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To target or not to target viral antigens in HBV related HCC?

To the Editor:

Chronic HBV is a leading cause of hepatocellular carcinoma (HCC) worldwide. Targeting viral antigens with immunotherapy seems an attractive treatment option. Bertoletti and colleagues have now taken a well-appreciated first step towards clinical application by treating an HBV-related HCC patient with autologous T cells engineered to recognize HBV surface antigen (HBsAg) [1].

The patient presented with recurrent HCC after liver transplantation. HBsAg was expressed by metastasized tumor cells but not donor-derived liver hepatocytes. Upon immunotherapy with HBsAg-specific T cells, a drop in blood HBsAg accompanied by T cell activation and degranulation was observed, from which the authors concluded tumor cells were recognized and attacked. With this result they claim to "confirm the feasibility of providing autologous TCR-redirected therapy against HCC and advocate this strategy as a novel therapeutic opportunity in hepatitis B-associated malignancies". Although we acknowledge HBsAg-specific T cell activation occurred, we wonder whether sufficient evidence was provided to conclude that T cells really acted on tumor cells and whether this therapeutic approach is feasible. Furthermore, based on previous reports on (limited) HBV antigen-expression by tumor cells and the very restricted eligible patient group, we challenge the therapeutic opportunity presented.

A primary question is whether HBV antigens represent feasible targets for immunotherapy against HCC. For a long lasting therapeutic effect, HBV antigens need to be; (1) stably expressed by tumor cells and presented in major histocompatibility complex (MHC)-I and; (2) absent/low on non-tumor tissue to prevent collateral damage. We have serious doubts these conditions are met in sufficient patients to justify further development of HBV antigen-targeted therapies for HCC, in the context of liver transplantation or not.

Although the tumors of the patient described by Qasim and colleagues were positive for HBsAg and MHC-HBsAg peptide complexes, they did not test if the tumor evolved to an HBsAg negative state once under immune attack. Importantly, a recent study by Faria and colleagues showed that despite presence of HBV DNA in most HCC recurrences, HBsAg (and other HBV antigens) are only detected in a minority of recurrent tumors [2]. Although HBV DNA integrates into the host genome in 85–90% of patients, integrations often lead to disruption of viral proteins and expression of HBV-host fusion constructs [3,4]. Moreover, in HCC, genes coding for viral proteins are often methylated (mostly C and S) or mutated (mostly X), augmenting or even fully abrogating expression, reducing the chance of presentation of regular HBV sequences in MHC [5,6].

In light of expression of HBV antigens on non-tumor liver tissue, it was rightly argued by Qasim and colleagues that only after transplantation (a minority of HCC cases), the liver is free of HBV antigens, and not subject to "on-target" effects. After liver sparing treatments, HBV continues to reside in the liver, rendering these patients (the vast majority) at risk for collateral damage by HBV-targeted therapy. Liver function in these patients is often severely compromised by chronic HBV and thus the chance that such therapy will destroy any remaining liver function is a high and unacceptable risk. This, in our opinion already represents a major obstacle, limiting widespread application. Moreover, Faria *et al.* observed the recurrence of HBV infection in 7 out of 8 patients with recurrent HCC after transplantation [2], indicating the high likelihood of HBV antigen presence in healthy liver tissue, possibly even higher than in tumors. Thus even in this most favourable setting there is considerable risk of collateral damage. Although in this case the transplanted liver was claimed to be free of HBV, the reported high prevalence of recurrent HBV infection in recurrent HCC [2], together with the observed rise in ALT values (indicating treatment-induced liver damage), suggest some level of undetected HBV infection may still have been present in the liver graft. Adding this to the lack of tumor shrinkage, we have serious doubts whether T cells attacked tumor cells rather than transplanted liver cells and thus if therapy feasibility, as stated by the authors, was really demonstrated.

In summary, the lack of evidence that HBV antigens are stably expressed on HBV-related HCC cells together with the high collateral damage risk, in our opinion, do not plead for targeting of regular HBV antigens for HCC immunotherapy.

So what should be targeted? Many so called "tumor antigens" have been reported for (HBV-related) HCC which may represent attractive targets (e.g., glypican 3, AFP). Similar to HBV antigens however, these are often also expressed elsewhere in the body and therefore also dangerous targets [7]. Recently, Bertoletti and colleagues argued that the safest targets may be mutated tumor proteins (e.g., neoantigens) [8]. In addition, we envision also tumor-specific HBV antigen mutations could be safe targets, provided stable expression. Screening for the most prevalent mutations in host and HBV antigens, together with mapping the tumor HLA-peptidome could lead to a panel of neoantigens to target or to patient specific targets.

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Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Author's contributions

S.I.B. drafted the manuscript and D.S. and A.M.W. critically revised the manuscript for important intellectual content.

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Reply to: "To target or not to target viral antigens in HBV related HCC?"

To the Editor:

We welcome a discussion about the therapeutic potential of HBV-specific TCR redirected (HBV-TCR) T cells in HBV-related HCC offered by Buschow *et al.*, who express strong reservations about the conclusions of our recent work [1] and about the use of HBV antigen as a target of HCC immunotherapy.

First, Buschow *et al.* challenged our idea that HBV antigen can be used as a target for HCC immunotherapy, suggesting that in our HBV-TCR T cell treated patient we "lack evidence to conclude that T cells really acted on tumor cells". They are instead proposing that the drop of HBsAg observed in the patient could be explained by the T cells targeting "some level of undetectable HBV infection of the transplanted liver" and not the HCC metastases.

In the liver-transplanted patient with extrahepatic HCC metastases described in our report (see Supplementary Material of our paper for the detailed clinical history) [1], liver biopsies were obtained from the transplanted liver and from the extrahepatic HCC metastases. HBsAg was found only in HCC metastases and not in the liver.

Furthermore, despite not being on anti-viral therapy, the patient sera was HBsAg positive but consistently HBV-DNA negative (a test performed monthly for the first 3 years after transplantation and every 3 months thereafter). HBV-DNA was also not found in the biopsy of the transplanted liver, while a truncated HBV-DNA coding only for HBsAg was detected in the biopsy material of the HCC metastasis. We sequenced this integrated section of HBV-DNA and demonstrated that it was coding for a non-mutated sequence of the HBs183-91 region that is recognized by our HBs183-91-directed TCR. We further characterized the extrahepatic HCC metastasis by staining them with a T cell receptor-like antibody specific for HBs183-91/HLA-A2 [2], demonstrating that HCC cells of this patient presented these specific HBV-peptide/HLA-class I complexes on their surface. Thus, we have provided extensive experimental evidence showing that HCC metastasis can process and present HBsAg in a form recognizable by our adoptively transferred HBV-TCR T cells. In contrast we failed to find any evidence of the presence of HBV and/or HBsAg expression in the transplanted liver of this patient.

Based on these results we have difficulty understanding how Buschow could hypothesize that the HBsAg drop and the HBV-TCR T cell expansion observed after adoptive transfer derived from T cell recognition of HBV-infected hepatocytes (that we cannot detect) and not, simply, from the recognition of extrahepatic HBsAg-expressing HCC cells.

Buschow *et al.* may say that only "seeing is believing" and we have to admit that we do not have a direct *in vivo* visualization of adoptively transferred T cells interacting with HCC cells in the patient. Nevertheless, we prefer to base our interpretation on the experimental evidences and not on speculation.

Buschow *et al.* then criticized the use of HBV antigen as an HCC-tumor antigen for immune intervention, providing arguments of limited quantitative and temporal HBV antigen expression in HCC cells and stressing the supposed "rarity" of our reported case. They argued that "For a long lasting therapeutic effect, HBV antigens need to be stably expressed by tumor cells and presented by MHC-I molecules" and point out that we did not test in our report if the tumor evolved to an HBsAg negative status under immune attack. To support the limited HBV antigen expression in HCC they quote a study of Faria *et al.* [3] that reported a reduced expression of HBsAg in tumors despite the presence of HBV-DNA in most HCC recurrences.

The question of stability of HBsAg expression under immunotherapy is puzzling and, we have argued in our report that, HBV-TCR T cells could not only lyse but also modulate HBsAg expression. Nevertheless, in previous experiments in animal models, adoptive transfer of HBV-TCR T cells did not suppress HBsAg production but resulted in lysis of HCC cells [4,5].