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 injury1. 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 HBeAg2, the age of infection and the liver environment3 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 function4. 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-15.

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 short term11. 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 fatal12-15. These
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. 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 and Eastern 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. PD-1 blockade showed limited antiviral activity, and no patient exhibited HBsAg seroconversion. 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, 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. The withdrawal of NAs does not always lead to a relapse. In a recent study, 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 | 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 | Vaccine 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 | NCT02407377 | ± 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 | - | - | - | - | - |
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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