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HBV-specific T cells as a Biomarker for Discontinuation of Nucleos(t)ide Analogue Therapy for Chronic Hepatitis B

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Hepatitis B virus (HBV) can cause severe liver diseases and is a major health problem. It chronically infects approximately 250 million people worldwide. Nucleos(t)ide analogs (NUCs), including lamivudine, adefovir, entecavir, tenofovir and telbivudine, are the most frequently used drugs for treating chronic hepatitis B (CHB) patients. NUCs can efficiently suppress HBV DNA replication and reduce viral load, leading to the loss of the HBV e antigen (HBeAg), the appearance of antibodies against HBeAg, and the normalization of the alanine aminotransferase (ALT) level. However, they cannot target the HBV genomic DNA, also known as the covalently closed circular DNA (cccDNA), in infected hepatocytes. For that reason, the discontinuation of the NUC therapy can lead to viral rebound and the development of hepatic flares, and a lifelong treatment of this therapy is often required for CHB patients. Nevertheless, there are also some patients for whom the treatment with NUCs can be safely stopped. Unfortunately, the biomarkers for the identification of this subset of patients are currently lacking. Recently, Rivino et al. found that the population of PD1⁺ HBV-specific T cells was much higher in chronic HBV patients who successfully discontinued the NUC therapy without developing hepatic flares than in those patients who developed hepatic flares (1). Their finding indicated that PD1⁺ HBV-specific T cells might serve as the much-needed biomarker for safe discontinuation of the NUC therapy for CHB patients.

Because of the potential drug toxicity, the emergence of drug-resistant mutants or other reasons, the NUC therapy may have to be stopped for some CHB patients. This termination of treatment poses a risk of viral rebound and the development of hepatic flares (2). The guidelines published by the American Association for the Study of Liver Diseases (AASLD)

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Conflict of Interest

The authors declare no conflict of interest.

in 2016 indicate that the best endpoint of the NUC therapy is the loss of the HBV surface antigen (HBsAg). However, this loss of HBsAg is infrequently achieved (3). Thus, there is a strong need for alternative biomarkers for safe stopping of the NUC therapy.

Instead of focusing on virological parameters, Rivino et al. focused on the identification of immunological biomarkers to predict whether the NUC therapy can be safely discontinued (1). They conducted a longitudinal analysis on CHB patients during and after the termination of the NUC therapy using different assays to understand immune profiles associated with the control of HBV or viral relapse upon NUC discontinuation. Their attention on immune profiles is sensible, as both clinical and animal studies indicate that the control of HBV infection is associated with effective innate and adaptive immune responses (4, 5).

Rivino et al. studied a cohort of 29 HBeAg-negative CHB patients who received initially the lamivudine and tenofovir combination therapy but subsequently only the lamivudine monotherapy for 48 weeks. After the exclusion of patients who experienced an increase in HBV DNA and those who chose to remain on the lamivudine therapy, patients were separated into 2 groups based on the presence (i.e., ALT levels >80 IU/ml) or absence (i.e., ALT levels ≤ 80 IU/ml) of hepatic flares during the 6-month period after the termination of the therapy. Patients with hepatic flares displayed in general 100-fold higher levels of HBV DNA than patients without flares during viral rebound after the therapy. Rivino et al. collected the peripheral blood mononuclear cells (PBMCs) from patients before and after the withdrawal of therapy and treated those cells with HBV peptides for ten days to expand HBV-specific T cells, which were then analyzed by the ELISPOT assay and flow cytometry. They found that patients who did not have hepatic flares after the withdrawal of the therapy had higher levels of HBV core and polymerase-specific T cells during the NUC therapy than those who had flares. By conducting intracellular cytokine staining, they found that HBV-specific T cells were mostly contained within the CD8⁺ T cell subset. Their studies indicated that, during the NUC therapy, the detection of core and polymerase-specific T cells that possessed *in vitro* proliferative ability could predict whether patients may safely withdraw from the NUC therapy.

Rivino et al. validated their finding in a second cohort of 27 HBeAg-negative CHB patients who received the monotherapy of entecavir, tenofovir or telbivudine. The same as in the first cohort, patients in the second cohort that did not flare after the withdrawal of the NUC therapy had a higher frequency of HBV core or polymerase-specific T cells during the NUC therapy than patients who did. By combining the studies of both cohorts, Rivino et al. concluded that if patients displayed an HBV core or polymerase-specific T cell response that generates over 40 spot-forming cells per 10⁵ cells in an ELISPOT assay, they would have a high probability of controlling HBV without developing severe hepatic flares after the termination of the NUC therapy. Recently, it was reported that a high level of antibodies against the HBV core antigen (HBcAg) at the end of NUC treatments was associated with a reduced risk of clinical relapse (6). It will be interesting to determine whether this higher level of antibodies against HBcAg is related to the higher HBV-specific T cell activities.

The analysis to characterize HBV-specific T cells is tedious. For that reason, Rivino et al. conducted Cytometry by Time-of-Flight (CyTOF) analysis to examine immune cell populations using the first patient cohort to identify additional biomarkers that might be associated with HBV control. Unfortunately, they found that patients with and without hepatic flares displayed similar frequencies and functional properties of T, B and natural killer (NK) cells, with only one exception that the frequency of CD4⁺ T cells was increased in patients with no flares. Such difference in frequency of CD4⁺ T cells was not observed in the second cohort. They also studied the transcriptional profiles of selected immune genes in CD4⁺ and CD8⁺ T cells during the NUC therapy by NanoString analysis and found that patients with no flares had increased expression of genes encoding costimulatory proteins/inhibitory receptors, including PD-1, TIM3, EGR2, KLRC1 and SLAMF1, in CD8⁺ T cells. The higher expression of PD-1 and TIM3 was also detected by CyTOF in CD8⁺ T cells of patients that did not flare. However, this differential expression of PD-1 in CD8⁺ T cells was not observed between the two groups of patients in the second cohort. Nevertheless, they found a positive correlation between the levels of PD-1 in total CD8⁺ T cells and the frequencies of *in vitro*-expanded HBV-specific T cells. They also found that HBV-specific T cells were enriched in the PD-1⁺ fraction of T cells after cell sorting and that PD-1⁺ CD8⁺ T cells from patients that did not flare had the tendency to produce higher levels of cytokines than those from patients that flared. The observation that PD-1 upregulation was associated with HBV-specific CD8⁺ T cells in patients without flares is interesting. Based on a previous report that PD-1 may play a role in the long-term survival of antigen-specific CD8⁺ T cells (7), Rivino et al. suggested that the expression of PD-1 in T cells might help to preserve a population of HBV-specific T cells, which otherwise would be depleted by chronic viral antigen stimulation.

The studies of Rivino et al. identified PD-1⁺ HBV-specific T cells as a potential biomarker for the safe discontinuation of the NUC therapy for CHB patients. This is an important step forward for improving the clinical care for CHB patients, and additional clinical trials to test this biomarker are warranted. As the analysis of PD-1⁺ HBV-specific T cells involves complicated procedures, additional improvements to simplify the assay will be needed for this biomarker to become useful in the clinics. The studies of Rivino et al. also raised some interesting questions. For example, since the magnitude of the HBV-specific T cell responses in patients with hepatic flares was low, it is curious regarding what causes the hepatic flares after the cessation of the NUC therapy in this group of patients. Similarly, it is unclear whether the inclusion of treatment that blocks the PD-1 and PD-L1 interaction will enhance HBV clearance in patients with PD-1⁺ HBV-specific T cells. These are the questions that deserve further attention.

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