Is PD-1 blockade a potential therapy for HBV?

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Immune tolerance and immune-mediated injury during HBV infection

HBV tolerance is partially understood. HBV is a non-cytopathic virus and its pathogenesis lies in immune-mediated liver injury[1]. Most people in the HBsAg-positive population are infected as children. For years or decades, HBV is tolerated with high levels of viral replication. The role of HBeAg[2], the age of infection and the liver environment[3] all appear to be crucial features of this specific immunotolerance. After this stage, immunotolerance and viral replication decrease, HBeAg escape mutations occur and a frequent scenario is HBsAg persistence, low replication and mild or no liver disease. Subsequently HBsAg can be cleared, or liver disease may progress to chronic hepatitis and cirrhosis. The main complication of HBV infection is hepatocellular carcinoma (HCC). Although oncogenic pathways are related to the life cycle of HBV (transactivation by the HBx protein and DNA viral integration into the liver genome), one very important risk factor for the development of HCC is liver inflammation and regeneration, both of which are mediated by adaptive immunity.

T-cell exhaustion and immune checkpoint proteins during HBV infection

Programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) are receptors of the CD28 family of co-stimulatory molecules that provide inhibitory signals to T-cells. In chronic viral hepatitis, upregulation of PD-1 and CTLA-4 is associated with T-cell exhaustion and persistent viral infection, favouring the chronicity of viral disease but limiting immunopathogenesis. Intrahepatic T-cells also upregulate BTLA and produce IL-10 that inhibits effective T-cell function[4]. HBsAg-specific B cells that are unable to mature *in vitro* into antibody secreting cells, and that display an increased expression of PD-1, could be partially boosted by the addition of anti-PD-1[5].

The study by Martinez and colleagues published in this issue of *JHEP Reports* shows for the first time that the upregulation of the PD-1:PD-L1 axis in patients with chronic HBV is not normalised in patients treated with nucleoside analog reverse-transcriptase inhibitors (NAs) with undetectable viremia for long periods of time. Furthermore, anti-PD-L1 blockade increased both the number of IFN-γ-producing T-cells and the amount of IFN-γ produced per cell in all patients with detectable HBV reactivity, independently of their clinical and treatment status. *Ex vivo* studies using blood from individuals with chronic HBV infection have demonstrated that inhibition of PD-1, CTLA-4 and TIM3 leads to enhanced HBV-specific CD8+ T-cell function[6–9]. In view of the pathogenesis of HBV and of recent achievements with immune checkpoint inhibitors (ICI) in cancer therapy, the latter may potentially enhance HBV-specific CD8+ T-cell activity and even the production of antibodies against HBsAg.

ICI for a HBV cure: At no risk?

One obvious issue is that ICI-induced immune restoration can lead to severe immune-mediated liver damage and inflammation, increasing the risk of HCC and liver failure. Furthermore, the HBV-DNA that is integrated in the DNA of most hepatocytes, transcribes the mRNA of viral proteins which are translated into viral proteins. One could imagine hepatocytes free of covalently closed circular DNA (cccDNA) which express viral proteins and could be the target of an uncontrolled and specific immune response. Under this hypothesis, transgenic HBsAg-Tg mice whose immunotolerance had broken through the TIGIT blockade, developed liver disease and HCC[10]. Immune-related adverse events due to the ICI response are delayed, long-lasting and not always controlled by corticosteroids.

In populations treated for HCC and thus frequently infected by HBV, checkpoint inhibitors have shown encouraging safety: an open label study of nivolumab (anti-PD-1 mAb) involving 20 European participants with virally suppressed chronic HBV infection, suggested that it was safe and well tolerated, at least in the short term[11]. In patients treated with ICI, few cases of hepatitis B reactivation have been observed in the event of concomitant immunodeficiency (chemotherapy, untreated HIV, bone marrow transplantation) and they resolved after HBV antiviral treatment. Larger populations and longer follow-up periods are required. However, these patients with HCC, liver disease and previous therapy with sorafenib clearly differ from those who should have an indication for ICI in HBV but not in HCC: *i.e.* younger patients without cancer or significant liver disease.

One important concern is non-HBV-related adverse effects of ICI, particularly since a combination of ICIs may be necessary to break down immune tolerance. In the CheckMate 067 phase III trial of patients with metastatic melanoma, more than 90% of those who received combined anti-CTLA4 and anti-PD-1 mAb experienced at least one immune-related adverse event, and about 50% had a serious immune-related adverse event. Although still rare, the cardiovascular, liver or neurological toxicity of immunotherapy may be serious and potentially fatal[12–15]. These

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adverse effects are acceptable in the context of rapidly fatal cancer but not in other cases.

ICI for HBV: Does it work?

The first trial of ICI for HBV infection was recently reported in virally suppressed HBeAg-negative patients[16]. During this phase I study, evaluating nivolumab with or without vaccination (GS 4774), a modest decline of HBsAg levels was frequently observed and 1/12 treated patients experienced HBsAg seroconversion. This trial concerned patients with neither HCC nor significant liver disease and no serious adverse event was reported. As depicted in Table 1, no phase II trials have been notified, either in the clinicaltrial.gov site or in meeting abstracts.

Because HBV is not a contraindication in most ICI trials, many HBV-positive cancer patients have been treated and the eventual, if any, long-term occurrence of HBsAg seroconversion can be monitored. The CheckMate 040 trial explored the administration of nivolumab in both Western[17] and Eastern[18] patients with HCC, including over a hundred HBsAg-positive individuals. Although no HBV reactivation was detected, 9-11% of patients with HBV exhibited an HBV-DNA increase >1 log from baseline[18]. PD-1 blockade showed limited antiviral activity, and no patient exhibited HBsAg seroconversion[17]. Thus, one could hypothesise that the rate of HBsAg seroconversion in treated patients is not significant. There are major limitations when studying the long-term effects of ICI in HBV-infected patients with cancer: previous or concomitant immunosuppressive therapies and the limited survival of relevant patients. In terms of safety and efficacy endpoints, the current HBV-infected population in which ICI are given differs from that which should be targeted by any HBV cure strategy.

Finally, the most encouraging data on efficacy and safety came from a relatively old paper on inhibition of the PD-1/PD-L1 and PD-L2 pathways in a woodchuck hepatitis virus model[19] which resulted in complete viral clearance in some animals. The addition of anti-PD-L1, entecavir and a therapeutic vaccine led to an enhanced immunological and clinical response, that was not associated with hepatotoxicity. These data suggested significant enhancement of antiviral effects for ICI.

HBV cure: A change of mind is necessary in the field

As recommended by most consensus guidelines and conferences, the large population of immunotolerant individuals, or those with so-called inactive disease, are currently not treated with NAs. The principal argument is that NAs need to be lifelong in these patients, thus stressing the need for the development of a strategy for HBV cure. The control of HBV replication by NAs has a clear impact on fibrosis, decreases the risk of HCC but rarely leads to HBsAg seroconversion. The relapse of HBV replication is due to hepatocyte HBV cccDNA which is not sensitive to NAs and is the most relevant target for new anti-HBV agents[20]. The withdrawal of NAs does not always lead to a relapse[21]. In a recent study[22], the authors showed that the population of HBV-specific T-cells present in the blood of patients with HBV who had successfully discontinued NAs without a hepatic flare are enriched for PD-1, but that these cells are functional in their proliferative and IFN-γ secretion capacities. These observations are in line with the lymphocytic choriomeningitis virus model showing that PD-1 expression on T-cells may be a stable form of functional differentiation with limited, recoverable, cytokine production and antiviral functions, which avoids T-cell deletion due to high

Table 1. Clinical trials on chronic HBV (excluding NA)

Therapy	Clinical trial	Combination	Last updated	Company/country	Results
GS-4774 vaccine	NCT01943799	Interferon ± GS-4774	2015	Gilead	No decline of HBsAg
TLR7 agonist GS-9620	NCT02166047	NUC	2016	Gilead	No decline of HBsAg
PD-1 inhibitor ± GS-4774	no NCT	PD-1 ± GS-4774	2017	BMS	Modest decline of HBsAg
PD-1 inhibitor	ACTRN12615001133527 [16]	NUC	2019	Gilead	Modest decline of HBsAg
Intestinal microbiota transplantation	NCT03429439	Interferon	2017	China	
TG1050: adenovirus vector	NCT02428400	-	2018	Transgene	
Myrcludex	NCT02881008	NUC	2018	Hepatera	No decline of HBsAg
GS-4774 vaccine	NCT02174276	TDF ± GS-4774	2019	Gilead	No decline of HBsAg
TLR8 agonist GS-9688	NCT03491553	NUC	2019	Gilead	
Hepadvax	NCT03038802	Engerix	2019	Vaxine Pty Ltd Australia	-
RIG-I agonist inarigivir	NCT03932513	NUC	2019	Gilead	
siRNA	NCT03772249	-	2019	Dicerna Pharmaceuticals	
DV 601 vaccine	NCT01023230	NUC	2019	Dynavax Technologies Corporation	
ABI-H0731 core inhibitor	NCT03109730	NUC	2019	Assembly Biosciences	
NVR 3778 core inhibitor	NCT02401737	± Interferon	2019	Novira Therapeutics, Inc.	Modest reduction of HBV-DNA, no effect on HBsAg
Hepatitis B immune globulin	NCT03575208	Interferon	2019	NIH USA	
BTLA-4 inhibitor	-	-	-	-	-
CTLA-4 inhibitor	-	-	-	-	-
CART cells	-	-	-	-	-

antigenic load in chronic infections[23]. Indeed, long-term NA treatment partially restores HBV-specific T-cell function[24 25].

An HBV cure of any type should have a very favourable risk-benefit ratio. The potential harms and effectiveness[26–28] of ICI blockade will require careful tailoring and monitoring. The development of *ex vivo* immunological assays, as described in the paper by Martinez, will be useful. Awaiting the HBV cure, it would be a good idea to broaden the indications for our "good old" NAs.

Conflict of interest

The authors have no conflict of interest.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhepr.2019.07.007.

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