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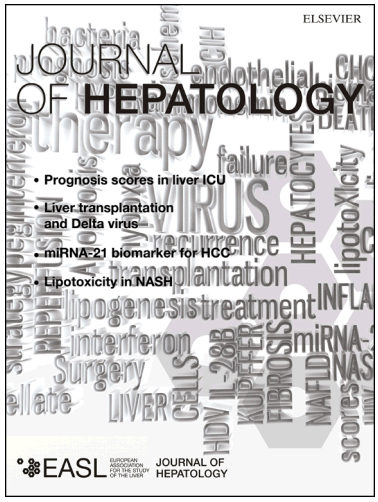

Letter to the Editor

Reply to: " To target or not to target viral antigens in HBV related HCC? by Buschow et al

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Reply to: " To target or not to target viral antigens in HBV related HCC? by Buschow et al.

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We welcome the possibility for a frank 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+ but consistently HBV-DNA negative (a test performed monthly for the first 3 years after transplantation and every 3 monthly 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 don’t have a direct in vivo visualization of adoptively transfer 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 didn’t 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].

However stable HBV antigen expression and MHC-class I presentation (before therapy) was not only detected in the HCC cells of our treated patient but also on other natural HCC cell lines with HBV-DNA integration[4].
Thus, at the moment, the experimental evidence shows that, at least at the time of therapy, HBV antigen was stably expressed in HCC cells, while there was no experimental evidence of the opposite. Indeed Buschow’s argument that “ HBsAg expression” was not detected in most HCC cells with HBV-DNA

integration or that HBV-DNA integration leads to disruption of the viral proteins or expression of host-viral chimeric protein is misleading since it does not take into consideration the difference between viral antigen recognition by T cell receptors or by antibodies. The fact that antibodies would fail to detect HBsAg in cells with HBV-DNA integration (as quoted by Faria et al[3]) shows that the whole HBsAg, with the correct conformation specific for the used antibody, was not produced in these HCC cells. However, negative detection with an antibody cannot exclude that the HBsAg with a different conformation or only a selected section of the antigen are expressed. T cells recognize short fragments of viral proteins presented by HLA-class I molecules and such fragments can perfectly result from the processing of truncated proteins. This is why, in contrast to what Buschow argued, we consider the high incidence of HBV-DNA integration [3,6](truncated HBV-DNA or hybridized with host protein[7]) in HBV related HCC not as a drawback but as a great opportunity to TCR-mediated immunotherapy. Certainly, as we discussed in a recent commentary (Bertoletti et al, Oncoimmunology 2015 in press), more work needs to be done to understand the difference between T cell recognition of HCC transformed hepatocytes or of HBV infected hepatocytes and to map the landscape of HBV epitopes expressed in HCC with HBV-DNA integration. Also Buschow's suggestion that methylation or mutation of the integrated HBV genes will result in reduced viral protein expression is, in our opinion, weak. T cells can be activated by very few viral peptide/HLA complexes present on the target cell surface and our virus-specific TCR are of high avidity, being able to recognize target pulsed with femtomolar concentrations of peptide[2,4]. More importantly, recent work characterized the whole transcriptome of different viral related tumors and demonstrated the presence of HBV related mRNA in more than 30% of analyzed HCC[8]. If we consider that in this analysis, liver tumors were not necessarily HBV-related, it appears difficult to agree with Buschow 's assertion that expression of HBV products in HCC represents an exceptional event.

Last, Buschow et al argued that our therapy is characterized by high and unacceptable risk for the patient. Patient safety is our primary concern and this is why we treated our patient only after repetitive testing showing that

HBV was undetectable in the transplanted liver. We agree that such therapy could carry risk to non-liver transplanted HCC patients, where HBV-specific TCR can also target non-malignant HBV infected hepatocytes. Nevertheless, immunotherapeutic approaches to boost T cell responses in patients with ongoing HBV replication in the liver are being actively considered by a number of groups; discussion of this is beyond the scope of this response. However, we are developing a strategy involving mRNA TCR electroporation to shorten the length of TCR-expression on T cells with the goal of reducing the risk of liver damage in HCC patients who have not received a liver transplant [5]. A further alternative would be to include a suicide gene mechanism to allow transduced T cells to be eliminated in case of unwanted effects in vivo.

Here, we would like to focus the discussion on the setting of HCC relapses occurring in liver-transplanted patients. Bushow et al state that since HBV re-infection of the transplanted liver can supposedly occur frequently, the risk of liver damage due to the recognition of HBV infected hepatocytes by the adoptively transfer HBV-TCR T cell remains " unacceptably high". However, what Buschow et al do not consider is the possibility to use therapeutic TCR that recognize HBV antigens presented by HLA alleles expressed in the metastatic HCC lesions but not in an HLA-mismatched liver transplant. This level of personalized construction of T cells results in the adoptive transfer of HBV-specific T cells that cannot recognize HBV infected hepatocytes of the transplanted liver. This is why we reject Buschow's statement that TCR-HBV based therapy would subject liver transplanted patients to a "considerable risk of collateral damage". Certainly, results obtained from a single case should be taken with caution and we agree that immunotherapy carries inherent risks[9]. However the targeting of normal HBV infected hepatocytes in liver transplanted patients can be theoretically eliminated after a careful characterization of the patients' pathology, HLA-profile and the subsequent precise and selective engineering of HBV-TCR T cells.

In conclusion, despite the criticisms expressed by Buschow et al., we are convinced that HBV antigen can be a target of HCC immunotherapy. Our data[1], combined with the characterization of the frequency of HBV-RNA [8]

and HBV-DNA integration in early [10] and late-onset[3,6] HCC, support this possibility. Certainly we are far from concluding that HBV-TCR T cells constitute " the cure" for such devastating diseases, but we derived a "glimpse of hope" from our first attempt to target HBV antigen in HCC using engineered T cells.

Ironically, after having argued about the un-suitability of HBV as an HCC target, Buschow proposes to target " HBV mutated protein" in HCC, an option that is in agreement with our conclusion to evaluate HBV antigen as a target for HCC. However, it is not clear how HBV mutated sequences could be selectively expressed in HCC and not in normal HBV infected hepatocytes and why, differently from non mutated sequences, these should be stably expressed. Nevertheless, we welcome any discussions that boost research in this area and help design new therapies that can provide hope to the patients affected by HCC.

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