cells in viral infections is increasingly recognized and studied. Preliminary findings suggest that CHB can diminish the anti-body producing function of HBsAg-specific B-cells.[97] B-cells produce the antibodies anti-HBs against HBsAg, the cardinal sign of a functional cure. In CHB the amount of antibodies is insufficient to counteract the high level of circulating HBsAg, indicating impaired global and/or HBsAg-specific memory B-cell function. Results from flow cytometry studies showed that HBsAg-specific B-cells were present in patients with CHB, but did not produce sufficient levels of anti-HBs to be detectable in serum, whereas vaccinated healthy controls exerted a strong anti-HBs production. The HBsAg-specific B cells had features of an

atypical memory phenotype and expressed - similar to the findings for T-cells - co-inhibitory molecules, such as PD-1. Gene expression profiling of intrahepatic B-cells with RNA-sequencing demonstrated B-cell impairment in the immune active phase of CHB.[98]

NK-cell function and production of cytokines IFN-gamma and TNF-alpha is reduced. Nucleos(t)ide analogue (NA) therapy can restore NK-cells to a quiescent state, which indicated a functional HBV-specific T-cell response.[99,100]

Kupffer cells, the liver resident macrophages within the sinusoids, comprise 20% of liver cells, are non-parenchymal liver cells and do not migrate. Upon sensing pathogens, macrophages secrete cytokines (TNF-alpha, IL1 and IL6) that induce tolerogenic or T-cell responses, expand the population of macrophages and monocytes in the liver, and start to differentiate cells based on the phase of injury. Macrophages can have divergent responses, ranging from pro-inflammatory as well as antiviral, depending on experimental conditions. Most of these results come from mouse models, which often do not fully mimic the full scope of the human immune system. Macrophage population in the liver can expand promptly with circulating monocytes. IFN-gamma stimulates macrophages to differentiate into inflammatory cells. Infection with HBV prompts macrophages to active NK-cells, expand their population in the liver and secrete high levels of cytokines.[101] Among these cytokines are IL-10, programmed death-ligand 1 and 2 (PD-L1/2) that can suppress the CD8+ T-cell response, therefore inducing immune tolerance in the liver.

Additionally, hepatic macrophages play an important role in inflammation, fibrogenesis and hepatocarcinogenesis. Macrophages presumably have anti-fibrotic properties, as has been demonstrated in two studies in which patients with NASH and fibrosis were treated with the Apoptosis Signal-regulating Kinase 1 [ASK1] inhibitor selonsertib[102] or the dual C-C motif chemokine receptor [CCR]2/CCR5 inhibitor cenicriviroc.[103]

Tumor-associated macrophages are abundantly present in explanted or resected HCC, and express PD-L1, thereby suppressing CD8+ T-cell function.[104] HBsAg can promote differentiation of myeloid-derived suppressor cells (MDSC), thereby contributing even more to suppressing antiviral T-cell responses.[105] Little is known about the role of dendritic cells during HBV infection.

Effects of therapy on immune reconstitution

Because the deficient host immunity often fails to clear an HBV infection spontaneously, one of the rationales for therapy is to reconstitute the immune system to enable immune control. Currently approved therapies for CHB provide partial immune restoration. In HBeAg positive patients, lamivudine (LAM) therapy induced a transient improvement in HBV-specific CD4+ and

CD8+ T-cells that lasted up to 6 months during treatment.[106] HBeAg negative patients have been studied longer and demonstrated that HBsAg decline was associated with structurally improved T-cell function, especially in patients who achieved HBsAg loss (almost similar to resolved acute HBV infection).[107]

T-cell restoration during NA-induced viral load decline is partial and transient compared to people with a resolved acute infection. The restoration should be complemented by strategies that further restore the T-cell activity, either directly or indirectly (by blocking co-inhibitory signals for example). If T-cell function is restored, additional measures such as T-cell vaccines or TLR7/9 agonists could further improve immune control. Animals pre-treated with anti-PD1 therapy responded better than animals who received ETV alone.

In HBeAg negative patients IFN-alpha could not restore the peripheral blood T-cell response, only in the late phase during therapy. Moreover, the anti-proliferative effect of IFN-alpha could also diminish T-cell activity. The partial and transient restoration of the T-cell response suggests that NA or IFN therapy alone is insufficient. Targeting multiple steps in the immune reconstitution pathway could engage the host immune response stronger, improving the potential for immune control over HBV.

Clinical outcome and prognosis

Patients with CHB may remain asymptomatic, but can develop HBV-related clinical manifestations of end-stage liver disease, such as cirrhosis and HCC.[108] These outcomes are not directly caused by HBV, since the virus has no cytopathic properties, but to the host immune response that mediates hepatocyte injury, persistent liver inflammation and insufficient virological clearance.[109] Extrahepatic manifestations may also develop.[66] Cirrhosis and HCC are the main determinants of liver-related morbidity and mortality. Cirrhosis may lead to hepatic decompensation, HCC or death. The clinical event rate largely depends on the disease phase of CHB. In the inactive carrier phase cirrhosis and HCC develops slowly (<1% per year), whereas the rate reaches 2-10% in the immune active or HBeAg negative hepatitis phase.

Cirrhosis

Cirrhosis represents the most common CHB-related complication. Compensated cirrhosis has a relatively good prognosis and often progresses silently for years. Nonetheless, the all-cause cirrhosis burden in Europe is very high and is responsible for 170,000 annual deaths. The risk of cirrhosis in CHB is associated with viral genotype and HBV DNA. In addition, untreated HBeAg positive patients have a twofold higher cirrhosis risk than HBeAg negative patients.[110] Ongoing

liver inflammation primarily contributes to the development of cirrhosis, which is histologically defined by the presence of liver nodule formation, loss of architecture and fibrosis, colloquially known as scarring of liver tissue.[111] These pathophysiological processes destroy liver tissue, leading to deteriorating liver functions and increased portal hypertension. The clinical appearance varies from jaundice to ascites, variceal bleeding or hepatic encephalopathy. This overt clinical stage, designated as decompensated cirrhosis, indicates a systemic dysfunction with adverse prognosis.[112] Patients with decompensated cirrhosis respond worse to pegylated interferon (PEG-IFN) treatment and sometimes NA than patients with compensated disease, and progress more rapidly to death (median survival decrease from 12 to 4 years) or liver transplantation.[113]

Hepatocellular carcinoma

HBV infection constitutes the main risk factor for development of HCC[2,114], which constitutes 90% of liver cancer diagnoses in 2017 (854,000 people worldwide) and led to 810,000 deaths.[115] Half of these HCC cases occurred in China, reflecting the distinct geographical association with HBV prevalence. The natural history study Risk Evaluation of Viral Load Elevation and Associated Liver Disease (REVEAL)-HBV from Taiwan established HBeAg positive status and increased viral load as the key predictors of HCC development. A higher viral load (≥10,000 copies/mL) was associated with a high risk of HCC, after adjustment for HBeAg status, liver cirrhosis and ALT.[36] Other risk factors for HCC among patients with CHB include older age, male sex (men over 40 or women over 50 years of age), HBV genotype C and F[116], basal core promoter mutations, HBsAg[117,118], alcohol use, metabolic syndrome, presence of cirrhosis, a family history of HCC, co-infection with HIV, HCV or HDV, and aflatoxin B1 exposure.[119,120] These factors contribute to HBV-related HCC through direct (HBV DNA integration, leading to down-regulation of tumour suppressor genes) and indirect mechanisms (persistent liver necro-inflammation). One-third of cirrhotic patients will develop HCC.[121] The cumulative HCC risk at 5 years in cirrhotic HBV patients varies between 10-17%. Although HCC most frequently occurs in cirrhotic livers, some non-cirrhotic patients also progress to HCC, probably because of viral genome integration leading to oncogenesis.[122] Despite the availability of several treatment options, the overall survival of patients diagnosed with HCC remains low. The cumbersome figures underline the need for improved rates of diagnosis and therapy for HBV-related HCC.[123]

TREATMENT OPTIONS AND ENDPOINTS

Defining goals and endpoints for treatment

The treatment for HBV infection aims to improve survival and quality of life by preventing disease progression and reducing the HCC risk. Since most of these clinical outcomes take decades to develop, biomarkers are often used that correlate with disease progression and predict occurrence of clinical events at an earlier stage. The nature of HBV infection prohibits currently approved therapies to achieve eradication of the virus, because antiviral treatment does not target cccDNA, the durable viral transcript template, or integrated HBV DNA.

As a result, the loss of HBsAg (± occurrence of anti-HBs) is currently considered the closest to 'cure' and is often referred to as 'functional cure'[124] (**Table 1-1**). Achieving functional cure is associated with excellent long-term and cancer-free survival and enables patients to stop treatment. Functional cure therefore indicates a very important and attainable goal for therapy, but occurs in less than 5% of patients.[110,119] In addition, the risk for HCC remains higher in patients with HBsAg loss compared to healthy controls[125], possibly reflecting ongoing oncogenesis by viral genome integration or a persistently elevated risk from pre-existing liver injury. Also, HBV reactivation may occur in HBsAg negative patients who received immunosuppressive therapy, giving testimony to the persistent presence of HBV infection.

The level of HBV DNA is another important marker with a major role in monitoring treatment efficacy and predicting disease progression and long-term outcome in CHB. Treatment-induced virologic suppression is associated with reduced fibrosis, HCC risk and clinical outcome. Partial cure is defined as undetectable HBV DNA.

Another surrogate endpoint for treatment response in CHB is HBeAg seroclearance ± development of antibodies to HBeAg (anti-HBe). HBeAg loss induced by PEG-IFN treatment occurs in a larger proportion of patients than HBsAg loss. Furthermore, HBeAg loss has proven serological and clinical benefits, as it is associated with a greater proportion achieving virological suppression, HBsAg loss, lower HCC risk and improved survival.[110,126]

The endpoint HBeAg loss has been frequently used in trials that investigated the effect of PEG-IFN and NA therapy in CHB. The durability of HBeAg loss and seroconversion should be evaluated after stopping treatment.[127]

Alternatively, quantitative HBsAg could be used as an early surrogate marker of response, since a decline in HBsAg is associated with HBsAg loss.[128] HBsAg derives from cccDNA, intrahepatic HBV DNA and from integrated DNA. While several studies have used quantitative HBsAg to predict response, HBsAg decline as primary endpoint has been used mainly in more recent trials.

Table 1-1. Definitions of cure for chronic hepatitis B.

Nomenclature	Definition	Outcome	Comment
Partial cure	HBV DNA undetectable HBsAg + cccDNA + Integrated HBV DNA +	Necro-inflammation reduced HCC risk persistent	Achievable, but possibly insufficient to withdraw therapy
Functional cure	HBV DNA undetectable HBsAg - cccDNA + Integrated HBV DNA +	Fibrosis regression HCC risk reduced	Achievable with current therapies, but rare
Complete or sterilizing cure	HBV DNA undetectable HBsAg - cccDNA - Integrated HBV DNA -	Restored liver to normal HCC risk eliminated	Not achievable with currently approved therapies

cccDNA, covalently closed circular DNA; HBV DNA, Hepatitis B virus DNA; HBsAg, Hepatitis B surface Antigen; HCC, hepatocellular carcinoma.

Treatment indication

The decision to start treatment for CHB is primarily based on serum ALT and HBV DNA levels. The major international clinical practice guidelines differ in thresholds for ALT and HBV DNA to initiate treatment.[64–66]

The 2017 EASL guideline advises to start antiviral treatment in the immune active phase, based on moderately increased levels of serum ALT (>ULN), HBV DNA (>2,000 IU/mL) and the severity of liver disease (moderate liver inflammation ± cirrhosis in liver biopsy).[66] HBeAg status has no role in treatment indication. Patients with HBV DNA >2,000 IU/mL and evidence of moderate fibrosis may also start treatment, regardless of the level of ALT. The requirement for a liver biopsy is omitted for patients with HBV DNA >20,000 IU/mL combined with ALT >2x ULN who can start therapy immediately.

Less conservative thresholds for treatment start are promoted by the 2018 AASLD guidance and 2015 APASL guidelines.[64,65] Treatment is recommended for HBeAg positive patients with ALT ≥2x ULN plus HBV DNA >20,000 IU/mL, and for HBeAg negative patients with ALT ≥2x ULN with HBV DNA >2,000 IU/mL. The argument for the comparatively higher ALT threshold was the lack of evidence for any other value.

All guidelines advocate starting treatment in patients with decompensated cirrhosis. Other factors to be considered in the decision to treat include age, family history of cirrhosis or HCC, previous use of therapy and extrahepatic manifestations. During the IT phase antiviral therapy is generally not recommended because response rates are suboptimal and antiviral resistance might develop.

Host and viral predictors of treatment response

The disease stage and several host and viral characteristics largely determine the treatment response. For PEG-IFN therapy, female sex, higher baseline (start of treatment) ALT, lower baseline HBV DNA, HBV genotype A/B vs. C/D are independently associated with response in HBeAg negative patients. (PEG)-IFN-induced HBeAg seroconversion was associated with higher serum ALT, lower levels of HBV DNA and a higher activity in liver biopsy samples.[129–134]

Genetic determinants of response to IFN-alpha have been evaluated in two independent genome wide-association studies (GWAS). The first GWAS, a mixed cohort of Caucasian and Asian HBeAg positive and HBeAg negative patients (n=1,058), identified associations between TRAPPC9, PRELID2 and G3BP2 with both short and long-term response to PEG-IFN across ethnicities, although none of the top-hit single nucleotide polymorphisms reached genome-wide significant ($P < 10^{-6}$).[135] In the second GWAS, the single nucleotide polymorphism FCER1A on chromosome 1 was associated with HBsAg loss ($p=2.65 \times 10^{-8}$) in 1,636 East-Asian patients treated with IFN-alpha 2a. The alpha subunit of the immunoglobulin E receptor is encoded by FCER1A.[136] Full results of the GWAS study will be published shortly.

Nucleos(t)ide analogue treatment

Nucleos(t)ide analogues are a class of direct acting antivirals (DAA) that block viral replication and are used to treat HIV, herpes viruses and HBV. Because NAs resemble naturally occurring nucleos(t)ides in uptake and metabolism, the viral DNA polymerase incorporates NAs in newly synthesized DNA strands at the 3' end, which terminates the strand completion.[137] The direct inhibition of the DNA polymerase (reverse transcription of pgRNA to DNA) decreases the viral load. However, chain termination by NAs blocks a late step in the viral lifecycle and does not immediately target the cccDNA pool. The NA agents tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF) and entecavir (ETV) are third-generation antiviral treatment agents for CHB with a good tolerability and high barrier to resistance in previously untreated patients.[138,139] These oral antiviral agents are preferred over older generation NAs adefovir (ADV), lamivudine (LAM) and telbivudine (TBV) which have higher rates of viral resistance and produce less durable response.[64–66]

Ongoing observational studies have confirmed effective viral suppression up to eight years of therapy. Long-term treatment with TDF leads to a durable and effective viral suppression (HBV DNA <69 IU/mL in 99.3% of patients on treatment) and ALT normalization. In the European VIRGIL study (n=744, 64% HBeAg negative) sustained virological response to ETV (HBV DNA <80 IU/mL) at 48, 96 and 144 weeks was reached by 89%, 98% and 99% of patients.[140–142] HBsAg loss occurred in two patients, none of

whom developed drug resistance. After 7 years of TDF therapy, HBsAg loss was 11.8%, drug resistance did not occur and tolerance was high.[143] Survival after 8 years of TDF or ETV therapy was comparable to the general population, but the risk of HCC remained slightly higher.[125,142,144]

A significant advantage to NA therapy is the excellent tolerability. The reported studies did not observe significant differences in renal (renal dysfunction, hypophosphatemia) or bone outcomes (bone mineral density) for TDF compared to ETV. A real-life study on ETV use described occurrence of lactic acidosis in 5/16 cirrhotic patients with CHB and reduced liver function.[145] Evidence for TDF-induced nephrotoxicity derived from *in vitro* and animal studies, and from studies on HIV/HBV co-infected patients. A feasible option for patients with, or at risk for, renal impairment is the use of an off-label reduced dose of TDF or switch to ETV if no LAM resistance is present.[146]

Since TDF may occasionally lead to renal impairment[143,144,147–149], the TDF prodrug TAF was developed and approved in 2017. TAF reaches greater intrahepatic and lower systemic concentrations of the active metabolite, thereby reducing the risk for renal or bone disease compared to TDF. The registration trials for TAF demonstrated a significant difference compared to TDF in several renal and bone function markers at week 48.[150,151] The decrease in estimated Glomerular Filtration Rate (eGFR) for TAF vs. TDF in HBeAg positive patients was 0.6 mL/min vs. 5.4 mL/min ($p<0.005$) and in HBeAg patients 1.8 mL/min vs. 4.8 mL/min ($p<0.005$). Although statistically significant, these differences might be clinically less relevant. TAF also normalizes ALT levels more rapidly than TDF, which is possibly related to metabolic effects. No virologic resistance has been reported for TDF or TAF. To verify whether these data translate into better outcomes over time should be evaluated in long-term clinical studies.

Despite the excellent efficacy and tolerability of NAs, the low rate of HBsAg loss and lack of immunological control exposes the need for better therapeutic regimens. One approach would be to stop NA therapy before HBsAg loss is reached. Other strategies could focus on combination therapy of NA with PEG-IFN or novel compounds. These approaches will be discussed in more detail below.

Immunological effects of nucleos(t)ide analogue therapy

Innate immunity

The limited research on effects of NA therapy on the innate immune system has focused on NK-cell function. ETV, LAM and ADV may partially restore NK-cell activity. However, during long-term TDF-induced virologic suppression the number, phenotype and activation status of NK-cells in the liver did not change.[100] In comparison, the frequency of NK-cells is lower in untreated patients

with CHB than in healthy controls. NK-cells have similar cytotoxicity, but less activation and production of IFN-gamma, and therefore a weaker Th1 response.[99]

Adaptive immunity

The T-cell response to HBV infection is functionally exhausted and narrow, but HBV remains susceptible when the immune response is boosted. T-cell function may transiently improve *in vitro* even though patients have been exposed to long-term viral load.[107,152]

B-cells have been studied less extensively, but also appear to have functionally impaired response to CHB infection.[153] HBs-specific B cells exert functional defects and share phenotypic characteristics with atypical memory B cells that express PD-1. Blocking PD-1 could increase the anti-HBs response of these cells *in vitro*.[154] The use of checkpoint inhibitors could thus be favourable to both B- and T-cells. During PEG-IFN add-on to NA therapy the frequency of peripheral B-cells decreased, and major repartition occurred (transitional B-cells and plasmablasts increased during treatment), possibly representing a higher B-cell generation and differentiation rate.

Clinical impact of nucleos(t)ide analogue treatment

Oral antiviral agents reduce liver fibrosis, risk of hepatocellular carcinoma (adjusted sub-hazard ratio: 0.63; 95%CI 0.49–0.80; P < 0.001) and death (only significant OR in patients who have received surgical resection), and have relatively few side effects.[36,123,142,143,155–157] However, functional cure, defined as HBsAg loss, occurs in <5% of patients receiving NA therapy, which often prompts long-term, if not life-long, NA treatment. Limited sustained immune control occurs after stopping NA therapy. Many patients relapse if NA therapy is discontinued. Long-term therapy may increase the risk of side effects, non-adherence, antiviral resistance and costs.

Long-term ADV therapy reduced the cccDNA pool (-0.8 log copies/mL), possibly because the virological suppression stopped nucleocapsid recycling to the nucleus and reduced incoming virions from the blood.[158] A newer study has confirmed that long-term NA treatment decreased levels of cccDNA (2.94 log [99.89%] reduction).[159] NA-induced HBsAg loss is as durable as spontaneous HBsAg loss. HBsAg seroreversion occurred in 2.9% vs. 2.1% of patients with NA-induced vs. spontaneous HBsAg loss, all of whom had consolidation therapy 6-12 months.[160,161]

Interferon-based treatment

IFN has antiviral and immunomodulating effects on several viral infections, including HBV[43,162,163]. IFN upregulates several interferon-stimulated genes (ISGs) that target viral replication and

cccDNA at different steps, and IFN stimulates NK-cell activity. Immunological studies have elucidated the effects of IFN-alpha that include transcriptional, post-transcriptional and epigenetic, antiviral adjuvant-inducing effects.[164–170] Antigen-presenting cells (APCs) are the most important source of innate IFN-alpha production, which expands the CD4+ and CD8+ T-cell response to viral infections. In the early phase of an infection with viruses or other intracellular pathogens, APCs and macrophages are stimulated to secrete high levels of IFN-a. IFN-alpha from antigen-presenting T-cells provides a signal to render CD4+ Th1 cells responsive to IL-12, which leads to the production of IFN-gamma, an important component of the Th1 immune response. In addition, IFN-alpha strengthens the CD8+ effector T-cell response and increases the number of CD4+ and CD8+ T-cells. IFN-alpha therefore has an important role in antigen presenting cell interactions to elicit a Th1 response.

In vitro and *in vivo* experiments that determined the effect of IFN-alpha on cccDNA showed a predominantly epigenetic effect. IFN-alpha inhibited transcription of cccDNA and decreased the acetylation of cccDNA-linked histones, but did not reduce cccDNA production.[164,171,172]

On the downside, IFN-alpha has anti-proliferative properties, which account for side effects such as neutropenia or reduced hypersensitivity. One of the larger risks during PEG-IFN therapy is the development of hepatitis flares, which may require frequent monitoring, dose reduction or treatment cessation. All major HBV treatment guidelines have therefore restricted the use of IFN-based therapy to patients with compensated liver disease.[64–66]

The linking of polyethylene glycol (pegylated) to conventional IFN-alpha has increased the half-life of IFN and enabled once weekly injections rather than 2-3 times per week. PEG-IFN therapy boosts the innate immunity, triggers T-cell-mediated immune responses, prevents the formation of HBV proteins, and depletes the intrahepatic cccDNA pool, which leads to more HBsAg loss than with NA therapy. Treatment with PEG-IFN leads in 20-40% of patients to sustained off-treatment immune control (HBeAg seroconversion) after 48 weeks of therapy.[173] Thirty percent of patients reach immune control after completing one year of PEG-IFN treatment. Of these patients, 30-50% will reach HBsAg loss during long-term off-treatment follow-up.[174] However, tolerability issues and the inability to accurately predict treatment responders limit the use of PEG-IFN. Careful selection of patients that respond to treatment is needed but remains challenging.

Clinical impact of pegylated interferon treatment

The impact of PEG-IFN a2a/a2b on serological and clinical endpoints has been the subject of several studies.[175,176] HBeAg seroconversion was associated with durable treatment response to PEG-IFN. A prospective follow-up study in PEG-IFN-alpha treated and untreated HBeAg positive

patients with compensated cirrhosis demonstrated that HBeAg seroconversion occurred in 27/40 (67%) vs. 30/50 (60%) of treated vs. untreated patients, of which 11 vs. 5 also achieved HBsAg loss. While HBeAg loss developed more rapidly in the treated group, the 5-year HBeAg loss rates were similar. The incidence of clinical events (hepatic decompensation, HCC or death) did not differ markedly between groups. However, no events occurred during 6 years of follow-up in the patients with HBeAg seroconversion and ALT normalization to PEG-IFN therapy. Age and ALT normalization during follow-up were predictive of survival; HBeAg loss was not.

Similar results were demonstrated in a long-term follow-up study of IFN-alpha-treated patients. HCC occurred less often in patients who achieved HBeAg loss than patients with persistent HBeAg positive status.[177] Another study in non-cirrhotic patients with CHB claimed that IFN-alpha-induced HBeAg loss was associated with better survival during 4 years of follow-up.

The PEG-IFN-alpha registration trial in HBeAg negative patients described that biochemical and virological response at week 24 were reached by 60% and 44% of patients.[178] Three years after the end-of-therapy, 31% and 28% had sustained biochemical and virological outcome, respectively. Real-life studies have reported a low rate of HBsAg loss during PEG-IFN therapy, which improved to 12% five years after the end of PEG-IFN. Thirty percent of sustained responders achieved HBsAg loss during long-term follow-up.

Optimizing the therapeutic regimen

In search of the optimal pegylated interferon regimen and endpoint

The suboptimal response rates encouraged researchers to determine the optimal parameters of PEG-IFN therapy to maximize response. Earlier approaches focused on dose and duration of PEG-IFN, whereas later studies have examined different combinations of NA and PEG-IFN.

Because of the large number of approved therapies (7 NAs and PEG-IFN) that can be prescribed for different durations or doses, many therapeutic combinations are possible which also have different expected treatment responses. The therapeutic heterogeneity complicates the difficult task of finding the optimal therapeutic management for CHB.

Dose and duration of pegylated interferon therapy

A phase II RCT from Asia compared different doses of PEG-IFN-a2a (90, 180 and 270 µg) to conventional IFN-a2a (4.5 MIU) for 24 weeks in HBeAg positive patients.[179] Twenty-four weeks after the end of treatment, combined response (defined as HBeAg loss, HBV DNA < 500,000

copies/mL and ALT normalization) was highest for patients treated with PEG-IFN alpha 90 and 180 µg (27% and 28%) compared to 270 µg (19%) and IFN-alpha (12%) (all PEG-IFN arms vs. IFN, p=0.036). All patients had rapidly reducing serum HBeAg levels, with a median HBeAg close to zero in the first four weeks of therapy. HBeAg loss occurred more often in patients receiving 90 µg (37%) or 180 µg (35%) than 270 µg (29%) or conventional IFN-a (25%). Similarly, the largest decline in HBV DNA and ALT normalization rate was observed in patients receiving 180 µg PEG-IFN, which persisted throughout follow-up.

The few studies that evaluated prolonged duration of PEG-IFN therapy revealed contrasting results that vary by HBeAg status and may be subject to insufficient statistical power. An Italian proof-of-concept RCT suggested that extending a 48 week PEG-IFN a2a regimen for another 48 weeks improved virologic (HBV DNA <2,000 IU/mL at End-Of-Follow-Up [EOF]) and biochemical response in 128 HBeAg negative genotype D patients.[180] Interestingly, the withdrawal rate (20%) did not increase during the extended treatment period. These findings should be interpreted cautiously because a lower-than-expected inclusion rate reduced the statistical power, thereby increasing the likelihood of false-negative results (type II error).

In contrast, a larger phase IV RCT from China in 264 HBeAg positive patients indicated no benefit to extending PEG-IFN treatment from 48 to 96 weeks. In this study therapy was extended for early non-responders (HBsAg > 1500 IU/mL or HBV DNA >log 5 IU/mL at week 24). The extended PEG-IFN treatment had similar response rates compared to 48 weeks of PEG-IFN. HBeAg loss, combined HBeAg loss with HBV DNA <2,000 IU/mL and HBsAg loss occurred in 34.3% vs. 31.3%, 23.9% vs. 17.9%, and 1.5% vs. 0.0% of patients receiving PEG-IFN for 96 vs. 48 weeks (all P-values >0.05). The withdrawal rate (4/264) was remarkably low during 48 weeks of PEG-IFN, but increased considerably (17.2%-22.4%) among patients with extended PEG-IFN treatment.[181] The per-protocol results were concordant with the modified intention-to-treat (mITT) results, implying a negligible underestimation of the true effect of extended PEG-IFN treatment. A lower dose PEG-IFNa2a (90 vs. 180 mcg) and shorter duration (24 vs. 48 weeks) was inferior for the endpoint HBeAg seroconversion 6 months post-treatment.[182]

Results from post-hoc analyses of the PEG-IFN a2a registration trials for PEG-IFN suggested completing 48 weeks of PEG-IFN if patients reached serum HBsAg <1,500 IU/mL at week 24, since HBeAg seroconversion and HBsAg loss rates were 55% and 7-12%, respectively.[182,183]

Monotherapy, sequential, combination, add-on or stop therapy

The suboptimal response to approved therapies for HBV and need for long-term administration of NAs motivated researchers to investigate whether a combination of NA and/or PEG-IFN therapy

could achieve higher response rates than monotherapy with either one. Such a combination approach could increase the number of patients with sustained response and facilitate stopping therapy.

The rationale for the use of combination therapy stems from enhancing the effect of one therapy on the other, and vice versa. Long-term NA-induced virologic suppression allows the immune system to partially restore and may decrease the level of cccDNA. Adding pegylated (PEG)IFN to ongoing NA therapy could boost the innate (NK cell) and adaptive (T-cell) immune response, prevent formation of HBV proteins (HBV RNA, HBsAg, HBeAg) and further decrease intrahepatic cccDNA levels.[91,99,107,158,159,163,184–187] Such an add-on strategy could allow patients to sustain response and facilitate stopping treatment. In addition, shorter therapies can limit the potential side effects of NA therapy.

The various therapeutic strategies can be largely divided into combination, sequential, add-on and stop therapy (**Table 1-2**).

Combination therapy

The major clinical practice guidelines for HBV do not support combination treatment of PEG-IFN with NA. Robust evidence is lacking that demonstrates that combination therapy is superior to PEG-IFN or NA monotherapy for different groups of patients: both for HBeAg positive[134,188] or negative[183], as well as treatment naïve or experienced patients. Limitations of these early studies were the use of first or second-generation NA agents, a short duration of follow-up or a small sample size.

Two recent studies from Europe that examined combination therapy with PEG-IFN and NA had opposing findings. An RCT compared PEG-IFN a2a plus TDF for 48 weeks to PEG-IFN or TDF alone in 740 treatment naïve patients (58% HBeAg positive)[174]. This study was the first to show a significantly higher rate of HBsAg loss at week 72 achieved by combination therapy compared to PEG-IFN or TDF monotherapy (9.1% vs. 2.8% vs. 0%; $p<0.003$). Adverse events occurred at a similar rate in the combination and PEG-IFN monotherapy arm. It is important to note that secondary outcomes were not significantly different between treatment arms. The TDF retreatment rate was 54% vs. 61%, HBeAg seroclearance was 29% vs. 25%, HBeAg seroconversion was 25% vs. 24% and proportion of patients with HBV DNA <15 IU/mL was 7% vs. 3.2%. Furthermore, this study did not report occurrence of sustained response off-treatment, which is a clinically more meaningful endpoint.

The Low Viral Load Study from the Netherlands randomized patients to PEG-IFN a2a add-on plus TDF vs. PEG-IFN add-on plus ADV or no treatment for 48 weeks, after which all patients discontinued treatment and were followed until week 72.[189] Interestingly, this trial included patients with a low viral load (<20,000 IU/mL) and HBeAg negative status; 96% was IFN-naïve and none had received NA therapy in the past 6 months. A substantial proportion of patients (n=29) did not receive or complete the treatment to which they were randomized. Despite relatively low HBsAg levels at randomization, the primary endpoint HBsAg loss at week 72 was reached by 4% vs. 4% vs. 0% of 134 patients in the PEG+ADV, PEG+TDF and no treatment arm (p=0.377) in the ITT analysis. HBsAg decline at week 48 (-0.61; -0.61; -0.06; p<0.0001) and week 72 (-0.53; -0.59; -0.15; p=0.001) was significantly different between the respective treatment arms, but rebounded slightly from week 48 to week 72. In multivariable analysis, lower week 12 HBsAg levels (OR: -1.03; SE: 0.31; p=0.001) and maximum ALT (OR: 5.02; SE: 1.31; p=0.0001) were independently associated with HBsAg decline >1 log IU/mL at week 48. In a post-hoc per-protocol analysis HBsAg declined stronger in the two PEG-IFN add-groups compared to the no treatment group. A high rate of adverse events and treatment discontinuation was reported.

Another study on combination therapy (PEG-IFN plus ADV for 48 weeks) used paired liver biopsies (before and after therapy) that provide valuable insights in the intrahepatic compartment.[185] This study showed that combination therapy reduced serum HBV DNA (-4.9 log copies/mL), intrahepatic HBV DNA (-2.2 log) and cccDNA (-2.4 log), although the lack of a monotherapy group makes it difficult to determine which therapy contributed to response.

Based on the accumulating evidence for combination therapy, PEG-IFN plus NA therapy can still not be recommended, since most serological outcomes were not reached, initial HBsAg levels declines during therapy rebounded in the off-treatment phase and the rate of adverse events was high. The differences in results between these studies could be attributed to various factors including baseline HBV DNA level, HBeAg status, ethnicity and HBV genotype.

Sequential therapy

Response to NA-IFN sequential or switch therapy appears to be confined to treatment-experienced HBeAg positive patients with low serological levels at the start of therapy. Evidence supporting sequential treatment for HBeAg negative patients is limited. Most of these studies have been performed in Asian patients.

In the NEW-SWITCH study[190–192] treatment-experienced, HBeAg positive patients who had received ETV pre-treatment for 9-36 months were randomized to 48 weeks of PEG-IFN a2a 180

µg/week or continued ETV 0.5mg OD. HBeAg seroconversion occurred in 14/94 (15%) vs. 6/98 (6%) of PEG-IFN vs. ETV patients. HBsAg loss and seroconversion and HBsAg <10 IU/mL at week 48 occurred also significantly more often in PEG-IFN arm. In a follow-up study of these patients, 7/23 (30%) achieved HBsAg loss of which 4 had HBsAg seroconversion one year after the end of PEG-IFN a2a therapy.[193]

In another study from China, treatment-naïve, HBeAg positive patients received either PEG-IFN a2a monotherapy, PEG-IFN + ETV add-on for 13 weeks or ETV + PEG-IFN sequential therapy for 48 weeks and were followed for another 48 weeks.[194] Neither ETV add-on nor ETV pretreatment revealed superior response rates (HBeAg loss or seroconversion, HBeAg decline or HBsAg decline) compared to PEG-IFN alfa-2a monotherapy after 48 weeks of therapy or 24 weeks after end of therapy.

Sequential therapy has been studied rarely in HBeAg negative patients and trials often employed previous generation antiviral agents (LAM, IFN-b). Sustained response (ALT normalization with suppressed HBV DNA) was not significantly different between LAM + IFN-a2b and LAM monotherapy after 48 weeks of therapy, but was higher 24 weeks after end-of-therapy.[195,196]

Another sequential therapy strategy, IFN followed by NA therapy, has been studied in mainly HBeAg positive cohorts. Included suboptimal responders to (PEG-)IFN with long on-treatment follow-up (up to 232 weeks of therapy).[197,198] After twenty-four to 48 weeks of (PEG)-IFN patients were switched to TBV or ETV. Rates of HBeAg loss and seroconversion were significantly higher in patients who received sequential therapy, but response to the same NA varied per study.

Add-on therapy

PEG-IFN can be added at an 'early' or a 'late' stage to ongoing NA therapy. Early add-on generally applies if patients have received a few weeks of NA therapy whereas late add-on indicates that patients have been treated for at least one year. Determining whether add-on or switch to PEG-IFN is preferable is still the subject of debate since study populations and endpoints differ markedly.

Early add-on has been studied in HBeAg positive patients in two prospective controlled trials[194,199] and in one RCT in HBeAg negative patients.[200] In these RCTs that compared PEG-IFN add-on to continuing NA therapy, decline rates were significantly higher in add-on patients, although primary endpoints (HBsAg loss at week 96; combined HBeAg loss with HBV DNA <200 IU/mL at week 96) were not reached in these, possibly underpowered, studies.

The multicentre ARES study randomly assigned HBeAg positive patients with compensated liver disease to either PEG-IFN add-on for 24 weeks (n=85) or continued ETV monotherapy (n=90).[199]

The primary endpoint response was defined as HBeAg loss combined with HBV DNA <200 IU/mL at week 48. The patients that responded received ETV consolidation therapy for 24 weeks and were followed until week 96. Response to add-on vs. monotherapy was observed in 16/85 (19%) vs. 9/90 (10%; p=0.095). PEG-IFN add-on therapy resulted in a significantly greater percentage of patients achieving HBsAg decline, HBeAg seroconversion and HBV DNA decline (p<0.001). Off-treatment disease remission was reached by 11/14 (79%) vs. 2/8 (25%) of patients in the add-on vs. monotherapy group (p=0.014).

The trial from China randomly allotted 210 patients with HBeAg positive CHB to 48 weeks of PEG-IFN a2a, PEG-IFN with add-on ETV 0.5 mg from week 13-36, or 24 weeks of ETV lead-in with switch to PEG-IFN from week 20 to week 68.[194] All patients were followed for another 24 weeks off-treatment at which time the primary endpoint HBeAg decline from baseline was determined. HBeAg reductions 24 weeks off-treatment were significant compared to baseline, but comparable across treatment arms (monotherapy: −1.4 PEIU (1.8); add-on: −1.6 (1.8); switch: −1.3 (1.7)). Off-treatment rates of HBsAg loss and HBeAg seroconversion and HBV DNA reductions were also similar between therapies, indicating no superior effect of ETV addition or ETV lead-in to PEG-IFN monotherapy.

The PEGAN RCT investigated PEG-IFN add-on in HBeAg negative CHB and was powered to detect a difference in HBsAg loss of 9.5% (10% vs. 0.5%) between add-on and monotherapy.[200] A total of 92 patients received 48 weeks of PEG-IFN add-on to NA therapy and 93 continued NAs. In the intention-to-treat analysis, HBsAg loss at week 96 occurred in 7/90 (7.8%) vs. 3/93 (3.2%) patients in the add-on vs. NA monotherapy group (p=0.15). At week 144, 10% vs. 4% achieved HBsAg loss, respectively (p=0.11). The only independent predictor of HBsAg loss at week 96 was level of HBsAg at randomization (OR of HBsAg loss per 1 log HBsAg increase: 0.36 (95%CI: 0.17-0.76); p=0.006). Results from the Fibrotest, a non-invasive measure of fibrosis, were not significantly different between treatment arms at week 144. Remarkably, 41% of patients in the add-on group discontinued the drug because of serious adverse events.

The few trials that explored late addition of PEG-IFN in HBeAg positive patients in Asia or Saudi-Arabia shared similar findings. In the multicentre PEGON trial the primary endpoint (defined as HBeAg seroconversion with HBV DNA <200 IU/mL 24 weeks after NA cessation) was observed in 7/39 (18%) of patients who were allocated to PEG-IFN a2b add-on vs. 3/38 (8%) allocated NA monotherapy (p=0.31).[201] HBeAg seroconversion (30% vs. 7%; p=0.03) and response at week 96 (26% vs. 7%; p=0.07) were highest among IFN-naïve patients in the add-on group vs. monotherapy. Patients in the add-on compared to the NA monotherapy group achieved greater HBsAg decline from week 0 to week 48 (-0.40 vs. -0.15 log IU/mL; p=0.005), and from week 0 to week 72 (-0.35

vs. -0.20 log IU/mL; p=0.01). However, among those who stopped treatment at week 48, HBsAg increased from week 72 to week 96 and was similar between add-on and NA monotherapy (0.28 vs. 0.22 log IU/mL; p=0.84).

A small RCT in 48 patients (46% genotype D, 38% HBeAg positive) from Saudi-Arabia corroborated these findings.[202] After TDF lead-in for at least 6 months, PEG-IFN a2a add-on treatment for 48 weeks was assigned to 23 patients and TDF monotherapy to 25, after which all patients continued TDF. The primary endpoint HBsAg loss at week 96 was achieved by one patient in the add-on group. HBsAg declines from week 0 to week 96 were small in the add-on (-0.3 log IU/mL; p=0.03) and TDF monotherapy group (-0.1 log IU/mL; p=0.09). HBeAg seroconversion was reached by one TDF-treated patient. Importantly, off-treatment response was not evaluated since TDF was continued in both groups.

Evidence for late PEG-IFN add-on in HBeAg negative patients is limited. The OSST study from China demonstrated that PEG-IFN added to long-term ETV therapy (n=97) was significantly associated with HBeAg seroclearance and seroconversion, compared to ETV monotherapy (n=100).[203] The outcomes from this trial could be subject to selection bias, since patients had very low HBeAg levels (<100 PEIU/mL) at randomization. This study identified a baseline HBsAg <1500 IU/mL as independent predictor of HBsAg seroconversion and HBsAg loss, although a rigorous grid search of cut-off values was not presented.

The PADD-ON study from Germany and the SWAP study from Singapore are RCTS that have presented interim results at international conferences. The PADD-ON study randomized 165 HBeAg negative patients (75% Caucasian, all HBsAg 100 IU/mL, NA-treated ≥1 year) to receive PEG-IFN add-on for 48 weeks (n=110) or continue NA therapy (n=58) who were then followed for another 24 weeks.[204] At baseline HBsAg was 6,548 vs. 8,427 IU/mL, HBV DNA was 5.3 vs. 10.7 IU/mL. The primary endpoint (HBsAg decline ≥1 log at week 48) was achieved by 26% of patients assigned add-on vs <2% assigned monotherapy (p<0.001). Remarkably, a high proportion of patients lost HBsAg by Week 48 (16% vs. 5% for add-on vs. monotherapy). Drop-out due to adverse events was 8%.

The SWAP study conducted in Singapore enrolled 254 viral suppressed, HBeAg positive and negative patients on >1 year of NA treatment.[205] A total of 124 patients received 48 weeks of PEG-IFN add-on, followed by 24 weeks of NA therapy; 124 switched to PEG-IFN and 62 patients continued NA therapy. The primary endpoint HBeAg loss ± HBsAg decline > 1 log IU/mL was evaluated at week 72. The preliminary findings indicated a superior effect of both add-on and switch arm to continued NA monotherapy at week 72. HBsAg loss at week 72 occurred in 6/53

(11%) vs. 7/57 (12%) vs. 0/32 (0%) ITT-analysed patients (overall p=0.052). However, the primary endpoint, HBsAg loss and HBsAg seroconversion did not differ significantly between add-on and switch therapy. Notably, the switch arm had significantly more frequent virological (15.5%) and clinical relapse (17.5%) that required retreatment in 11 patients, compared to the add-on (2.2%; 1.1%) or monotherapy arm (2.1%; 0.0%). In multivariable analysis for HBsAg loss (n=19), older age, baseline HBsAg, week 12 HBsAg <63 (1.8 log) IU/mL, week 12 HBsAg decline ≥1 log and ALT increase were independent predictors. The confidence intervals in this preliminary analysis were very wide and the analysis included multiple HBsAg covariates that correlated with each other, limiting the interpretation of results.

Uncontrolled studies in both HBeAg positive and negative patients favour the use of PEG-IFN add-on.[206–209]

New hepatitis B cure trials

While virtually all novel antiviral compounds effectively drive down viral and protein loads, these agents alone did not achieve sustained immune control to clear HBV. Almost all companies have therefore adapted HBV cure trial designs to add short-term PEG-IFN arms in the studies designed in 2020 and 2021. This holds for nucleic acid polymers (NAPs: REP 2139; REP 2165), capsid assembly modulators (CpAM: NVR 3-778), antisense oligonucleotide (ASO: GSK3228836), farnesoid X Receptor Modulator (FXR: EYP001a), RNA inhibitors (RNAi: JNJ-73763989 + JNJ-56136379); and NTCP-receptor blockers (MYR203 in HBV/HDV-coinfection).[210–215] The immunomodulatory and antiviral properties of PEG-IFN could broaden and lengthen immunological control through innate (NK cell) and adaptive (CD4, CD8 T-cell) immune responses, prevent formation of HBV proteins (HBV RNA, HBeAg, HBsAg) and further decrease intrahepatic cccDNA.[91,107,163,184–187] Another advantage to using PEG-IFN in future trials is the well-known safety profile.

Stopping nucleos(t)ide analogue therapy

Discontinuing NA therapy has been the subject of debate in several recent trials. NA therapy cessation aims to sustain virologic suppression while potentially clearing HBsAg, which may or may not be related to ALT flares. Current guidelines suggest that HBeAg positive can discontinue NA therapy after HBeAg seroconversion.[64–66] Evidence to support stopping NAs in HBeAg negative patients is limited and highlights the need for long-term consolidation therapy and close follow-up monitoring. Patients with advanced liver fibrosis should continue NA therapy indefinitely.

The results published thus far suggest that stopping NAs is difficult because many patients experience virological relapse (up to 45%), sometimes with potentially fatal flares.[216–226] Although an off-treatment hepatitis flare might signal or induce HB sAg loss, it has been challenging to distinguish these 'good' flares from the 'bad' ones [227]. Limitations of these studies were the small sample size or retrospective study design.

The 6-year cumulative HBsAg loss rate ranged widely from 13-55% in HBeAg negative patients.[216,219,224,228–231] However, prediction of HBsAg loss, virological relapse or sustained virological suppression remains challenging. Low levels of serum HBsAg (<3.0 log IU/mL), higher ALT and interferon-inducible protein 10 (IP-10) were associated with HBsAg loss. A propensity score matching analysis of roughly 686 patients from the natural history study REVEAL-HBV and an HBeAg negative off-NA cohort supported these results and found that baseline HBsAg <100 IU/mL and treatment cessation were associated with HBsAg loss.[232] HBsAg titers at the end of therapy were also indicative of sustained response after stopping treatment, defined as persistently undetectable HBV DNA, or HBV DNA <2,000 IU/mL + normal ALT.[219] Other studies also claimed that serum low HBsAg levels at the end of LAM therapy could predict HBsAg loss or sustained response (HBV DNA ≤200 IU/mL at 12 months after NA stop).[222,223] Specifically, the individual studies found that HBsAg loss was best predicted by cut-off values of HBsAg ≤117.3 IU/mL (sensitivity: 95%; specificity: 76%; AUC: 0.91)[222], HBsAg <100 IU/mL or >1 log reduction (sensitivity: 78%; specificity: 96%; AUC: 0.91)[223] and ≤1,000 IU/mL (sensitivity, specificity and AUC unknown).[219] After discontinuing NA treatment markers of T-cell activation in serum (IP-10, TNF-alpha, IL-12, IL-10) increased, suggesting an improvement in T-cell function.[233] A promising global initiative, the continuing RETRACT-B cohort study, reported 8.1% HBsAg loss after NA withdrawal among 1,509 CHB patients (87% Asian, 11% Caucasian). Chance of HBsAg loss was 2.6 times greater among Caucasians than Asians (OR: 1.7; 95% CI: 1.1-2.6; p=0.03).[234] The key message gathered from the diverging HBsAg thresholds is that long-term antiviral treatment can be stopped when patients reach a low HBsAg level, although how low exactly remains to be answered.

Further questions to be answered concern the optimal duration of NA consolidation therapy and the association between HBsAg loss and ALT flares. The APASL guideline suggests consolidation therapy for at least 1 year after HBeAg seroconversion, but others (DARING-B, 60 patients) have advocated continuing NA therapy for at least 4 years).[220] Several larger RCTs that are currently completing follow-up could elucidate the potential benefits of NA treatment withdrawal.

Table 1-2. Effect of current therapeutic regimens for chronic hepatitis B by HBeAg status.

HBeAg positive							
	NA mono-therapy	**PEG-IFN mono-therapy**	**Combination NA + NA**	**Combination PEG-IFN + NA**	**NA switch to PEG-IFN**	**PEG-IFN add-on to NA**	**NA cessation**
Virological suppression*	Very high 99.3%	Moderate	High 80% at 2 years	70% at 1 year 9% at 1.5 years	Moderate 32%	Good 57%	45% relapse
HBeAg seroconversion	Low Increases up to 53% at 5 years	Moderate 30-40% after 1 year	Moderate 22%	Moderate 27-30%	Low 10-15%	Moderate 26%	N/A
HBsAg loss	<5%	3-7% at 3 years off-therapy	5%	7--9%	7-11%	1%	Up to 39% in those with low HBsAg
ALT normalisation	40-70%	30-41%	65%	35-39% at 2 years	59%	88% at 2 years	Possible flares
Clinical use	Standard of care	Young or treatment naïve patients	Viral resistance	Low baseline HBsAg & week 12 HBsAg decline >1 log IU/mL	Baseline HBsAg <1,500 IU/mL	Undetermined	Low HBsAg (unknown threshold)
HBeAg negative							
	NA mono-therapy	**PEG-IFN mono-therapy**	**Combination NA + NA**	**Combination PEG-IFN + NA**	**NA switch to PEG-IFN**	**PEG-IFN add-on to NA**	**NA cessation**
Virological suppression*	Very high 95% at 5 years of therapy	Moderate 44% at 6 months off-therapy	Very high 90% at 2 years	Very high 87% at 2 years	8.3%	High	45% relapse
HBeAg seroconversion	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HBsAg loss	<1%	Up to 12% at 5 years off-therapy	<1%	5%	7-11%	10%	Up to 39% in those with low HBsAg
ALT normalisation	71-83%	59%	80%	60-79%	53%	Unknown	Moderate Possible flares
Clinical use	Standard of care	Young or treatment naïve patients	Viral resistance	Low baseline HBsAg & week 12 HBsAg decline >1 log IU/mL	Baseline HBsAg <1,500 IU/mL	Undetermined	Low HBsAg (unknown threshold)

These results do not stem from head-to-head studies and depend on the duration of follow-up.
* HBV DNA <20 IU/mL. ALT, alanine aminotransferase; HBeAg, Hepatitis B e Antigen; HBsAg, Hepatitis B surface Antigen; NA, nucleos(t)ide analogue; N/A, not applicable; PEG-IFN, pegylated interferon.

AIMS AND OUTLINE OF THE THESIS

Aims

The aim of this thesis is to improve immune control in patients with chronic hepatitis B through attempts to cure and modifications to the treatment paradigm.

Part I focuses on two approaches to achieve cure. The first strategy, NA therapy withdrawal, was investigated in the Toronto STOP Study and described in **Chapter 2**. This randomized controlled trial evaluated safety and efficacy of stopping NA therapy in end-of-therapy HBeAg negative patients. A significant risk of NA therapy discontinuation is the development of ALT flares. **Chapter 3** details a post-hoc analysis on the incidence, severity, outcome, and predictors of flares after NA withdrawal which was subsequently validated in an external cohort. Another approach to reach cure is detailed in **Chapter 4.** A pooled analysis of two trials was performed to investigate whether PEG-IFN add-on could increase response compared to NA monotherapy, and whether pre-treatment factors could identify the best treatment responders.

Part II outlines treatment modifications aimed at improving clinical outcome. **Chapter 5** compares the prevalence of viral and biochemical breakthrough and renal function kinetics in renally impaired patients with CHB that used reduced or full dose tenofovir disoproxil fumarate. Global migration is changing the epidemiology of CHB, especially in low-endemic regions, which could also influence the use of and perceptions on conventional Western medicine and complementary and alternative medicine. **Chapter 6** addresses the influence of individual complementary and alternative medicine modalities in patients with CHB and explores determinants of use, in particular factors related to migration, socio-economic status, and clinical outcome.

Finally, **Chapter 7** provides a brief overview of key findings, integrates these in recent literature and presents future directions to achieve functional cure in CHB.

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CHAPTER 2

LIMITED SUSTAINED RESPONSE AFTER STOPPING NUCLEOS(T)IDE ANALOGUES IN HBEAG NEGATIVE PATIENTS WITH CHRONIC HEPATITIS B: RESULTS FROM A RANDOMIZED CONTROLLED TRIAL (STOP STUDY)

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ABSTRACT

Objective: Although most patients with chronic hepatitis B (CHB) reach effective virologic suppression with long-term nucleos(t)ide analogues (NA) therapy, some might not need to continue treatment for life. In this randomized, controlled, phase IV trial we evaluated off-therapy outcomes in patients after discontinuing long-term NA therapy.

Design: Patients who had received NA therapy for ≥1 year and achieved virologic suppression (HBeAg seroconversion combined with undetectable HBV DNA ≥12 months in HBeAg positive patients, or undetectable HBV DNA ≥36 months in HBeAg negative patients) were randomized 2:1 to stop or continue NA therapy for 72 weeks. Sustained disease remission (HBeAg negative, HBV DNA <2,000 IU/mL and normal ALT) was evaluated at 72 weeks after stopping NA therapy.

Results: Among 67 enrolled patients, sustained disease remission was observed in 13/45 (29%) stop vs. 18/22 (82%) continue patients. HBsAg loss occurred in two patients (1 in each group). The median HBsAg decline from randomization to week 72 was similar in both groups (0.2 [0.0-0.4] vs. 0.1 [0.0-0.2] log IU/mL in stop vs. continue patients; p=0.04).

Among patients who stopped, 15/45 (33%) had virologic or biochemical relapse and 17/45 (38%) were retreated according to pre-defined criteria. A total of 11/18 (61%) pre-treatment HBeAg positive vs. 6/27 (22%) HBeAg negative patients required retreatment (p=0.01). Fourteen (21%) patients developed ALT >10x ULN and another 7 (10%) had ALT >5x ULN. No patients experienced liver decompensation or died.

Conclusion: The findings of this prospective study suggest limited benefit of stopping NA therapy in chronic hepatitis B.

INTRODUCTION

The goals of treatment for patients with chronic hepatitis B (CHB) – improving survival and quality of life – can be achieved by nucleos(t)ide analogues (NA).[1] However, since NA interfere late in the viral lifecycle and do not directly inhibit or degrade cccDNA, even with long-term therapy, patients rarely achieve functional cure.[2] Because of this, long-term NA therapy has become a prerequisite for most patients to improve their clinical outcomes. On the other hand, prolonged NA therapy increases risks of treatment non-adherence and adverse events (renal impairment, bone loss, virologic resistance), as well as greater health-care expenditures and inconvenience to patients.[3] There has thus been great interest in determining whether patients can stop NA therapy before achieving HBsAg loss.

The early studies had ambiguous results, showing the potential of stopping NA therapy, with relatively high HBsAg loss rates, but also highlighting a wide range of virologic relapse which sometimes led to dangerous flares.[4–7] The current hepatitis B virus (HBV) clinical practice guidelines have included considerations for NA discontinuation that were based on low-level evidence from several retrospective or small prospective studies.[8–10]

To clarify the potential benefit of NA treatment withdrawal, we performed a randomized controlled trial to evaluate the safety and efficacy of stopping long-term NA therapy in HBeAg negative patients.

MATERIALS AND METHODS

Trial design

This was an investigator-initiated, single center, randomized, open-label, phase IV clinical trial (STOP study; registered at ClinicalTrials.gov, Identifier: NCT01911156) conducted at the Toronto Centre for Liver Disease (University Health Network, Canada) from May 2016 to May 2018. Patients were randomized (baseline) 2:1 to discontinue (stop group) or continue NA monotherapy (continue group) after which patients were followed for 72 weeks (End-of-Follow-Up [EOF]).

The random allocation sequence was computer-generated in blocks of six by a biostatistician. After an eligible patient provided written informed consent, a research nurse who was not involved in the trial opened the next numbered sealed envelope with assigned intervention. Due to the nature of the intervention, patients, clinicians and study team could not be blinded to treatment allocation.

Patients in the stop group had study visits at week 4, 6, 12, 18, 24, 36, 48, 60 and 72, whereas patients in the continue group had visits at week 24, 48 and 72. The latter group continued using the NA they had been prescribed or received TDF 300 mg once daily if treatment continuation was not possible for logistic or financial reasons.

Patients in the stop group were retreated if they fulfilled one of the following criteria: HBeAg seroreversion (reappearance of HBeAg in serum); HBV DNA >2,000 IU/mL and ALT >600 IU/mL at any visit; HBV DNA >2,000 IU/mL and ALT >5x upper limit of normal (ULN: 40 IU/mL) on two consecutive visits; HBV DNA >2,000 IU/mL and ALT >200 IU/mL but <600 IU/mL for >6-8 weeks; or HBV DNA >20,000 IU/mL on two consecutive visits at least 4 weeks apart. The final decision to restart antiviral treatment was at the discretion of the treating physician.

ALT flare management was also at the discretion of the treating physician and was based on the severity of flares and signs of (impending) liver failure. In an ALT flare event, clinical assessments were done at least once a month until ALT levels reached <200 U/L. During these visits, patients underwent physical examination and routine biochemical, virologic and haematological testing. Retreated patients then continued follow-up according to the scheduled study visits.

An independent Data Safety Monitoring Board reviewed all safety data on a regular basis and advised the study team on requirements to amend or terminate the study. This study was approved by the research ethics board of University Health Network in Toronto and performed in concordance with Good Clinical Practice guidelines and the Declaration of Helsinki (2013). All patients provided written consent.

Study population

Patients with chronic hepatitis B (HBsAg positive >6 months), aged 18 years or above, were eligible if they had received NA monotherapy for ≥ one year prior to screening, had documented HBeAg status at start of NA therapy and achieved virologic suppression on NA therapy. Pre-treatment HBeAg positive patients were included if they were HBeAg negative, anti-HBe positive and HBV DNA undetectable for at least 12 months prior to screening, and pre-treatment HBeAg negative patients if they had undetectable HBV DNA for at least 36 months prior to screening. Patients were excluded for the following reasons: treatment with any investigational drug within 30 days of screening; ALT >10x ULN; creatinine clearance <70 mL/min; presence of cirrhosis as documented by biopsy within 5 years, liver stiffness measurement (FibroScan) >9kPa, and/or Fibrotest >0.48; neutropenia (neutrophils <1,000/mm3); co-infection with hepatitis C or D virus and/or human immunodeficiency virus (HIV); other acquired or inherited causes of liver disease

(alcoholic liver disease, fatty liver disease, drug related liver disease, auto-immune hepatitis, hemochromatosis, Wilson's disease or α-1 antitrypsin deficiency); α-fetoprotein >50 ng/mL; hyper- or hypothyroidism ; immune suppressive treatment within the previous 6 months; pregnancy and/or lactation; significant pulmonary dysfunction in the previous 6 months, malignancy other than skin basocellular carcinoma in previous 2 years, immunodeficiency syndromes (e.g. HIV positivity, autoimmune diseases, organ transplants other than cornea and hair transplant); any medical condition requiring, or likely to require chronic systemic administration of steroids, during the course of the study; substance abuse (alcohol (>80g/day) and intravenous or inhaled drugs (past 2 years); any other condition which in the opinion of the investigator would make the patient unsuitable for participation, or could interfere with the patient participating in and completing the study.

Efficacy analysis

The efficacy analysis comprised all patients who were randomized. The primary endpoint of sustained response was defined as HBV DNA <2,000 IU/mL 48 weeks after baseline. Secondary outcomes included virologic outcomes (HBV DNA <20/200/2,000 IU/mL); serological endpoints (HBeAg seroreversion, sustained disease remission [HBeAg negative, HBV DNA <2,000 IU/mL and ALT normalization], HBsAg loss, HBsAg decline from baseline); biochemical measures (ALT normalization, clinical relapse [HBV DNA >2,000 IU/mL + ALT >1.5x ULN]); and histological responses (liver stiffness).

Study follow-up and measurements

At every study visit, a physical exam and routine biochemical and haematological tests were performed. Plasma and serum research samples were collected and stored at -80 degrees Celsius. An assessment of liver fibrosis was done at baseline and week 48 by FibroScan or Fibrotest measurement. Serum ALT values were standardized by dividing the value by the ULN (40 IU/mL). Virologic and serological tests were analysed at the core laboratory of University Health Network (Toronto, Canada). Serum HBV DNA was measured by Cobas TaqMan 48 polymerase chain reaction assay (lower limit of detection: 20 IU/mL; Roche Diagnostics, Basel, Switzerland). Serum HBeAg, anti-HBe and HBsAg were analysed by Architect (Abbott Laboratories, North Chicago, IL, USA; lower limit of detection: 0.05 IU/mL). If HBV genotype could not be assessed due to undetectable HBV DNA levels at baseline, historic HBV genotype results were used, where possible. The presence of cirrhosis was defined by Ishak stage 6 on liver biopsy.

Safety analysis

The safety analysis included all patients who were randomized. Safety measures included recording and grading of adverse events (AEs; vital signs, and chemistry and hematology data, analyzed according to the modified World Health Organization (WHO) grading system, adapted for chronic liver disease). The causality of AEs was determined by the investigator.

Statistical analysis

The power analysis was based on the primary endpoint, which was estimated to occur in 60% of patients who discontinued NA monotherapy and in 95% of patients who continued NA monotherapy. After correction for duration of HBeAg loss and 2:1 weighted randomization, power fixed at 80% and a two-sided α-level of 0.05, the required sample size was 58 patients. Taking into account a 10% drop-out rate, a total of 66 patients were required to reach the minimum sample size: 44 patients in the stop group and 22 patients in the continue group.

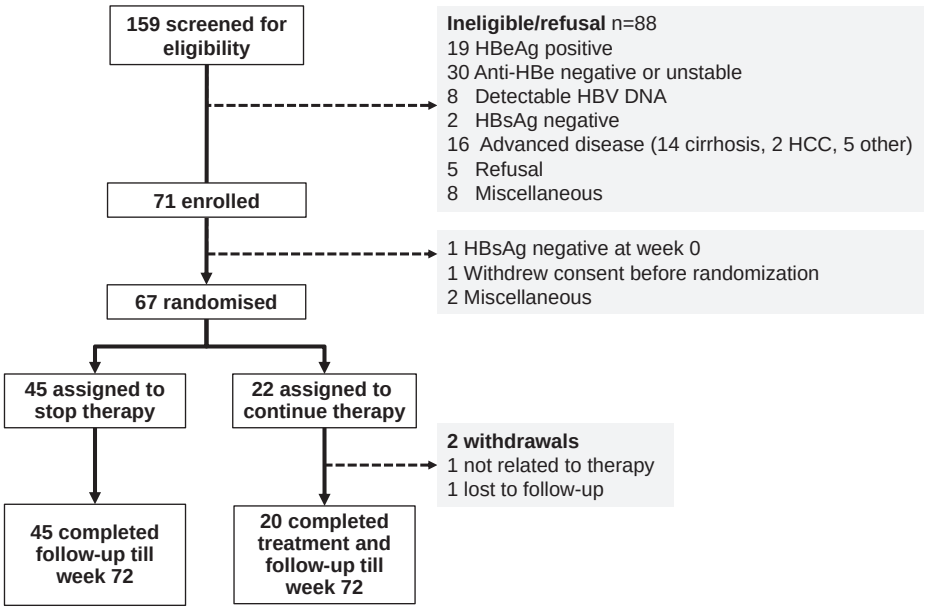
A data analysis plan was specified prior to freezing the database. No imputation was performed for missing data for the primary endpoint or any secondary outcomes. At the time of a missing visit or at the time of retreatment, patients were coded as not having achieved a virologic, serological or biochemical outcome. Variables are summarized by mean ± SD or frequency (percentage). Non-normally distributed variables were log-transformed. Outcomes were compared by chi-squared test, Student's t-test or Mann-Whitney test, where appropriate. Cumulative rates of relapse were evaluated by Kaplan-Meier method and tested by log-rank test and Cox proportional hazards regression. Analyses were performed in SPSS (v. 25.0, Chicago, IL) and SAS v. 9.4 (SAS Institute Inc., Cary, NC). Two-sided p-values <0.05 were considered significant.

RESULTS

Study cohort

A total of 159 patients were screened, of which 88 patients declined participation or were ineligible (Figure 1). One patient was excluded due to HBsAg loss at baseline and two patients due to withdrawing consent prior to randomization. Of the 67 enrolled patients, 45 (67%) patients were randomly allocated to stop and 22 (33%) to continue NA therapy.

Figure 1: Flow chart of patient inclusion.



The baseline characteristics were balanced between the treatment arms (Table 1). The mean (SD) age was 49 (10) years, 60% of patients were male and all but two patients were of Asian ethnicity. Forty (60%) patients were HBeAg negative and twenty-seven (40%) patients HBeAg positive at the start of NA therapy. Sixty-one (92%) patients were anti-HBe positive at baseline. After inclusion of the first 11 subjects who were allowed to be anti-HBe positive or negative at baseline, the study protocol was amended to only include patients if they were anti-HBe positive at baseline. The mean duration of NA therapy was 7.3 (2.9) years and HBsAg level was 3.0 (0.7) log IU/mL at baseline.

Table 1. Characteristics per treatment arm of 67 HBeAg negative patients with chronic hepatitis B.

	Stop (n=45)	Continue (n=22)
Randomization		
Age (years)	49 (10)	50 (9)
Male sex, n (%)	26 (58)	14 (64)
Asian/Caucasian race, %	98/2	95/5
Body mass index (kg/m^2)	25 (5)	25 (5)
HBV genotype: A/B/C/D, %*	0/18/27/2	0/14/32/0
TDF/ETV monotherapy, n	41/4	21/1
Duration of NA therapy (years)	7.6 (3.1)	6.8 (2.4)
Prior use of other NA agents, n (%)**	15 (33)	9 (41)
Prior (PEG-) interferon therapy, n (%)	4 (8.9)	3 (14)
Duration of HBeAg seroconversion (years)	3.8 (2.3)	4.1 (2.2)
Duration of undetectable HBV DNA (years)***	6.0 (2.3)	5.1 (2.4)
ALT (x ULN)	0.6 (0.2)	0.7 (0.2)
Anti-HBe positive, n (%)	41 (91)	21 (95)
HBsAg (log IU/mL)	3.1 (0.6)	3.0 (1.0)
METAVIR fibrosis score 0/1/2/3/4, %	30/34/30/7/0	32/27/18/14/9
Liver stiffness (kPa)	4.9 (1.0)	5.2 (1.6)
Start of therapy		
ALT (x ULN)	2.5 (2.8)	2.3 (1.8)
HBV DNA (log IU/mL)	6.1 (1.8)	6.0 (1.3)
HBeAg negative, n (%)	27 (60)	13 (59)

Continuous data presented as mean (standard deviation); *HBV genotype was known for 31 (46%) patients; ** Other NA agents comprised adefovir dipivoxil, emcitrabine, lamivudine or telbivudine; *** Undetectable HBV DNA was defined as <20 IU/mL. ALT, alanine aminotransferase; AST, aspartate transaminase; ETV, entecavir; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; PEG, pegylated; TDF, tenofovir disoproxil fumarate; ULN: upper limit of normal; WBC, white blood cell count.

Sustained response, HBsAg loss and retreatment

The primary endpoint of HBV DNA <2,000 IU/mL at week 48 was observed in 12/45 (27%) stop vs. 21/22 (95%) continue patients (p<0.005; Table 2). A total of 12/45 (27%) stop patients vs. 19/22 (86%) continue patients remained in disease remission with HBV DNA<2,000 IU/mL and ALT<1.5 x ULN (p<0.005) at week 48. At week 72, HBV DNA <2,000 IU/mL was observed in 13/45 (29%) stop patients and in 20 (91%) continue patients (p=0.04). Throughout 72 weeks of follow-up, 28/45 (62%) stop patients remained off-treatment. These patients had used NA therapy on average for 6.5 (2.3) years, 25% were HBeAg positive at start of therapy and 96% was anti-HBe positive at baseline.

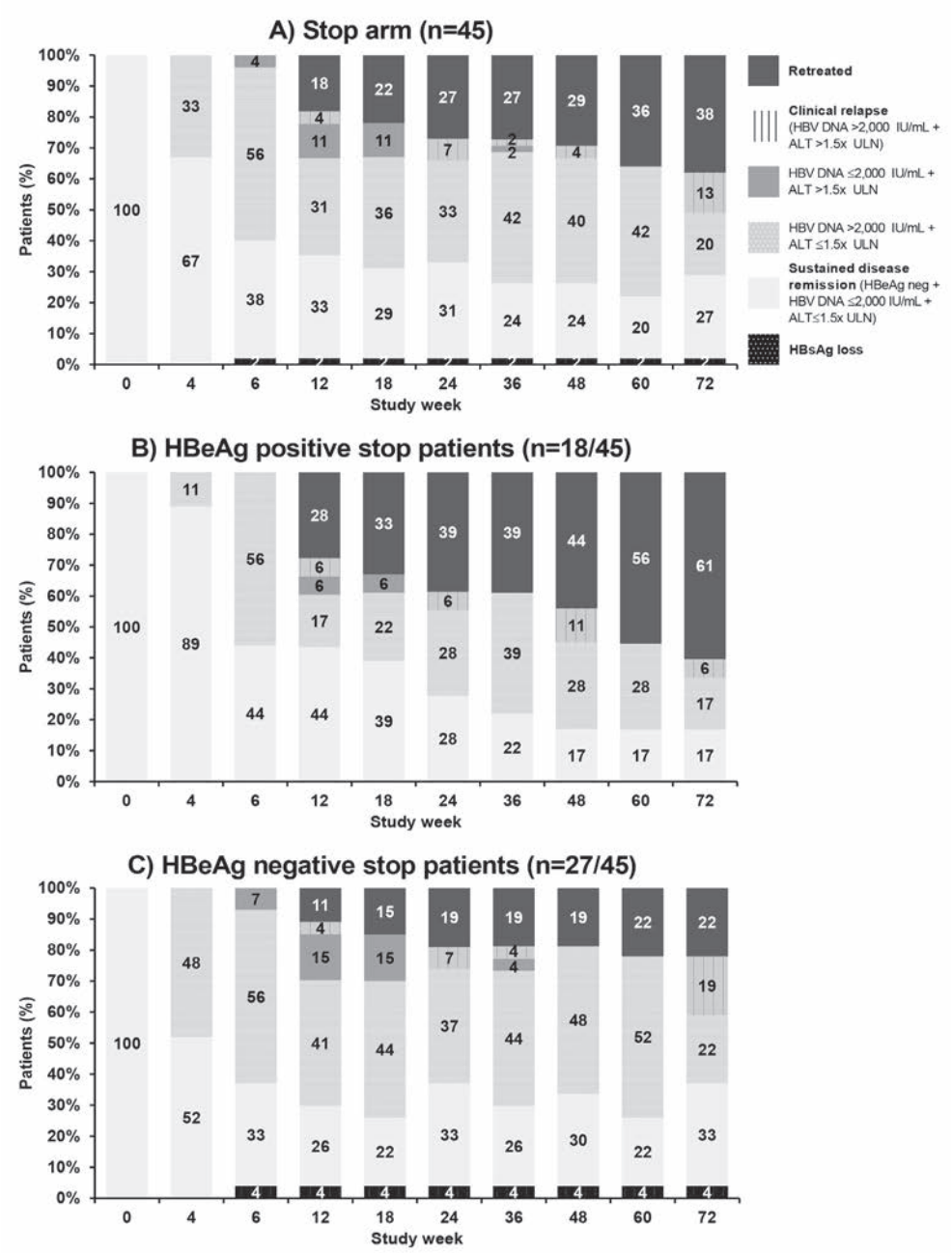
HBsAg loss occurred in two patients, one in each randomized arm. Of these two, only the stop patient developed antibodies to HBs. This patient was a 66-year old Asian female who had been

Table 2. Primary and secondary outcomes at Week 24-72 (n=67).

	Week 24			Week 48			Week 72		
Mean (SD) or n (%)	**Stop (n=45)**	**Continue (n=22)**	**P**	**Stop (n=45)**	**Continue (n=22)**	**P**	**Stop (n=45)**	**Continue (n=22)**	**P**
Virologic outcome									
HBV DNA <2,000 IU/mL	15 (33)	20 (91)	<0.005*	12 (27)	21 (95)	<0.005*	13 (29)	20 (91)	0.04*
HBV DNA <200 IU/mL	9 (20)	20 (91)	<0.005*	7 (16)	21 (95)	<0.005*	5 (11)	20 (91)	<0.005*
Undetectable HBV DNA (<20 IU/mL)	6 (13)	19 (86)	<0.005*	4 (8.9)	21 (95)	<0.005*	1 (2.2)	20 (91)	<0.005*
Serological outcome									
HBeAg seroreversion	0 (0.0)	0 (0.0)	1.00	2 (4.4)	0 (0.0)	1.00	1 (2.2)	0 (0.0)	1.00
HBeAg neg + HBV DNA <2,000 IU/mL + ALT ⩽ULN	15 (33)	16 (73)	<0.005*	12 (27)	19 (86)	<0.005*	13 (29)	18 (82)	<0.005*
HBsAg loss	1 (2.2)	1 (4.5)	1.00	1 (2.2)	1 (4.5)	1.00	1 (2.2)	1 (4.5)	1.00
HBsAg decline >1 log IU/mL from Wk 0	1 (2.2)	0 (0.0)	1.00	1 (2.2)	0 (0.0)	1.00	4 (8.9)	0 (0.0)	0.29
Biochemical outcome									
ALT normalisation (⩽ULN)	28 (62)	16 (73)	0.40	26 (58)	19 (86)	0.03*	21 (47)	18 (82)	0.01*
ALT ⩽ULN + HBV DNA<2,000 IU/mL	15 (33)	16 (73)	<0.005*	12 (27)	19 (86)	<0.005*	13 (29)	18 (82)	<0.005*
Histological outcome									
LSM (kPa)	-	-	-	5.1 (1.6)	5.1 (2.2)	0.98	5.2 (1.6)	5.3 (1.6)	0.90
LSM change from Wk 0 (kPa)**	-	-	-	0.2 (-0.7;1.0)	0.1 (-0.8;0.7)	0.53	0.0 (-0.9;1.0)	0.1 (-0.5;0.9)	0.83
Retreatment									
Retreated	12 (27)	-	-	13 (29)	-	-	17 (38)	-	-

Data presented as mean (SD) or n (%). At the time of a missing visit (2 patients in continue group) or at the time of retreatment, patients were coded as not having achieved a virologic, serological or biochemical outcome. Groups with low cell frequencies (<5) were compared with Fisher's exact test. * p<0.05; ** Median (interquartile range). ALT, alanine aminotransferase; HBV, hepatitis B virus; LSM, liver stiffness measurement; ULN, upper limit of normal.

Figure 2: Beneficial and adverse outcomes in 45 NA stop patients



Panel B and C show results of pre-treatment HBeAg positive and negative patients, respectively. The last-value-carried-forward method was used in case of missed study visits.

treated with TDF for 11.5 years, was HBeAg negative at start of therapy, anti-HBe positive at baseline and had a HBsAg at baseline of 2.8 log IU/mL. In comparison, HBsAg loss in the NA continuation group occurred in a 59 year-old Caucasian male who had used TDF for 9.4 years. The patient was HBeAg negative at start of therapy, anti-HBe negative at baseline and had a very low HBsAg value at baseline (0.1 log IU/mL). Both patients exhibited small fluctuations in quantitative HBsAg after an initial negative HBsAg test.

Among patients who stopped NA therapy, 17/45 (38%) required retreatment by week 72 (Figure 2). The median time to retreatment was 12 weeks. One (6%) patient was retreated due to HBeAg seroreversion on two consecutive visits, 12 (71%) patients for virologic relapse >20,000 IU/mL twice and 4 (24%) patients because of combined virologic and biochemical relapse, of which 2 had concurrent imaging suggestive of fibrosis/cirrhosis development. The median (interquartile range) time to retreatment was 12 (10-30) weeks. Retreatment led in all 17 stop patients to prompt virologic suppression, HBeAg negative status and ALT normalization. Six (13%) other stop patients developed clinical relapse (HBV DNA >2,000 + ALT >1.5 x ULN) by the EOF that did not meet the study requirements for retreatment and for which the investigator did not restart therapy.

Virologic, serological and ALT kinetics

Virologic, serological and biochemical kinetics are depicted in Figure 3. Virtually all NA stop patients had a virologic relapse within the first 12 weeks of NA discontinuation with peak HBV DNA values of 8 log IU/mL. Continuously suppressed HBV DNA was observed in the NA continuation group throughout follow-up.

The median HBsAg decline from randomization to week 72 was 0.2 (0.0-0.4) in the stop group and 0.1 (0.0-0.2) log IU/mL in the continuation group (p=0.04) and was not associated with peak ALT or HBV DNA values after stopping (p>0.05). Similarly, among the 17/45 retreated patients the development of ALT flares did not influence HBsAg decline from randomization to week 72. The median [IQR] HBsAg decline was 0.4 (0.3 - 0.7) vs. 0.2 (-0.1 - 0.3) log IU/mL in 13 retreated stop patients with and 4 without ALT flare (p=0.09). Among these retreated patients, HBsAg declines were not associated with ALT values at the time of retreatment or peak ALT values throughout off-therapy follow-up (p>0.05). Four (8.9%) stop patients achieved HBsAg decline >1 log IU/mL from baseline to week 72 compared to none of the continue patients (p=0.16). Six (13%) stop and 2 (10%) continue patients achieved HBsAg <100 IU/mL at the EOF (p=1.00).

Because retreatment may have altered the disease course of patients stopping therapy, we compared the difference in peak HBsAg decline and its rate by retreatment. Neither the mean

(SD) peak HBsAg decline, nor the proportion of patients with > 1 log IU/ml HBsAg decline was significantly different according to retreatment (data not shown).

Since only 5/67 (7.4%) patients had baseline HBsAg <100 IU/mL, the small number of patients with low baseline HBsAg values reduced the strength of a stratified analysis on HBsAg decline. After determining category thresholds for baseline HBsAg based on the histogram, 11/27/25 patients were categorized into baseline qHBsAg groups 0.0-2.3; 2.3-3.3; and >3.3 log IU/mL. HBsAg decline at week 72 was 0.4 (0.5), 0.4 (0.7) and 0.1 (0.1) log IU/mL, respectively, and was not significantly different between groups (p=0.11).

Liver stiffness measurements

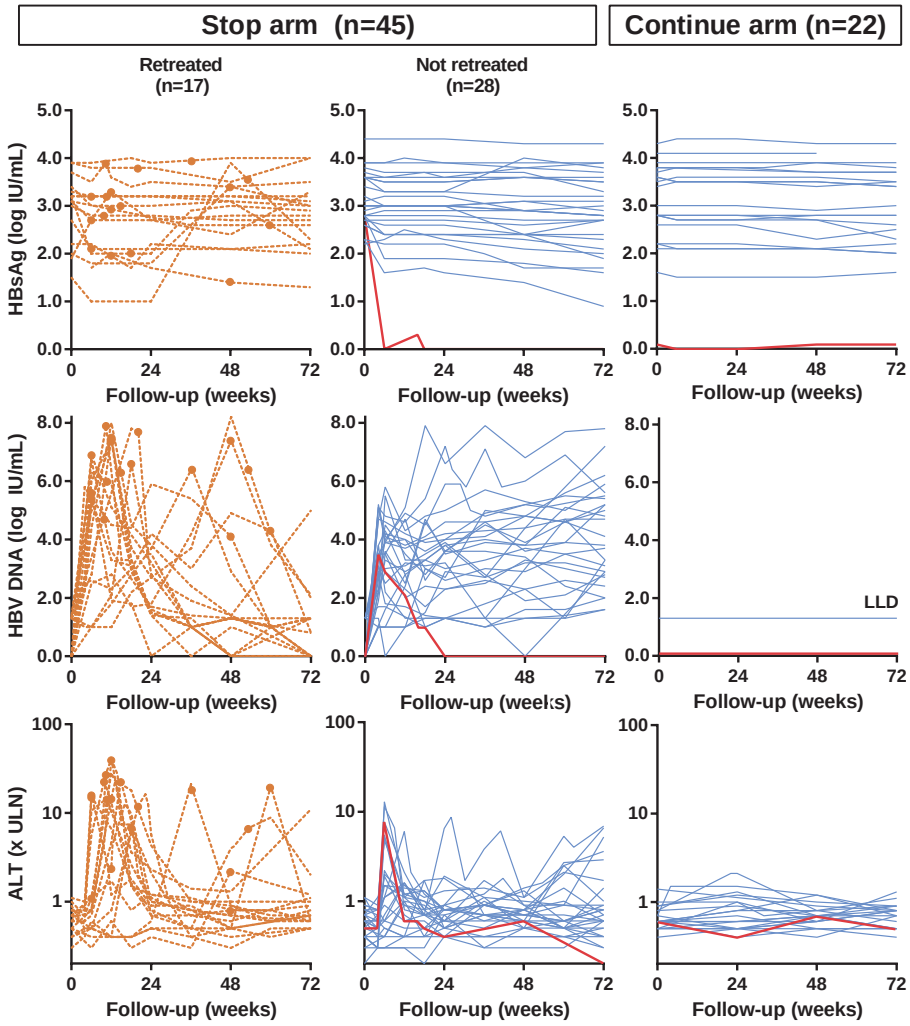
At week 72, liver stiffness measurements by FibroScan were 5.2 (1.6) vs. 5.3 (1.6) in the NA stop vs. continue group (p=0.90; Table 2); the liver stiffness decline at week 72 from baseline was 0.0 (-0.9 – 1.0) vs. 0.1 (-0.5 – 0.9; p=0.83).

Outcomes according to pre-treatment HBeAg status

Baseline values according to pre-treatment HBeAg status are shown in Supplementary Table 1. The primary endpoint was achieved by 3/18 (17%) initially HBeAg positive vs. 7/24 (29%) initially HBeAg negative stop patients (p=0.10; Figure 2). A greater proportion of pre-treatment HBeAg positive compared to HBeAg negative stop patients was retreated (11/18 [61%] vs. 6/27 [22%]; log-rank p=0.01). The median time to retreatment was similar for pre-treatment HBeAg positive and negative patients (12.3 [10.7-49.9] vs. 12.7 [9.3-23.1] weeks; p=0.37). In a post-hoc, multivariable, logistic regression analysis that included relevant baseline factors, HBeAg positive status at start of treatment was the only independent predictor of relapse (odds ratio [95% confidence interval]: 7.4 [1.3-42.6]; p=0.03). Importantly, end-of-therapy qHBsAg, HBV genotype, ALT, METAVIR fibrosis score or the duration of NA consolidation therapy were not associated with relapse (p>0.05).

Furthermore, HBeAg seroreversion occurred at least transiently in 8/45 (18%) NA stop patients. These patients met retreatment criteria other than HBeAg seroreversion first, but had HBeAg seroreversion after retreatment. All were HBeAg positive at start of therapy and 3/8 (38%) patients were anti-HBe negative at EOT. HBeAg seroreversion persisted for at least 2 consecutive visits in 4 stop patients, of which 3 had simultaneous clinical relapse. The 4 other stop patients experienced HBeAg seroreversion at a single visit with concurrent clinical relapse, all of whom achieved HBeAg negative status, HBV DNA <20 IU/mL and ALT normalization upon retreatment.

Figure 3: Virologic, serological and biochemical kinetics in patients who stopped or continued NA treatment (n=67).



The median HBsAg change from randomization to week 72 was 0.0 (-0.1-0.2) vs. 0.2 (0.0-0.3) log IU/mL in HBeAg positive vs. negative stop patients (p=0.04) (Supplementary Figure 1). The median peak ALT (13.7 [2.8-21.2] vs. 5.0 [1.7-8.5] x ULN; p=0.03) and mean peak HBV DNA values (6.1 [1.8] vs. 5.0 [1.3] log IU/mL; p=0.02) were significantly different between pre-treatment HBeAg positive and negative stop patients. However, these differences disappeared when the results were stratified according to retreatment (p>0.05 in all groups).

We also analyzed outcome according to anti-HBe status at treatment discontinuation. However, the small number of patients (n=5) who were anti-HBe negative at stopping treatment limited a comprehensive analysis. A subgroup analysis that excluded these 5 baseline anti-HBe negative patients showed results similar to the overall group.

Safety

Safety data are reported in Table 3. The vast majority of grade 3 adverse events comprised ALT elevations >5X ULN. Among patients who stopped therapy 14 (21%) developed ALT >10x ULN (peak ALT: 41x ULN) and another 7 (10%) patients had ALT >5x ULN. No flares occurred in those who continued NA therapy. One of the flares occurred in a pre-treatment HBeAg positive patient whose bilirubin increased to 68 µmol/L after stopping NA therapy without developing signs of decompensation. One other patient in the stop group underwent an elective cataract surgery during post-treatment follow-up. None of the patients developed hepatic decompensation, serious adverse events or died.

Table 3. Safety profile up to Week 72.

Adverse Event	n (%)	Stop (n=45)	Continue (n=22)
Grade 1	Total	38 (84)	10 (45)
Grade 2		11 (24)	0
Grade 3	Total	22 (49)	0
	ALT >5.0 x ULN	22 (49)	0
	Hyperbilirubinemia (>66 umol/L)	1 (2.2)	0
	Elective cataract surgery	1 (2.2)	0
Grade 4 (SAE)		0	0

ALT, alanine aminotransferase; SAE, Serious Adverse Event; ULN, upper limit of normal.

DISCUSSION

In this largest prospective RCT to date on NA withdrawal in CHB, 27% of patients had sustained response, 71% relapsed and 2% achieved HBsAg loss 72 weeks after stopping therapy. HBsAg levels in the stop group declined only marginally and were not different from the NA continuation group. These findings suggest that stopping NA therapy confers little benefit in our population of mainly Asian patients.

Our findings that very few patients achieved HBsAg loss and had minimal HBsAg decline contradicts results from previous studies. In a RCT that randomized 42 HBeAg negative mainly Caucasian patients to stop or continue NA therapy, 19% of stop patients achieved HBsAg loss by week 144 off-therapy (6.3% annually), which was significantly more than in the continuation group. The median HBsAg change was -0.59 log IU/mL.[4] A large retrospective study from Taiwan reported HBsAg loss in 13% of 691 patients at 6 years off-therapy follow-up (1.8% annually).[5] Although the follow-up of our study was 1.5 years, the minute HBsAg decrease over time in all patients suggests that few will achieve HBsAg loss during longer follow-up. In addition to ethnicity, the discrepant HBsAg findings between studies may be due to differences in end-of-therapy HBsAg values, HBV genotype, the duration of NA consolidation therapy and retreatment criteria.[11,12]

Remarkably, pre-treatment HBeAg positive patients were three times more likely than HBeAg negative patients to require retreatment for relapse after stopping. These results challenge previous claims of a more sustained off-therapy response for HBeAg positive than negative patients.[13] Importantly, even though pre-treatment HBeAg positive patients only required 1 year of NA therapy following HBeAg seroconversion for study entry, the mean (SD) duration of consolidation therapy was 3.4 (1.7) years and did not differ between those who did and did not require retreatment. In a large, prospective cohort study from China, the cumulative virologic relapse rate at 10 years off-therapy was 31% vs. 62% in 138 pre-treatment HBeAg positive and 85 HBeAg negative patients.[14] In this study however, 218/223 (98%) patients had used older generation NA therapies (adefovir dipivoxil, emtricitabine, lamivudine or telbivudine); the EOT fibrosis stage was unknown; and since HBV DNA was measured with a less sensitive assay (lower limit of quantification: 200 IU/mL) a low residual viral load could have influenced relapse rates. Notably, different studies have used different definitions for relapse and for retreatment criteria, which could account for the discrepant results. Furthermore, at least transient HBeAg seroreversion occurred in 8/45 (18%) patients in our study, raising concern that stopping in this population is not only not helpful, but potentially harmful.

The host's immune system plays an integral role in viral control during and after NA therapy. Long-term NA consolidation treatment partially restores the liver-specific cellular immune response and reduces the risk of relapse off-therapy.[12,15,16] The patients in our study had received substantially longer NA consolidation therapy (3.8 years for HBeAg positive patients; 6.0 years for HBeAg negative patients) than the 6-12 months as recommended by the current guidelines.[8–10] Nonetheless, the high rate of relapse and of retreatment that occurred predominantly within the first 24 weeks suggests that the recommended duration of consolidation therapy might be too short or not useful at all. A previous study from our group found that prolonging consolidation therapy beyond 3 years reduced the risk of persistent virologic relapse.[12] We were not able to confirm these findings in the current study because almost all patients had greater than 3 years of consolidation therapy at study entry and a relatively high rate of relapse was still seen.

Some authors have suggested that virologic rebound off-therapy precipitates HBsAg loss.[17] This pattern was also visible in the single stop patient who achieved HBsAg loss after a simultaneous decline in HBsAg and rise in viral load. However, overall, we saw no change in HBsAg levels after stopping therapy and HBsAg decline was not associated with peak ALT or HBV DNA values. The overall lack of HBsAg decline in our study precluded any further exploration. A critical point to discuss with patients who consider stopping NAs is the small but significant risk of adverse outcomes. In a systematic review of NA withdrawal studies hepatic decompensation occurred in 0.8-3% of patients.[18] At least 11 deaths (0.2-1.6% of patients) have been reported due to off-therapy flares in various studies, at least some in the presumed absence of cirrhosis.[7,1319] Since none of the published studies have been able to find reliable predictors of relapse, a visitintensive monitoring schedule must be reinforced. Essentially all episodes of retreatment in our study were preceded by lone virologic relapse in the previous study visit. Further exploration of the predictive value of HBV DNA and new virologic biomarkers, such as HBcrAg or HBV RNA, holds promise to improve identifying those at highest risk for adverse events.

An important strength of the present study, which is the largest randomized controlled trial on NA discontinuation, was the inclusion of both pre-treatment HBeAg positive and negative patients. Furthermore, the retreatment criteria of our study were not as stringent as in other studies, which allowed us to better evaluate the offtherapy viral and biochemical kinetics during close-visit monitoring. We balanced the retreatment criteria such that we allowed the viral load to rebound but not at the costs of dangerous flares.

A limitation to our study was the inclusion of a predominantly Asian cohort. This prohibited us from studying the influence of HBV genotype or race on off-treatment outcomes. In addition to further exploring ethnicity-related questions in a heterogeneous population, future studies

should ideally be designed with well-balanced, predefined retreatment criteria that are not based on current guideline recommendations and investigate long-term clinical outcomes.

In conclusion, the findings from this predominantly Asian cohort suggest that NA discontinuation before HBsAg loss has limited benefits, especially for pre-treatment HBeAg positive patients.

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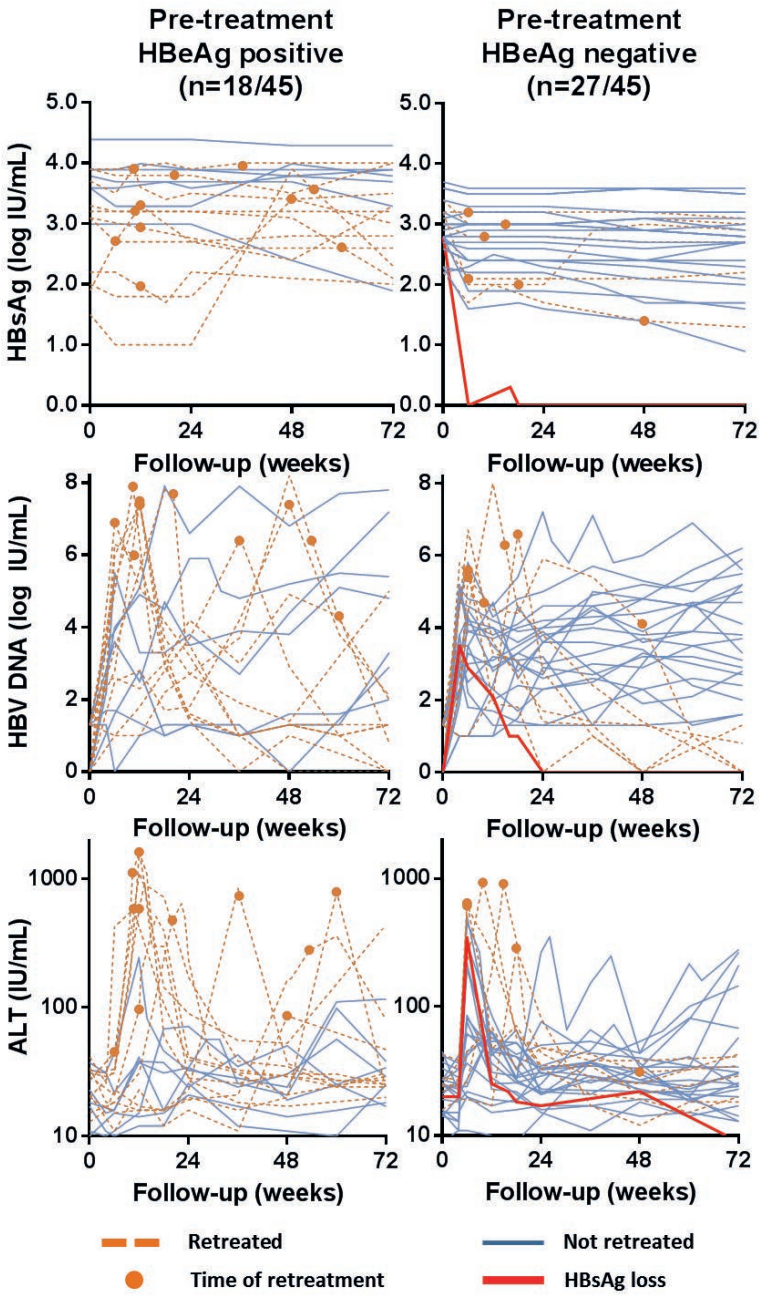
SUPPLEMENTARY FILES

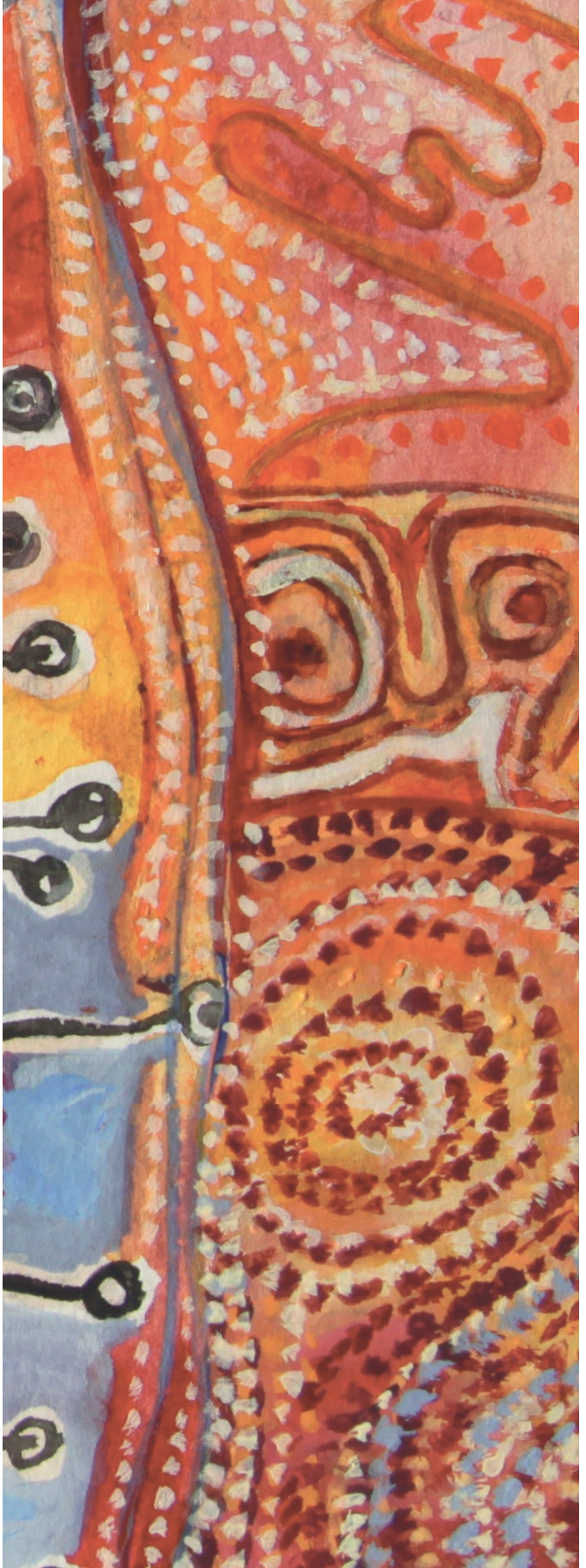
Supplementary Table 1: Characteristics in 45 nucleos(t)ide analogue therapy discontinuation patients according to pre-treatment HBeAg status.

	Pretreatment HBeAg positive (n=18)	Pretreatment HBeAg negative (n=27)
Randomization		
Age (years)	46 (9)	52 (11)
Male sex, n (%)	11 (61)	15 (56)
Asian/Caucasian race, %	94/6	100/0
Body mass index (kg/m^2)	24 (4)	26 (5)
HBV genotype: A/B/C/D, %*	0/0/50/0	0/30/11/3.7
TDF/ETV monotherapy, n	89/11	82/18
Duration of NA therapy (years)	7.9 (2.9)	7.4 (3.2)
Prior use of other NA agents, n (%)**	9 (50)	6 (22)
Prior (PEG-) interferon therapy, n (%)	2 (11)	2 (7.4)
Duration of HBeAg seroconversion (years)	3.4 (1.7)	7.1 (4.5)
Duration of undetectable HBV DNA (years)***	5.7 (2.0)	6.2 (2.5)
ALT (x ULN)	0.6 (0.2)	0.6 (0.2)
Anti-HBe positive, n (%)	14 (78)	27 (100)
HBsAg (log IU/mL)	3.2 (0.9)	3.0 (0.6)
METAVIR fibrosis score 0/1/2/3/4, %	17/28/39/17/0	33/44/22/0/0
Liver stiffness (kPa)	4.5 (0.9)	5.1 (1.1)
Start of therapy		
ALT (x ULN)	3.6 (4.0)	1.9 (1.5)
HBV DNA (log IU/mL)	7.5 (1.4)	5.3 (1.4)

Continuous data presented as mean (standard deviation); *HBV genotype was known for 21 (47%) patients; ** Other NA agents comprised adefovir dipivoxil, emcitrabine, lamivudine or telbivudine; *** Undetectable HBV DNA was defined as <20 IU/mL. ALT, alanine aminotransferase; AST, aspartate transaminase; ETV, entecavir; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; PEG, pegylated; TDF, tenofovir disoproxil fumarate; ULN: upper limit of normal; WBC, white blood cell count.

Supplementary Figure 1: Virologic, serological and biochemical kinetics in 45 patients who stopped NA treatment according to pre-treatment HBeAg status.





CHAPTER 3

MALE SEX AND EARLY VIROLOGIC RELAPSE PREDICT ALT FLARES AFTER NUCLEOS(T)IDE ANALOGUE WITHDRAWAL IN HBEAG NEGATIVE PATIENTS WITH CHRONIC HEPATITIS B: RESULTS FROM THE TORONTO HBV STOP STUDY

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Provisionally accepted

ABSTRACT

Background and Aims: When HBeAg-negative patients with chronic hepatitis B (CHB) stop nucleos(t)ide analogue (NA) therapy before achieving HBsAg loss, flares often ensue. Early prediction of these potentially detrimental events remains challenging. We determined the incidence, severity, outcome and predictors of flares after NA withdrawal.

Methods: Forty-five non-cirrhotic, HBeAg negative patients previously enrolled and closely monitored in an RCT were included; 107 patients from an external, prospective cohort were used for validation. Retreatment criteria were pre-defined. Pre- and post-treatment predictors of ALT flare (>5x ULN) and adverse outcomes were evaluated by Cox proportional-hazards regression.

Results: Seventy-two weeks after NA withdrawal, 23/45 (51%) patients had developed >5x ULN and 14 (31%) >20x ULN. Median time to develop ALT >5x ULN was 12 weeks after NA withdrawal. Clinical relapse (HBV DNA >2,000 IU/mL + ALT >2x ULN) occurred in 27/45 (60%). No patients decompensated or died.

Independent predictors of ALT>5xULN were male sex (HR [95%CI] 3.2 [1.2-8.9]; p=0.03) and serum HBV DNA (1.2 [1.0-1.8]; p=0.03) at week 6 off-therapy. Specifically, week 6 HBV DNA >10,000 IU/mL predicted ALT >5xULN (3.4 [1.4-8.4]; p=0.01), which was externally validated. End-of-therapy HBsAg values were not associated with ALT flares (p=0.76).

Conclusion: A detailed analysis of post-treatment flares in a prospective RCT revealed a high cumulative incidence of 52% in HBeAg negative CHB. Male sex and week 6 HBV DNA >10,000 IU/mL independently predicted flares. The proposed threshold enables prediction of imminent flares in patients who may benefit from closer monitoring and earlier retreatment.

INTRODUCTION

Patients with chronic hepatitis B (CHB) often require long-term nucleos(t)ide analogue (NA) therapy for their active disease. Although this therapy is very effective, prolonged treatment duration carries considerable costs and could also result in side effects, therapy non-adherence and virologic resistance. Discontinuing NA therapy to achieve functional cure (HBsAg loss) has therefore been evaluated in several retrospective studies and a few small prospective studies.[1–6] These studies showed widely ranging rates of relapse, some of which led to hepatic decompensation and even death.

Predicting off-therapy ALT flares that require retreatment according to pre-defined criteria at the end-of-therapy (EOT) or during the early off-treatment follow-up could aid the clinical decision-making process and prevent potentially dangerous flares. The current HBV clinical practice guidelines suggest that NA discontinuation can be considered in selected cases, but provides little guidance to identify these patients in clinical practice.[7–9] The timing of retreatment is crucial because retreating too early might not be necessary, while retreating too late might result in hepatic decompensation or even death.[2]

We previously described the results of a randomized, controlled trial on NA cessation.[4] In this current study, we aimed to describe the incidence and outcome of ALT flares and determine predictors thereof in two independent cohorts of HBeAg negative patients with CHB who stopped NA therapy.

MATERIALS AND METHODS

Study population and design

The study population included 2 separate cohorts of HBeAg negative patients with CHB. Cohort 1 included 67 patients who were randomized to stop (n=45) or continue NA therapy (n=22) for 72 weeks as part of an investigator-initiated, randomized controlled trial (Toronto STOP study; ClinicalTrials.gov, Identifier: NCT01911156).[4] The study was performed at the Toronto Centre for Liver Disease (University Health Network, Canada) from May 2016 until May 2018. Since the NA continuation group did not experience any ALT flares, this study focused on the 45 NA cessation patients. Cohort 2, a validation cohort, originated from a prospective, observational study conducted at Nanfang Hospital (Guangzhou, China; November 2012 until July 2015) where 107 patients stopped NA therapy and were prospectively followed for at least six months.[10] The methods of both studies have been described in detail elsewhere and are largely comparable.[4,10]

In brief, patients from Cohort 1 were included if they were non-cirrhotic, had received NA monotherapy for ≥12 months, had documented pre-treatment HBeAg status and were virologically suppressed on NA therapy. Pre-treatment HBeAg positive patients had to be HBeAg negative, anti-HBe positive and HBV DNA undetectable on treatment for at least 12 months before screening. Pre-treatment HBeAg negative patients had to maintain undetectable HBV DNA for at least 36 months before screening. No patient had a co-infection with hepatitis C virus or human immunodeficiency virus (HIV) or any other concomitant liver disease. This study was approved by the research ethics board of University Health Network and conducted in agreement with Good Clinical Practice guidelines and the Declaration of Helsinki (2013). All patients provided written consent. All authors had access to the study data and reviewed and approved the final manuscript.

Endpoints

ALT flare was the major outcome of interest. For prediction analysis, ALT flare was defined as >5x the upper limit of normal (ULN) or >3x baseline value, whichever was higher, according to definitions described in several landmark papers and recent expert meetings (NIH CTCAE; Hepatitis B Research Network; HBV forum).[11,12] Other outcomes included analyzed ALT flares at a different cut-off (>2x/10x/20x ULN) and clinical relapse (combined HBV DNA >2,000 IU/mL with ALT >2x ULN) at a single visit.

Study follow-up and measurements

NA stop patients had study visits at week 4, 6-8, 12, 18, 24, 36, 48, 60 and 72. During these visits, a symptom-directed physical exam and routine biochemical and haematological tests were also performed in addition to HBV markers. Data from the start-of-therapy were obtained from electronic medical charts according to a pre-defined protocol. The presence of cirrhosis was defined by Ishak stage 6 on liver biopsy, liver stiffness (FibroScan) >9kPa, or Fibrotest >0.48.

Laboratory analysis

Serum ALT was standardized by dividing the ALT value by the ULN (40 IU/mL). Virologic and serological values were analysed at the core laboratory of University Health Network (Canada). Serum HBV DNA was measured by Cobas TaqMan 48 polymerase chain reaction assay (lower limit of detection: 20 IU/mL; Roche Diagnostics, Basel, Switzerland). Serum HBeAg, anti-HBe and HBsAg were analysed by Architect (Abbott Laboratories, North Chicago, IL, USA; lower limit of detection: 0.05 IU/mL). HBV genotype was retrieved from medical charts, where possible.

ALT flare management and NA retreatment

Retreatment was initiated if one of the following criteria was fulfilled: HBeAg seroreversion (reappearance of HBeAg in serum); HBV DNA >2,000 IU/mL and ALT >600 IU/mL at any visit; HBV DNA >2,000 IU/mL and ALT >5x ULN (>200 IU/mL) on two consecutive visits; HBV DNA >2,000 IU/mL and ALT >200 IU/mL but <600 IU/mL for >6-8 weeks;or HBV DNA >20,000 IU/mL on two consecutive visits at least 4 weeks apart. The final decision to retreat was at the treating physician's discretion.

ALT flare management was also at the discretion of the treating physician and was guided by the severity of flares and signs of (impending) liver failure. Clinical assessments were performed at least monthly until ALT levels reached <200 IU/L. Once ALT fell below 200 IU/mL, follow-up continued according to visits scheduled in the study protocol.

Statistical analysis

Variables are summarized by mean ± SD or frequency (percentage). Non-normally distributed variables were log-transformed. Outcomes were compared by chi-squared test, Student's *t*-test or Mann-Whitney test, where appropriate. Cumulative rates of ALT flare were evaluated by Kaplan-Meier method and tested by log-rank test and Cox proportional hazards regression. The survival analysis was adjusted for pre-defined covariates that were likely to influence the outcome. Follow-up time was calculated from the date of NA withdrawal until an outcome was achieved or retreatment was initiated. The duration of consolidation therapy for HBeAg positive patients was calculated as time from HBeAg seroconversion and undetectable HBV DNA until randomization, whereas for HBeAg negative patients the time from undetectable HBV DNA until randomization was computed. Two-sided p-values <0.05 were considered significant. Analyses were performed in SPSS (v. 25.0, Chicago, IL) and SAS v. 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Study cohort

Among the 45 patients from cohort 1 who were randomly assigned to stop NA therapy, the mean (SD) age at NA discontinuation was 49 (10) years, 26 (60%) patients were male and all but two patients were of Asian ethnicity (Table 1). The mean duration of NA therapy was 7.6 (3.1) years and the duration of consolidation therapy was 3.8 (2.3) years. Nearly all patients (41/45 [91%]) were positive for anti-HBe at EOT. At the start-of-therapy, 24/45 (53%) patients were HBeAg negative. The 107 patients from cohort 2 had comparable characteristics at NA discontinuation (Table 1).

Table 1. Characteristics of HBeAg negative patients with chronic hepatitis B who stopped NA therapy.

	Cohort 1 (n=45)	Cohort 2 (n=107)
End-of-therapy		
Age	49 (10)	35 (8)
Male, n (%)	26 (58)	90 (84)
Asian/Caucasian race, %	98/2	100
Body mass index (kg/m^2)	25 (5)	-
HBV genotype: A/B/C/D, %*	0/18/27/2	-
TDF/ETV/ADV/LDT/LAM/dual therapy, %	91/9/0/0/0/0	1/40/25/18/2/14
Duration of NA therapy (years)	7.6 (3.1)	4.6 (2.7)
Prior (PEG-) interferon therapy, n (%)	4 (9.1)	22 (21)
Duration of HBeAg seroconversion (years)	3.8 (2.3)	2.9 (1.8)
Duration of undetectable HBV DNA (years)**	6.0 (2.3)	N/A
ALT (x ULN)	0.6 (0.2)	0.6 (0.3)
AST (IU/mL)	23 (5)	N/A
HBeAb positive, n (%)	41 (91)	97 (91)
HBsAg (log IU/mL)	3.1 (0.6)	2.4 (1.4)
Liver stiffness (kPa)	4.9 (1.0)	5.6 (1.5)
Start-of-therapy		
HBV DNA (log IU/mL)	6.1 (1.8)	5.9 (1.4)
HBeAg negative, n (%)	24 (53)	32 (30)

Continuous data presented as mean (standard deviation); *HBV genotype was known for 31 (46%) patients; ** Undetectable HBV DNA was defined as <20 IU/mL. ADV, adefovir dipivoxil; ALT, alanine aminotransferase; AST, aspartate transaminase; ETV, entecavir; HBV, hepatitis B virus; LAM, lamivudine; LDT, telbivudine; NA, nucleos(t)ide analogue; N/A, not available; PEG, pegylated; TDF, tenofovir disoproxil fumarate; ULN: upper limit of normal.

ALT flares

Among cohort 1, the cumulative incidence of ALT flares according to the pre-defined thresholds is depicted in Figure 1A. By week 24, 22/45 (49%) patients developed ALT flare >2x ULN, which continued to increase to 56% by week 48 and 65% by week 72. Almost half of all ALT flares >2x ULN (13/29 [45%]) occurred within 6 weeks after NA discontinuation. The cumulative incidence of ALT flares according to other definitions, defined as ALT >5, >10 and >20x ULN, was 52%, 32% and 15% by week 72. The median time to ALT flare >5x ULN was 12 weeks. The mean (SD) rate of ALT increase to its peak value was 0.8 (1.0) ULN per week. The mean rate of ALT increase to its peak value was 1.5 (1.0) ULN per week for patients with an ALT flare >5x ULN, 1.9 (1.0) for ALT >10x ULN and 2.2 (1.0) for ALT >20x ULN. The median (IQR) peak ALT was 6.2 (2.0-15.6) x ULN with an absolute peak ALT of 41x ULN (1626 IU/ml).

By week 72, the cumulative incidence of ALT >2/5/10/20x ULN was 72/60/47/25% in pre-treatment HBeAg positive patients compared to 60/45/32/8% in pre-treatment HBeAg negative NA stop patients (p-value for every definition: >0.05). Median peak ALT values were significantly higher in pre-treatment HBeAg positive than negative patients (median [IQR] ALT: 14.6 (2.8-21.1) vs. 5.0 (1.9-8.5) x ULN; p=<0.005).

Predictors of ALT flare >5x ULN

By univariable Cox proportional hazards regression, male sex, duration of NA therapy, and week 6 serum values of AST and HBV DNA were associated with ALT >5x ULN (p<0.10) (Figure 2). Quantitative HBsAg was not predictive (p=0.76). Independent predictors of ALT >5xULN comprised male sex (HR [95% CI]: 3.2 [1.2-8.9]; p=0.03) and serum HBV DNA (HR: 1.2 [1.0-1.8]; p=0.03) at week 6 off-therapy. Subsequently we estimated the optimal cut-off for week 6 HBV DNA to predict the risk of ALT flares. A week 6 HBV DNA >10,000 IU/mL significantly predicted a higher risk of ALT >5x ULN (HR: 3.4 [1.4-8.4]; p=0.01; Figure 2). The positive predictive value was 74% and the negative predictive value was 65%. The cumulative rates per subgroup are shown in Figure 3. ALT flare >5x ULN occurred in 100% of males with week 6 HBV DNA >10,000 IU/mL compared to 23% of females with HBV DNA values below the cut-off (HR: 8.8 [2.3-32.8]; p<0.005).

Figure 1: Cumulative incidence of (A) ALT flares and (B) virologic relapses in 45 NA stop patients by pre-treatment HBeAg status (Cohort 1).

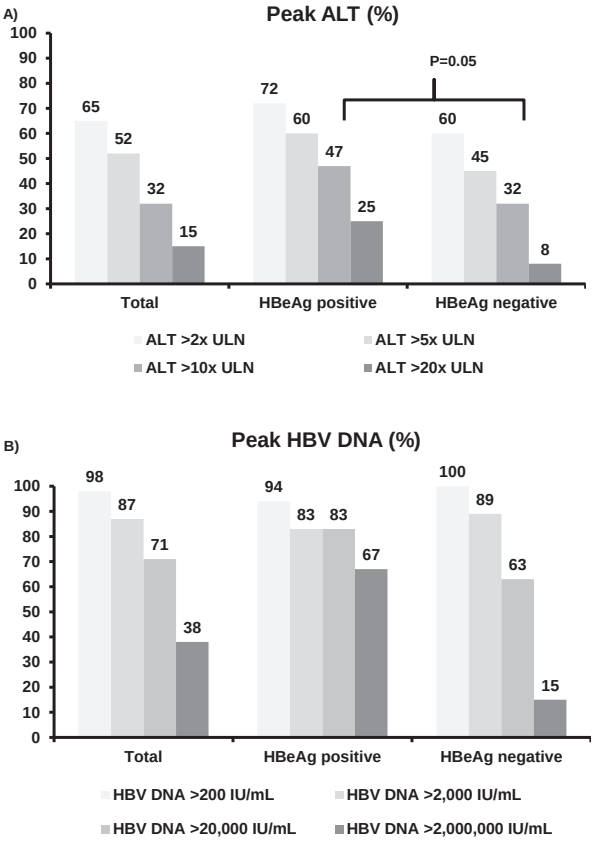
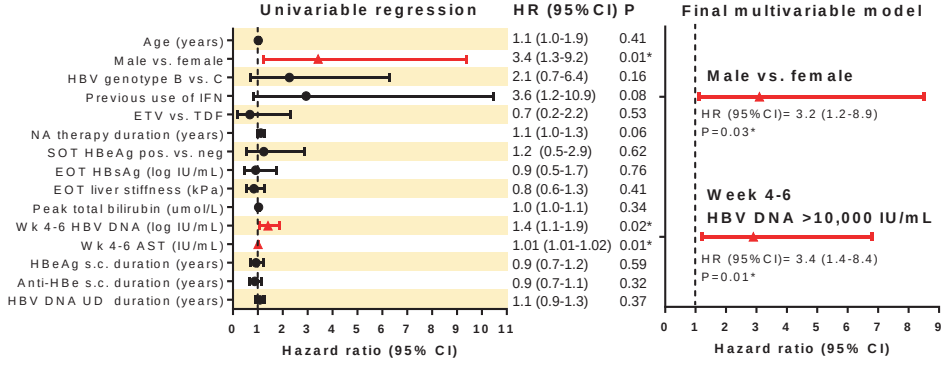


Figure 2: Predictors of ALT >5x ULN in 45 stop patients with Cox proportional hazards regression in Cohort 1.



Validation of the prediction model

Validation of the two predictors in cohort 2 confirmed only that week 8 HBV DNA >10,000 IU/mL significantly increased the risk of ALT >5x ULN (HR: 10.0 [95%CI: 2.9-34.5]; p<0.001); sex was not an independent predictor (p=0.91).

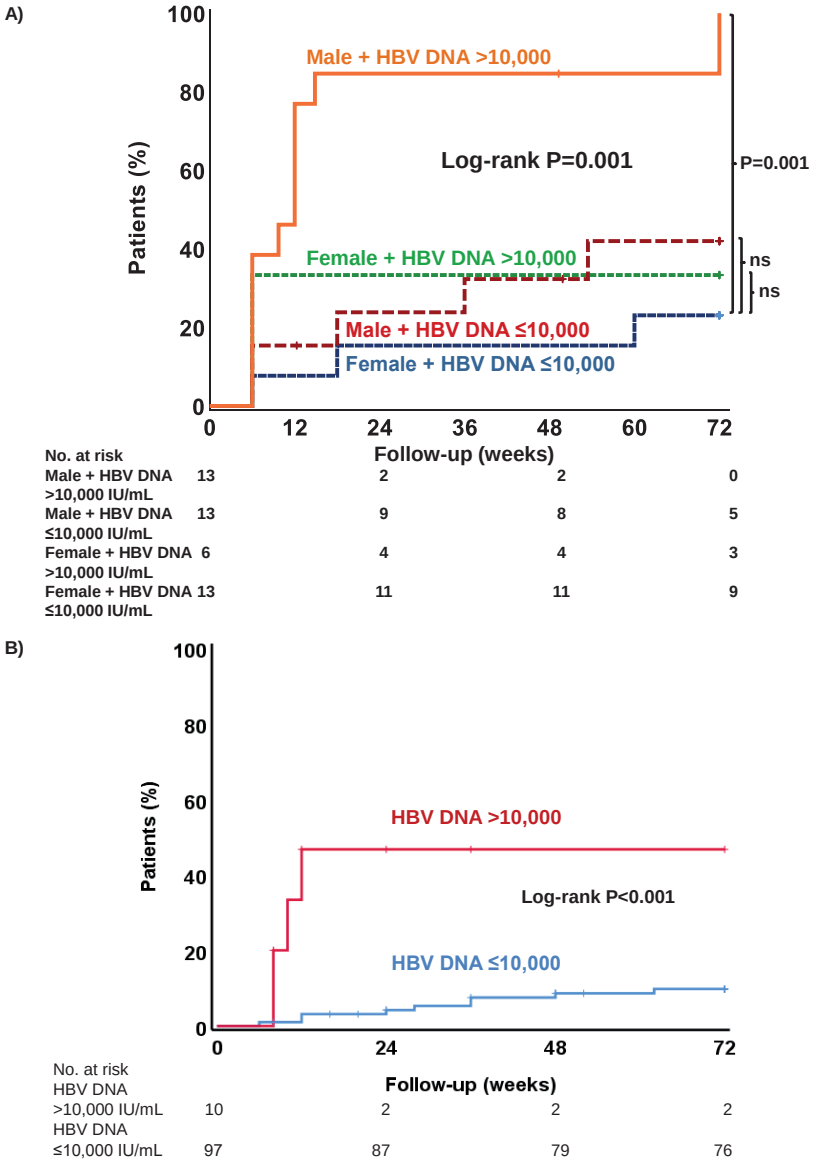
Virologic Outcome and Safety

After NA discontinuation, all patients had detectable HBV DNA and virtually all of them experienced virologic relapse (Figure 1B). HBV DNA >2,000 occurred in 39/45 (87%) patients by week 72. HBV DNA >2 million IU/mL was observed in 17/45 (38%) patients. The median peak HBV DNA was 5.6 (4.3-6.9) log IU/mL and was significantly higher in pre-treatment HBeAg positive than negative patients (6.8 vs. 5.1 log IU/mL; p<0.005). Clinical relapse at week 72 (HBV DNA >2,000 IU/mL combined with ALT >2x ULN) was reported in 27/45 (60%) patients.

HBsAg decline >1.0 log IU/mL was achieved by 5/45 (9%) patients, of which one patient achieved HBsAg loss. However, peak ALT values were not associated with HBsAg decline from baseline to week 72 (Pearson correlation: ρ=0.04; p=0.78) (Figure 4). Similarly, peak bilirubin values were not correlated to HBsAg decline (ρ=-0.02; p=0.91).

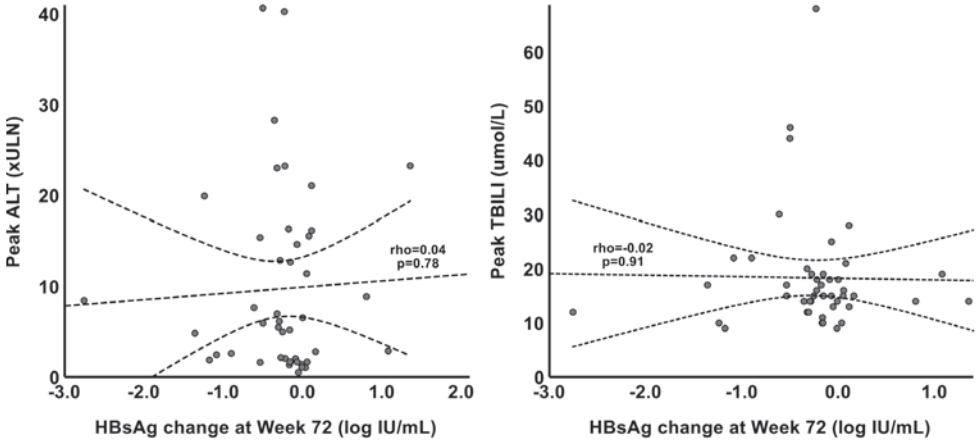
By week 72, 17/45 (38%) stop patients had received retreatment. Retreatment led in all 17 patients to prompt virologic suppression, HBeAg negative status and normalized ALT. Although in one patient bilirubin increased to 68 umol/L, hepatic decompensation or death did not occur. Overall, the median (IQR) peak total bilirubin was 15 (13-19) µmol/L. Three (6.7%) patients had a bilirubin >34 µmol/L.

Figure 3: Cumulative rate of ALT >5x ULN stratified by sex and week 6 HBV DNA in stop patients: (A) Cohort 1; (B) Cohort 2.



Event rate analysed by Cox proportional hazards regression.

Figure 4: Correlation of peak ALT and peak total bilirubin to HBsAg change from baseline to Week 72 in Cohort 1.



Negative values indicate a decline in HBsAg; positive values indicate an incline.

DISCUSSION

This study was a pre-specified analysis of HBeAg negative patients with CHB stopping long-term NA therapy as part of a large, prospective RCT.[4] The present work showed that by week 72, 52% of patients had experienced an ALT flare and 38% was retreated. Male sex and week 6 HBV DNA >10,000 IU/mL independently predicted an ALT flare >5x ULN. This HBV DNA cut-off value remained significantly associated after validation in an external cohort. Although the rate and peak value of ALT flares were high during follow-up for 72 weeks, the proposed viral load threshold facilitates identifying patients with a high risk of flares who may require closer follow-up and earlier retreatment.

In our study, EOT HBsAg values were not associated with ALT flares, nor was HBsAg decline associated with peak ALT, peak total bilirubin or HBV DNA values. Several studies have proposed that post-withdrawal ALT flares or virologic relapse may precede HBsAg loss.[13–15]

Most patients in this study had significant ALT elevations, most of which occurred within 12 weeks after NA withdrawal, which is in line with other studies.[5,16] Even so, not all patients may need (immediate) retreatment. Despite high peak ALT values (ALT >10x and >20x ULN in 32% and 15%, respectively) and nearly universal occurrence of virologic rebound, 38% of patients was retreated and none developed hepatic decompensation. However, patients with detectable HBV DNA continue to develop adverse outcomes that require retreatment.[17] Moreover, as the currently approved NA agents are highly effective, affordable, have very few safety issues, and prediction of

AEs remains difficult, one could argue that most patients should remain on therapy until HBsAg loss is achieved. However, economic and practical limitations of many healthcare systems require otherwise.

The heart of the matter is therefore predicting with high sensitivity and specificity which flares herald a severe or even fatal outcome. Our finding that week 6 HBV DNA levels predicted hepatitis flares is concordant with a prospective cohort study from Taiwan, in which off-treatment HBV DNA >100,000 IU/mL predicted subsequent clinical flares.[13] Similarly, in the cohort study from Nanfang Hospital, patients with HBV DNA >200,000 IU/mL or with persistently increased HBV DNA (>2,000 IU/mL) after NA cessation had a significantly higher risk of biochemical flares (ALT >2x ULN).[10] In other studies, which also reported off-therapy flares in 50-75% of patients, predicting flares or retreatment was difficult.[1,6,14,18] Older age and higher EOT HBsAg levels were associated with relapse.[18,19] Different definitions of outcomes and retreatment criteria and a lack of external validation may have increased the heterogeneity in results.[6]

This is the first study describing that males had a higher risk of flares than females. The 18-months cumulative incidence of ALT flare >5x ULN and clinical relapse was 69% and 82% in males compared to 26% and 32% in females, respectively ($p=0.01$ and $p<0.005$). The association with sex was also present when we used the ALT ULN according to the AASLD Guidelines (data not shown), which substantiates the robustness of this finding.[7] Although previous papers on NA discontinuation did not find a significant association between sex and ALT flares, another study on peginterferon-α treatment showed that fewer males had a sustained response than females.[20] These findings warrant validation in other cohorts.

Interestingly, the duration of NA consolidation therapy was not associated with the risk of flares. Even though most patients in this current study had received consolidation therapy for more than 3 years, the risk of flares remained high. The results are in line with a study from Taiwan, in which consolidation therapy beyond 1.5 years did not lower the rate of relapse.[21] Remarkably, an analysis of 2 RCTs on tenofovir disoproxil fumarate showed that even after at least 8 years of tenofovir disoproxil fumarate treatment, 29% of patients developed ALT >5xULN in a relatively short treatment-free follow-up of 24 weeks.[5]

In lieu of validated predictors of flares, the most reliable safeguard remains intensive monitoring. Since most flares occurred in the first few months following NA cessation, monitoring should take place especially frequent in the first year. Flare prediction in our analysis was dependent on the level and off-treatment month, which was also observed by another group.[22] However, even 12 months after NA withdrawal ALT flares continued to occur which underscores the need

for continuous follow-up, albeit at a lower rate than during the first year off-therapy. Based on rapid off-therapy development of severe ALT flares and virologic relapse in our study, we suggest measuring ALT, AST, INR and bilirubin monthly during the first 6 months, and HBsAg, HBeAg and HBV DNA every 3 months for the first year.

Important strengths of the current study were the comprehensive follow-up of a prospectively investigated cohort from a recent RCT and validation in an external cohort. Another strong point is that retreatment criteria in this study were not as stringent as in other studies, which allowed us to better evaluate the off-therapy viral and biochemical kinetics during close-visit monitoring. Our study may be limited by the inclusion of predominantly Asian patients. Future studies with longer follow-up should evaluate whether novel biomarkers, such as HBV RNA, HBcrAg or anti-HBc, improve the prediction of clinical outcomes.

To summarize, after stopping long-term NA therapy, 52% of HBeAg negative patients experienced ALT flare of >5x ULN and 87% had virologic relapse. Flares were not associated with maintained clinical remission of disease nor with HBsAg decline after stopping therapy. In this predominantly Asian cohort, male patients with rapid viral load increase after NA discontinuation have the greatest risk of subsequent severe flares, which may prompt closer monitoring or immediate retreatment.

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CHAPTER 4

LOW HEPATITIS B SURFACE ANTIGEN AND HBV DNA LEVELS PREDICT RESPONSE OF PEGYLATED INTERFERON ADDITION TO ENTECAVIR IN HEPATITIS B E ANTIGEN POSITIVE CHRONIC HEPATITIS B

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ABSTRACT

Background: Various treatment combinations of peginterferon (PEG-IFN) and nucleos(t)ide analogues have been evaluated for chronic hepatitis B (CHB), but the optimal regimen remains unclear.

Aims: We studied whether PEG-IFN add-on increases response compared to entecavir (ETV) monotherapy, and whether the duration of ETV pre-treatment influences response.

Methods: Response was evaluated in HBeAg positive patients previously treated in two randomized controlled trials. Patients received ETV pre-treatment for at least 24 weeks and were then allocated to 24-48 weeks of ETV + PEG-IFN add-on, or continued ETV monotherapy. Response was defined as HBeAg loss combined with HBV DNA <200 IU/mL 48 weeks after discontinuing PEG-IFN.

Results: Of 234 patients, 118 were assigned PEG-IFN add-on and 116 continued ETV monotherapy. Response was observed in 38/118 (33%) patients treated with add-on therapy and in 23/116 (20%) with monotherapy (p=0.03). The highest response to add-on therapy compared to monotherapy was observed in PEG-IFN naïve patients with HBsAg levels below 4,000 IU/mL and HBV DNA levels below 50 IU/mL at randomization (70% vs. 34%; p=0.01). Above the cut-off levels, response was low and not significantly different between treatment groups. Duration of ETV pre-treatment was associated with HBsAg and HBV DNA levels (both p<0.005), but not with response (p=0.82).

Conclusions: PEG-IFN add-on to ETV therapy was associated with higher response compared to ETV monotherapy in patients with HBeAg positive CHB. Response doubled in PEG-IFN naïve patients with HBsAg below 4,000 IU/mL and HBV DNA below 50 IU/mL, and therefore identifies these as the best candidates for PEG-IFN add-on.

INTRODUCTION

The achievement of functional cure for chronic hepatitis B infection (CHB) remains difficult due to a persistent infection of hepatocytes with covalently closed circular DNA (cccDNA).[1,2] CccDNA is a minichromosome that serves as a transcription template for hepatitis B virus (HBV) antigen and virion production. Nucleos(t)ide analogue (NA) therapy only marginally reduces levels of cccDNA such that cccDNA depletion would require years of NA treatment.[3,4]

NA therapy effectively suppresses the hepatitis B virus (HBV) up to eight years with few side-effects, but serological response rates remain low. The discontinuation of NA therapy leads to frequent virological relapse and patients therefore require long-term, if not indefinite NA therapy.[5–10] In contrast, a finite course of pegylated interferon (PEG-IFN) achieves more sustained immune response than NA therapy.[9,11,12] PEG-IFN is also able to directly target cccDNA and induce cccDNA decline in combination with NA therapy.[13,14] PEG-IFN monotherapy however induces sustained response in only 30-40% of patients and has limited tolerability.[15,16]

These limitations of CHB therapy have led to the evaluation of various treatment combinations of NAs and PEG-IFN to maximize response rates, among which is the strategy of adding PEG-IFN to NA treatment (PEG-IFN add-on). One of the rationales for the PEG-IFN add-on strategy is that long-term NA treatment enables partial restoration of the liver-specific immunology of both the adaptive (T-cells) and innate immune system (natural killer cells).[17–20] Viral load suppression could thus increase the immunomodulatory effect of PEG-IFN therapy resulting in increased HBsAg loss and HBeAg loss or accelerated HBsAg decline rates.[11]

Several randomized controlled trials (RCT) employed a PEG-IFN add-on strategy in HBeAg positive and negative patients on long-term NA monotherapy.[21–23] PEG-IFN add-on increased HBeAg seroconversion and viral antigen decline, but primary efficacy endpoints were not reached, possibly because of insufficient power or because the effect was limited to a subgroup of patients only. Clinical practice could benefit substantially if these responsive patients can be identified at the start of PEG-IFN therapy with readily available laboratory markers. Other remaining issues concern the optimal duration of PEG-IFN add-on and of NA pre-treatment.

We therefore evaluated whether PEG-IFN add-on to ETV treatment increases serological response compared to ETV monotherapy in CHB, and whether the duration of ETV pre-treatment or the length of PEG-IFN addition therapy influenced response. To this purpose, we performed an analysis in a large HBeAg positive CHB population that was previously treated in two global RCTs.

MATERIALS AND METHODS

Combined study design

We conducted a post-hoc analysis of two international RCTs (ARES and PEGON; registered at ClinicalTrials.gov, Identifier: NCT00877760, NCT01532843).[21,23] Detailed inclusion and exclusion criteria have been previously described. In short, patients with CHB were eligible if they were HBeAg positive at randomization (baseline) and had a serum alanine aminotransferase (ALT) between 1.3 and 5 times the upper limit of normal (ULN). Patients had received pre-treatment with ETV for at least 6 months. The main exclusion criteria were history of decompensated liver disease, co-infection with hepatitis C virus or HIV, other concomitant liver disease, and any contra-indication for interferon therapy.

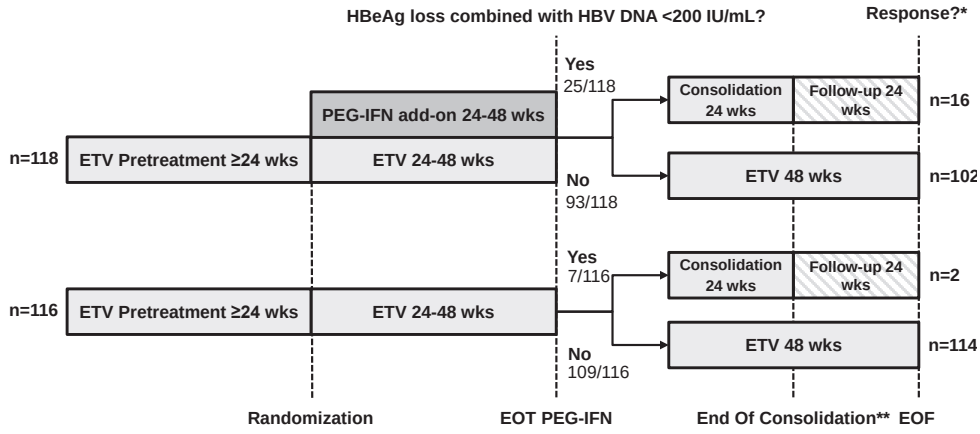
After initial treatment with ETV (Baraclude, 0.5 mg once-daily), patients were randomized to either 6-12 months of PEG-IFN addition or of continued ETV monotherapy (Figure 1). Patients treated within the ARES trial received PEG-IFN a2a (Pegasys, 180 µg once-weekly) and patients in the PEGON study PEG-IFN a2b (PegIntron, 1.5 µg/kg once-weekly). If patients achieved HBeAg seroclearance in combination with an HBV DNA level below 200 IU/mL at the end of PEG-IFN treatment (EOT) or at the corresponding time point for patients allocated to ETV monotherapy, ETV was discontinued after a minimum of 24 weeks consolidation therapy. Otherwise, ETV was continued until the end of follow-up (EOF), which was 48 weeks after EOT for all patients regardless of treatment response.

Several patients within the ARES study did not reach the designated primary endpoint at the end of treatment. These patients were allowed to enroll in the subsequent PEGON trial and were then randomized again to PEG-IFN add-on or ETV monotherapy. This study was approved by local ethics boards of all centers and performed in concordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written consent.

Study endpoints

Response was defined as combined HBeAg loss with HBV DNA <200 IU/mL at EOF. We analyzed the modified intention-to-treat population, which includes all patients who received at least one dose of the allocated treatment after baseline. Patients were considered non-responders in case of missing HBeAg status or HBV DNA at EOF. To assess the potential for functional cure, as studied with therapeutic compounds now in development, we also investigated specific other virological and serological outcomes (Table 2).

Figure 1: Combined study design.



* Response: HBeAg loss in combination with HBV DNA <200 IU/mL at end of follow-up for the intention-to-treat population. ** Only for responders. Non-responders were treated with ETV until EOF. Out of the 32 patients who reached response at EOT, 16/25 patients assigned PEG-IFN add-on and 2/7 patients assigned ETV monotherapy discontinued treatment after 24 weeks of consolidation therapy. Of these patients 12/16 vs. 2/2 patients allocated to PEG-IFN add-on vs. ETV monotherapy sustained response at EOF. EOT, end of treatment; EOF, end of follow-up.

Study follow-up and measurements

During PEG-IFN treatment, routine examination and laboratory testing were performed every 4 weeks. After PEG-IFN treatment was stopped, patients visited the outpatient clinic every 12 weeks until EOF. Patients on ETV monotherapy had study visits every 12 weeks throughout the entire study period. Routine biochemical and hematological tests were assessed locally at every visit. Serum ALT levels were standardized according to the ULN per center and gender. Serum HBV DNA was measured with the Cobas TaqMan 48 polymerase chain reaction assay (lower limit of detection: 20 IU/mL; Roche Diagnostics, Basel, Switzerland). Serum HBeAg, anti-HBe and HBsAg were evaluated with Architect (Abbott Laboratories, North Chicago, IL, USA) or Cobas Elecsys 411 (lower limit of detection 0.30 IU/L and 0.05 IU/mL, respectively; Roche Diagnostics). HBV genotyping was performed with the INNO-LiPA HBV genotype assay (Fujirebio Europe, Ghent, Belgium). If HBV genotype could not be assessed due to undetectable HBV DNA levels at baseline, we reviewed HBV genotype data in medical charts where possible. The presence of cirrhosis was defined by Ishak stage 6 on liver biopsy, or an aspartate aminotransferase to platelet ratio index (APRI) score >1.0.[24]

Statistical analysis

Variables are summarized with mean ± SD or frequency (percentage). Non-normally distributed variables were log-transformed. Differences in outcomes were evaluated by chi-squared test, Student's *t*-test or Mann-Whitney test, where appropriate. To study the influence of PEG-IFN addition on response and adjust for confounders, we performed logistic regression analysis. Predefined covariates included age, gender, HBV genotype, cirrhosis, previous use of PEG-IFN, duration of ETV pre-treatment, ALT, HBV DNA and HBsAg. The duration of ETV pre-treatment and HBV DNA were categorized due to a skewed distribution. Predictors that were significantly associated with response in univariable regression (p-value <0.10) were further evaluated in multivariable regression (backward stepwise selection). Interactions between response and baseline variables included in the final model were explored.

Cut-off values for HBV DNA and HBsAg at baseline were evaluated to find clinically useful starting rules for PEG-IFN add-on. HBsAg levels were dichotomized at thresholds between 2.7 and 5.0 log IU/mL in steps of 0.1. HBV DNA was categorized at 50, 100, 500 and 1,000 IU/mL. The likelihood-ratio test and sum of log-likelihood ratios of the two treatment groups were calculated. We selected optimal cut-off values based on a minimum response difference of 15% between add-on and monotherapy; a significant likelihood ratio test of add-on vs. monotherapy below the cut-offs, but not above; and the lowest sum of likelihood ratios. For each threshold Receiver Operating Characteristic (ROC) curves were constructed and AUCs were calculated and compared to each other. Furthermore, a sensitivity analysis was performed among non-responding patients within the ARES study who subsequently received retreatment in the PEGON study by modeling the correlated data in a generalized estimating equation.[25] Analyses were performed in SPSS (v. 22.0, Chicago, IL) and SAS v. 11.2 (SAS Institute Inc., Cary, NC). Two-sided p-values <0.05 were considered significant.

RESULTS

Patient population

A total of 234 patients met the inclusion criteria. Excluded were 5 patients assigned PEG-IFN add-on and 10 assigned ETV monotherapy who had achieved HBeAg loss at baseline (during ETV pre-treatment). At baseline, 118 patients were allocated to PEG-IFN add-on and 116 patients continued ETV monotherapy. Baseline characteristics were comparable between the two groups (Table 1). The mean age was 33 (SD 9) years, the majority of patients were male and of Asian ethnicity. HBV genotypes A/B/C/D/other were present in 4%, 17%, 41%, 24% and 1% of patients, respectively. In total, 80/118 (68%) patients received PEG-IFN add-on for 24 weeks and 38/118

(32%) patients received PEG-IFN add-on for 48 weeks. Among patients included in the ARES study, 36 non-responders were re-included in the subsequent PEGON trial. The baseline characteristics per trial are shown in Supplementary Table 1.

Table 1. Characteristics of the modified intention-to-treat population at randomization.

		PEG-IFN add-on (n=118)	ETV monotherapy (n=116)
Age, years (SD)		33 (10)	33 (9)
Male gender (%)		87 (74)	83 (72)
Ethnicity (%)	Asian	85 (72)	84 (72)
	Caucasian	31 (26)	31 (27)
	Other	2 (1.7)	1 (0.9)
HBV genotype (%)†	A	3 (2.5)	6 (5.2)
	B	22 (19)	17 (15)
	C	45 (38)	51 (44)
	D	30 (25)	26 (22)
	Other/unknown†	18 (14)	16 (14)
Cirrhosis (%)‡		3 (2.5)	5 (4.3)
Previous (PEG-)IFN therapy (%)		16 (14)	20 (17)
ETV pre-treatment (%)	6-12 months	80 (68)	79 (68)
	1-2 years	12 (10.2)	9 (7.9)
	2-3 years	16 (22)	28 (24)
Alanine aminotransferase, ULN (IQR)		0.5 (0.3-0.9)	0.5 (0.4-0.9)
HBV DNA, IU/mL (%)	Undetectable§	38 (32)	42 (36)
	20-100	16 (14)	27 (23)
	100-1,000	27 (23)	18 (16)
	>1,000	37 (31)	29 (25)
Quantitative HBsAg, log IU/mL (SD)		3.7 (0.7)	3.6 (0.7)
Quantitative HBeAg, log IU/mL (IQR)		1.1 (0.5-2.0)	1.0 (0.4-1.9)
PEG-IFN duration (%)	24 weeks	80 (68)	-
	48 weeks	38 (32)	-

† HBV genotyping was not possible for 32 patients (all Asian) due to undetectable HBV DNA at randomization; ‡ Cirrhosis was defined as Ishak stage 6 on liver biopsy; all 81 patients with unavailable biopsy data had an APRI score <1.0, which suggests absence of cirrhosis; § <20 IU/mL; IQR: interquartile range; SD: standard deviation; ULN: upper limit of normal.

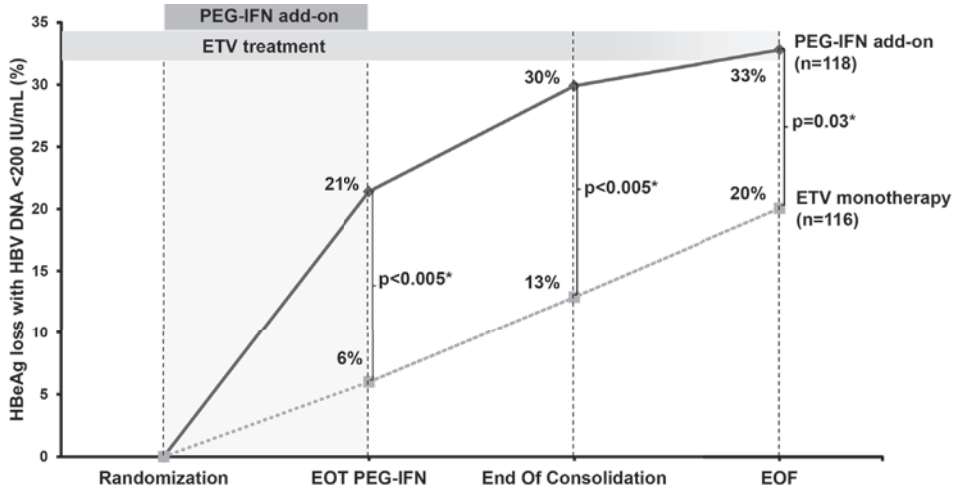
Response

Response was reached in 38/118 (33%) patients allocated to add-on therapy and in 23/116 (20%) patients with ETV monotherapy (p=0.03; Figure 2 and Table 2). Other serological, virologic and biochemical outcomes are reported in Table 2. HBeAg seroconversion rates at EOF were also significantly higher in PEG-IFN add-on patients. The response group comprised significantly more males (84 vs 69%, p=0.03), and had a higher frequency of genotype B (26% vs 13%) and fewer genotype D (12% vs 28%) compared to non-responders. Furthermore, responders had significantly

lower ALT (0.4 vs. 0.6 x ULN, p=0.01), HBsAg (3.3 vs. 3.8, p<0.005) and HBeAg (0.5 vs. 1.4, p<0.005) levels at baseline, and a higher frequency of undetectable HBV DNA at baseline (53% vs. 28 %, p<0.005) than non-responders. Other baseline characteristics were comparable between patients with and without a response. Response occurred in 12/16 patients assigned to PEG-IFN add-on vs. 2/2 assigned to ETV monotherapy (p=0.42) in the subgroup that achieved HBeAg loss in combination with HBV DNA <200 IU/mL at EOT.

The two sensitivity analyses (cohort without 36 retreated non-responders and whole cohort with adjustment for correlated data) were consistent with our findings indicating that PEG-IFN add-on significantly increased response to ETV monotherapy (Supplementary table 1).

Figure 2: Response.



* P <0.05. Out of 32 patients who reached combined HBeAg loss and HBV DNA <200 IU/mL at week 48, 18 discontinued treatment after ETV consolidation therapy. EOT, end of treatment; EOF, end of follow-up.

HBsAg decline and loss

HBsAg decline >0.5 log IU/mL occurred more often in the PEG-IFN add-on group compared to the ETV monotherapy group at EOF (25 [23%] vs. 11 [9.6%]; p=0.01). HBsAg <1,000 IU/mL was reached by 35/118 (30%) patients with PEG-IFN add-on and by 28/116 (24%) with ETV monotherapy (p=0.32) at EOT, which increased to 27% at EOF in both groups (p=0.97). The proportions of patients with HBsAg <100 IU/mL in PEG-IFN add-on vs. ETV monotherapy were 1 (1%) vs. 5 (4%) at baseline (p=0.09), and 6 (5%) vs. 5 (4%) at EOF (p=0.77). The proportion of patients in the add-on group with HBsAg <100 IU/mL increased from baseline to EOF (p=0.06). HBsAg loss was observed in one patient assigned to PEG-IFN add-on.

Sustained response after ETV discontinuation

Among the EOT responders, 16 (64%) of 25 PEG-IFN add-on patients vs. 2 (29%) of 7 ETV monotherapy patients discontinued ETV treatment after 24 weeks of ETV consolidation therapy. The remaining EOT responders continued ETV treatment despite response due to protocol violations. After ETV discontinuation, 12/16 vs. 2/2 patients allocated to PEG-IFN add-on vs. ETV monotherapy had a sustained response (p=0.42). Within the total cohort, response was sustained 24 weeks after stopping ETV in 12/118 (10%) vs. 2/116 (0.2%) patients assigned PEG-IFN add-on vs. ETV monotherapy (p=0.01). Similarly, disease remission (combined HBeAg loss, HBV DNA <200 IU/mL and ALT normalisation at EOF) in PEG-IFN add-on vs ETV monotherapy was achieved by 12/16 vs. 2/2 patients (p=0.42).

Response prediction

By univariable analysis, response was associated with PEG-IFN add-on (odds ratio [OR]: 1.9; 95% confidence interval [CI]: 1.1-3.5; p=0.03), male sex (OR: 2.3; 95%CI: 1.1-4.9; p=0.03), HBV genotype (p=0.02), lower ALT (OR: 0.3; 95%CI: 0.1-0.7; p=0.01), lower HBV DNA level (OR: 0.5; 95%CI: 0.3-0.7; p<0.005) and lower HBsAg level at baseline (OR: 0.4; 95%CI: 0.2-0.6; p<0.005; Table 3). The duration of ETV pre-treatment was associated with HBsAg and HBV DNA at baseline (both p<0.005), but not with response (1-3 years vs. 0-1 year, OR: 1.1; 95%CI: 0.6-2.2; p=0.76), nor was duration of the PEG-IFN add-on regimen (p=0.92). In multivariable analysis, PEG-IFN add-on remained independently associated with response (OR: 2.5; 95%CI: 1.3-4.8; p=0.01, when adjusted for HBV DNA and HBsAg level at baseline). Response rates to PEG-IFN add-on compared to ETV monotherapy increased especially in PEG-IFN naïve patients with lower serum HBV DNA and HBsAg at baseline (Supplementary figure 1).

Table 2. Outcome over time in 234 HBeAg positive patients.

	Baseline Randomization			End of PEG-IFN Week 24-48			End of consolidation Week 72			End of follow-up Week 96		
n (%)	Add-on n=118	ETV Mono n=116	P	Add-on n=118	ETV Mono n=116	P	Add-on n=118	ETV Mono n=116	P	Add-on n=118	ETV Mono n=116	P
Response												
HBeAg loss + HBV DNA <200 IU/mL	-	-	-	25 (21)	7 (6.0)	<0.005*	35 (30)	15 (13)	<0.005*	38 (33)	23 (20)	0.03*
Virological outcomes												
HBV DNA <2,000 IU/mL	89 (75)	92 (79)	0.48	111 (95)	100 (86)	0.02*	104 (89)	102 (88)	0.82	99 (85)	104 (90)	0.24
HBV DNA <200 IU/mL	64 (54)	75 (65)	0.11	102 (87)	88 (76)	0.03*	93 (80)	92 (79)	0.97	95 (82)	97 (84)	0.62
HBV DNA undetectable†	38 (32)	41 (35)	0.61	37 (32)	41 (35)	0.55	37 (32)	41 (35)	0.55	36 (31)	40 (35)	0.54
Serological outcomes												
HBeAg loss	-	-	-	25 (22)	7 (6.0)	<0.005*	36 (32)	15 (13)	<0.005*	40 (36)	23 (20)	0.01*
HBeAg seroconversion	-	-	-	19 (16)	2 (1.7)	<0.005*	26 (22)	5 (4.3)	<0.005*	28 (24)	11 (9.6)	<0.005*
HBsAg loss	0 (0.0)	0 (0.0)	NS	1 (0.8)	0 (0.0)	NS	1 (0.8)	0 (0.0)	NS	1 (0.8)	0 (0.0)	NS
HBsAg <1,000 IU/mL	22 (19)	22 (19)	0.95	35 (30.0)	28 (24)	0.32	33 (28)	32 (28)	0.92	31 (27)	31 (27)	0.97
HBsAg <100 IU/mL	1 (0.8)	5 (4.3)	0.09	10 (8.5)	4 (3.4)	0.10	6 (5.1)	4 (3.4)	0.53	6 (5.2)	5 (4.3)	0.77
HBsAg decline >0.5 log IU/mL	-	-	-	30 (26)	2 (1.7)	<0.005*	30 (26)	6 (5)	<0.005*	25 (23)	11 (9.6)	0.01*
Biochemical outcome												
ALT normalization	96 (81)	90 (78)	0.56	73 (63)	94 (82)	<0.005*	104 (91)	98 (86)	0.21	103 (92)	99 (86)	0.16

* P <0.05. † <20 IU/mL. NS: not significant

Table 3. Logistic regression on response at end of follow-up.

	Univariable regression			Multivariable regression		
Variable	**OR**	**95%CI**	**P**	**OR**	**95%CI**	**P**
Age, years	1.02	0.99-1.05	0.24			
Gender, male vs. female	2.31	1.09-4.90	0.03*			NS
HBV genotype‡			0.02*			NS
C	Ref.					
A vs. C	1.50	0.35-6.47	0.59			
B vs. C	2.09	0.95-4.59	0.07			
D vs. C	0.43	0.17-1.07	0.07			
Other vs. C	1.44	0.61-3.37	0.41			
Cirrhosis	1.76	0.41-7.59	0.45			
Duration of ETV, months			0.79			
0-1 yr	Ref.					
1-3 yrs vs. 0-1 yr	1.12	0.56-2.23	0.76			
>3 yrs vs. 0-1 yr	1.28	0.46-3.54	0.64			
PEG-IFN experienced vs. naïve	0.64	0.27-1.56	0.33			
PEG-IFN duration, 12 vs. 6 mo	0.96	0.41-2.20	0.92			
PEG-IFN add-on, compared to ETV monotherapy	1.92	1.06-3.49	0.03*			
within PEG-IFN naïve				3.72	1.76-7.87	<0.005*
within PEG-IFN experienced				0.24	0.04-1.66	0.15
ALT, x ULN	0.32	0.14-0.74	0.01*			NS
HBV DNA, IU/mL†			<0.005*			0.02*
Undetectable	Ref.			1.00		
20-100 vs. undetectable	0.67	0.30-1.49	0.33	0.62	0.26-1.47	
100-1,000 vs. undetectable	0.53	0.24-1.17	0.12	0.47	0.19-1.16	
>1,000 vs. undetectable	0.10	0.03-0.29	<0.005*	0.12	0.04-0.42	
HBsAg, log IU/mL	0.38	0.24-0.60	<0.005*	0.51	0.29-0.89	0.02*

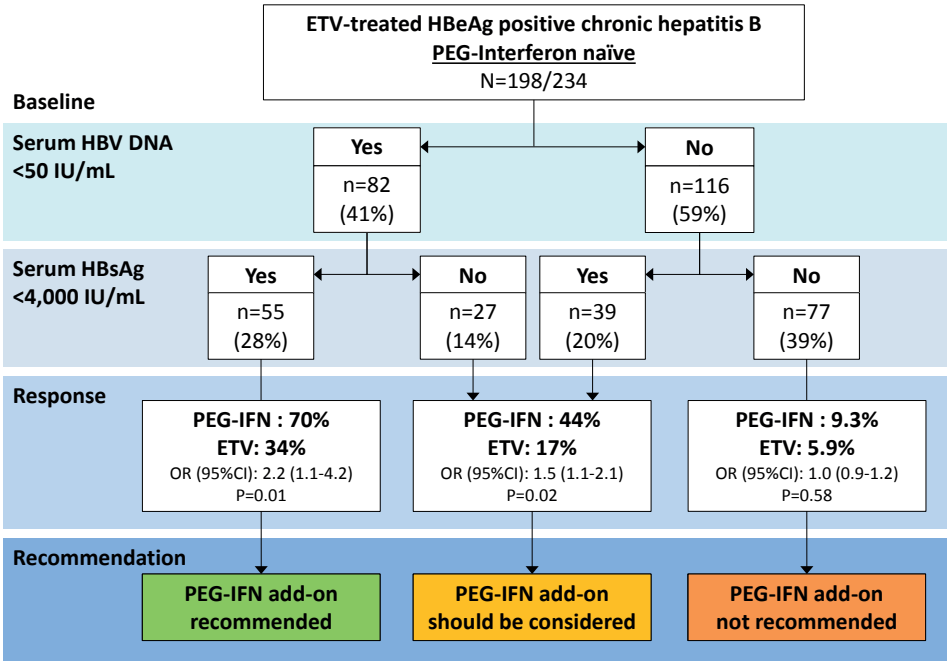
* P <0.05. † HBV DNA groups: < lower limit of detection (<20 IU/mL); 20-100 IU/mL; 100-1,000 IU/mL; ≥1,000 IU/mL NS: not significant; Ref: reference; ULN: upper limit of normal.

Response-guided therapy using HBV DNA and HBsAg

To establish clinical starting rules for PEG-IFN add-on, the relationship between different cut-off values of HBsAg and HBV DNA at baseline and likelihood of response was evaluated (Suppl. Figure 2 and Suppl. Table 3). As previous use of PEG-IFN was strongly associated with a lack of response, we evaluated all PEG-IFN naïve patients (198/234 (85%)). Based on this analysis, PEG-IFN naïve patients with an HBsAg level below 4,000 IU/mL (3.6 log) and HBV DNA level below 50 IU/mL (1.7 log) at baseline achieved the largest gain in probability of response with PEG-IFN add-on compared to ETV monotherapy (70% vs. 34%, p=0.01; Figure 3). Patients who met one of the above criteria achieved a moderate gain in response from PEG-IFN add-on, compared to ETV monotherapy (44% vs. 17%; p=0.02). Above the proposed HBsAg and HBV DNA cut-off levels,

response was very low and not significantly different between treatment groups (PEG-IFN add-on vs. ETV monotherapy: 9.3% vs. 5.9%; p=0.58). The cut-off values combined had an AUC of 0.79 (95%CI: 0.72-0.86) for probability of response.

Figure 3: Algorithm for probability of response at end of follow-up based on HBV DNA and HBsAg at baseline.



DISCUSSION

In this combined analysis of two global RCTs, PEG-IFN add-on to ETV increased response compared to ETV monotherapy in HBeAg positive patients with CHB. Response was 33% for add-on patients versus 20% for ETV monotherapy. HBeAg seroconversion rates at EOF were also significantly higher in add-on patients. The response to PEG-IFN add-on was especially high (up to 70%) among patients who were naïve to PEG-IFN therapy and had low HBV DNA (< 50 IU/ml) and HBsAg levels (< 4000 IU/ml) at the start of PEG-IFN therapy.

This is the first study demonstrating a higher response in patients allocated to PEG-IFN add-on compared to ETV monotherapy. The strengths of this study are inclusion of a large multi-ethnic cohort of patients comprising treatment naïve and experienced patients who after ETV treatment did not reach HBeAg seroconversion. These patients are representative of the majority of

treatment eligible patients seen in clinical practice who would otherwise continue NA therapy for longer duration. A finite PEG-IFN add-on regimen offers disease remission and discontinuation of treatment, thereby preventing additional costs and the potential of non-adherence and resistance associated with long-term or indefinite NA therapy.

To avoid unnecessary side-effects and costs of PEG-IFN it is essential to identify the optimal candidates for add-on therapy as only a subset will respond. The current HBV clinical practice guidelines only broadly mention the usefulness of quantifying HBV DNA and HBsAg to decide when and in whom to start PEG-IFN. Evidence to support one cut-off value over another is limited.[26,27] We established clinical starting rules for PEG-IFN add-on based on widely available biomarkers. Based on results from this study, we recommend starting PEG-IFN add-on in PEG-IFN naïve patients with an HBsAg level below 4,000 IU/mL (3.6 logs) and HBV DNA below 50 IU/mL (1.7 log) at randomization. A sufficiently large subgroup (28% of PEG-IFN naïve patients) had laboratory levels below these thresholds. PEG-IFN add-on response rates were nearly twice as high as the average PEG-IFN response in previous studies.[15,16] In patients with values below either of the cut-off values, PEG-IFN add-on should be considered, as these patients have a moderately high response to PEG-IFN. PEG-IFN add-on is not recommended in patients with both HBsAg and HBV DNA levels above the cut-off values, because of the low probability of response. Our HBsAg threshold is concordant with a threshold found in another study which showed that HBsAg <1500 IU/mL predicted response.[28] Moreover, the higher and thus more lenient HBsAg cut-off value established in this study would allow practitioners to identify even more candidates for PEG-IFN add-on at an earlier stage in their disease course. None of the previous add-on studies provided a comprehensive grid search to establish response-guided therapy. Apart from response, the side effects and cost-effectiveness should to be taken into consideration when deciding on a treatment strategy.

In recent RCTs that compared PEG-IFN add-on to continuing NA monotherapy, HBsAg decline rates were significantly higher in the add-on group, yet the primary endpoints (HBsAg loss at week 96; combined HBeAg loss with HBV DNA <200 IU/mL at week 96) were not reached, potentially due to a type II error.[21–23] In the ARES study response was achieved in 19% of patients in the add-on arm vs. 10% in the monotherapy arm (p=0.095); declines in HBsAg, HBeAg and HBV DNA were also larger in the add-on group (all p<0.001).[21] Uncontrolled studies in HBeAg positive and negative patients reported similar findings.[29,30]

The PEGAN study in HBeAg negative patients did not find a significant effect of PEG-IFN add-on on HBsAg loss at week 96, but was possibly underpowered and included older-generation NAs.[22] This study showed that PEG-IFN add-on treatment resulted in significantly greater HBsAg declines and, within patients who received a full 48 week course, larger proportions of HBsAg

loss and seroconversion. Within patients with an HBsAg titre below 3 log IU/mL at baseline, 6/26 (23%) achieved HBsAg loss (full dose analysis). The PEGAN study suggested using add-on only in patients with baseline HBsAg levels of less than 3 log IU/mL. Other regimens of PEG-IFN and NA therapy, such as sequential or combination therapy have been evaluated in CHB, but the optimal strategy remains unclear.[28,31]

The optimal duration of ETV pre-treatment or PEG-IFN add-on therapy has not yet been established. Prolonged NA pre-treatment partially restores immune function (NK and T cells).[17–20] In our study the duration of ETV pre-treatment correlated to baseline HBV DNA and HBsAg, but not to response. This suggests that levels of HBsAg and HBV DNA at the start of PEG-IFN therapy are more important in considering which patients to treat than the actual duration of ETV pre-treatment. The duration of PEG-IFN add-on treatment did not correlate with response. A post-hoc analysis in a previous study revealed larger HBsAg decline after 24 weeks of PEG-IFN add-on to ETV therapy compared to 52 weeks of combined PEG-IFN and LAM therapy.[32] This suggests that a PEG-IFN course of 24 weeks is at least as effective as 52 weeks, while the shorter regimen would reduce the risk of IFN-related adverse events and treatment costs. Our analysis lacked a comparison to PEG-IFN monotherapy. However, the focus of this study was to investigate PEG-IFN add-on in the large population of patients currently on NAs, and not treatment naïve patients. Furthermore, the relation between the type of PEG-IFN (a2a or a2b) and the respective PEG-IFN add-on trials could potentially have influenced response rates.

The endpoint of HBeAg seroclearance is clinically relevant because it is associated with a lower risk of HCC and improved survival.[9] Since only a subset of patients stopped ETV therapy after receiving consolidation therapy the durability of sustained response after treatment discontinuation could not be studied in further detail. Long-term follow-up studies could focus on the effect on HBsAg loss or development of important clinical outcomes (decompensation, HCC and death), although such studies will be difficult to perform. Due to the fact that part of the patients had received long-term HBV suppressive therapy HBV genotype and cirrhosis status was not known for some patients. Nevertheless, the sensitivity analyses performed to adjust for these partially missing baseline characteristics also showed higher response and HBsAg decline achieved by PEG-IFN add-on compared to ETV monotherapy. It is important that our findings will be validated in new PEG-IFN add-on studies.

In conclusion, PEG-IFN add-on to ETV therapy was associated with a higher probability of response and HBeAg seroconversion compared to ETV monotherapy in HBeAg-positive CHB. Response was highest in patients who were naïve to PEG-IFN therapy with levels of HBsAg below 4000 IU/ml and HBV DNA below 50 IU/ml. In particular these patients should be offered PEG-IFN add-on therapy.

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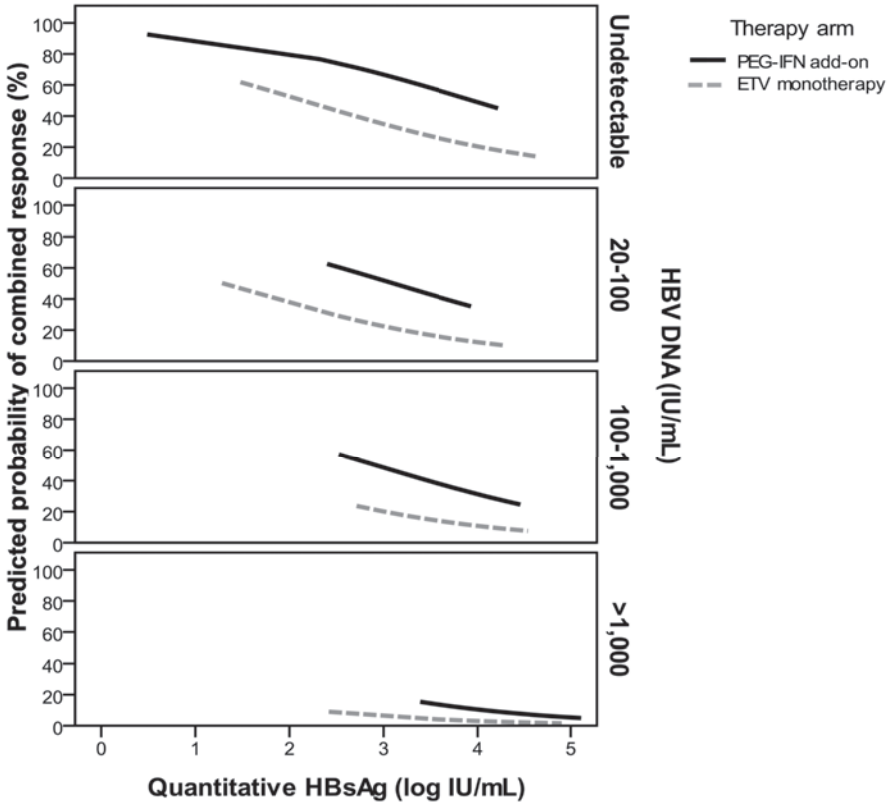
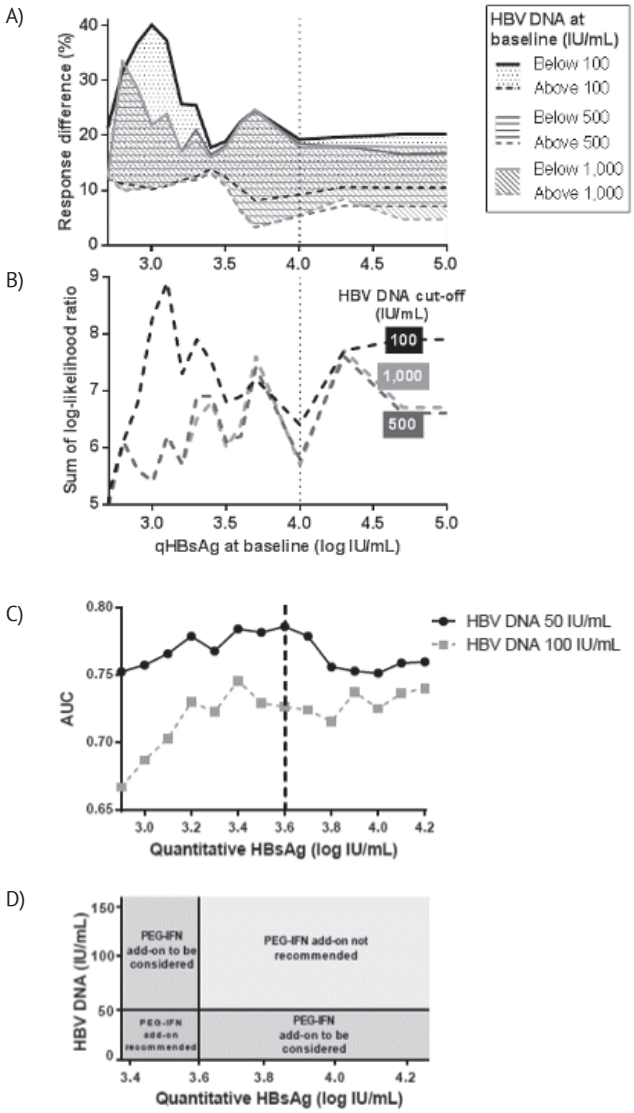
SUPPLEMENTARY FIGURES

Supplementary Figure 1: Predicted probability of response at end of follow-up according to treatment, serum HBV DNA and HBsAg levels at baseline in PEG-IFN naïve patients.



Supplementary Figure 2: Classification methods used for optimal cut-off value selection.



Panel A) Response difference at EOF between PEG-IFN add-on and ETV monotherapy; panel B) Sum of log-likelihood ratios; panel C) AUC for predicted probability of response; and panel D) PEG-IFN add-on treatment recommendation based on cut-off values of HBV DNA and HBsAg at baseline in PEG-IFN naïve patients.

SUPPLEMENTARY TABLES

Supplementary Table 1: Patient characteristics of ARES and PEGON trial patients at randomization.

		ARES (n=159)	PEGON (n=75)
Age, years (SD)		32 (9)	35 (9)
Male gender		114 (72%)	56 (75%)
Ethnicity	Caucasian	59 (37%)	3 (4.0%)
	Asian	97 (61%)	72 (96%)
	Other	3 (1.9%)	0 (0.0%)
HBV genotype	A	9 (5.7%)	0 (0.0%)
	B	30 (19%)	9 (12%)
	C	67 (42%)	29 (39%)
	D	53 (33%)	3 (4.0%)
	Other	0 (0.0%)	34 (45%)
Cirrhosis		8 (5.2%)	-
PEG-IFN naive		141 (89%)	57 (76%)
ETV pre-treatment	6-12 months	159 (100%)	-
	1-3 years	-	55 (73%)
	>3 years	-	20 (27%)
Alanine aminotransferase, x ULN (IQR)		0.7 (0.4-1.0)	0.4 (0.3-0.5)
HBV DNA, IU/mL	Undetectable[§]	25 (16%)	55 (73%)
	20-100	29 (18%)	13 (17%)
	100-1,000	44 (28%)	2 (2.7%)
	>1,000	61 (38%)	5 (6.7%)
Quantitative HBsAg, log IU/mL (SD)		3.9 (0.7)	3.3 (0.7)
Quantitative HBeAg, log IU/mL (SD)		1.2 (1.0)	1.1 (0.8)
Therapy arm	PEG-IFN add-on	80 (50%)	38 (51%)
	ETV monotherapy	79 (50%)	37 (49%)
PEG-IFN duration	24 weeks	80 (50%)	-
	48 weeks	-	38 (51%)

§ <20 IU/mL. IQR: interquartile range; SD: standard deviation; ULN: upper limit of normal.

Supplementary Table 2: Outcome over time excluding initial non-responders who received retreatment.

	Baseline (Randomization)			End of PEG-IFN Week 24-48			End of consolidation Week 72			EOF Week 96		
n (%)	Add-on (n=100)	ETV Mono (n=98)	P	Add-on (n=100)	ETV Mono (n=98)	P	Add-on (n=100)	ETV Mono (n=98)	P	Add-on (n=100)	ETV Mono (n=98)	P
Response												
HBeAg loss + HBV DNA <200 IU/mL	-	-	-	19 (19)	5 (5.1)	<0.005*	29 (29)	14 (14)	0.01*	31 (32)	22 (22)	0.15
Virological outcomes												
HBV DNA <2,000 IU/mL	72 (72)	74 (76)	0.58	93 (94)	82 (84)	0.02*	87 (88)	85 (87)	0.81	83 (85)	87 (89)	0.40
HBV DNA <200 IU/mL	47 (47)	57 (58)	0.12	84 (85)	71 (72)	0.03*	77 (78)	75 (77)	0.84	79 (81)	80 (82)	0.86
HBV DNA undetectable†	9 (9.0)	16 (16)	0.12	46 (47)	36 (37)	0.17	42 (42)	40 (41)	0.82	46 (47)	46 (47)	0.99
Serological outcomes												
HBeAg loss	-	-	-	19 (19)	5 (5.1)	<0.005*	29 (30)	14 (14)	0.01*	33 (35)	22 (23)	0.06
HBeAg seroconversion	-	-	-	15 (15)	2 (2.0)	<0.005*	21 (21)	5 (5.1)	<0.005*	22 (22)	11 (11)	0.04*
HBsAg loss	1 (1.3)	0 (0.0)	0.32	1 (1.3)	0 (0.0)	0.32	1 (1.3)	0 (0.0)	0.32	1 (1.3)	0 (0.0)	0.32
HBsAg <1,000 IU/mL	18 (18)	17 (17)	0.90	30 (30)	21 (21)	0.16	27 (27)	22 (22)	0.43	27 (28)	22 (22)	0.41
HBsAg <100 IU/mL	0 (0.0)	0 (0.0)	0.30	9 (9.1)	2 (2.0)	0.03*	6 (6.1)	2 (2.0)	0.15	6 (6.1)	3 (3.1)	0.31
HBsAg decline >1 log IU/mL	-	-	-	9 (9.2)	0 (0.0)	<0.005*	7 (7.2)	0 (0.0)	0.01*	6 (6.4)	2 (2.1)	0.14
HBsAg decline >0.5 log IU/mL	-	-	-	24 (25)	2 (2.0)	<0.005*	26 (27)	5 (5.2)	<0.005*	23 (25)	10 (10)	0.01*
Biochemical outcome												
ALT normalization	80 (80)	72 (74)	0.28	57 (58)	76 (78)	<0.005*	87 (88)	80 (82)	0.22	88 (90)	84 (86)	0.38

* p<0.05; † <20 IU/mL. EOF, end of follow-up; NA: Not applicable.

Supplementary Table 3A. Performance of different HBsAg and HBV DNA thresholds at randomization for response prediction in PEG-IFN naïve patients.

Cut-off		n (%)	Response %		OR	P	Sens	Log LR	AUC
HBsAg log IU/mL	HBV DNA log IU/mL		PEG add-on	ETV mono					
≤2.9	1.7	15 (8)	100	55.6	0.6	0.06	55	5.0	0.56 (0.47-0.65)
>2.9	1.7	67 (34)	53.1	22.9	1.6	0.01	68	6.7	
≤3.0	1.7	20 (10)	100	45.5	NA	0.01	64	9.3	0.59 (0.50-0.68)
>3.0	1.7	62 (31)	48.3	24.2	1.5	0.05	64	3.9	
≤3.1	1.7	26 (13)	100	43.8	NA	<0.005	59	11.6	0.60 (0.51-0.69)
>3.1	1.7	56 (28)	46.4	21.4	1.5	0.05	68	4.0	
≤3.2	1.7	30 (15)	83.3	50.0	3.0	0.06	53	3.7	0.62 (0.53-0.70)
>3.2	1.7	52 (26)	50.0	15.4	1.7	0.01	76	7.4	
≤3.3	1.7	37 (19)	78.6	43.5	2.6	0.04	52	4.6	0.61 (0.52-0.70)
>3.3	1.7	45 (23)	50.0	14.3	1.7	0.01	80	6.8	
≤3.4	1.7	42 (21)	70.6	44.0	1.9	0.09	53	2.9	0.62 (0.54-0.71)
>3.4	1.7	40 (20)	52.4	10.5	1.9	0.01	85	8.7	
≤3.5	1.7	49 (25)	71.41	39.3	2.1	0.03	58	5.1	0.61 (0.53-0.70)
>3.5	1.7	33 (17)	47.1	12.5	1.7	0.03	80	4.9	
≤3.6	**1.7**	**55 (28)**	**69.6**	**34.4**	**2.2**	**0.01**	**59**	**6.8**	**0.62 (0.53-0.70)**
>3.6	**1.7**	**27 (14)**	**46.7**	**16.7**	**1.6**	**0.10**	**78**	**2.8**	
≤3.7	**1.7**	**62 (31)**	**66.7**	**31.4**	**2.1**	**0.01**	**62**	**7.7**	**0.62 (0.53-0.70)**
>3.7	**1.7**	**20 (10)**	**45.5**	**22.2**	**1.4**	**0.29**	**71**	**1.2**	
≤3.8	**1.7**	**70 (35)**	**60.6**	**29.7**	**1.8**	**0.01**	**65**	**6.8**	**0.61 (0.53-0.70)**
>3.8	**1.7**	**12 (6)**	**60.0**	**28.6**	**1.8**	**0.28**	**60**	**1.2**	
≤3.9	1.7	73 (37)	62.9	31.6	1.8	0.01	65	7.3	0.65 (0.57-0.73)
>3.9	1.7	9 (5)	33.3	16.7	1.3	0.57	50	0.3	
≤4.0	1.7	75 (38)	61.1	30.9	1.8	0.01	65	7.1	0.64 (0.56-0.72)
>4.0	1.7	7 (4)	50.0	20.0	1.6	0.43	50	0.6	
≤4.1	1.7	76 (38)	61.1	32.5	1.7	0.01	63	6.3	0.65 (0.57-0.73)
>4.1	1.7	6 (3)	50.0	0.0	2.0	0.12	100	2.6	
≤4.2	1.7	78 (39)	61.1	31.0	1.8	0.01	63	7.2	0.66 (0.58-0.74)
>4.2	1.7	4 (2)	50.0	0.0	2.0	0.25	100	1.7	

Supplementary tables 3A and 3B depict the various quantitative measures that were used to select the optimal cut-off values for HBV DNA and HBsAg to predict response. The underscored and bolded values designate the values that were considered to have the best combination of all diagnostic measures. OR, odds ratio; P, P-value; Sens, sensitivity; Log LR, log-likelihood ratio; AUC, Area Under the Curve.

Supplementary Table 3B. Performance of different HBsAg and HBV DNA thresholds at randomization for response prediction in PEG-IFN naïve patients.

Cut-off		n (%)	Response %		OR	P	Sens	Log LR	AUC
HBsAg log IU/mL	HBV DNA log IU/mL		PEG add-on	ETV mono					
⩽2.9	2.0	19 (9.6)	100	41.7	-	0.01	55	8.7	0.59 (0.49-0.68)
>2.9	2.0	81 (41)	50.0	22.2	0.6	0.01	68	6.9	
⩽3.0	2.0	75 (38)	100	40.0	0.4	<0.005	64	12.5	0.62 (0.52-0.71)
>3.0	2.0	25 (13)	45.5	21.4	1.4	0.03	64	4.9	
⩽3.1	2.0	68 (34)	91.7	40.0	7.2	<0.005	59	9.4	0.63 (0.54-0.72)
>3.1	2.0	32 (16)	45.2	18.9	1.5	0.02	68	5.5	
⩽3.2	2.0	63 (32)	78.6	43.5	2.6	0.04	53	4.6	0.66 (57-0.75)
>3.2	2.0	37 (19)	48.3	14.7	1.6	<0.005	76	8.6	
⩽3.3	2.0	55 (28)	76.5	39.3	2.6	0.02	52	6.1	0.65 (0.56-0.74)
>3.3	2.0	45 (23)	46.2	13.8	1.6	0.01	80	7.2	
⩽3.4	2.0	52 (26)	70.0	47.8	2.0	0.04	53	4.3	0.67 (0.58-0.76)
>3.4	2.0	48 (24)	51.9	8.0	1.8	<0.005	85	10.3	
⩽3.5	2.0	39 (20)	68.0	36.1	2.0	0.01	58	6.1	0.66 (58-0.76)
>3.5	2.0	61 (31)	44.4	9.5	1.6	0.01	80	6.5	
⩽3.6	2.0	33 (17)	66.7	32.5	2.0	0.01	59	7.7	0.66 (0.57-0.75)
>3.6	2.0	67 (34)	43.8	11.8	1.6	0.04	78	4.4	
⩽3.7	**2.0**	**26 (13)**	**64.5**	**30.2**	**2.0**	**<0.005**	**59**	**8.7**	**0.66 (0.58-0.75)**
>3.7	**2.0**	**74 (37)**	**41.7**	**14.3**	**1.5**	**0.12**	**78**	**2.5**	
⩽3.8	**2.0**	**17 (8.6)**	**59.5**	**28.3**	**1.8**	**<0.005**	**62**	**8.3**	**0.66 (0.57-0.74)**
>3.8	**2.0**	**83 (42)**	**50.0**	**18.2**	**1.6**	**0.17**	**71**	**1.8**	
⩽3.9	**2.0**	**12 (6.1)**	**60.0**	**29.2**	**1.8**	**<0.005**	**65**	**8.6**	**0.68 (0.59-0.76)**
>3.9	**2.0**	**88 (44)**	**33.3**	**11.1**	**1.3**	**0.37**	**60**	**0.72**	
⩽4.0	2.0	8 (4.0)	58.5	27.5	1.8	<0.005	65	9.2	0.66 (0.58-0.75)
>4.0	2.0	92 (46)	50.0	16.7	1.7	0.35	50	0.82	
⩽4.1	2.0	7 (3.5)	58.5	28.8	1.7	<0.005	63	8.4	0.67 (0.59-0.76)
>4.1	2.0	93 (47)	50.0	0.0	2.0	0.09	100	3.0	
⩽4.2	2.0	5 (2.5)	58.5	27.8	1.7	<0.005	63	9.2	0.68 (0.59-0.76)
>4.2	2.0	95 (48)	50.0	0.0	2.0	0.17	100	2.2	

CHAPTER 5

MAINTAINED VIROLOGIC SUPPRESSION AND RENAL FUNCTION WITH REDUCED DOSE TENOFOVIR DISOPROXIL FUMARATE IN RENALLY IMPAIRED CHRONIC HEPATITIS B PATIENTS

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ABSTRACT

Background: Tenofovir disoproxil fumarate (TDF) effectively suppresses viral replication in chronic hepatitis B (CHB), but occasionally leads to renal impairment. We evaluated the prevalence of viral and biochemical breakthrough and renal function kinetics in renally impaired CHB patients on reduced and on full dose TDF.

Methods: This clinic-based longitudinal cohort study included patients receiving full and reduced dose TDF (due to eGFR (Cockcroft-Gault) <60mL/min/1.73m^2). Viral and biochemical breakthroughs were assessed 1 month after starting full and reduced TDF dose until the end of follow-up. Breakthroughs were studied in full and reduced dose TDF, and renal function (MDRD) longitudinally before and after dose reduction within patients starting on full dose TDF.

Results: Of 750 patients on TDF, 78 (10%) had reduced dose and 672 (90%) full dose. At the time of dose reduction, 36 (46%) patients had chronic kidney disease stage G3B. A viral breakthrough occurred in one cirrhotic dialysis-dependent patient (dosed 300 mg weekly) which resolved without signs of decompensation, and in one patient on full dose which resolved spontaneously. One biochemical breakthrough occurred during dose reduction and resolved naturally without viral breakthrough. The MDRD improved within the first year of dose reduction (+3.0 (2.5) mL/min per year; p<0.005) and remained stable thereafter. Fifty-three (79%) patients reached an MDRD >50mL/min during dose reduction.

Conclusions: Low dose TDF maintains renal function and viral suppression in most renally impaired CHB patients, even in those with advanced liver disease. This useful, yet simple strategy could be particularly viable in resource-constrained settings.

INTRODUCTION

Chronic hepatitis B (CHB) remains a major cause of morbidity and mortality worldwide.[1] To prevent disease progression, patients with CHB are treated with nucleos(t)ide analogues (NA). These first-line antiviral agents can effectively suppress the hepatitis B virus (HBV), resulting in a lower risk of hepatocellular carcinoma (HCC) and improved survival.[2–5] However, since patients rarely achieve functional cure during NA treatment and often relapse upon stopping NAs, long-term or indefinite antiviral therapy is often necessary, which also requires a proper safety profile.[6–8] One of the most potent NAs, tenofovir disoproxil fumarate (TDF), has a high barrier to drug resistance, is effective against lamivudine-resistant strains of HBV and is generally very safe, but may occasionally lead to renal impairment or bone loss.[5,9]

Recently, the prodrug tenofovir alafenamide (TAF) was approved for the treatment of CHB.[10,11] Compared to TDF, TAF is administered at a lower dose of 25 mg and reaches higher intrahepatic but lower systemic concentrations which may reduce the risk of renal and bone side effects. These potential benefits should be weighed against the high costs of a drug, unavailability in low—and middle-income countries and unknown long-term safety effects, all of which might preclude the reimbursement of TAF and favor TDF.

Another approach to prevent TDF-induced renal impairment, as suggested by practice guidelines for CHB, is reducing the dose if creatinine clearance (CrCl) levels fall below 50mL/min/1.73m^2.[12,13] To date, no study has investigated whether the dose modification can actually prevent further renal function decline and maintain effective virologic suppression. TDF pharmacokinetic studies that could have elucidated dosing effects were not conducted in patients with CHB. Since the dose of 300 mg received prior approval for the treatment of HIV/HBV co-infection, this dose was subsequently selected for HBV mono-infection without further investigating the efficacy of lower doses.[14,15] Long-term follow-up of patients on renally dosed TDF could shed light on the prevalence and consequences of renal side-effects.

To clarify the efficacy of renally dosed TDF we examined the frequency of viral and biochemical breakthroughs, and studied renal function in patients with CHB. These findings may determine the value of adjusting the TDF dose as a suitable therapeutic strategy for renally impaired patients.

MATERIALS AND METHODS

Study design

In this clinic-based retrospective longitudinal cohort study, patients were included if they were aged 18 years or above, had chronic hepatitis B (Hepatitis B surface Antigen positive on two

separate occasions ≥6 months apart), and received TDF treatment at the Toronto Center for Liver Disease (Canada). All TDF-treated consecutive patients between January 2008 and December 2017 were eligible. Patients were excluded if TDF was prescribed prophylactically during immunosuppressive or anti-cancer therapy, co-infection with hepatitis C virus, hepatitis D virus or HIV was present, or follow-up was available for less than 1 year. Patients with renal impairment were identified based on an estimated Glomerular Filtration Rate (eGFR, Cockcroft-Gault [CG] equation) <60mL/min/1.73m^2, which was the criterion for dose adjustment of TDF. Unadjusted body weights were used for the eGFR calculation. All patients were followed from the start of TDF treatment (baseline) until the End-Of-Follow-Up (EOF). For patients on full dose TDF (both patients continuing full dose and reducing dose afterwards) the baseline was defined as the start of TDF treatment, and for patients starting with reduced dose TDF as the start of dose reduction. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and approved by the ethical review board of the University Health Network, Toronto, Canada.

Data collection

Data was collected from electronic hospital records on demographics, treatment characteristics, clinical outcomes and risk factors for chronic kidney disease. As follow-up during routine clinical care did not occur at pre-determined intervals, the number of visits and duration of follow-up varies between patients. Clinical outcomes included HCC, liver cirrhosis, hepatic decompensation (defined by presence of jaundice, bleeding esophageal varices, ascites, hepatic encephalopathy and/or spontaneous bacterial peritonitis), hepatorenal syndrome, liver, kidney or solid organ transplantation and non-alcoholic fatty liver disease. Risk factors for chronic kidney disease comprised diabetes mellitus (diagnosed before inclusion or use of antidiabetic agents), hypertension (diagnosed before inclusion or use of antihypertensive agents), obesity, dyslipidemia and metabolic syndrome. Laboratory data included serum liver enzyme levels (alanine aminotransferase (ALT), aspartate transferase (AST), HBV virology and serology (HBsAg, HBeAg, anti-HBe, HBV DNA), phosphate and creatinine clearance. Serum HBV DNA was measured by COBAS Amplicor (Lower limit of detection: 20 IU/mL, Roche, Switzerland). Proteinuria was determined in urinalysis, where available.

Study endpoints

Virological breakthrough was defined as a confirmed increase in HBV DNA >1 log IU/mL compared to nadir on-therapy or HBV DNA ≥100 IU/mL on NA with previously undetectable HBV DNA (<10

IU/mL).[12] Biochemical breakthrough was defined as a confirmed ALT >1.5xULN (upper limit of normal: 40 IU/mL). Viral blips were defined as HBV DNA increase between 1 to 2 logs on 1 occasion with undetectable HBV DNA in a follow-up sample.

To assess renal function, serum creatinine clearance was measured and eGFR was calculated by the Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), and CG equation. Other renal markers and endpoints that were assessed comprise fluctuations in serum creatinine, renal impairment (confirmed ⩾20% eGFR decrease from baseline), renal improvement (confirmed ⩾20% eGFR increase from baseline)[16–18], glycosuria, hypophosphatemia (<0.8 mmol/L), and proteinuria. Chronic kidney disease was present if eGFR values were lower than 60mL/min/1.73m2 for at least three months.[1919]

Statistical analysis

Variables are reported with mean (SD) or frequency (percentage). Variables were compared by X^2-test, Student's *t*-test or their non-parametric equivalent, where appropriate. Correlated data were analysed by paired *t*-test or repeated measures ANOVA. The person-years of follow-up were computed from the date of TDF dose reduction to the date of death, switch to another antiviral agent or last follow-up visit, whichever came first. Subjects who did not develop an event were censored at the last visit date. Any acute kidney injury events (e.g. prerenal impairment after dehydration or postrenal obstruction) that were identified from electronic medical records were also censored. Risk rates were calculated by dividing the number of events by person-years of follow-up. The cumulative incidence of GFR improvement and hepatic decompensation by follow-up year was calculated.

Taking into account within-subject variability of GFR, a General Linear Model repeated measure was modeled with random intercept and slope, adjusted for the presence of cirrhosis and diabetes mellitus. The MDRD slope was calculated for before dose reduction (intercept + ($slope_1$ * year)), after dose reduction (intercept + (($slope_1$ + $slope_2$) * year), for follow-up >0 year) and after 1 year after dose reduction ($intercept_{year1}$ + (($slope_1$ + $slope_2$ + $slope_3$) * year), for follow-up >1 year). The difference in slope before vs. after dose reduction was calculated. Analyses were done in SPSS v25.0 (Chicago, IL) and SAS v11.2 (SAS Institute Inc., Cary, NC). Two-sided p-values were considered significant at the 0.05 level.

RESULTS

Patient characteristics

Out of 4204 patients with chronic hepatitis B, 757 patients met the inclusion criteria. A total of 672 (89%) patients had received TDF full dose and 85 (11%) had reduced dose. Among the reduced dose patients, seven were excluded due to viral coinfections (n=3), prophylactic antiviral treatment (n=2), or having had less than one year of follow-up (n=2), leaving 78 reduced dose patients for analysis. Of these 78 patients, 42 started initially with TDF full dose, but were later prescribed a reduced dose. At baseline, patients on reduced dose compared to full TDF dose were older (68 [11] vs. 45 [13] years; $p<0.005$), had more often risk factors for renal impairment (45% vs. 5%; $p<0.005$) and had comparable levels of serum HBV DNA and ALT ($p>0.05$) (Table 1). Patients that started on full dose TDF and either continued full dose or received dose reduction were not different from each other at baseline for clinical or virologic characteristics.

Further characterization of baseline values for patients with modified TDF doses showed the following: the MDRD was 51.3 (20.1) mL/min/1.73m^2; 46% had chronic renal disease stage 3B or higher and 13% had decompensated cirrhosis (Table 1 and Suppl. Table 1). Notably, the eGFR based on CG calculation was lower (43 [14] mL/min/1.73m^2) than the MDRD and was used for 9 (12%) patients to decide on dose reduction. All patients had received a dose adjustment due to eGFR <60mL/min/1.73m^2, four of whom also had hypophosphatemia at the time. Seven (10%) patients received dose adjustment for eGFR between 50-60mL/min/1.73m2, which is equivalent to CKD stage 2. This more conservative threshold than the label was sometimes used at the Toronto Centre for Liver Disease for patients with comorbidities (diabetes mellitus type 2, NAFLD) that may increase the risk of renal function decline. The majority of patients received TDF 300 mg Q48hr (range 75 mg OD to 300 mg every other day) for a mean duration of 3.4 (2.4) years. The total number of person-years of follow-up was 321.5 years with an average follow-up of 2.6 (2.3) years.

Table 1. Characteristics at the time of tenofovir disoproxil fumarate start.

		TDF		P
		Start of full dose (n=672)	Start of reduced dose (n=78)	
Demography				
Age, mean (SD), years		45 (13)	68 (10.5)	<0.005*
Sex, male, n (%)		470 (70)	54 (69)	0.45
Laboratory at start of TDF, mean (SD)				
ALT, x ULN†		1.2 (0.7-2.7)	0.6 (0.4-1.0)	0.39
HBV DNA, log IU/m†		4.9 (2.4-7.1)	1.1 (0.0-1.3)	0.77
HBeAg positive, n (%)		323 (48)	12 (15)	0.10
HBsAg, log IU/mL		2.5 (0.6)	2.0 (0.7)	0.01*
Creatinine, micromole/L		75 (34)	176 (203)	<0.005*
eGFR-CG, mL/min/1.73m^2		114.2 (51.2)	41.3 (11.5)	<0.005*
MDRD, mL/min/1.73m^2		107.3 (50.4)	51.3 (20.1)	<0.005*
CKD-EPI, mL/min/1.73m^2		90.4 (28.9)	39.7 (20.6)	<0.005*
Serum phosphate, mmol/L		-	1.0 (0.3)	-
Serum albumin, g/L		43 (5)	41 (4)	0.78
Body Mass Index, kg/m^2		24.7 (4.1)	25.7 (16.7)	0.21
Cirrhosis (biopsy and/or clinical evidence), n (%)		282 (42)	66 (84)	<0.005*
Liver stiffness, kPa		9.3 (8.0)	8.0 (2.4)	0.94
Treatment, n (%)				
TDF treatment duration, mean (SD), months		56 (37)	41 (29)	<0.005*
Full dose TDF treatment duration		-	36 (26)	-
TDF dose reduction†	300 mg Q48hr	-	49 (73)	-
	150 mg OD		12 (15)	
	300 mg Q72hr		9 (13)	
	300 mg once weekly		8 (12)	
Dose adjustment reason	GFR <60 mL/min	-	78 (100)	-
	Hypophosphatemia		4 (5.1)	
RAS inhibitor (angiotensin II receptor blocker or angiotensin-converting-enzyme inhibitor)		47 (7.0)	39 (50)	0.10
Beta blocker		-	14 (18)	-
Diuretic		-	24 (31)	-
Comorbidity, n (%)				
Diabetes mellitus		27 (4.0)	18 (23)	0.01*
Hypertension		45 (6.7)	33 (42)	<0.005*
Dyslipidemia		20 (3.0)	17 (22)	0.02*
Non-alcoholic fatty liver disease		-	8 (10)	-
CKD stages	G1 (⩾90 mL/min/1.73m^2)	311 (46)	0 (0.0)	-
	G2 (60-89)	361 (54)	0 (0.0)	
	G3a (45-59)	0 (0.0)	42 (54)	
	G3b (30-44)	0 (0.0)	24 (31)	
	G4 (15-29)	0 (0.0)	7 (9.0)	
	G5 (<15)	0 (0.0)	5 (6.4)	
Hepatic decompensation		51 (7.6)	10 (13)	<0.005*
Hepatocellular carcinoma		58 (8.6)	12 (16)	0.01*

Baseline for patients on full dose TDF was defined as the start of TDF treatment and for patients on reduced dose TDF as the start of dose reduction. †All TDF dose reductions were due to renal impairment; † median (IQR); *p<0.05. Abbreviations: ALT, alanine aminotransferase; CKD, chronic kidney disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR-CG, estimated Glomerular Filtration Rate, Cockcroft-Gault; HBV, hepatitis B virus; MDRD, Modification of Diet in Renal Disease; RAS, renin-angiotensin system; TDF, tenofovir disoproxil fumarate.

Virological and biochemical breakthrough

Throughout treatment with decreased TDF doses, one viral breakthrough (HBV DNA peak: 4,000 IU/mL, or 3.6 log IU/mL) occurred in a cirrhotic, dialysis-dependent patient who had used TDF 300 mg weekly for 3.1 years (Table 2). Without developing signs of decompensated cirrhosis, the patient reached undetectable HBV DNA four months after a dose increase to 300 mg three times weekly. Similarly, one patient on full TDF dose had, due to non-compliance, a viral breakthrough that resolved without adjusting treatment or clinical sequelae after three months. Viral blips occurred in 3 (7.1%) patients before dose reduction, in 2 (3.1%) after dose reduction, and in 19 (2.8%) patients on continuous full dose TDF (after dose reduction vs. continuous full dose, p=1.00). One patient on reduced dose had a biochemical breakthrough (ALT peak 2x ULN), which resolved spontaneously while HBV DNA levels remained undetectable.

Table 2. Virologic and biochemical breakthrough, and renal function kinetics during tenofovir disoproxil fumarate therapy.

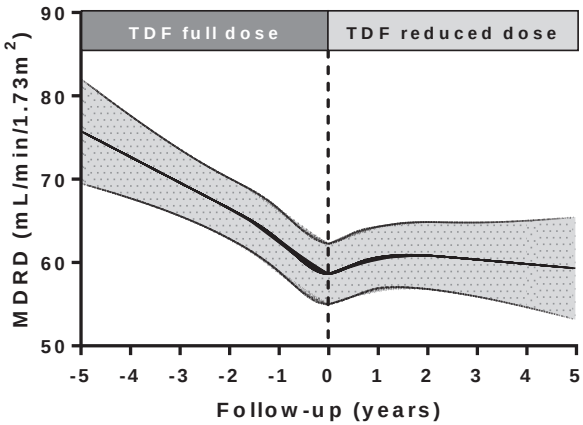
	TDF full dose (n=672)	TDF reduced dose (n=67)		P†
		During initial full dose (n=42/67)	After dose reduction (n=67)	
Breakthrough, n (%)				
Virologic breakthrough	5 (0.7)	0 (0.0)	1 (1.6)	0.44
HBV flare	1 (0.1)	0 (0.0)	1 (1.6)	0.17
Therapy non-compliance	4 (0.6)	0 (0.0)	0 (0.0)	0.38
Viral blipping	19 (2.8)	3 (7.1)	2 (3.1)	1.00
Biochemical breakthrough	93 (13.8)	0 (0.0)	1 (1.6)	<0.005*
Combined viral-biochemical breakthrough	0 (0.0)	0 (0.0)	0 (0.0)	-
Treatment adjustment due to HBV flare	0 (0.0)	0 (0.0)	1 (1.6)	0.09
Renal function, mean (SD)				
MDRD at EOF (mL/min/1.73m^2)	-	-	57.4 (27.4)	-
MDRD change from baseline to EOF (mL/min/1.73m^2)	-	-	+3.4 (14.4)	0.06
MDRD >50mL/min/1.73m^2 at EOF, n (%)	-	-	49 (73)	-

Patients with reduced dose TDF were censored from this analysis if they had daily GFR measurements (hospital admission for non-hepatitis-related malignancy or admission to Intensive Care Unit, n=4) or were treated with hemodialysis for kidney disease deemed unrelated to HBV or TDF (n=7). †Frequencies were compared between TDF full dose and TDF reduced dose group with χ^2-tests, or with Fisher's exact test in case of low cell frequencies; *P<0.05. Abbreviations: EOF, End-Of-Follow-Up; HBV, hepatitis B virus; MDRD, Modification of Diet in Renal Disease; TDF, tenofovir disoproxil fumarate.

Renal outcomes

The analysis of renal function over time was restricted to patients with a TDF dose reduction. Patients with daily GFR measurements (hospital admission for non-hepatitis-related malignancy or admission to Intensive Care Unit, n=4) or treated with hemodialysis for kidney disease deemed unrelated to HBV or TDF, (n=7) were censored in this analysis. The frequent measurements strongly influence the mean GFR over time, while the kidney function progression of these very sick patients with severe HBV-unrelated comorbidity differs from patients with CHB alone.

Figure 1. Predicted mean MDRD (95% CI) from 5 years before to 5 years after start of tenofovir disoproxil fumarate dose reduction in patients with chronic hepatitis B.

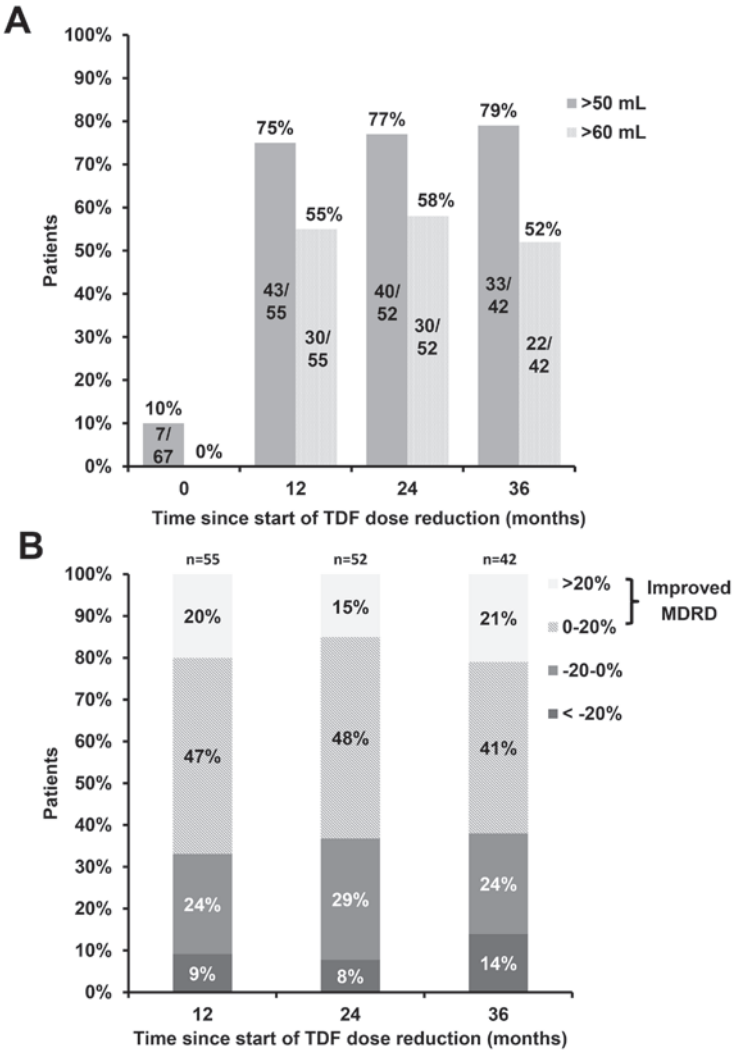


MDRD analysed by repeated measures General Linear Model with random slope and intercept, adjusted for cirrhosis and diabetes mellitus. Sixty-seven patients were followed for at least one year and 57 patients for up to 5 years after dose reduction. The declining slope before dose reduction changed 1 year after into an increase (p<0.005).

Changes in GFR were observed during follow-up and are illustrated in Figure 1 and 2. The declining MDRD during full dose TDF treatment changed into an increase within the first year of dose reduction (intercept: 58.5 mL/min; mean [95%CI] slope before vs. after: -3.7 (1.2) vs. +3.0 (2.5) mL/min per year; p<0.005). Thereafter, the MDRD remained stable throughout follow-up (intercept: 62.1 mL/min; slope: -0.6 (0.5); p=0.22)), resulting in an MDRD of 57.4 (27.4) mL/min at EOF (Table 2). During dose reduction the MDRD increased from baseline to EOF (+3.4 (14.4) mL; p=0.06) and 53 (79%) patients reached an MDRD >50mL/min. Similar GFR trends were observed for GFR calculated by CKD-EPI and CG formula. A subgroup analysis among the 42 patients who were on full dose TDF before dose reduction showed concordant results. Overall, none of the patients developed Fanconi syndrome or lactic acidosis through TDF treatment. The four patients

with hypophosphatemia at baseline remained hypophosphatemic at EOF while no other patients developed hypophosphatemia during treatment with lower dose TDF.

Figure 2. MDRD kinetics from start of tenofovir disoproxil fumarate dose reduction until End-Of-Follow-Up.



(A) Proportion of patients with MDRD above cut-off, and (B) change in MDRD during TDF dose reduction compared to baseline.

Antiviral treatment regimen alterations

Several patients had NA treatment modifications after the TDF dose was reduced. Eight patients were able to have their TDF dose (re-)escalated (of which seven to full dose) because of confirmed MDRD increases above 60mL/min. Conversely, for two patients the TDF dose was further reduced to 75 mg OD due to impaired, but stable renal function (MDRD at follow-up visit: 43mL/min and 56mL/min). Lastly, six patients switched from TDF reduced dose to other antiviral treatment (5 to entecavir 0.5/1.0 mg OD; 1 to lamivudine 100 mg every other day).

Other clinical outcomes

Among the patients with reduced dosed TDF the 5-year cumulative incidence of cirrhosis was 9.4%. Throughout follow-up, none of the patients developed hepatic decompensation or hepatocellular carcinoma or underwent a liver transplantation. A total of six (7.7%) patients died during follow-up (1 liver-related death).

DISCUSSION

In this clinic-based real-world study in patients with CHB, renal function decline was largely reversed after renal dose adjustment of TDF, while few viral or biochemical breakthroughs occurred. These findings suggest that a modified dose of TDF is virologically effective and renally safe, and should be considered a viable option in renally compromised patients with CHB.

Renal impairment associated with TDF mainly stems from proximal tubulopathy. TDF is primarily eliminated by the kidneys through glomerular filtration and proximal tubular secretion.[20,21] The proximal tubular cells actively take up the tenofovir metabolite which may cause mitochrondrial toxicity through inhibition of mitochondrial DNA polymerase. The subsequent tubular dysfunction manifests through increased serum creatinine and decreased serum phosphate levels. NA-induced nephrotoxicity was especially evident with older generation NAs such as adefovir, but has been rare in the ongoing follow-up studies for TDF.[9] The best evidence for TDF-induced nephrotoxicity has been derived from *in vitro* and animal studies, as well as from studies on HIV/HBV co-infection.[22,23] Kidney function was stable throughout follow-up, which was also evident from no additionally occurring hypophosphatemia or other renal events, and more patients who had improved CKD.

The optimal dose adjustment or dosing interval of TDF in renally impaired patients with CHB remains unclear. This study included dose reductions ranging from 75 mg OD to 300 mg every other day. Virologic suppression was maintained in 94% of patients on reduced doses. The only

viral breakthrough, which resolved without signs of decompensation upon increasing the dose to 300 mg biweekly, occurred in a cirrhotic patient on TDF 300 mg weekly. While this could suggest that a weekly TDF dose was sub-therapeutic, preliminary conclusions were based on a single patient, and thus, the lowest effective dose remains unclear. All virologic events occurred in patients with GFR <50 mL/min/1.73m2. Whether patients were dose reduced according to the drug label (<50mL/min/1.73m2) or at a GFR according to CKD stage 2 (<60 mL/min/1.73m2) therefore did not affect the incidence of virologic events. The indication for dose reduction in previous studies differs depending on the kidney function formula used.[24] Similarly, in our study 9 (12%) patients in our cohort were put on TDF dose reduction when the eGFR calculated with CG formula was used, instead of the MDRD as per current guidelines.

The few studies that investigated the effect of TDF dose reduction on viral and renal outcome showed concordant results. The change in creatinine clearance was similar between HIV patients with TDF dose adjustment versus TDF full dose for 12 months, while a greater gain in CD4 count occurred after dose adjustment.[25] A case series in 11 HBeAg negative patients with CHB and advanced liver disease revealed no viral breakthrough during treatment with TDF 75 mg OD for a median (range) duration of 80 (24-576) weeks.[26] These retrospective studies however were small, lacked analyses of important confounders, and were subject to indication bias.

Clinically, a reduced dose TDF regimen could be particularly useful in resource constrained settings or in countries where tenofovir alafenamide or entecavir are not widely available. Given the availability of TDF as effective and low-cost generic worldwide and reimbursement restrictions for TAF both in low- to high-income countries, it is unlikely that TAF will fully supersede TDF. Resorting to reduced dose TDF for the treatment of renal impairment could also prevent emergence of multidrug (lamivudine) resistance that can develop during sequential monotherapy of lamivudine and entecavir. Our extensive experience with reduced dose TDF instead of entecavir therapy in patients with renal dysfunction stems from the inability to prescribe entecavir due to specific reimbursement restrictions within the Ontario healthcare system. This approach could additionally benefit patients with bone disease.

Strengths of this study include the selection of patients with a TDF dose reduction solely indicated for renal function decline, long-term follow-up of a heterogeneous group of patients, ranging from patients with relatively mild to significant comorbidity and of older age. The majority of patients was older, had cardiovascular comorbidity and advanced liver disease. These factors predisposed patients to renal impairment regardless of TDF use. Conversely, limitations of this study include the retrospective design, the lack of urinalysis data to examine urine markers of renal impairment, and dose adjustments that were not always applied according to guidelines. Because this study

did not aim to compare but merely describe virologic events in full and reduced dose TDF, and event rates were very low, analyses were not matched for baseline differences nor was statistical testing for subgroup comparisons done.

In conclusion, TDF dose adjustment was renally safe and virologically effective for CHB patients with impaired renal function, and could be a feasible option to reverse further renal decline during long-term antiviral treatment.

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SUPPLEMENTARY TABLE

Suppl. Table 1. Characteristics at the time of tenofovir disoproxil fumarate dose reduction in 78 CHB patients.

		TDF dose reduction	
		Started full dose (n=42)	Started reduced dose (n=36)
Demography			
Age, mean (SD), years		72 (8)	68 (12)
Sex, male, n (%)		27 (65)	27 (74)
Ethnicity, Asian/Caucasian/Black, %		76/17/7	81/19/0
Laboratory, mean (SD)			
ALT, x ULN†		0.6 (0.4-0.8)	0.7 (0.4-1.1)
HBV DNA, log IU/m†		1.0 (0.0-1.3)	1.1 (0.0-2.1)
HBeAg positive, n (%)		10 (24)	2 (5.6)
HBsAg, log IU/mL		2.2 (0.4)	1.3 (1.6)
Creatinine, micromole/L		188 (230)	137 (82)
eGFR-CG, mL/min/1.73m^2		41 (11)	46 (17)
MDRD, mL/min/1.73m^2		51 (20)	51 (18)
CKD-EPI, mL/min/1.73m^2		51 (21)	51 (18)
Serum phosphate, mmol/L		1.0 (0.3)	1.0 (0.30)
Serum albumin, g/L		41 (4)	40 (6)
Body Mass Index, kg/m^2		26.7 (19.6)	23.1 (4.4)
Cirrhosis (biopsy and/or clinical evidence), n (%)		35 (83)	31 (86)
Liver stiffness, kPa		7.7 (2.9)	8.7 (2.1)
Treatment, n (%)			
TDF treatment duration, mean (SD), months		48 (32)	40 (26)
Full dose TDF treatment duration		36 (26)	-
TDF dose reduction†	300 mg Q48hr	19 (45)	30 (83)
	150 mg OD	10 (24)	2 (5.6)
	300 mg Q72hr	8 (19)	1 (2.8)
	300 mg once weekly	5 (12)	3 (8.3)
Dose adjustment reason	GFR <60 mL/min	42 (100)	36 (100)
	Hypophosphatemia	3 (7.1)	1 (2.8)
RAS inhibitor (ARB or ACEi)		15 (36)	24 (67)
Beta blocker		5 (12)	9 (25)
Diuretic		9 (21)	15 (42)
Comorbidity, n (%)			
Diabetes mellitus		8 (19)	10 (28)
Hypertension		13 (31)	20 (56)
Dyslipidemia		6 (14)	11 (31)
Non-alcoholic fatty liver disease		2 (4.8)	6 (17)
CKD stages	G1 (⩾90 mL/min/1.73m^2)	0 (0.0)	0 (0.0)
	G2 (60-89)	0 (0.0)	0 (0.0)
	G3a (45-59)	23 (55)	19 (53)
	G3b (30-44)	11 (26)	13 (36)
	G4 (15-29)	6 (14)	1 (2.8)
	G5 (<15)	2 (4.8)	3 (8.3)
Hepatic decompensation		4 (9.5)	6 (17)
Hepatocellular carcinoma		5 (12)	7 (19)

† Median (IQR); ‡ all TDF dose reductions were due to renal impairment; *p<0.05. Abbreviations: ACEi, angiotensin-converting-enzyme; ALT, alanine aminotransferase; ARB, angiotensin II receptor blocker inhibitor; CKD, chronic kidney disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR-CG, estimated Glomerular Filtration Rate, Cockcroft-Gault; HBV, hepatitis B virus; MDRD, Modification of Diet in Renal Disease; RAS, renin-angiotensin system; TDF, tenofovir disoproxil fumarate.

CHAPTER 6

PREVALENCE AND PREDICTORS OF COMPLEMENTARY AND ALTERNATIVE MEDICINE MODALITIES IN PATIENTS WITH CHRONIC HEPATITIS B

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ABSTRACT

Background & Aims: The use of complementary and alternative medicine (CAM) in patients with chronic hepatitis B (CHB) can interact with antiviral treatment or influence health-seeking behavior. We aimed to study the use of individual CAM modalities in CHB and explore determinants of use, particularly migration-related, socio-economic and clinical factors.

Methods: A total of 436 CHB outpatients who attended the Toronto Centre for Liver Disease in 2015-2016 were included in this cross-sectional study. Using the comprehensive I-CAM questionnaire and health records, data were collected on socio-demographic and clinical variables and on usage of 16 CAM modalities in the last year.

Results: Sixty percent of patients were male, 74% were Asian and 46% were using antiviral treatment. Three-hundred nine (71%) patients used CAM. Vitamin/mineral preparations (45% of patients) were most commonly used. Overall CAM use and the specific use of potentially injurious CAM, such as green tea extract (9.2%) and St. John's wort (0.2%), were not associated with liver disease severity. Female sex, family history of CHB, lower serum HBV DNA, and higher socio-economic status were independently associated with bio-holistic CAM use, the clinically most-relevant CAM group ($p<0.05$); ethnicity, antiviral therapy use and liver disease severity were not.

Conclusions: CAM use among CHB patients was extensive, especially use of vitamin and mineral preparations, but without direct influence on liver disease severity. Bio-holistic CAM use appeared to be associated with socio-economic status rather than with ethnicity or liver disease severity. Despite the rare use of hepatotoxins, physicians should actively inquire about it.

INTRODUCTION

Chronic hepatitis B (CHB) affects approximately 240 million people world-wide and the associated liver-related morbidity and mortality continue to rise.[1–3] Global migration is changing the epidemiology of CHB, especially in low-endemic regions (North-America, Europe) with a high immigration rate from highly-endemic areas.[4,5] These epidemiological shifts increase the ethnocultural diversity, and could therefore influence the use of and perceptions on conventional Western medicine and on complementary and alternative medicine (CAM).

Patients with chronic diseases increasingly use CAM in addition to, or as a replacement of conventional treatments.[6] CAM is defined as "a group of diverse medical and health care systems, practices, and products that are not generally considered part of conventional medicine".[7] Examples of CAM include Traditional Chinese Medicine, acupuncture and dietary supplements. The proportion of patients with chronic liver disease that uses CAM varies widely from 27% to 80%.[8–11] Patients use CAM both for disease-related symptoms as well as for general well-being.[6] The identification of patterns in CAM use could be of great relevance to health care providers, since CAM products may interact with antiviral treatment or influence the health care-seeking behavior of patients.[12,13] Insight on CAM use is especially important for an ethnically diverse population such as those with CHB, where ethnic and acculturation factors can enlarge differences in CAM use and clinical outcomes.

The prevalence and predictors of individual CAM modalities in patients with CHB have not been well characterized. Previous studies on CAM use in CHB focused on specific types of CAM, were restricted to subgroups of patients, or evaluated few clinically important determinants.[9,14,15] We evaluated the use of various CAM modalities and its relation to clinical, socio-economic and migration-related factors in a large, multi-ethnic CHB cohort.

PATIENTS AND METHODS

Study population

Patients with CHB aged 18 years or above who attended the hepatology outpatient clinic of the Toronto Centre for Liver Disease, Canada, between January 1, 2015 and October 31, 2016 were invited to participate in this cross-sectional study. Both new patients and those in follow-up were eligible. The Toronto Centre for Liver Disease is the only specialized Liver Unit in the city of Toronto and comprises a wide variety of immigrants from around the globe. We excluded patients with a history of hepatocellular carcinoma, HIV co-infection, liver decompensation and organ- or bone marrow transplant. The Research Ethics Board of University Health Network approved this

study which was performed in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written consent.

Data collection

To address CAM use in patients with CHB, we developed a modified version of the International Complementary and Alternative Medication Questionnaire (I-CAM-Q).[16] The modified I-CAM-Q is a standardized comprehensive survey that comprises demographic, ethnic, socio-economic and clinical data, and use of 16 CAM therapies as classified by the National Center for Complementary and Alternative Medicine. The I-CAM-Q was designed for use across different populations and countries, but has not been validated. Both an English and Mandarin version of the modified I-CAM-Q was available. Patients completed the questionnaire at the time of an outpatient visit with the help of a research coordinator and if needed a translator. Any omissions or ambiguities in responses were followed up during the day of clinic visit or with telephone calls. Ethnicity-related questions involved country of birth, ethnicity, primary language, immigrant status, and time since immigration. Ethnicity was categorized into five groups: Chinese (China, Hong Kong, Taiwan), South-East Asian (Philippines, Korea, Vietnam, Thailand, Cambodia, Laos, Indonesia, Malaysia), South-Asian (India, Pakistan, Afghanistan, Bangladesh, Tibet), Caucasian (Europe, Russia, Turkey, Middle-East, North-Africa, Hispanic/Latino) and Black (Africa, Haiti, Jamaica). Socio-economic information included annual household income over the last 12 months, highest level of education, employment status and private insurance coverage. Clinical data (body height and weight, duration of hepatitis B virus (HBV) infection, serum ALT, serum HBV DNA, HBeAg status, cirrhosis (defined as Metavir F4/Ishak stage 6 on liver biopsy, or radiographic evidence of cirrhosis), current and past CHB treatment, duration of CHB treatment, as well as family history of CHB and/or hepatocellular carcinoma were retrieved from patient medical records and the questionnaire.

CAM therapies have been categorized by the National Center for Complementary and Alternative Medicine of the National Institutes of Health.[7] We obtained information on the following CAM domains and modalities from the survey: holistic therapies (homeopathy, acupuncture, naturopathy), biologically-based practices (herbal products, vitamin and mineral preparations, dietary supplements), manipulative and body-based therapies (chiropractic, massage, manipulation), mind-body medicine (meditation, spiritual therapy, visualization/guided imagery, health prayers, attendance of a traditional healing ceremony, qi gong, tai chi, yoga). For every type of CAM, patients reported visits to CAM providers, the use of CAM products, the frequency and duration of use, the primary aim (treatment of acute or chronic symptoms of CHB, general well-being, other reason), the efficacy of CAM practice, (reasons for) non-disclosure and physician inquiry about of CAM use.

Statistical analysis

Baseline characteristics are reported in means ± standard deviations (SD) for continuous variables, or frequency (percentage) for categorical variables. Differences in baseline characteristics and outcomes were analyzed using chi-squared test, Fisher's exact test, Student's t-test or Mann-Whitney test, where appropriate. Current CAM use was defined as annual or more frequent use of at least one of the CAM modalities. To evaluate whether ethnicity, antiviral treatment and hepatitis activity were associated with use of CAM, predictors that were univariably associated with CAM use in logistic regression (p-value <0.10) were analyzed in multivariable logistic regression. For this analysis, the clinically most relevant CAM groups (holistic and biologically-based therapies) were included. These bio-holistic CAM therapies were selected for further analysis because of potentially relevant clinical interactions. In addition there was insufficient statistical power to include other CAM modalities. Covariates included age, sex, ethnicity, duration of CHB, current antiviral treatment, previous use of pegylated (PEG-) interferon, previous use of nucleos(t)ide analogues (NA), cirrhosis, serum ALT level, serum HBV DNA level, HBeAg status, body mass index (BMI), family history of CHB and/or liver cancer, time since immigration, immigrant status, highest level of education, employment status, annual income and private drug plan coverage. All p-values were two-sided with a significance level of 0.05. Analyses were performed in SPSS (v. 22.0, Chicago, IL).

RESULTS

Patient characteristics

A total of 600 patients were approached in the inclusion period, of whom 436 (73%) patients completed the survey. Patients were excluded due to the following: HBsAg negative (n=7), relevant co-morbidity (n=7), acute HBV (n=2), or refusal to participate (n=148). Sociodemographic and clinical characteristics of enrolled patients according to CAM use are shown in Table 1. The mean (SD) age was 49 (14) years, 263 (60%) patients were male, and 201 (46%) currently used antiviral treatment for CHB. Two hundred eight (48%) patients were Chinese, 86 (20%) South-East Asian, 28 (6.4%) South-Asian, 72 (17%) Caucasian, 39 (8.9%) Black and 3 (0.7%) patients had more than one race/ethnicity. Two-hundred nine (48%) patients born abroad had lived for 20 or more years in Canada. Two-hundred sixty-four (61%) patients had finished college or higher education and 235 (54%) did not have any private insurance plan. Fifteen percent was HBeAg positive, the mean ALT was 1.5 (0.3) log IU/mL, median HBV DNA 1.8 (0.0-3.6) log IU/mL, and 65 (15%) patients were cirrhotic. Two-hundred three (47%) patients had a family history of CHB.

Several characteristics were significantly different between CAM users and CAM non-users. Notably, CAM users were predominantly female, South-Asian or Black, had a higher socio-economic status, and more often a family history of CHB. Other baseline characteristics were comparable between CAM users and CAM non-users.

Table 1. Patient characteristics according to CAM use in the last year.

		Overall CAM (n=309)	No CAM (n=127)	Bio-holistic CAM† (n=235)
Demographics				
Age, *years*		49 (14)	48 (14)	51 (14)
Sex, *male*		177 (57)	86 (68)	125 (53)
Race/ethnicity	*Chinese*	134 (43)	74 (58)	113 (48)
	South-East Asian	65 (21)	21 (17)	48 (20)
	South-Asian	22 (7.1)	6 (4.7)	11 (4.7)
	Caucasian	52 (17)	20 (16)	44 (19)
	Black	34 (11)	5 (3.9)	17 (7.2)
	Mixed	2 (0.6)	1 (0.8)	2 (0.9)
Socio-economic factors				
Married		210 (68)	91 (72)	160 (68)
Duration of stay in Canada	*0-3 years*	28 (9.1)	3 (2.4)	12 (5.1)
	4-19 years	107 (35)	60 (48)	80 (34)
	≥20 years	174 (56)	63 (50)	143 (61)
Residency status	*Citizen*	252 (82)	20 (16)	201 (86)
Primary language	*English*	187 (61)	58 (46)	137 (59)
	Mandarin/Cantonese	85 (28)	51 (40)	75 (33)
	Other	33 (11)	18 (14)	19 (8.2)
Education level	*⩽ High school*	110 (36)	62 (49)	80 (34)
	College/Bachelor	161 (52)	53 (42)	122 (52)
	Master/Doctorate	38 (12)	12 (9.4)	33 (14)
Employment status	*Employed*	222 (72)	94 (75)	164 (70)
	Unemployed	32 (10)	17 (14)	21 (8.9)
	Retired	55 (18)	15 (12)	50 (21)
Annual income, Canadian dollar	*<$25.000*	69 (28)	42 (40)	49 (21)
	$25.000-$49.999	63 (25)	32 (31)	46 (20)
	$50.000-$99.999	77 (31)	18 (17)	60 (26)
	≥$100.000	41 (16)	13 (12)	35 (15)
Private drug plan	*None*	146 (54)	89 (73)	111 (52)
	50-79%	34 (13)	7 (5.8)	29 (14)
	80-99%	49 (17)	13 (11)	38 (18)
	≥100%	43 (16)	12 (10)	33 (15)
Clinical data				
Duration of HBV infection, years		17 (12)	16 (10)	18 (12)
Current CHB therapy		140 (45)	61 (48)	114 (49)
Duration, *years*		5.3 (9.4)	4.6 (3.1)	5.5 (10.4)

	Overall CAM (n=309)	No CAM (n=127)	Bio-holistic CAM† (n=235)
Previous CHB therapy	135 (44)	53 (42)	107 (46)
Nucleos(t)ide analogue	128 (42)	51 (40)	100 (43)
(PEG-)interferon	24 (7.8)	10 (7.9)	19 (8.1)
Family history of CHB	156 (51)	47 (37)	126 (54)
Family history of liver cancer	52 (17)	20 (16)	45 (19)
Laboratory			
ALT, *log IU/L*	1.5 (0.3)	1.5 (0.2)	1.5 (0.3)
HBV DNA, *log IU/mL**	1.9 (0.0-3.5)	1.6 (0.0-3.6)	1.6 (0.0-3.0)
HBeAg positive	43 (14)	21 (17)	26 (11)
BMI, kg/m^2	25 (4.2)	25 (4.2)	25 (4.1)
Cirrhosis	50 (16)	15 (12)	39 (17)

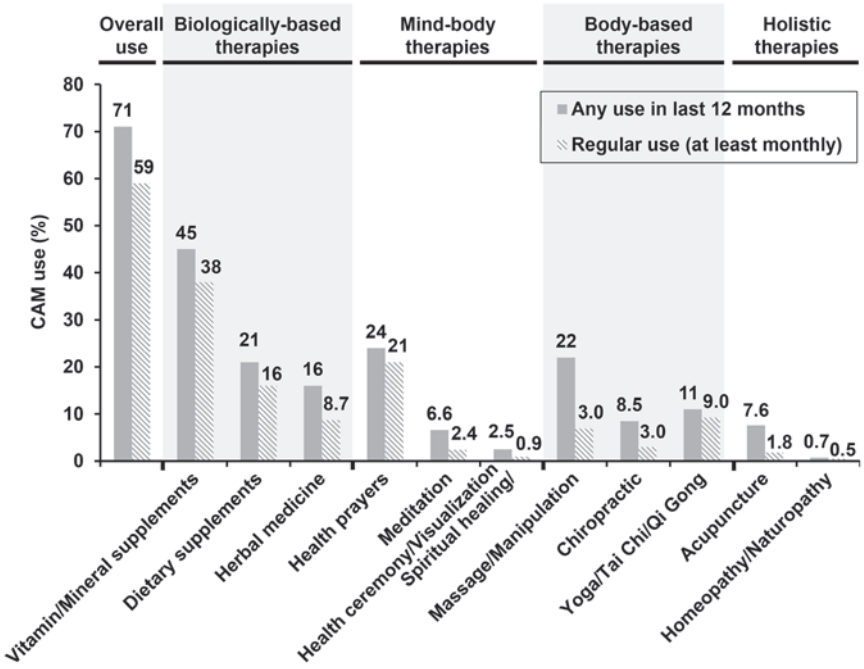
Data represented as n (%) or mean (standard deviation); † Use of biologically-based and holistic therapies combined. * Median (interquartile range); ALT: alanine aminotransferase; CAM: complementary and alternative medicine; CHB: chronic hepatitis B; HBV: hepatitis B virus; PEG: pegylated.

Patterns of CAM use

Three hundred nine (71%) patients had used CAM at least once during the past 12 months, and two hundred fifty-six (59%) patients had used CAM regularly (at least monthly; Figure 1). Biologically-based (51%) and mind-body therapies (35%) were the most frequently utilized CAM domains. Within these domains, vitamin and mineral preparations (45%), spiritual practices (29%), and dietary supplements (21%) were the most common CAM modalities. The use of body-based therapies (24%) was moderate and the use of holistic practices (8.9%) was low.

CAM use was significantly different among different ethnicities (64% in Chinese, 72% in Caucasians, 76% in SE-Asians, 79% in South-Asian, and 87% in Blacks; p=0.03 (Table 1 and Supplementary Table 1). Specifically, mind-body medicine was practised more often by South-Asian (54%) and Black patients (77%) than other ethnic groups (22%; p<0.005). Homeopathy (2.8%) and naturopathy (1.4%) were more often used by Caucasian than other groups (p=0.03). The overall use of vitamin and mineral preparations (45%) and herbal product use (16%) did not differ significantly among ethnic groups. Vitamin and mineral supplements predominantly comprised of vitamin D (39%), multivitamins (38%), calcium (26%), vitamin C (19%), and omega-3 fatty acid (19%). Ginger extract (34%), milk thistle (15%) and ginseng (5.9%) were the most commonly used herbal preparations.

Figure 1: Use of CAM modalities in the last 12 months in 436 patients with CHB.



The use of CAM products with a reported hepatoprotective or hepatitis B infection-altering effect (milk thistle and ginger extract) was very low (3%) and was not related to subjects' liver disease severity, as was reflected by no association with serum ALT, HBV DNA or presence of cirrhosis ($p>0.05$). The use of green tea extract (9.2%) and St. John's wort (0.2%), the only known potentially harmful CAM products in this study, was not associated with liver disease severity ($p>0.05$).

Attitudes toward CAM use

The main reason to use herbal products was to improve general well-being (63%; Table 2). Thirty-two percent of patients used herbal products for liver-related symptoms, compared to 2.6% of vitamin and mineral product users ($p<0.005$). Homeopathy and spiritual therapies were rated predominantly as very helpful, whereas most other CAM therapies were considered helpful. A quarter of patients rated vitamin/mineral (24%) and other supplements (25%) as not helpful at all. The majority of patients (87%) started CAM therapy before they were diagnosed with CHB and had been using it for at least 5 years, especially acupuncture (71%), visualization (57%) and herbal medicine (67%).

Forty-three percent of physicians had actively inquired about CAM use (Supplementary Table 1). Doctors had inquired about CAM use less often in Caucasian patients (33%) than in Chinese patients (46%; p=0.06). Fifty-two percent of patients had not informed their physicians about CAM use, ranging from 46% (Black patients) to 64% (South-Asian patients), and this did not differ between ethnic groups. The main reasons for patients not to disclose CAM use were: not considered important to inform treating physician, non-liver related CAM use, not inquired by physician, anticipated physician disinterest or disapproval, and already informed general practitioner or other treating physician.

Table 2. Pattern of use, and attitudes towards individual CAM modalities.

CAM modality	Number of users (n)	Started after CHB diagnosis (%)	Duration of use (%)	Frequency of use (%)	Reason for use (%)	Helpfulness (%)
			0-1/ >1-5/ >5 years	When symptomatic/ daily/ weekly-monthly/ annually	Liver-related symptoms/ general well-being/ other	Very helpful/ helpful/ moderately-minimally/ not helpful
Biologically-based therapies						
Vitamin/mineral supplements	193	11	29/33/38	1/79/5/215	2.6/79/23	12/28/33/24
Dietary supplements	92	3.3	11/13/76	8/45/24/24	1/5/94	9/43/12/25
Herbal medicine	67	33	42/31/27	3/34/23/40	32/66/10	12/39/31/7
Mind-body therapies						
Health prayer	103	21	1/7/92	1/69/34/14	11/67/28	45/43/8/2
Meditation	28	14	18/25/57	0/29/47/25	0/6/94	50/36/11/4
Visualization	7	43	0/43/57	0/14/29/57	0/2/98	0/50/34/17
Spiritual healing	4	0	50/25/25	25/0/25/25	0.2/0.2/99	0/67/0/33
Healing ceremony	3	0	0/0/100	0/33/0/67	0/1/99	0/50/0/50
Yoga	28	14	28/21/52	0/14/62/24	0/6/94	35/38/20/7
Tai chi	15	13	13/33/53	0/33/60/7	0/3/97	33/33/34/0
Qi gong	12	25	18/27/55	0/33/50/17	0.2/2/98	33/33/33/0
Body-based therapies						
Massage	95	9.5	46/25/28	3/0/32/65	0/10/90	19/38/38/5
Chiropractic	37	16	60/19/22	8/0/35/56	0.2/3/97	22/35/41/3
Manipulation	4	0	75/25/0	0/0/25/75	0/0/2/99	25/50/25/0
Holistic therapies						
Acupuncture	29	12	79/14/7	3/0/28/69	0/1/99	24/45/20/10
Homeopathy	7	13	17/50/33	17/17/17/34	0/2/98	100/0/0/0
Naturopathy	2	0	0/50/50	0/50/50/0	0/100/0	50/0/50/0

CAM: Complementary and alternative medicine; CHB: chronic hepatitis B.

Determinants of bio-holistic CAM use

Determinants for the use of the clinically most relevant CAM group, (bio-holistic CAM, were studied with logistic regression (Table 3). The bio-holistic CAM therapies were selected because of possible clinical interactions and limited statistical power to study other CAM products. Female sex (OR for female versus male: 2.18; 95%CI: 1.35-3.59; p<0.005), higher education level (Master's degree vs. ≤ High school, OR: 2.95; 1.40-6.20; p<0.005), employment status (OR for retired vs employed: 5.22; 2.72-10.03; p<0.005), higher private drug plan coverage (80-100% vs. none, OR: 2.07; 1,98-3,94; p=0.02), lower HBV DNA (OR: 0.89; 0.81-0.98; p=0.02) and a family history of CHB (OR: 1.65; 95%CI: 1.07-2.55; p=0.03) were independently associated with use of bio-holistic CAM modalities. Age, ethnicity, immigrant status, time since immigration, and primary language were not associated.

Table 3. Logistic regression on bio-holistic CAM use†.

		Univariable			Multivariable		
		OR	**95%CI**	**P**	**OR**	**95%CI**	**P**
Demographics							
Age, *years*		1.03	1.02-1.05	<0.005*			ns
Sex, female vs male		2.13	1.44-3.14	<0.005*	2.18	1.35-3.59	<0.005*
Race/ethnicity	*Chinese*	1.00		0.10			ns
	South-East Asian	1.64	0.99-2.73				
	South-Asian	0.63	0.26-1.49				
	Caucasian	1.57	0.91-2.69				
	Black	0.98	0.49-1.98				
Socio-economic factors							
Married		0.86	0.57-1.30	0.48			
Duration of stay in Canada	*0-3 years*	1.00		<0.005*			ns
	4-19 years	1.23	0.53-2.86				
	≥20 years	2.55	1.13-5.77				
Residency status	*Citizen vs non-citizen*	1.95	1.15-3.32	0.01*			ns
Primary language	*English*	1.00		0.17			
	Mandarin/Cantonese	0.75	0.49-1.14				
	Other	0.53	0.28-1.02				
Education level	*≤ High school*	1.00		<0.005*	1.00		<0.005*
	College/Bachelor	1.89	1.24-2.86		2.03	1.23-3.34	0.01*
	Master/Doctorate	2.64	1.39-5.02		2.95	1.40-6.20	<0.005*
Employment status	*Employed*	1.00		<0.005*	1.00		<0.005*
	Unemployed	0.88	0.47-1.65		1.45	0.72-2.96	0.30
	Retired	4.14	2.35-7.29		5.22	2.72-10.03	<0.005*
Annual income, Canadian dollar	*<$25.000*	1.00		0.02*			ns
	$25.000-$49.999	1.00	0.57-1.78				
	$50.000-$99.999	2.09	1.19-3.65				
	≥$100.000	1.7	0.94-3.45				

		Univariable			Multivariable		
		OR	**95%CI**	**P**	**OR**	**95%CI**	**P**
Private drug plan	*None*	1.00		<0.005*	1.00		0.02*
	50-79%	2.86	1.45-5.66		2.81	1.31-6.07	0.01*
	80-99%	2.08	1.18-3.67		2.07	1.09-3.94	0.03*
	≥100%	1.64	0.91-2.97		1.63	0.83-3.19	0.16
Clinical data							
Duration of HBV infection	*0-9 yr*	1.00		0.12			
	10-19 yr	1.41	0.89-2.21				
	20-29 yr	1.53	0.90-2.61				
	≥30 yr	2.18	1.07-4.45				
Current CHB therapy		1.13	0.77-1.65	0.53			
Previous CHB therapy		1.25	0.85-1.83	0.26			
(PEG-)interferon		1.78	0.88-3.61	0.11			
Nucleos(t)ide analogue		1.12	0.76-1.64	0.57			
Family history of CHB		1.93	1.32-2.84	<0.005*	1.65	1.07-2.55	0.03*
Family history of liver cancer		1.53	0.92-2.53	0.10			ns
Laboratory							
ALT, *log IU/L*		0.47	0.22-1.03	0.06			ns
HBV DNA, *log IU/mL*		0.89	0.82-0.97	0.01*	0.89	0.81-0.98	0.02*
HBeAg positive		0.48	0.27-0.85	0.01*			ns
BMI, *kg/m2*		0.99	0.94-1.03	0.58			
Cirrhosis*		1.09	0.64-1.86	0.74			

* P<0.05; † Use of biologically-based and holistic therapies combined, at least monthly. ALT: alanine aminotransferase; CAM: complementary and alternative medicine; CHB: chronic hepatitis B; HBV: hepatitis B virus; ns: not significant; PEG: pegylated.

DISCUSSION

In this clinic-based study we reported the prevalence of CAM use and individual CAM modalities in a large multi-ethnic CHB cohort, and examined factors that determined CAM usage. A majority of patients used CAM in the past year (71%), ranging from 64% for Chinese to 78% in non-Asian patients. Vitamin and mineral preparations were used most frequently, followed by spiritual healing practices, body-based therapies and herbal medicine. Variables significantly associated with bio-holistic CAM use were female sex, higher socio-economic status, lower serum HBV DNA, and a family history of CHB; ethnicity and migration-related factors were not.

The use of CAM in our study was extensive compared to previous studies in CHB, but was not associated with the use of antiviral treatment or disease severity. Two previous studies in CHB reported that 46% of children used CAM, and 32% of patients in Hong Kong ever used Traditional Chinese Medicine, compared to 19% among Chinese patients in our study.[14,15] Other epidemiological studies in non-CHB chronic liver disease showed substantial variation in CAM

use rates (27% to 80%).[8,9,11,17] The comparatively high rate of CAM use in this study could be due to the comprehensive definition of CAM, the population under study, and the setting where patients were investigated (tertiary referral centre versus family practice).[18,19]

Prolonged and/or frequent use of presumed noxious CAM compounds can adversely impact clinical disease markers in liver disease, due to herb-drug interactions or influence of cytochrome P450 systems. These findings mainly stem from studies in liver diseases other than CHB.[20–22] In our study, patients were taking mainly 'western style' CAM products (mostly vitamins) and hardly any herbals or supplements with possible beneficial effects for HBV or liver disease. The use of potentially harmful CAM products such as green tea extract or St. John's wort was very low and not associated with liver disease severity, although this should be interpreted cautiously as few participants used these CAM products and no follow-up data was available. We are concerned when patients take a mix of herbs that are difficult to identify, but this did not occur frequently in our population, which was probably biased because all patients visited western style practitioners in a hospital. Alternatively, it might be possible that patients used CAM products which contained hepatotoxins but that they did not consider these as CAM. Nonetheless, this was the largest multi-ethnic clinic-based study in CHB and therefore probably indicative of real world CAM use in CHB in North-America. In order to monitor the (safe) use of CAM, physicians should be encouraged to actively ask about CAM use and specific harmful products, which was currently only done by less than half of the treating physicians.

This study was the first to investigate the influence of ethnicity on CAM modalities in North-America, which contains a predominantly immigrant population with CHB. The demographics of Toronto, one of the most multicultural and multiracial cities worldwide where 52% of the population is composed of visible minorities, enabled us to comprehensively evaluate the role of ethnicity in CAM use.[23] CAM use in general differed by ethnicity, specifically for spiritual therapy, yoga, tai chi and homeopathy. The use of spiritual therapy was higher in South-Asian and Black patients compared to other patients. Vitamin and mineral preparation use was surprisingly similar between ethnic groups, possibly because these products have become popular among the population at large in Western countries. Earlier studies on CAM use in ethnic subgroups in Canada combined healthy subjects and patients with chronic conditions, thereby mixing different motives and patterns of use.[17,24] Remarkably, ethnicity and migration-related factors were not associated with oral CAM use after adjustment in multivariable analysis. Other determinants, such as higher socio-economic status, were either much stronger predictors of CAM use or correlated with migration-related factors, so that any effect of ethnicity and migration-related factors might be unobservable, as seen in prior research.[18] The high cost of CAM products likely restricted access to the more affluent patients, regardless of ethnic background. These findings suggest that

health care providers of CHB should focus on socio-economic status rather than ethnic or cultural factors when inquiring about CAM use.

The use of CAM is widespread and growing in populations where evidence-based medicine is dominant.[25,26] U.S. adults spent $33.9 billion out-of-pocket annually on CAM visits and products, whereas one in every two European citizens uses CAM, which underlines the breadth of CAM use nowadays.[25,26] Apart from reporting CAM use rates in chronic liver disease patients, it is equally important to gain insight in why patients opt for non-conventional medical therapies. This study showed that most patients used CAM for reasons unrelated to their chronic liver disease, except for herbal medicine. A possible explanation is that the most commonly used herbal products milk thistle (*Silybum marianum*) and several Traditional Chinese Medicine products have been associated with hepatoprotective effects, while the efficacy of other CAM therapies is less clear.[12,27–30] Additionally, the non-liver related use of CAM could reflect an increasing demand for 'salutogenesis', an approach that focuses on determinants of well-being, rather than on determinants of disease, and is key to the CAM paradigm.[31]

Strong aspects of this study are the inclusion of a large, multi-ethnic cohort of CHB patients who completed an extensive survey on CAM-related factors. Conversely, the inherent recall bias for questionnaires and cross-sectional design restricted us to study long-term consequences of CAM use. Future studies on CAM use in CHB could focus on these long-term effects and associated factors.

In summary, CAM use in this clinic-based population of CHB patients was common and the CAM products that patients used, primarily vitamin and mineral preparations, appeared to be safe. Few patients had used CAM products that were considered to be harmful. CAM use was associated with female sex, higher socio-economic status, lower HBV DNA and a family history of CHB; not with ethnic background, antiviral treatment or liver disease severity. Most treating physicians had not inquired about the use of CAM, neither had most of the patients discussed its use.

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SUPPLEMENTARY FILE

Supplementary Table 1: CAM use in the last 12 months by ethnic group.

		Ethnic group						
	Total (N=436)	**Chinese (n=208)**	**SE-Asian (n=86)**	**S-Asian (n=28)**	**Caucasian (n=72)**	**Black (n=39)**	**Mixed (n=3)**	**P**
Overall use	309 (71)	134 (64)	65 (76)	22 (79)	52 (72)	34 (87)	*2 (67)*	0.03*
Biologically-based therapies	223 (51)	103 (50)	49 (57)	10 (36)	42 (58)	17 (44)	*2 (67)*	0.17
Vitamins/minerals	196 (45)	83 (40)	46 (54)	10 (36)	39 (54)	17 (44)	*1 (33)*	0.09
Dietary supplements	92 (21)	3 (1.4)	24 (28)	16 (57)	31 (43)	17 (44)	*1 (33)*	<0.005*
Herbal medicine	68 (16)	40 (19)	12 (14)	2 (7.1)	11 (15)	2 (5.1)	*1 (33)*	0.13
Mind-body therapies	152 (35)	45 (22)	33 (38)	16 (57)	27 (38)	30 (77)	*1 (33)*	<0.005*
Health prayers	105 (24)	17 (8.2)	28 (33)	15 (54)	16 (22)	29 (74)	*0 (0.0)*	<0.005*
Meditation	29 (6.6)	6 (2.9)	4 (4.7)	3 (11)	10 (14)	3 (7.7)	*1 (33)*	0.02*
Visualization	6 (1.4)	3 (1.4)	1 (1.2)	0 (0.0)	1 (1.4)	1 (2.6)	*0 (0.0)*	0.94
Spiritual healing	4 (0.9)	3 (1.4)	1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	*0 (0.0)*	0.74
Healing ceremony	3 (0.7)	1 (0.5)	1 (1.2)	0 (0.0)	1 (1.4)	0 (0.0)	*0 (0.0)*	0.85
Yoga	29 (6.7)	11 (5.3)	2 (2.3)	2 (7.1)	11 (15)	3 (7.7)	*0 (0.0)*	0.02*
Tai chi	15 (3.4)	12 (5.8)	2 (2.3)	0 (0.0)	1 (1.4)	0 (0.0)	*0 (0.0)*	0.05*
Qi gong	12 (2.8)	11 (5.3)	0 (0.0)	0 (0.0)	1 (1.4)	0 (0.0)	*0 (0.0)*	0.14
Body-based therapies	106 (24)	49 (24)	24 (28)	3 (11)	19 (26)	10 (26)	*1 (33)*	0.43
Massage	95 (22)	46 (22)	20 (23)	3 (11)	17 (24)	8 (21)	*1 (33)*	0.68
Chiropractic	37 (8.5)	18 (8.7)	8 (9.4)	1 (3.6)	7 (9.7)	3 (7.7)	*0 (0.0)*	0.89
Manipulation	4 (0.9)	2 (1.0)	1 (1.2)	0 (0.0)	1 (1.4)	0 (0.0)	*0 (0.0)*	0.93
Holistic therapies	39 (8.9)	20 (9.6)	3 (3.5)	2 (7.1)	11 (15)	2 (5.1)	*1 (33)*	0.10
Acupuncture	33 (7.6)	20 (9.6)	3 (3.5)	2 (7.1)	8 (11)	0 (0.0)	*0 (0.0)*	0.10
Homeopathy	3 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.8)	0 (0.0)	*1 (33)*	0.04*
Naturopathy	2 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)	1 (2.6)	*0 (0.0)*	0.16
Physician asked patient about CAM use	187 (43)	96 (46)	34 (40)	10 (36)	24 (33)	20 (51)	*3 (100)*	0.22
Patient informed physician about CAM use	206 (48)	96 (46)	44 (51)	10 (36)	35 (49)	21 (54)	*3 (100)*	0.59

* P<0.05 (mixed ethnic group excluded from statistical testing due to small number). CAM: complementary and alternative medicine; SE-Asian: South-East Asian; S-Asian: South-Asian.

CHAPTER 7

SUMMARY AND DISCUSSION

Adapted from:

Liem KS, September 26, 2018. Effect of Pegylated Interferon Add-On Therapy to Achieve Disease Remission in Patients with Chronic Hepatitis B. Master's thesis, University of Toronto, Toronto, Canada. Retrieved from https://tspace.library.utoronto.ca/handle/1807/91715
Liem KS, Janssen HLA. Aliment Pharmacol Ther. 2019;49:610-611
Liem KS, Gehring AJ, Feld JJ, Janssen HLA. Gastroenterology 2020;158:1185–1190
Liem KS, Gehring AJ, Janssen HLA. Gastroenterology 2020;159:1187-1188
[1–3]

SUMMARY AND DISCUSSION

Despite effective vaccination, chronic hepatitis B (CHB) poses a substantial hazard to public health with limited options for finite treatment. The goals of treatment for patients with CHB – improving survival and quality of life – can be achieved by nucleos(t)ide analogues (NA). However, since NA therapy does not directly target key sources of intrahepatic virion production (cccDNA and integrated DNA), patients rarely achieve functional cure. Finite treatment approaches before patients achieve HBsAg loss and other treatment regimens have therefore been studied.

This thesis centers on treatment optimization for patients with CHB: maintaining response after withdrawing or dose reducing NA treatment, improving response by modifying the NA treatment regimen with PEG-IFN add-on, and studying response and health-seeking behaviour in relation to use of complementary and alternative medicine.

To this end, the thesis was divided in two parts: in part I, we studied the sustainability of response after discontinuing long-term NA therapy and evaluated predictors of off-therapy flares. Furthermore, we investigated the effect of PEG-IFN add-on to NA monotherapy on serological response and aimed to identify the best candidates for PEG-IFN add-on therapy in clinical practice. In part II, we investigated how miscellaneous treatment modifications may influence renal function or health-seeking behavior.

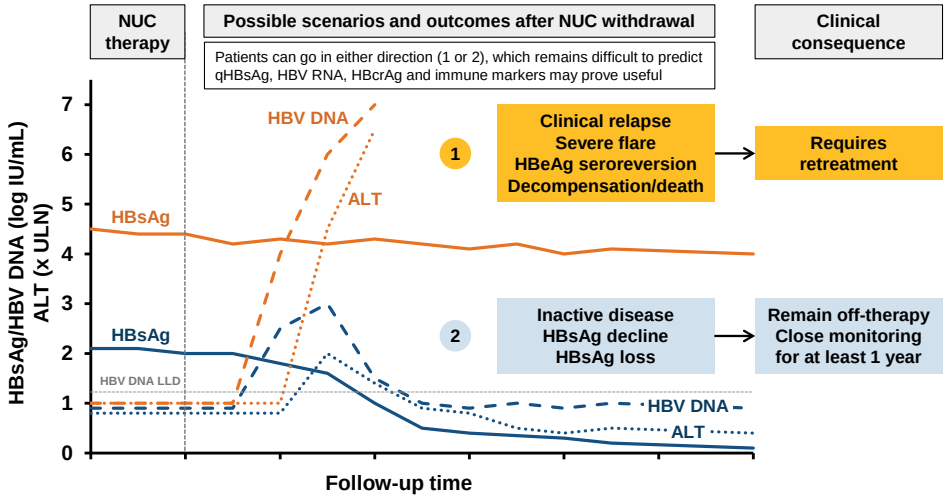
ATTEMPTS TO HBV CURE

Nucleos(t)ide analogue therapy withdrawal

Previous studies have suggested that stopping NA therapy in HBeAg negative patients may lead to functional cure.[4–8] However, the current evidence has also underscored inconsistent findings because of widely ranging relapse rates and potentially dangerous flares (Figure 1). To clarify the potential benefit of NA treatment withdrawal, we investigated in **Chapter 2** whether HBeAg negative patients could achieve a response with HBsAg loss after NA therapy cessation. We therefore performed an RCT in patients who had received NA therapy for at least 1 year and achieved virologic suppression. This study revealed that stopping NA therapy led to high rates of relapse and retreatment and a low rate of HBsAg loss. A post-hoc analysis showed that pre-treatment HBeAg positive patients had worse off-therapy outcomes than HBeAg negative patients. These results suggest limited benefits to stopping NA therapy, especially if patients were HBeAg positive at the start of therapy. The findings of this Asian majority cohort are not in line with other studies. Although follow-up was shorter than in the previously cited studies, HBsAg kinetics differed considerably from the Taiwanese study despite both studies including mainly

Asian patients with genotypes B and C. Important differences were the duration of NA therapy and length of consolidation therapy, which were both longer in the Canadian study. Counterintuitively, longer consolidation therapy did not decrease the relapse risk or increase HBsAg decreases in the Canadian trial.

Figure 1: Clinical scenarios after nucleos(t)ide analogue withdrawal in patients with chronic hepatitis B.



To further examine the characteristics and severity of flares after NA withdrawal, we examined 2 cohorts of patients stopping NA therapy in **Chapter 3**: the first was a cohort derived from the Canadian RCT and the second an observational cohort from a prospective study from China. Predicting ALT flares that require retreatment according to pre-defined criteria at the end-of-treatment (EOT) or during early off-treatment follow-up could greatly aid the clinical decision-making process and prevent potentially dangerous flares. The timing of retreatment is crucial because retreating too early might not be necessary, while retreating too late might result in hepatic decompensation or even death. Off-therapy flares occurred at a high cumulative incidence of 52%. Even though most patients in this study had significant ALT elevations, most of which occurred within 12 weeks after NA withdrawal, not all patients may need (immediate) retreatment. The most important determinants of flares were male sex and HBV DNA values measured 6 weeks after NA cessation. These results were partially validated in the external cohort. The proposed threshold in Chapter 3 thus enables predicting imminent flares in patients who may benefit from closer monitoring and earlier retreatment.

The low rates of on-therapy functional cure (hepatitis B surface antigen [HBsAg] loss), which is regarded as the optimal end point to withdraw therapy, preclude many patients from stopping therapy. Additionally, other concerns of long-term therapy such as adherence, side effects, and, particularly, costs, spurred a worldwide scientific debate on NA discontinuation before patients reach functional cure. In a recent Commentary from our group, we argue that the current evidence on NA withdrawal lacks robustness and seems to underlie premature suggestions that patients can stop NA therapy before they achieve HBsAg loss.[2] Results are inconsistent because of different definitions for outcome, retreatment, and monitoring frequency, as well as the inclusion of highly selected populations with varying durations of consolidation therapy. NA therapy is very effective and has minimal side effects; nonadherence can be identified before virologic resistance/breakthrough occurs; costs of treatment may not be offset by costs of continued and frequent monitoring for undetermined duration, particularly as NAs have become generically available; and, most important, relapse is common and its occurrence and clinical consequence remain highly unpredictable.

The current data which suggest a beneficial effect of stopping NA are largely based on observational cohort studies whereas the published RCTs show very little benefit. HBsAg loss rates were not consistently increased, neither in Asian nor Caucasian populations.[2] For example, a recent international cohort study in 178 HBeAg negative patients (44% Asian, 49% Caucasian) reported that NA therapy withdrawal did not influence HBsAg loss rates.[9] Most observational studies were not designed to investigate differences in HBsAg loss rates and should therefore be cautiously interpreted. Most studies were from Asia, of which the majority were from Taiwan where the main reason for stopping was a lack of continued reimbursement by national health insurance after only a few years of NUC therapy. Even in studies with longer off-treatment follow-up, rates of HBsAg loss varied from 0% to 19%, which at best reflect that only highly selected patients have higher chances of achieving HBsAg loss.

Without the tools for proper patient selection, potential benefits of NA discontinuation do not outweigh limitations of long-term NA therapy for most patients in clinical practice. The road forward involves evaluating biomarkers shortly after, or preferably, before stopping NA therapy to predict outcomes. Work on virologic (quantitative HBsAg, HBV RNA, HBcrAg and anti-HBc) biomarkers continues and may increase our predictive power for safely stopping NUC therapy.[10–15] An interesting example of risk stratification for NA cessation according to HBV RNA and quantitative HBsAg values was recently described in an Asian cohort.[16]

Additionally, immunologic biomarkers that discriminate between antiviral responses and pathologic inflammation after stopping therapy could facilitate clinical decisions, limit adverse

outcomes, and identify mechanisms leading to functional cure. However, specific mechanisms driving viral control and inflammation in the human liver have not been completely defined. Initial serum cytokine studies, in small cohorts, could not predict off-therapy relapse, but confirmed associations between liver disease and chemokine production.[17,18] Investigation of cellular responses to NA withdrawal revealed increased natural killer cell function during ALT elevation, which supports a role for natural killer cells in biochemical reactivation.[19] In the adaptive compartment, HBV-specific T-cell expansion increased after NA cessation, and patients with the most expanded (HBV core and polymerase specific, PD-1 positive) T cells before stopping therapy did not experience biochemical reactivation.[20–22] Inflammation and viral control are temporally regulated networks of responses after stopping therapy. Refining this series of immunologic events requires longitudinal investigation of not only blood but also human liver samples with analytical approaches expanding beyond conventional immunologic effectors. Thus far, no biomarker has proven robust through prospective, external validation and several are not yet commercially available for clinical decision making.

Nonetheless, economic and practical limits of health care systems will continue to challenge the HBV management paradigm. Evidence for future guidelines should be reinforced by prospective randomized controlled trials that stratify the analysis by HBeAg status, include heterogeneous populations and are powered to detect differences in HBsAg loss rate. The optimal duration of NA consolidation therapy and hepatocellular carcinoma risk after NA withdrawal should also be examined. Meanwhile, reanalyzing published data could hopefully provide more nuanced views on when and in whom to stop NA treatment.

Adding on peg-interferon to nucleos(t)ide analogue therapy

Various treatment combinations of PEG-IFN and NA agents have been evaluated to treat CHB, but the optimal regimen remains unclear. In **Chapter 4**, we aimed to study whether PEG-IFN add-on increases response compared to ETV monotherapy in HBeAg positive patients treated within two previously published RCTs (ARES and PEGON trial). Patients received ETV pretreatment for at least 24 weeks and were then allocated to 24-48 weeks of ETV + PEG-IFN add-on, or to continue ETV monotherapy. Serological response was observed in 38/118 (33%) patients treated with add-on therapy and in 23/116 (20%) with monotherapy (P = 0.03). The highest response to add-on therapy compared to monotherapy was observed in PEG-IFN naive patients with HBsAg levels below 4000 IU/mL and HBV DNA levels below 50 IU/mL at randomization (70% vs 34%; P = 0.01). Above the cut-off levels, response was low and not significantly different between treatment groups. Duration of ETV pretreatment was associated with HBsAg and HBV DNA levels (both P < 0.005), but not with response (P = 0.82).

The debate about the value of PEG-IFN add-on has not been settled. It has been proposed that the endpoint for a PEG-IFN add-on regimen should be durable response after stopping entecavir (ETV) therapy, such as HBeAg seroclearance or seroconversion combined with HBV DNA <2,000 IU/mL at 24 or 48 weeks off-therapy.[1] While we acknowledge the value of determining response off-therapy, the aim of this study was arguably different and our definition of response has, regardless of cessation of ETV therapy, great clinical relevance by itself beyond increasing cost-effectiveness. Specifically, this study focused on identifying responders to PEG-IFN add-on to ETV, and not on the results of finite ETV therapy. In contrast to the response rates to PEG-IFN monotherapy (25-35%) in prior CHB studies, we identified a subgroup of patients with a considerably higher response rate (up to 70%) 48 weeks after stopping PEG-IFN. Identifying these responders at the start of PEG-IFN add-on therapy will, independently of ETV discontinuation, influence clinical practice, reduce unnecessary exposure to PEG-IFN, and improve cost-effectiveness.

In recent RCTs that evaluated adding on PEG-IFN to continued NA monotherapy, rates of HBsAg decline were significantly higher in the add-on arm, although the primary endpoints (HBsAg loss at week 96; combined HBeAg loss with HBV DNA <200 IU/mL at week 96) were not achieved, which may be due to type II errors.[23–25] In the ARES study response was achieved in 19% of patients in the add-on arm vs 10% in the monotherapy arm (P = 0.095); declines in HBsAg, HBeAg, and HBV DNA were also larger in the add-on group (all P < 0.001).[23] In line with these studies, the very recently published multicenter SWAP RCT also did not reach its primary endpoint.[26] This study randomly allocated HBeAg positive and negative patients to add-on (PEG-IFN to NA), switch therapy (NA switch PEG-IFN) or continued NA monotherapy. Although the main endpoint (HBeAg loss or >1log reduction in qHBsAg) and HBsAg loss at week 72 were comparable between arms, especially HBeAg negative patients appeared to benefit. Uncontrolled studies in HBeAg positive and negative patients reported similar findings.[27,28]

Long-term follow-up allows detecting side-effects that were considered unrelated to the study drug or did not occur in the trial population, which is generally much smaller in size than cohorts in subsequent follow-up studies and has therefore a lower ability to detect minor effects. In addition, follow-up studies can shed light on long-term therapy outcomes, which is especially informative for patients with CHB that often receive treatment for decades.

A follow-up study of the above cohort (n=96) revealed that early response benefit achieved by PEG-IFN add-on was lost during follow-up beyond 96 weeks.[29] While PEG-IFN add-on seems to increase the rate of response early on, the long-term response rates are comparable. The lack of a long-term PEG-IFN add-on effects could be related to treatment duration or the loss of treatment responders in the follow-up cohort, which skewed response rates towards each

other. Although initial non-responders were overrepresented in the PEG-IFN add-on group, add-on therapy might induce accelerated HBeAg loss rather than higher HBeAg loss rates compared to ETV monotherapy in the long-term. Since this retrospective study mainly included initial non-responders (86/96 [90%]), especially in the add-on group, response possibly reflected the late effects of add-on therapy, rather than the off-therapy durability of early effects. Many patients who achieved response in the initial study were lost-to-follow-up, which likely biased outcomes negatively. A post-hoc analysis of the paper indicated that, after extrapolation of response sustainability of original ARES subjects, response rates of PEG-IFN add-on would indeed remain higher than of ETV monotherapy.

The persistent role of PEG-IFN alpha in the treatment of CHB was highlighted in another long-term follow-up study, which described that early HBeAg loss was associated with a higher probability of HBsAg loss (cumulative 5 and 10-year incidence of 14% and 32%, respectively) and improved clinical outcomes, particularly in patients of male sex, Caucasian race, age ≥40 years, with genotype A, and pre-existing cirrhosis.[30]

Apart from response, side effects should be considered when deciding on a treatment strategy. Importantly, almost all patients in the add-on group experienced AEs during randomized therapy compared to less than 10% in the monotherapy. The rate of SAEs was low and comparable between groups. Even though the 2 reported SAEs that were considered related to PEG-IFN were reversible, the high rate of AEs during PEG-IFN therapy marks an important caveat for initiating PEG-IFN add-on treatment and the need for on-treatment monitoring of laboratory markers and subsequent dose reduction or therapy discontinuation. The high rate of PEG-IFN related AEs has also been cited in previous PEG-IFN monotherapy and combination studies.[31–34]

Additionally, cost-effectiveness should be considered during treatment selection. As described in the Introduction, CHB is associated with high health-care costs.[35] Finite therapy with PEG-IFN might be more cost-effective than long-term or indefinite NA monotherapy, especially if physicians carefully select patients based on host and viral markers at the start of therapy or early on-treatment. Response-guided therapy, such as the clinical decision model proposed in this thesis, could significantly increase the cost-effectiveness of PEG-IFN. The wholesale acquisition costs (WAC) for PEG-IFN α2a and α2b for a 48-week supply in Canada were CAD $37,000 and $33,600, respectively. In comparison, the WAC of TDF or ETV treatment for 48 weeks is considerably lower: $430 – $1350 for TDF and $1,600 - $6,600 for ETV.

More comprehensive cost-effective studies on antiviral therapy for CHB have also been performed and have the added benefit of including both direct and indirect costs. PEG-IFN, especially using

response-guided therapy using week 12 stopping rules, was most effective and cost-effective in HBeAg positive and, in some models, for HBeAg negative patients in the UK and in China.[36–38] The Markov model based study from Hong Kong simulated lifetime clinical and economic events, and reported Quality Adjusted Life-Years (QALYs) and cost-effectiveness ratios (CERs) for patients with HBeAg positive and negative CHB. The CER for HBeAg positive patients for 48 weeks of conventional PEG-IFN treatment was US $9,664/Quality Adjusted Life-Year (QALY), and $10,621/QALY for ETV monotherapy. Conversely, for HBeAg negative patients the CERs were $38,474/QALY and $34,310/QALY for PEG-IFN and ETV therapy, respectively. The CERs for HBeAg negative patients were higher than for HBeAg positive patients, because the former group more often requires lifelong therapy. All CERs were well below the threshold of $50,000/QALY. Notably, PEG-IFN treatment based on the week 12 stopping rules was the most cost-effective strategy for HBeAg positive disease ($9,501/QALY). The proposed PEG-IFN add-on response-guided therapy from this thesis could increase the CER even more, and therefore favor this approach from an economical perspective. Remarkably, the very low NNT (2.9 among PEG-IFN naïve patients with levels below the proposed cut-offs) revealed the easily reached efficacy threshold for this strategy. In other words, 3 patients would need to be treated with PEG-IFN add-on to achieve response.

The present study is limited by the lack of a PEG-IFN monotherapy arm. We could therefore not determine whether the addition of PEG-IFN or the ongoing ETV therapy, or a synergistic effect of both, attributed to improved response. Although we considered adding a historical control arm of PEG-IFN monotherapy, we chose not to do so because the available data were derived from a study with a markedly different design (no NA pre-treatment or consolidation therapy). The added heterogeneity would have further complicated elucidating the true driver of response. The discordancy was further shown when a preliminary inverse probability of treatment weighting (IPTW) analysis, which aims to correct baseline differences due to heterogeneous study designs, included the PEG-IFN monotherapy arm but failed to adjust sufficiently.

TREATMENT MODIFICATION

Dose-adjusted tenofovir disoproxil in renally impaired hepatitis B

Because first-line NA therapy with tenofovir disoproxil fumarate (TDF) is safe, efficacious and reduces the risk of clinical events, research has focused on further therapy improvements, such as lowering side effects during long-term therapy.[39,40] Throughout a decade of experience with TDF in treating CHB and HIV/HBV co-infection occasional reports have surfaced on renal impairment.

To prevent renal impairment due to TDF treatment, the HBV treatment guidelines suggest dose adjustments in renally impaired patients (eGFR <50mL/min).[41–43] The dosing recommendations are largely derived from pharmacokinetic studies in HIV, but have not been studied in CHB.[44] In **Chapter 5**, the influence of a renally adjusted TDF dose was evaluated on the prevalence of viral and biochemical breakthrough and renal kinetics of CHB patients with impaired kidney function. This study was the first to longitudinally investigate the effect of a reduced TDF dose on virologic and renal parameters in CHB. The renal function decline that was observed during full TDF dose was halted during reduced TDF dosing. Virologic suppression was maintained in most renally impaired CHB patients, even in those with advanced liver disease. This useful, yet simple strategy could be particularly viable in resource-constrained settings. Additionally, this study underlines the importance of monitoring renal function during NA treatment.

Mechanistically, TDF-induced nephrotoxicity is attributed to a mitochondriopathy of the renal tubular cells. Risk factors for nephrotoxicity comprise age, concomitant nephrotoxic medication, comorbidity such as diabetes mellitus and hypertension and HBV-specific factors. In general, TDF not only inhibits viral DNA polymerase, but also to a lesser extent the host cell α and β DNA polymerases and mitochondrial γ DNA polymerase.[45] Mitochondrial toxicity was shown by preclinical and clinical models.[46–48] TDF treatment led to impaired mitochondrial DNA function and glycogen accumulation in the oxidative respiratory chain, which might damage tubular cell function. The lack of ATP reduces the reabsorption of potassium, phosphate and glucose and other small molecules, which may lead to clinical conditions such as Fanconi syndrome.[49] The potential severity of NA-induced mitochondrial toxicity was gravely illustrated by fialuridine, a promising drug for CHB in 1993, that led to acute liver failure, two liver transplantations and the death of five out of fifteen study patients.[50]

The incidence of renal impairment during TDF treatment in CHB varies in the literature. Whereas an increased serum creatinine of ⩾5 mg/dL was observed in 1% of patients receiving TDF for 5 years in clinical trials, large population-based studies have not always corroborated this

finding.[51–55] More importantly for clinical practice, the incidence of hard renal endpoints (renal failure or renal-replacement therapy) was not significantly different between NA-exposed and naïve patients.[56] These differences could be explained by unmeasured confounders such as concomitant nephrotoxic agents, fluctuations in creatinine values and different definitions of renal impairment. To minimize the influence of minor creatinine fluctuations our study therefore defined renal impairment as a confirmed ≥20% decrease in eGFR from baseline, as per the KDIGO CKD Guideline.[57]

Similar virologic and renal outcomes were observed during reduced TDF dose regimens in other studies. Most of these retrospective studies however were small, lacked correction for relevant confounders and were at risk for indication bias.[58,59] A recent non-inferiority RCT was one of the first attempts to prospectively study TDF dose adjustment and virologic and renal kinetics. Forty-six CHB patients with moderate TDF-induced renal impairment (mean eGFR at randomization 55 mL/min) were randomized to receive TDF Q48hr and Q72hr.[60] After 1 year of follow-up no viral breakthroughs had occurred. Renal function did not change significantly during follow-up and was comparable between the study arms (Q48hr vs. Q72hr: 55.6±5.0 vs. 54.6±5.5 mL/min at randomization to 62.5±10.3 vs. 62.3±8.6 mL/min at month 12, p>0.05). This study implies that even a Q72hr dosing regimen maintains adequate virologic suppression while renal function did not further deteriorate. Unfortunately, the lack of a power calculation, absence of correction for confounders and lack of a (historical) control arm with full dose TDF limits the generalizability of the results.

The present study limitations include the retrospective nature and low event rates of virologic and renal events. The latter precluded proposing an optimal dose adjustment or interval. Furthermore, we could not perform prediction analyses to identify patients at risk for renal impairment that could benefit most from an off-label TDF dose reduction. Future studies could investigate effects of TDF dose titration, identify risk factors in treatment selection or externally validate results, although this is unlikely to occur given the recent market approval of tenofovir alafenamide (TAF).

The addition of TAF to the antiviral therapy playbook addressed the need for an effective NA agent with a lower risk of bone and renal side effects than TDF. The HBV treatment guidelines recommend starting TAF in eligible patients at risk for renal (eGFR <60 mL/min) or bone disease.[41–43] Real-world cohort studies from Canada (Canadian Hepatitis B Network), the U.S. and Asia confirmed that TAF was effective and safe and that switching TDF to TAF halted kidney function decline in patients with and without impaired kidney function.[61,62] Among patients with baseline eGFR<90 (CKD stage ≥2), the significant decline in mean eGFR during TDF therapy (p=0.029) stopped after switch to TAF (p=0.90). By week 96, 21% (55/267) of patients with CKD stage 2 at

switch improved to stage 1, 35% (30/85) of CKD stage 3-5 patients improved to stage 2 and 1.2% (1/85) to stage 1. Nonetheless, the potential benefits should be balanced against the high costs of a newly patented drug and incomplete reimbursement in low- and middle-income countries, which might limit the availability of TAF and favour TDF.

Complementary and alternative medicine use

The use of complementary and alternative medicine (CAM) may interact with antiviral treatment efficacy and safety and influence health-seeking behavior. Globally, the use of CAM is common and grows in populations where evidence-based medicine is dominant.[63,64] In parallel with this trend, the incidence of CAM-induced liver injury (ranging from self-limiting side effects to severe hepatotoxicity or even acute liver failure) has also increased.[65,66]

In **Chapter 6**, we therefore studied the use of individual CAM modalities in CHB and examined factors that determined CAM use, particularly those associated with migration, socio-economic status, and clinical factors. The use of CAM among CHB patients was extensive, especially the use of vitamin and mineral supplements. The prevalence of CAM use in our study was higher than in other studies in CHB, which may be due to the comprehensive definition of CAM, the setting where patients were investigated (tertiary referral center versus family practice) and the study population.[67,68]

CAM use did not have any direct influence on liver disease severity. The use of potentially harmful CAM products such as green tea extract (*Camellia sinensis*) or St. John's wort was very low and not associated with liver disease severity. This observation should be interpreted cautiously as few patients used such CAM products and the cross-sectional nature of the survey prevented analyzing longitudinal effects and health behavior. Our concern lies in patients taking a mix of herbs that are difficult to identify. However, this did not occur commonly in our cohort, which was probably biased because all patients visited practitioners in a western style hospital. Alternatively, patients may have used CAM products which contained hepatotoxins but which patients did not consider as CAM or that contents of CAM products were mislabeled.[69] Despite the rare use of hepatotoxins, physicians are encouraged to address CAM use and specific harmful products as part of standard practice, which was currently done by less than half of the treating physicians.

A strong point was that the inclusion of a study population from one of the most ethnically diverse metropoles worldwide enabled us to comprehensively evaluate the association between CAM use and ethnicity. Unsurprisingly, the use of CAM was significantly different across ethnicities. Specifically, mind-body medicine was conducted more often by Black (77%) and South-Asian

patients (54%) than other ethnicities (22%; P < 0.005). Homeopathy (2.8%) and naturopathy (1.4%) were more frequently used by Caucasians than other groups (P = 0.03). The overall use of vitamin and mineral preparations (45%) and herbal product use (16%) did not differ significantly among ethnic groups. An interesting finding was that the use of bio-holistic CAM modalities was associated with socio-economic status rather than with ethnicity or liver disease severity. The significance of socio-economic indicators was echoed by the comprehensive European Social Survey on use of CAM in 33,000 participants from 21 countries.[70] This observation may imply that health care practitioners should focus on socio-economic status rather than cultural or ethnic determinants when asking about CAM use.

CONCLUSIONS AND RECOMMENDATIONS

In this thesis we have aimed to investigate whether NA therapy may be withdrawn before patients achieve functional cure, whether the addition of PEG-IFN to NA therapy improves serological response, whether a reduced TDF dose affects viral and renal kinetics and whether use of CAM influences response or health-seeking behavior.

The withdrawal of NA therapy in an Asian majority cohort suggested limited benefit, especially if patients were HBeAg positive at the start of therapy. Patients with an early and rapid viral load increase after NA discontinuation had the greatest risk of subsequent severe flares, which may prompt more intensive monitoring or immediate retreatment. Secondly, PEG-IFN add-on to ETV therapy was associated with higher response compared to ETV monotherapy in patients with HBeAg positive CHB. Response doubled in PEG-IFN naive patients with HBsAg below 4000 IU/mL and HBV DNA below 50 IU/mL, and therefore identifies these as the best candidates for PEG-IFN add-on therapy. Thirdly, the dose reduction of TDF in renally impaired CHB patients largely maintains renal function and viral suppression, even in those with advanced liver disease. This useful, yet simple strategy could be particularly viable in resource-constrained settings. Lastly, the use of complementary and alternative medicine was common in a clinic-based population with CHB, especially vitamin and mineral preparations, whereas specific hepatotoxic compounds were rarely consumed.

Future perspectives

Elimination of viral hepatitis as public health threat by 2030 has received global awareness when the World Health Organization added this ambitious goal to the Agenda for Sustainable Development. To reach this objective, five core intervention areas have been outlined, one of which is expanding and improving effective therapy regimens. Following the 'call to arms', this

thesis centers on optimizing and tailoring the current treatment paradigm with NA and PEG-IFN therapy and provides a backbone for future clinical trials aimed at achieving functional cure in CHB. Several issues concerning topics in this thesis warrant further investigation.

Tailoring strategies towards NA withdrawal

Carefully selecting patients for NA withdrawal, as emphasized by all guidelines, cannot be reliably done without validated serologic, virologic, or immunologic markers. Inconclusive findings from many studies imply that no single marker predicts relapse. Low end-of-treatment HBsAg values (<100 IU/mL) might predict HBsAg loss, but this finding has not been shown convincingly yet.[71–73] HBsAg levels and other promising predictors (older age, anti-HBc, HBV RNA, and hepatitis B core-related antigen) should be externally and prospectively validated. Risk scores or machine learning algorithms may further improve the precise identification of suitable patients.[74,75] Pretreatment fibrosis that might have regressed during treatment is another determinant that deserves further attention. Asian patients are potentially less likely to benefit from NA withdrawal. Furthermore, pretreatment HBeAg positive patients more often experienced relapse that required retreatment than pretreatment HBeAg-negative patients, all of whom had marginal HBsAg declines. Caucasian patients with HBV genotype D might benefit more, but prospective studies are needed to support these preliminary findings. Some, but certainly not all post-withdrawal ALT flares may increase HBsAg loss. Too little is known about predisposing flare characteristics to provide recommendations either before, or even after, flares start. Hence, clinicians are left trying to ensure that they do not retreat too early to miss benefits, while trying to avoid waiting too long, which could lead to decompensation or worse.

Three critical issues need to be addressed in future NA cessation studies. Retreatment criteria and monitoring frequencies should be standardized. Because essentially all patients experience virologic relapse, this criterion is not suitable for retreatment. Monitoring should probably include measuring ALT monthly and HBV DNA every other month for the first 6 months, and thereafter both every 3 months for the first 1–2 years. Second, biomarkers are needed to distinguish beneficial from detrimental flares as early as possible, which may be extremely challenging to identify. Third, well-defined criteria are needed to assess which patients are likely to decompensate. A threshold that only excludes cirrhotic patients from stopping NAs might be too liberal.

Optimizing response prediction for PEG-interferon

The current study established the value of widely available laboratory markers to identify the strongest PEG-IFN responders. More precise identification of treatment responders at the start of

PEG-IFN add-on therapy will, regardless of ETV discontinuation, influence clinical practice, reduce unnecessary exposure to PEG-IFN, and improve cost-effectiveness. Novel serum biomarkers such as HBV RNA, HBcrAg or intrahepatic cccDNA could be studied as well to enhance prediction modeling. Apart from clinical or laboratory markers, response prediction could also be explored with genetic markers, such as single nucleotide polymorphisms, although previous attempts did not find any strong hits, or epigenetic markers.

Future experimental studies could focus on the immunological effects of add-on therapy and investigate the intrahepatic compartment. How are levels of intrahepatic cccDNA affected by PEG-IFN add-on therapy compared to NA monotherapy? Is the higher rate of HBsAg loss to PEG-IFN add-on in HBeAg negative compared to HBeAg positive patients related to stronger reductions in cccDNA or to other sources of HBsAg production? A requirement for such studies is a validated and standardized assay to measure cccDNA, which is currently not available. Other questions concern whether PEG-IFN add-on could restore the HBV specific T-cell and B-cell response. T-cell exhaustion can be overcome, but to what extent and how to achieve this goal with therapeutic compounds approved or in development remains unknown. Are markers of T-cell exhaustion (PD-1, TIM-3) reduced during add-on treatment? Use of fine-needle aspiration-biopsies would be preferred over a customary liver biopsy. To gain insight into immune control or inflammation, a comprehensive phenotypic and functional analysis of intrahepatic T-cell responses should be done on the smaller number of cells obtained in the context of different disease stages and serum biomarkers.

PEG-interferon as back-bone for future trials

The discovery of a cure for HCV and new *in* vivo and *in vitro* models to study infection with HBV has shifted the attention of clinicians, researchers, policy makers and the pharmaceutical industry towards development of compounds to cure HBV. The definition of HBV cure has been debated extensively over the past years.[76] The strictest definition is sterilizing cure, which is defined as undetectable HBsAg, HBV DNA and removal of intrahepatic cccDNA and integrated HBV DNA. A sterilizing cure is presently not possible with current therapies, has not been observed during the natural course of acute or chronic HBV infection; and more importantly, is not needed to improve clinical outcome. A more feasible alternative is functional cure defined as HBsAg loss, which indicates immune control with sustained virologic remission. HBsAg loss remains the best indicator of long-term favorable outcomes.[43,77,78] A third alternative would be long-term inactive disease (eg, HBV DNA <2000 IU/ mL with normal ALT for ≥2 years), although this endpoint is far less sustainable than HBsAg loss, remains hard to predict and needs follow-up to confirm its robustness.

Numerous compounds are under development that target steps along the viral reproduction cycle or the host-immune response. A growing body of evidence shows that these agents (nucleid acid polymer, capsid assembly modulators, siRNA, Toll-like receptor-7 agonist, therapeutic vaccine) are generally safe and profoundly suppress HBV DNA, but only modestly reduce HBsAg values.[79–81] A promising study on siRNA showed that greater HBsAg declines were observed in treatment naïve HBeAg positive patients, but the sustainability of response beyond week 24 remains unclear.[82]

Since none of the novel compounds appear to cure CHB by itself, the key to functionally curing of HBV probably lies in combining agents with different mechanisms of action, which is conceptually similar to the PEG-IFN add-on strategy. These approaches should aim to reduce HBV DNA and antigens (NA, capsid assembly modulators, siRNA) followed by boosting host immune responses (TLR-7 agonist, RIG-I agonist, checkpoint inhibitors).[83–85] This knowledge would allow researchers to study whether immunological compounds, such as TLR agonists or checkpoint inhibitors, improve durability of response after NA pre-treatmen or to address immunological mechanisms and clinical sequelae of flares that may ensue.[86] Research should focus on agents that permanently silence cccDNA, reduce the number of cccDNA-containing cells and engage the HBV-specific host immune responses to mimic outcomes after spontaneous resolution of an acute HBV infection. The rate of on-therapy degradation of cccDNA indicates the duration of treatment. Promising candidates for such a novel add-on approach are capsid assembly modulators or siRNA agents, which could more efficiently reduce the HBV protein load or even target the persistent cccDNA pool than current NA agents. Combination therapy with immunomodulators and antivirals remains the best strategy in trials aiming to cure CHB.

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CHAPTER 8

SAMENVATTING EN DISCUSSIE

In **Hoofdstuk 1** wordt de achtergrond van dit proefschrift beschreven. Ondanks effectieve vaccinatie vormt chronische hepatitis B (CHB) wereldwijd een aanzienijk risico voor de publieke gezondheid. De huidige behandelopties verbeteren de overleving en kwaliteit van leven, maar vereisen vaak langdurig of zelfs levenslang gebruik, zoals met de virusremmers nucleos(t)ide analogen (NA). Deze behandelingen kunnen namelijk niet het virus volledig uit het lichaam verwijderen. Daarnaast leidt behandeling met immuunmodulator peg-interferon bij een klein deel van de patiënten tot adequate controle van het virus door het immuunsysteem. Deze controle wordt vaak omschreven als verlies van het hepatitis B surface antigen (HBsAg) of functionele genezing.

Dit proefschrift richt zich daarom op het optimaliseren van de behandeling van patiënten met CHB:

- het behouden van het behandeleffect als NA-behandeling wordt gestaakt voordat functionele genezing is bereikt;
- het verbeteren van het behandeleffect door peg-interferon toe te voegen aan de NA-behandeling;
- het effect op virologische en renale kinetiek bij dosisverlaging van NA;
- het bestuderen van het behandeleffect en gezondheidsgedrag bij patiënten die ook complementaire en alternatieve geneeswijzen gebruiken.

POGINGEN TOT GENEZING VAN HEPATITIS B

Stoppen met nucleos(t)ide analogenbehandeling

In **Hoofdstuk 2** onderzochten we in een gerandomiseerde, gecontroleerde studie of het stoppen van de NA-behandeling tot functionele genezing kan leiden bij HBeAg-negatieve ziekte. Deze studie werd uitgevoerd bij patiënten die minimaal 1 jaar behandeld waren met NA-therapie en hiermee het aantal virusdeeltjes adequaat onderdrukten. Deze studie toonde dat het stoppen van NA-therapie leidt tot een grotere kans op recidive en herbehandeling terwijl de kans op functionele genezing juist erg klein was. Met name patiënten die vóór NA-behandeling HBeAg positief waren, ondervonden weinig voordeel van het stoppen van de NA-behandeling. Deze uitkomsten verkregen uit een overwegend Aziatische groep patiënten komen niet overeen met andere studies. Mogelijke verklaringen zijn de langere duur van de NA-behandeling en een ander hepatitis B virus genotype in de huidige studie.

Een belangrijk risico van het stoppen van de NA-behandeling is het optreden van leverontsteking, ook wel hepatitis flare genoemd. Recent onderzoek heeft zich toegespitst op het inschatten van vóórkomen en ernst van deze flares, zodat we weten welke patiënten kunnen stoppen met behandeling en welke zo nodig herbehandeld moeten worden. In **Hoofdstuk 3** hebben we dit

onderzocht in twee verschillende patiëntengroepen: de eerste groep komt uit de Canadese gerandomiseerde studie en de tweede groep is afkomstig uit een beschrijvende studie uit China. De cumulatieve incidentie van flares was hoog (52%) en werd voornamelijk 12 weken na behandelstop gezien. De belangrijkste voorspellers van flares waren mannelijk geslacht en de virale load gemeten 6 weken na behandelstop. Deze resultaten werden deels gevalideerd in de Chinese patiëntengroep. De voorgestelde afkapwaarden in dit hoofdstuk kunnen helpen om in de kliniek in te schatten welke patiënten baat hebben bij meer monitoring en vroegere herbehandeling.

Effect van peg-interferon toevoeging

Hoewel meerdere behandelcombinaties van peg-interferon en NA zijn onderzocht, blijft het optimale regime voor het behandelen van CHB vooralsnog onduidelijk. In **Hoofdstuk 4** hebben we het behandeleffect geëvalueerd als peg-interferon wordt toegevoegd (add-on) aan behandeling met NA entecavir bij patiënten met HBeAg-positieve ziekte. Deze studie borduurt voort op werk uit twee eerder gepubliceerde RCT's (ARES en PEGON-studie). Patiënten werden minimaal 24 weken behandeld met entecavir waarna werd geloot of peg-interferon werd toegevoegd gedurende 24-48 weken of entecavir alleen werd doorgezet. Het behandeleffect, gemeten als serologische respons, trad op bij 33% patiënten met add-on behandeling vergeleken met 20% bij alleen entecavir (P=0.03). Zowel patiënt- als virusspecifieke eigenschappen beïnvloedden de respons. Het grootste effect van add-on behandeling (tot 70%) werd gezien bij patiënten die niet eerder peg-interferon hadden gebruikt, HBsAg-waarden onder 4000 IU/mL en HBV DNA <50 IU/mL hadden ten tijde van de loting. Boven deze afkapwaarden was het behandeleffect klein en niet significant verschillend tussen de studiearmen. De duur van voorbehandeling met entecavir was geassocieerd met HBsAg en HBV DNA, maar niet met het behandeleffect.

BEHANDELINGSAANPASSINGEN

Dosisvermindering van tenofovir bij CHB met verminderde nierfunctie

De veiligheid en effectiveit van eerste lijnsbehandeling met NA zoals tenofovir disoproxil fumaraat (TDF) is in de afgelopen 10 jaar uitvoerig beschreven, maar vanwege de lange behandelduur is het belangrijk om ook de langetermijneffecten in ogenschouw te nemen. Zo weten we bijvoorbeeld dat bij patiënten met HIV/HBV co-infecties die met TDF behandeld worden soms nierschade optreedt. Daarom wordt bij patiënten met CHB met een verminderde nierfunctie de TDF-dosering verlaagd. Het wetenschappelijk bewijs komt echter uit HIV-studies waardoor het de vraag is hoe dit zich vertaalt naar CHB. In **Hoofdstuk 5** beoordeelden we of een aangepaste TDF-dosering bij verminderde nierfunctie invloed heeft op het vóórkomen van

virale en biochemische opvlammingen en op de nierfunctie. De afnemende nierfunctie, wat zichtbaar was tijdens gebruik van de normale TDF-dosering, stopte nadat de TDF-dosering was verlaagd. Virologische onderdrukking bleef adequaat bij de meeste patiënten, zelfs bij diegene met gevorderde leverziekte. Deze bruikbare, doch eenvoudige strategie zou extra van pas kunnen komen in samenlevingen met beperkte financiële middelen. Eerdere studies toonden vergelijkbare uitkomsten, maar konden vanwege retrospectief studiedesign en kleine patiëntenaantallen minder sterke conclusies trekken. De recente toevoeging van tenofovir alafenamide (TAF) aan het therapeutisch arsenaal voor CHB speelde al in op het voorkomen van bot en nierbijwerkingen. Desalniettemin moeten de potentiële voordelen gewogen worden. De hoge kosten van een recent gepatenteerd medicijn en het onvolledige vergoedingsbeleid in lage- en middeninkomenslanden kunnen leiden tot een voorkeur voor TDF.

Complementaire en alternatieve geneeswijzen

Het gebruik van complementaire en alternatieve geneeswijzen (CAM) kan behandeleffecten, bijwerkingen en het gezondheidsgedrag beïnvloeden. Wereldwijd neemt het gebruik van CAM toe, evenals het optreden van CAM-gerelateerde leverschade (variërend van voorbijgaand tot acuut leverfalen). In **Hoofdstuk 6** hebben we bestudeerd hoe vaak verschillende soorten CAM worden gebruikt bij CHB. Daarnaast onderzochten we factoren die het gebruik van CAM beïnvloeden, zoals migratie, socio-economische status en klinische factoren. Onder patiënten met CHB was het gebruik van CAM hoog, met name van vitamine en mineraalsupplementen. Het hogere percentage dat CAM gebruikt kan verklaard worden door de ruime definitie van CAM in deze studie, de onderzoeksplaats (tertiair verwijzingscentrum versus huisarts) en de patiëntengroep. Aan de andere kant leidde het gebruik van CAM niet tot directe leverschade en werden potentieel gevaarlijke CAM-producten (groene thee-extract of sint-janskruid) weinig gebruikt. Desondanks dienen artsen bij patiënten met CHB het gebruik van CAM en schadelijke producten specifiek uit te vragen, wat momenteel door minder dan de helft van de ondervraagde artsen werd gedaan.

Tot slot beschrijft **Hoofdstuk 7** een discussie over de studies in dit proefschrift, suggesties voor verder onderzoek en implicaties voor kliniek en beleid.

CHAPTER 9

APPENDICES

ABBREVIATIONS

ASK1	Apoptosis Signal-regulating Kinase 1
ADV	Adefovir dipivoxil
ALT	Alanine aminotransferase
APC	Antigen-presenting cell
APRI	Aspartate aminotransferase-to-Platelet Ratio Index
BMI	Body mass index
cccDNA	Covalently closed circular DNA
CCR	C-C motif chemokine receptor
CD	Cluster differentiation
CER	Cost-effectiveness ratio
CHB	Chronic hepatitis B
CI	Confidence interval
CpAM	Core protein allosteric modifier
CTLA	Cytotoxic T-lymphocyte–associated antigen
eGFR	Estimated Glomerular Filtration Rate
EOC	End-Of-Consolidation
EOF	End-Of-Follow-Up
EOT	End-Of-Treatment
ETV	Entecavir
GEE	Generalized estimating equation
GWAS	Genome-wide association study
HBV	Hepatitis B virus
HBcAg	Hepatitis B core Antigen
HBcrAg	Hepatitis B core-related Antigen
HBeAg	Hepatitis B e Antigen
HBsAg	Hepatitis B surface Antigen
HBxAg	Hepatitis B x Antigen
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HIV	Human immunodeficiency virus
HSPG	Heparan sulphate proteoglycan
IA	Immune active
IC	Immune control
IL	Interleukin
IP	Interferon-inducible protein
IQR	Interquartile range
ISG	Interferon-stimulated gene
IT	Immune tolerant
LAM	Lamivudine
LCMV	Lymphocytic choriomeningitis
LLD	Lower limit of detection

MCAR	Missing completely at random
MDSC	Myeloid-derived suppressor cell
mITT	Modified intention-to-treat
NA	Nucleos(t)ide analogue
NAFLD	Non-alcoholic fatty liver disease
NAP	Nucleic acid polymer
NASH	Non-alcoholic steatohepatitis
NNT	Number-Needed-To-Treat
n.s.	Not significant
OR	Odds ratio
NK	Natural killer
NTCP	Sodium taurocholate polypeptide
PCR	Polymerase chain reaction
PD/PDL	Programmed death / Programmed death-ligand
PEG-IFN	Pegylated interferon
pgRNA	Pregenomic RNA
QALY	Quality Adjusted Life-Year
rcDNA	Relaxed circular DNA
REVEAL	Risk Evaluation of Viral Load Elevation and Associated Liver Disease
RIG-I	Retinoic acid-inducible gene I
SD	Standard deviation
SNP	Single nucleotide polymorphism
STAT	Signal transducer and activator of transcription
TAF	Tenofovir alafenamide
TBV	Telbivudine
TDF	Tenofovir disoproxil fumarate
TGF-beta	Tissue growth factor-beta
TLR	Toll-like receptor
TIM3	T-cell immunoglobulin and mucin-domain containing-3
TNF-alpha	Tissue necrosis factor alpha
TRAIL	TNF-related apoptosis-inducing ligand
ULN	Upper limit of normal
WAC	Wholesale acquisition cost
WHO	World Health Organization

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PORTFOLIO

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Promotors: Prof. Dr. H.L.A. Janssen, Prof. Dr. R.A. de Man

Co-promotor: Dr. B.E. Hansen

Oral presentations	Year	Workload (ECTS)
Addition of peginterferon alfa-2a increases HBsAg decline in HBeAg-negative chronic hepatitis B patients treated with long-term nucleos(t)ide analogue therapy: Results from a multicenter randomized controlled trial (PAS Study). Annual Meeting AASLD, Boston	2019	1.5
Higher relapse and retreatment rates in patients who started therapy as HBeAg positive than HBeAg negative after stopping long-term nucleos(t)ide analogue therapy: Results from the randomized controlled STOP Study. ILC EASL, Vienna	2019	1.5
Limited sustained response and lack of HBsAg decline after stopping long-term nucleos(t)ide analogue therapy in HBeAg negative patients with chronic hepatitis B: Results from the randomized controlled STOP Study. Annual Meeting AASLD, San Francisco	2018	1.5
Low serum HBsAg and HBV DNA predict response of peg-interferon addition to entecavir in HBeAg positive chronic hepatitis B. ILC EASL, Paris	2018	1.5
Sustained virological suppression and improved renal function with reduced dose tenofovir disoproxil fumarate in renally compromised patients with chronic hepatitis B. Sheila Sherlock Liver Research Day, Toronto	2018	1.5
Low serum HBsAg and HBV DNA levels identify strongest responders of pegylated interferon addition to entecavir in HBeAg positive chronic hepatitis B. Global Hepatitis Summit, Toronto	2018	1.5
Who is using CAM, and why? Complementary and alternative medicine modalities in chronic hepatitis B. Sheila Sherlock Liver Research Day, Toronto	2017	1.5
Addition of (pegylated) interferon to entecavir increases serological response in treatment naïve Hepatitis B e Antigen positive patients with chronic hepatitis B. Najaarscongres Digestive Disease Days, Veldhoven	2016	1.5
Addition of (pegylated) interferon to entecavir increases serological response in treatment naive, Hepatitis B e Antigen-positive patients with chronic hepatitis B. Sheila Sherlock Liver Research Day, Toronto	2016	1.5
Prevalence of Complementary and Alternative Medication (CAM) Usage in Chronic Hepatitis B Patients: A Preliminary Analysis. Sheila Sherlock Liver Research Day, Toronto	2015	1.5

Poster presentations	Year	Workload (ECTS)
Addition of (pegylated) interferon to entecavir increases response in treatment naive HBeAg-positive patients with chronic hepatitis B. ILC EASL, Amsterdam	2017	0.5
Use of various complementary and alternative medicine practices in an ethnically diverse chronic hepatitis B population: role of ethnicity and acculturation. Annual Meeting AASLD, Boston	2017	0.5
Prevalence and predictors of complementary and alternative medicine modalities in patients with chronic hepatitis B. Annual Meeting AASLD, Boston	2017	0.5
Prevalence and predictors of complementary and alternative medicine modalities in patients with chronic hepatitis B. CASL, Toronto	2017	0.5
Effect of (pegylated) interferon therapy to achieve disease remission in chronic hepatitis B. IMS scientific day, Toronto	2018	0.5
Low serum HBsAg and HBV DNA predict response of PEG-interferon add-on to entecavir in HBeAg positive chronic hepatitis B patients. ILC EASL, Paris	2018	0.5
Sustained virological suppression and improved renal function with reduced dose tenofovir disoproxil fumarate in renally compromised patients with chronic hepatitis B. ILC EASL, Paris	2018	0.5
Prevalence and predictors of complementary and alternative medicine use in a migrant-rich chronic hepatitis B population: socio-economics are more important than ethnicity. Global Hepatitis Summit, Toronto	2018	0.5
Low dose tenofovir disoproxil fumarate improves kidney function and sustains virologic suppression in renally compromised chronic hepatitis B patients. Annual Meeting AASLD, San Francisco	2018	0.5
Higher relapse and retreatment rates in patients who started therapy as HBeAg positive than HBeAg negative after stopping long-term nucleos(t)ide analogue therapy: Results from the randomized controlled STOP Study. CASL, Toronto	2019	0.5
Low rate of hepatitis B reactivation among patients with chronic hepatitis C during direct acting antiviral therapy. ILC EASL, Vienna	2019	0.5
Incidence and predictors of flares after discontinuing nucleos(t)ide analogue therapy in HBeAg negative patients with chronic hepatitis B: Results from the randomized controlled STOP Study. ILC EASL, Vienna	2019	0.5
Real-world effectiveness and renal safety of tenofovir alafenamide fumarate among chronic hepatitis B patients in Canada. ILC EASL, Vienna	2019	0.5

Master of Science IMS, University of Toronto, Canada	Year	Workload (ECTS)
MSC 1010H – MSc Seminars in Translational Research	2016-2018	120.0
CHL 5224H – Modern statistical genetics		
MSC 1090 – Introduction to Computational Biostatistics with R		
Module – Global health research		
Module – GREAT epidemiology		
Advanced writing 2		
Advanced writing 3		
Courses & workshops		
Introduction to Data-Analysis; Conceptual Foundation of Epidemiologic Study Design; Regression Analysis; Logistic Regression; Survival Analysis; Genomics in Molecular Medicine; Course on R (NIHES) Explore Statistics with R (Karolinska Institutet, digital)	2013-2016	7.0
Biweekly hepatology journal club (TCLD, Canada)	2015-2019	4.0
Good clinical practice – 'BROK' (NFU)	2015	2.0
Scientific Integrity (Erasmus MC)	2015	0.3
Hepatitis Masterclass (Virology Education, Utrecht)	2015	1.0
Phlebotomy workshop (CIMT, Canada)	2017	0.8
Protecting Human Research Participants (TCLD, Canada)	2018	0.3
Attended conferences, seminars and symposia		
Diner pensant hepatologie (Rotterdam)	2015-2016	0.8
2e Nationale hepatitisdag (Amsterdam)	2015	0.3
Annual Meeting (AASLD)	2015-2020	6.0
International Liver Congress (EASL)	2016-2021	6.0
Sheila Sherlock Liver Research Day (Toronto)	2016-2019	1.2
31st Erasmus Liver Day (Rotterdam)	2016	0.8
Najaarscongres Digestive Disease Days (Veldhoven)	2016	0.3
AASLD/EASL - HBV Treatment Endpoints Workshop (Washington DC)	2016	0.8
International Hepatitis B Cure Workshop (Toronto)	2017-2018	2.0
7th Canadian Liver Meeting (Toronto)	2018	0.3
The 16th Global Hepatitis Summit (Toronto)	2018	1.0
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Travel grant poster presentation (ILC, Amsterdam)	2017	
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Teaching		
Supervising research student Brandon Chan	2017-2018	2.0

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CURRICULUM VITAE

Kin Seng Liem was born on August 19th, 1989 in Rotterdam, The Netherlands. After graduating with honours from the Gymnasium at Krimpenerwaard College in Krimpen aan den IJssel in 2007, Seng started Medical School at Utrecht University. Early on, his scientific interest was aroused during an Honours Programme research project on chronic, unexplained pain in the UMC Utrecht Neurology department (supervisors dr. T.J. Snijders, prof. dr. J. van Gijn). This was followed by an extracurricular research internship in neurophysiology at the Technion Faculty of Medicine in the coastal town Haifa, Israel.

It was perhaps a Hepatology internship at the Academic Medical Center (prof. dr. U.H.W. Beuers), or even the notion that since Babylonian times not the brain but the liver was considered the site of the soul and central place of all forms of mental and emotional activity, that led Seng astray into the field of Hepatology.

After obtaining his medical degree in 2014, Seng embarked on an overseas PhD trajectory in Toronto, Canada, as part of a joint Rotterdam-Toronto research collaborative, supervised by prof. dr. H.L.A. Janssen, prof. dr. R.A. de Man and dr. B.E. Hansen. Four years down the road have enabled Seng to concurrently obtain a Master of Science in Medical Science at University of Toronto and to explore the Atlantic and Pacific coast of Canada.

Upon return to the Netherlands in 2019, Seng started as fledgling resident not-in-training in Internal Medicine at Haaglanden MC, advanced into the postgraduate training in Gastroenterology and Hepatology (programme director dr. A. Langers) and is currently working for two years at Internal Medicine at Groene Hart Ziekenhuis (programme director dr. T. Koster) before continuing at Haaglanden MC (programme director dr. H. van Soest). Seng lives in the Hague, together with Vivien Chung.